DOCTORAL THESIS

ASSESSING THE IMPACTS OF AQUATIC INVASIVE SPECIES IN FRESHWATER ECOSYSTEMS

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SUMMARY

English version

As more regions of the World and especially within Europe are becoming increasingly connected, more and more countries are subject to globalization and the threat of alien species getting a foodhold in even the furthest places is increasing. How will science, stakeholders and governments tackle this increasing danger to human well-being and natural ecosystems with their biodiversity? This thesis is organized in three parts: a) a summary of the current knowledge on impacts of invasive alien species and their assessments; b) 6 studies addressing single species impact, and c) 4 studies investigating interactions among alien and native species. As a result, the conducted studies contribute to improve the knowledge on the investigated species, building a base for future studies on some neglected species, such as the North American channel catfish *Ictalurus punctatus*. Moreover, the works conducted on the ecosystem level highlighted the different nature and diverse interactions among alien species. Although the studied species differ, the results indicate that a) an alien predator exerts a lower impact on the invaded ecosystem and the recipient fauna if prey items with the same life-history are abundantly available, and b) differing life-history and history of co-evolution are favouring the competition among predators with unforeseeable effects on other present species. Thus, this work highlights the needs for assessments on a case by case basis, as interactions among species vary according to ecosystem and present species.

Italian version

Poiché le regioni del mondo sono sempre più collegate, sempre più paesi sono soggetti alla globalizzazione; la minaccia delle specie aliene che possono trovare una possibilità di arrivare anche nei luoghi più lontani è in aumento. In che modo la scienza, i portatori di interesse e i governi affrontano questo crescente pericolo per il benessere umano e gli ecosistemi naturali con la loro biodiversità? La presente tesi è organizzata in tre parti: a) una sintesi delle attuali conoscenze sugli impatti delle specie aliene invasive e delle loro valutazioni; b) 6 studi riguardanti l'impatto di singole specie e c) 4 studi che indagano le interazioni tra specie aliene e native, che influenzano l'impatto delle rispettivi IAS. Di conseguenza, gli studi condotti hanno contribuito ad aumentare la conoscenza delle specie indagate, costruendo una base per studi futuri su specie poco conosciute, come il pesce gatto punteggiato nordamericano *Ictalurus punctatus*. Inoltre, i lavori condotti a livello di ecosistema hanno evidenziato la differente natura e le diverse interazioni tra le specie aliene presenti. Sebbene le specie studiate fossero diverse, i risultati indicano che a) un predatore alieno esercita un impatto minore sull'ecosistema invaso e sulla fauna nativa se prede aliene con le quali condivide la stessa storia evolutiva sono abbondantemente disponibili e b) una diversa storia evolutiva e la coevoluzione favoriscono la competizione tra predatori con effetti imprevedibili sulle altre specie presenti. Pertanto, questo lavoro ha evidenziato la necessità di valutare caso per caso, in quanto le interazioni tra le specie variano a seconda dell'ecosistema e delle specie presenti.

INTRODUCTION

Freshwater Alien Species and the Italian peninsula

Only 0.3% of the Earth surface and 3% of the totally available water is freshwater, although not entirely available to humans (Kibona et al. 2009; Cassardo and & Jones 2011; Lui et al. 2011). Moreover, aquatic ecosystems provide uncountable resources of utmost importance and human well-being (Postel & Carpenter 1997; Aylward et al. 2005; Dudgeon et al. 2006; Collen et al. 2014). In this context, sustaining freshwater ecosystems and protecting them from alteration is a priority concern for the future (Saunders et al. 2002; Dudgeon et al. 2006).

Aside from anthropogenic disturbances, the introduction of alien species through e.g. ornamental and aquarium trade (e.g. Padilla & Williams 2004; Mrugała et al. 2015) as well as fish stocking, aquaculture and recreational angling (Cambray 2003; Savini et al. 2010) has been considered as the main driver of biodiversity loss (Sala et al. 2000; Clavero & García-Berthou 2005; Clavero & García-Berthou 2006; Assessment 2005; Gherardi 2007; IUCN 2018) and ecosystem homogenization (Rahel 2002). Indeed, the current extent and rate of invasive alien species (IAS) introductions is dramatic (Ricciardi 2007, Hulme et al 2009). This was shown to be especially true for Europe (see Figure 1). Freshwater ecosystems have shown to be more vulnerable to IAS than marine or terrestrial ecosystems (Ricciardi & Rasmussen 1999; Sala et al. 2000; Gherardi 2007; Strayer 2010) due to the high intrinsic dispersal and invasion abilities of freshwater organisms (Ricciardi & Rasmussen 1999; Bohonak & Jenkins 2003), the geographic isolation of e.g. lakes (Lodge 1994) and the intensive human use (mostly linked to recreation, food sources or commercial purposes with an intentional trend to farm mostly alien species; see e.g. Silva et al. 2009). While the spread of species into a foreign region can be a natural occurrence, the increased intensity with which humans have exploited these ecosystems (Rahel 2000, 2001; Gherardi 2007), paired with increasing global climate change (e.g. Capinha et al. 2012), is likely increasing the frequency of global invasions and range expansions of alien species (Capinha et al. 2012; Capinha et al. 2013a, b; Mazza et al. 2014). In general, the exploitation of aquatic ecosystems has caused a high number of accidental contaminations and numerous introductions with often irreversible ecological damage (Gozlan 2008, Gozlan & Newton 2009). For instance, in England in the highly commercially used Thames River, 96 established alien species have been reported and approximately 55% of them were intentionally introduced (Jackson & Grey 2013). Additionally, unintentional introductions via e.g. ballast water, fishing gear or hull fouling (GISP 2008) are facilitating the increasing spread of alien species in freshwater ecosystems (Gherardi et al. 2009).

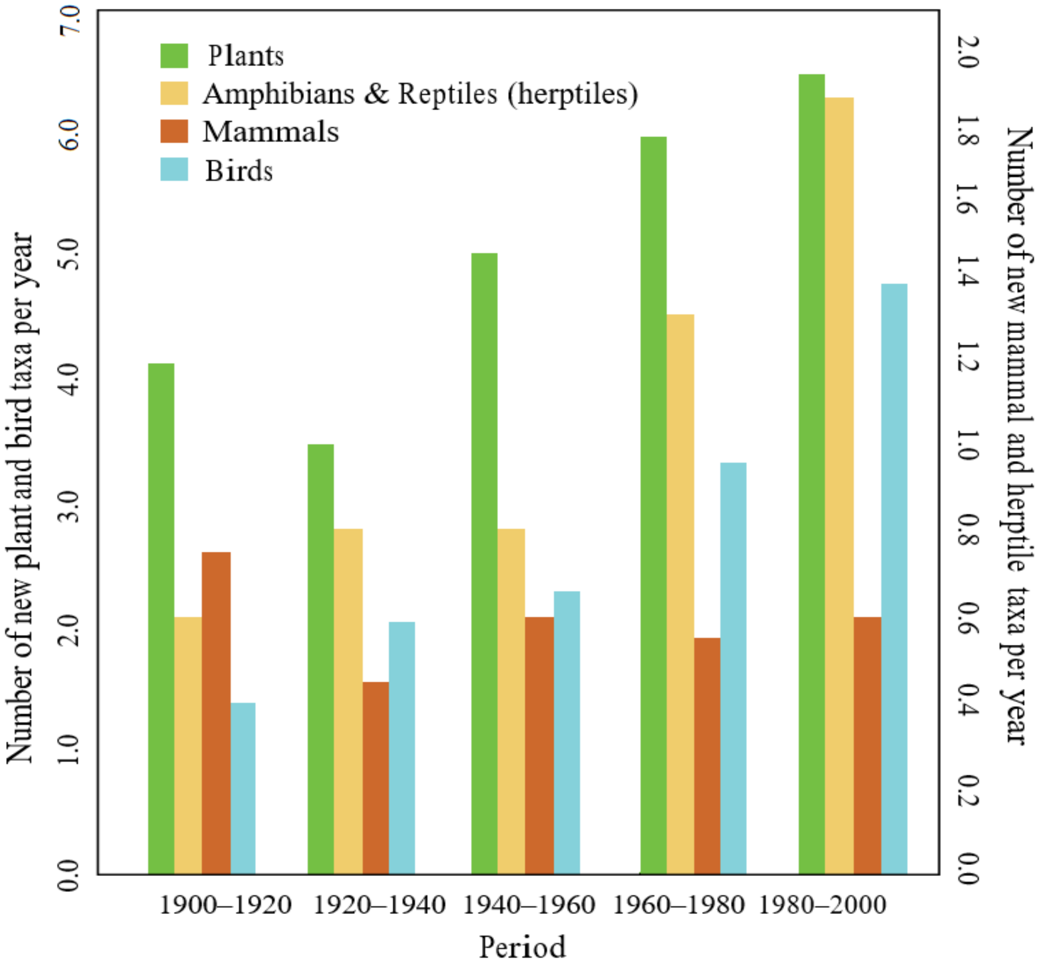

Figure 1: Alien taxa newly recorded as established in Europe per annum (adapted from Hulme et al. 2009).

When referring to "alien" species, it is generally alluded to a species that has naturally not been present in a certain area, but it was accidentally, or voluntary introduced by humans (IUCN 2018). In various European countries, year 1492, when Cristopher Columbus arrived in North America, was defined as the temporal divide between what has been considered as native or alien (Koch 2004). While this categorization may differ among countries, under this definition, various species in Europe, such as the common carp *Cyprinus carpio,* have been considered as a "naturalized" species (Garcia-Berthou et al. 2007; Van Kleunen et al. 2015; Vilizzi et al. 2015). In the case of many introduced fish species (e.g. for angling), no negative impacts have been recorded and thus, these species definition as either alien, native, or naturalized is of secondary importance. Nonetheless, after exerting documented negative impacts and being considered as invasive, a stringent time-based definition cannot always be applied (Sukopp 2000). According to the Convention on Biological Diversity states (UN 1992), "an invasive alien species is a species, that is established outside of its natural past or present distribution, whose introduction and/or spread threaten(s) biological diversity".

Not alien species per se are the main threat, but those that become invasive (Allendorf & Lundquist 2003; Gurevitch & Padilla 2004). Indeed, the difference between an alien and an invasive species can originate from a variety of factors, such as a lack of predators, a predatory advantage over other species, a superior defence mechanism or any other advantage over native species. Geographical and environmental barriers, biotic and abiotic factors can limit their establishment and successive spread. Especially local environmental conditions (e.g. climate, water chemistry, current etc.), ecological and behavioural traits of the potential invader, the life history and introduction history are additionally defining components determining the efficiency of an alien species to become invasive (Gallardo & Aldridge 2013). In the present work, the term” invasive” will refer to alien species that have been proven to cause negative ecological and/or socio-economic impacts (Gherardi 2006).

With the increasing need to investigate all aspects related to alien species, the scientific community adapted, resulting in the creation of the scientific study field called “invasion biology”, a research field that was theoretically founded by Charles Elton's book “The Ecology of Invasion by Animals and Plants” in 1958 (Richardson & Pyšek 2007). It is indeed a fairly new research field that gained much attention when invasive alien species like *Dreissena polimorpha* appeared, established and spread throughout freshwater systems in North America (Ogutu-Ohwayo 1990; Ricciardi & Rasmussen 1998; Zambrano et al. 2006). In scientific terms, the objectives of studies in this field are: i) predicting the likelihood, threats and pathways for introductions of alien species, ii) assessing the impacts of alien species onto recipient ecosystems and iii) develop ways to monitor, control or eradicate invasive alien species. Although Darwin (1881; 1992) considered the common earth worm *Lumbricus terrestris* as the most influential creature on the planet (“Worms have played a more important part in the history of the world than humans would at first suppose”) (Meysman 2006), it is now common knowledge that several species gained negative fame for their devastating impact on recipient environments. Overall, over 14,000 alien species are listed in the European database DAISIE (Nentwig 2009) with economic impacts recorded for 13 % and ecological impacts for 11 % (Vilà et al. 2009).

The recent EU regulation on IAS 1143/2014, providing a “list of invasive alien species of union concern”, foresees the increased engagement of Member States by implementing measures to decrease the introduction, detect, control or subsequently manage IAS. Risk assessments are foreseen in this EU regulation and thus, different assessment protocols to evaluate the impact of invasive species have been developed. Newer attempts aim at implementing protocols that can successfully assess the threat of potential invasive species on larger scales. Moreover, such tools are complimented by projects like the “Delivering Alien Invasive Species in Europe” (Nentwig 2009) which was funded by the sixth framework programme of the European Commission, serving as an information database developed by leading experts in the field of biological invasions. Its aim was i) the creation of a database and update on the situation of alien and invasive alien species that are prone to threaten terrestrial, freshwater and marine European environments, ii) provision of a basis for the applied prevention and control of invasions and iii) list and assess impacts and risks of the most widely spread invasive species. With DAISIE as a basis in combination with national knowledge throughout Europe, Europe-wide strategies can be developed. Overall, DAISIE became the largest database containing information on 12,122 alien species occurring in Europe, 2440 experts working with biological invasions in Europe and enabling the investigation of threats across Europe. As a result, using approximately 250 checklists, "100 of the worst" invasive species in Europe were identified and information on the most important aspects regarding these species were listed. Due to the importance of a prioritization, DAISIE, among other tools (NOBANIS; ESENIAS, Karachle et al. 2017; EASIN, Katsanevakis et al. 2012; etc.) became common tool used for the allocation of funds for projects aiming at tackling the threat of invasive species.

Aside from prioritization, previous studies have shown a demand for unified risk assessments (Figure 2) concerning the impact of alien species and a greater amount of detailed information about alien species' specific impacts (Anderson et al. 2004; Keller et al. 2007; Springborn et al. 2011; Jeschke et al. 2014; Pyšek & Richardson 2010; Hawkins et al. 2015). Indeed, for some invasive aquatic species impacts have been assessed on multiple scales, shedding light on processes underlying the success of these invaders. Since the threat of invasive species was widely recognized, several attempts to assess the impacts of IAS or their presence were performed on local, national or European level (European Union). However, standardized methods to assess impacts are difficult to produce due to the mentioned lack of data on per-capita effects and the variability in the nature of impacts (Parker et al. 1999; Ricciardi 2012). Moreover, information on species-specific impacts is in many cases anecdotal or even lacking entirely (Ricciardi 2007).

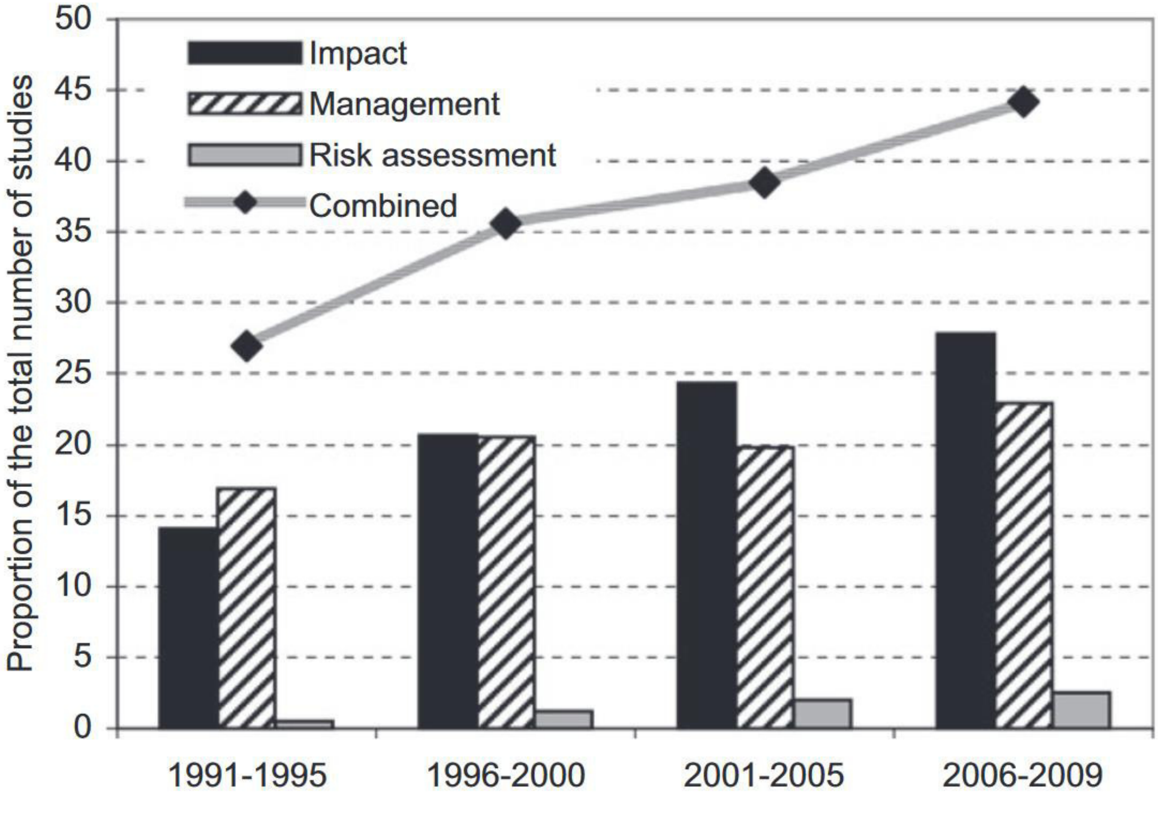

Figure 2: Trends in the studies in impact, management, and risk assessment over the last three decades. From Pyšek & Richardson (2010).

Therefore, to enable thorough and detailed risk assessments, impacts need to be investigated. However, newer schemes for the assessment of impacts and prioritization of alien species have made considerable improvements (Blackburn et al. 2014; Figure 3).

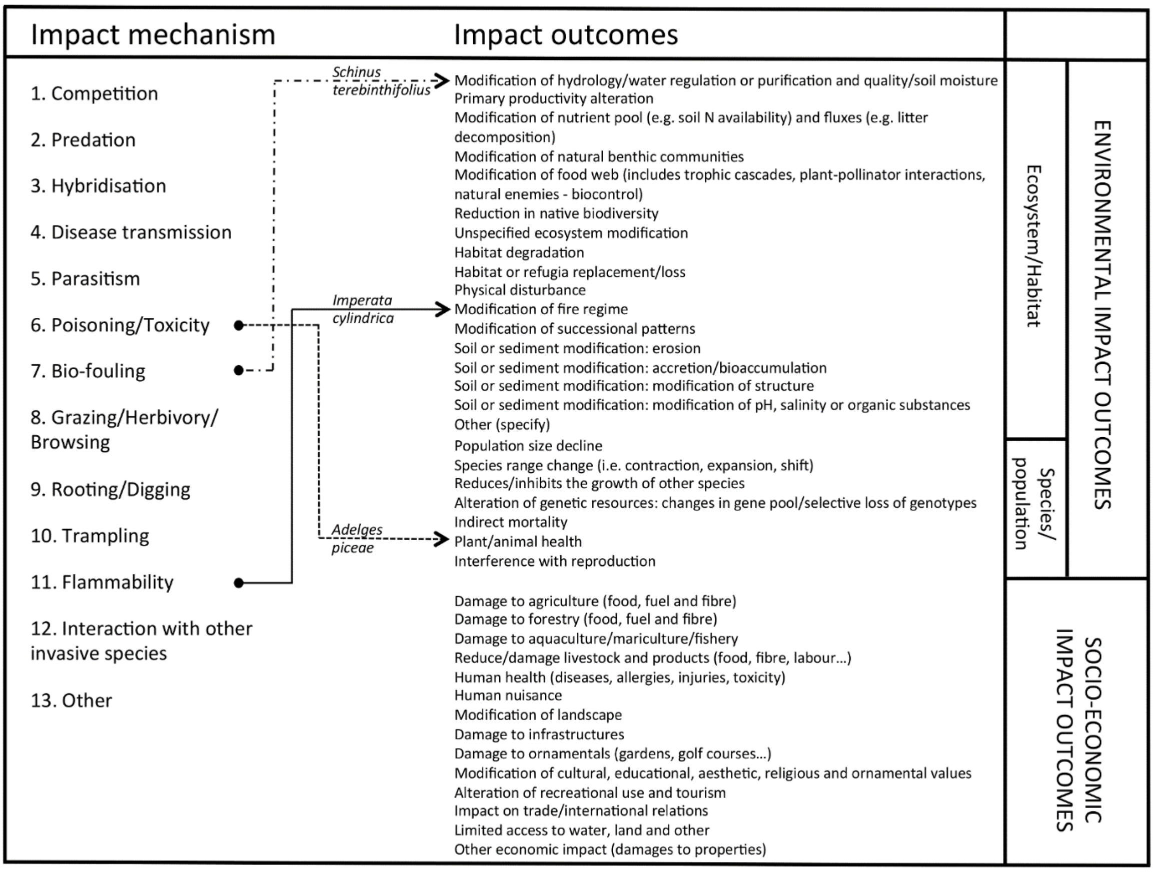

Figure 3: Impact scheme of the Global Invasive Species Database, implemented by the IUCN Species Survival Commission (SSC) Invasive Species Specialist Group. Blackburn et al. (2014)

Not only those impacts exerted by a specific single alien species need to be assessed. Moreover, arising interactions among simultaneously present species, alien or native, might alter the effect of a new invader (Simberloff & Von Holle 1999; Mooney & Cleland 2001; Liu et al. 2005; Simberloff 2006). Such IAS facilitating interactions, especially in highly invaded freshwater ecosystems as often found in Italy (Gherardi et al. 2008; Nocita & Zerunian 2007), have not been studied in depth and need to be addressed, as they likely affect the establishment of further species, impact on native environments and the possibility of management efforts targeting IAS to succeed.

Indeed, the Italian peninsula is a good example, emphasizing the need for impact assessments and successive prioritization, as it comprises the highest faunal biodiversity for all habitats in Europe (Jackson & Grey 2013). Many freshwater systems, and especially water bodies, have been subject to anthropogenic changes and have been affected by industrial and agricultural activities. The vulnerability of Italian freshwater ecosystems (Gumiero et al. 2009), although generally less related to the geographic isolation of the Italian peninsula due to the Alps in the North, can rather be considered as the result of the excessive anthropogenic use and linked IAS introductions (Gherardi et al. 2008; Colangelo et al. 2017). Although only *Cyprinus carpio* and *Carassius auratus* had been introduced into Italian freshwater ecosystems until the 18th century, the number of introductions has increased with about 0.5-1.5 species being introduced every ten years (Gherardi et al. 2008; Nocita & Zerunian 2007). With 48 native and 41 alien fish species, the ratio of alien to native fish species is uncommonly high. Additionally, five of these alien species are listed in the IUCN/ISSG list of the 100 of the World's Worst Alien Species (Lowe et al. 2000), one among the 100 of Europe's Worst Invasive Alien Species (Vilà et al. 2009) and four are known to impact threatened native species (Genovesi et al. 2015; IUCN 2018). Additionally, about 32% of these originated from America, 34% from other European countries, 15% from Asia, 12% from the Palearctic, and 7% from Africa (Nocita et al. 2017).

Thus, this displayed magnitude of invasions over time highlights the need for the assessment of previously unstudied alien species' impacts and interactions among species, enabling a future orientated management.

Due to the synergistic effects of climate change and habitat alteration, the presence of invasive aquatic species (IAS) is rapidly increasing. The impacts exerted by IAS upon recipient ecosystems can increase economic costs (e.g. for the maintenance), affect various ecological aspects (through biodiversity loss and homogenization) and even diminish the ecosystem services (food, tourism, water provision etc.). It becomes clear that the control and management of invasive species and successively increasing number of invaded ecosystems is and will be a worldwide priority.

The successful but challenging management of IAS is hence a process consisting of the following steps:

(1) Early Detection,

(2) identification of Routes of Introduction and Pathways of dispersal, and

(3) development of efficient Control measures; public awareness and stakeholder involvement are also critical for preventing new introductions and for mitigating the Impact of existing ones

For this purpose, the Marie Skłodowska Curie International Training Network (ITN) “AQUAINVAD-ed” project (*grant agreement no 642197*) was drafted, aiming at filling the current gaps using novel molecular advances combined with the power of crowd data sourcing (citizen science) to develop innovative methods of early detection, control and management of IAS. To achieve this goal, a multi-disciplinary consortium of experts in invasion biology, ecology, marine and freshwater biotechnology, citizen science and environmental policy, combined with the efforts of eight PhD students, worked together in UK, Spain and Italy (https://www.aquainvad-ed.com/).

Aquainvad-ED
Innovative Training Network

Studying indirect and direct biological impacts of AIS on native species and ecosystems, the overall objective of the presented work is to analyse factors that determine AIS establishment and expansion. Thus, it aims at quantifying the impact of several invasive species on the recipient freshwater ecosystems. Different methods were applied to achieve this objective. Trophic network of recipient ecosystems were studied, shedding light upon possible interactions between alien species. The results showed that the studied invasive species exert significant damage to flora and or fauna of the invaded surrounding ecosystems. Additionally, for the correct management of freshwater ecosystems when confronted with the problem of biological invasions, it is necessary to not only understand the biology of alien species, but also to understand the impacts and dynamics related to their introduction in order to mitigate their impact, prevent and / or control future invasions.

Control methods and impacts, as well as protocols to assess different types of impacts needed to be reviewed. Chapter 1 deals with the theoretical concept of aquatic invasive species' impacts and consists of two chapters. Chapter 1.1 can be considered as a broad review of specifically chosen high profile AIS in freshwater environments and their associated impacts. Chapter 1.2 targets existing protocols to assess the impacts of freshwater AIS while investigating the broad scale of globally used tools.

Aside from this theoretical reprocessing, several questions in regard to alien species were addressed. The first one assessed whether the burrowing activity of the North American crayfish *Procambarus clarkii* can affect the stability of artificial river levees (Chapter 2.1). Additionally, the impacts and potential threats of the North American channel catfish *Ictalurus punctatus*, being an alien species of increasing interest for the conservation of Italian freshwater ecosystems, were reviewed (Chapter 2.2). Relevant questions that were addressed in the following chapter were linked to the presence and associated impacts of this species on the recipient flora and fauna (Chapter 2.3; 2.4) and secondly the potentially arising competition with the also alien European catfish *Silurus glanis* in the Arno River (Chapter 2.5).

Significant information on whether or how the impact of invasive species on recipient ecosystems (and thus the present flora and fauna) is mitigated by the presence of other invasive species with or without common life-history traits and a common history of co-evolution remained understudied (but see Simberloff & Von Holle 1999 and Braga et al. 2018 on the "invasional meltdown hypothesis"). Therefore, different methods were applied to fill these gaps.

It was investigated how the interactions of invasive fish species can lower the impact on the recipient ecosystem and chance of successful eradication (Chapter 3.1). With this information, it was subsequently tested if Stable Isotope based modelling can be used to predict a) the outcome of biocontrol agent introductions and b) the response of reintroduced predators to newly arriving competition (Chapter 3.2). Additionally, the potentially facilitating interactions among aquatic invasive species from the same geographical origin, in this case between *L. catesbeianus* and *P. clarkii* present in a freshwater pond community, were analysed to identify potential interactions between these IAS, likely affecting the impact on native species (Chapter 3.3). Lastly, it was investigated how interspecies interactions in communities composed by alien species only are established, how the trophic web is structured and how species from different geographical regions interact (Chapter 3.4).

Three hypotheses were formulated and analysed in the context of chapters 2 and 3.

Hypothesis 1: **(Chapter 2)**	Data and studies on per-capita effects of intensively studied species are still needed. One species is the red swamp crayfish *Procambarus clarkii*. Despite being one of the most studied crayfish species, burrowing behavior is still poorly documented as it can induce relevant impacts on the invaded habitat. Secondly, many so far unstudied alien species (e.g. *Ictalurus punctatus*) also pose a significant threat to their recipient ecosystems.
Hypothesis 2: **(Chapter 2)**	The understudied alien catfish *Ictalurus punctatus* should be considered as an invasive species as it exerts a direct and negative impact on recipient Italian freshwater ecosystems.
Hypothesis 3: **(Chapter 3)**	The presence of multiple invasive species with or without common life-history and history of co-evolution can affect (i.e. lower or increase) the magnitude of exerted impacts, while these interactions might be specific to species and ecosystems.

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CHAPTER 1

IMPACTS AND IMPACT ASSESSMENTS

DELIVERABLES PRODUCED AS PARTA OF THE MARIE S. CURIE ITN "AQUAINVAD-ED" PROCESS ASSESSMENT.

Chapter 1.1

A review on impacts of high profile freshwater Aquatic Invasive Species

Part of the AQUAINVAD-ed Project Deliverable 1.7

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Abstract

Introductions of alien invasive species are said to have been beneficial in some cases. Such case was e.g. the introduction and use of new species to diversify agriculture and aquaculture in e.g. astaciculture. However, invasive species can directly affect indigenous species by competition, niche displacement or hybridisation potentially driving them extinct, or indirectly by changing the composition and structure of habitats, rendering a change in the functioning of the entire ecosystem while causing high economic costs. Through habitat alteration and exploitation, ecosystems have become more accessible for invading species. Furthermore, is the presence of an invasive species a potential gateway for several successive introductions by lowering the ecosystems resistance to invasions. These impacts can be reviewed and extrapolated to the specific situation of another ecosystem or quantified locally in so-called assessments. But while these assessments are increasingly important, they exist only for a variety of chosen species. Usually, Risk-assessments as management tools have been made for various high-profile species such as the North American crayfish *Procambarus clarkii* or the American Bullfrog *Lithobates catesbeianus*. However, there are many gaps such as the lack of a broader availability of non-species-specific assessment tools or effective eradication tools. Here, impacts, risk-assessments, control and eradication methods for a variety of freshwater and marine alien invasive species are reviewed. As a result, the urgent need for long-term studies on the impacts of invasive species in multiple-invaded areas with the associated intra- and interspecific relationships is emphasized. Additionally, as the unequal distribution of scientific efforts has led to a situation, where most often species are only receiving attention when they have already become invasive, it is called for an increased development of risk-assessment tools and precautious studies.

Introduction

Aquatic environments and the ecosystem services they provide are a widespread resource of utmost importance for human well-being. However, today invasive alien species are a major threat to them. In the European DAISIE database (DAISIE 2009), over 14 000 alien species, of which economic impacts are documented for 13% (1347) and ecological impacts for 11% (1094), are recorded. Most alien species are terrestrial with a smaller fraction in marine and freshwater environments (Vilà et al. 2009). Despite aquatic alien species being less then terrestrial ones, they are causing great damages, particularly in fresh waters (Ricciardi & Rasmussen 1999; Sala et al. 2000; Gherardi 2007).

Indeed, beside anthropogenic disturbances such as water quality deterioration, habitat destruction or exploitation of fragmented regions, the introduction of IAS to freshwater and marine ecosystems has been considered as the main driver of biodiversity loss (Sala et al. 2000; Clavero & García-Berthou 2005; Clavero & García-Berthou 2006; Millennium Ecosystem Assessment 2005; Gherardi 2007; IUCN 2009) and homogenization (Rahel 2002). Thus, drawing on ecology and economics to incorporate the impacts of invasive species on ecosystem services into decision-making is a crucial key to reestablishing and sustaining those life-support services that all organisms depend upon (Pejchar et al. 2009).

Invasive alien species

In contrast to a native or indigenous species, the term alien refers to a species that has previously not been present in a certain area where it was accidentally or voluntary introduced by humans (IUCN 2000), whereas both, an indigenous or IAS can become invasive by exhibiting negative impacts on ecosystems. In the present report, we will use the term invasive referring to alien species causing ecological and socio-economic impacts (Gherardi 2006).

These impacts can vary for species and region depending on abiotic and biotic factors (Hulme et al. 2006; Kestrup & Ricciardi 2009; Ricciardi et al. 2013). Importantly, IAS can cause a loss of biodiversity in a relatively short time, resulting in the likely extinction of e.g. endemic cave species to go unnoticed (Mazza et al. 2014). Although the scientific field of invasion biology gained much attention due to the great impacts caused by AIS such as *Dreissena polymorpha* or *Lates niloticus* (Ricciardi et al. 1998; Ogutu-Ohwayo 1990; Zambrano et al. 2006), the natural spread and non-natural distribution of a mammal like *Sus scrofa* into a foreign terrestrial ecosystem or the presence of alien insect pests (Paine & Miller 2002; Dukes et al. 2009) are most often accompanied by generally higher public attention (Cruz et al. 2005; Sáaez-Royuella & Telleríia 2008; Merino et al. 2009; Cuevas et al. 2010), resulting in aquatic habitats often being altered to a certain degree without public or scientific notice. Freshwater ecosystems have shown to be more vulnerable than marine or terrestrial ecosystems (Ricciardi & Rasmussen 1999; Sala et al. 2000; Gherardi 2007; Strayer 2010) due to higher intrinsic dispersal abilities of freshwater organisms (Ricciardi & Rasmussen 1999; Jenkins 2003), geographic isolation of e.g. lakes (Lodge 1994) and intensive human use. While the spreading of species into a foreign region can be a natural occurrence, the increased intensity with which humankind has exploited these ecosystems (Rahel 2000, 2001; Gherardi 2007), paired with rising global climate (e.g. Capinha et al. 2012), is likely to increase the frequency of global invasions and range expansions (Capinha et al. 2012; Capinha et al. 2013a, b; Mazza et al. 2014).

According to Olenin et al. (2010), in aquatic ecosystems, introduction can be divided in Primary introductions - appearance of a NIS in a new area (called introduced area) directly from its native range and Secondary introductions – consequential spread of NIS from the introduced area. Nonetheless, not all introduced alien species become invasive. Geographical and environmental barriers can limit the establishment and spread. Local environment conditions (e.g. climate, water chemistry, current), ecological and behavioural traits and the life history are last defining components of alien species establishments determining the efficacy of some species to become invasive (Gallardo & Aldridge 2013). Human activities play a key role as a major forecaster for the future distribution of alien species overcoming geographical borders and indirect cause of the invasion process (Pyšek et al. 2010; Pyšek & Richardson 2010). These human activities are mostly linked to recreation, food sources or commercial purposes with a trend to farm mostly alien species (De Silva & Turchini 2008). Ornamental/aquarium trade (e.g. Mrugala et al. 2014) as well as aquaculture and recreational angling (Cambray 2003; De Silva et al. 2009) are indeed recognised as major drivers of AIS introductions. In England, in the highly commercially used River Thames, 96 established alien species have been reported and approximately 55% of them were intentionally introduced (Jackson & Grey 2013). This exploitation and use of aquatic ecosystems have caused a vast amount of accidental contaminations and numerous introductions with often irreversible ecological damage (Gozlan 2008, 2009). Particularly, unintentional introductions via e.g. ballast water, fishing gear, hull fouling etc. (GISP 2008) are currently facilitating the growing spread of invasive species into freshwater (Gherardi et al. 2009) and marine environments (approx. 7000 coastal and marine species travelling through ballast water in tanks every day unnoticed, WWF 2009). For example, the EU project “ALIENS” dealt with the invasive seaweed distribution along the coastline of six European countries and detected 15 seaweed taxa in 90 l of ballast water (http://cordis.europa.eu/result/rcn/84185_en.html). Other important pathways for accidental introductions are the artificial canals, which connect previously isolated water bodies enabling species to spread beyond natural borders (Panov et al. 2009). The spread of the killer shrimp was indeed directly facilitated through the opening of the newly constructed Danube-Main-Rhine canal in 1992 (Kinzelbach 1995). The Suez Canal, an artificial connection between the Mediterranean Sea and the Red Sea, led to the introduction of over 440 NIS in the Mediterranean with a variety of ecological effects on native species (Galil et al. 2015).

Another key player in invasive species biology with uncertain outcomes due to its less predictability will be global change. For invasive species, it is generally accepted that, besides ways of transport and introduction facilitated by anthropogenic activities, an increase in temperature will change the establishment, impact and distribution (Hellmann et al. 2008). As the establishment and spread of a NIS likely varies with physical environmental gradients (Ricciardi 2003; Jokela & Ricciardi 2008; Gallardo & Aldridge 2013), a rising global temperature will potentially clear constraints by altering ecosystems and making them less resistant to invasions. The altered state will potentially increase the persistence of NIS thriving in warmer environmental conditions (Dukes & Mooney 1999; Walther et al. 2009) and facilitate the colonization of new areas (Lee & Chown 2007) by enabling NIS to dominate indigenous species due to a tolerance towards higher temperatures (Byers 2002; Hellmann et al. 2008) while simultaneously exercising negative influence on alien species that require colder habitats. Together, invasive species and climate change are the double trouble for ecosystems (Dukes 2011).

Current regulations on alien species

The growing concern of governmental institutions on NIS is not only due to the ecological impacts, but also for economic reasons such as high costs of management and control. Therefore, it was inevitable to introduce and enforce legislative measures to target and prevent the occurrence of AIS while simultaneously managing them. As in the world Biodiversity Strategy, the EU Strategic Plan for Biodiversity 2011-2020 puts emphasis on IAS, encouraged by the EU-Commission and participating member states to establish measures of prevention, monitoring, eradication and management for invasive plants and animals. While all measures are equally important in a globalized world, the prevention of invasions was recognized as the most cost-effective and environmentally desirable measure (European Commission 2014). Especially, the recent *EU Regulation No 1143/2014* on invasive alien species is the first EU regulation completely dedicated to IAS and associated management, based on the hierarchical approach suggested by the CBD in 2002. The Regulation underlines the high importance of contributing „an adequate knowledge to address the problems raised by invasive species" and, among other measures, to carry out a risk assessment (assessment of potential pathways, introduction, establishment and spread of IAS in relevant biogeographical regions). UK government paid particular attention to the problem of NIS since 2003 with a policy review that has been the starting point for writing the first "GB Invasive Non-indigenous Species Strategy" (GB INSS) in 2008. This document was an important step for the IAS management. It encompasses the (1) establishment of a non-indigenous Species Information Portal (NNSIP), providing a central repository for non-indigenous species information and distribution data; (2) risk assessments for 60 species; (3) raise of awareness through two campaigns focused on aquatic plants ("Be Plant Wise") and recreational water users ("Check Clean Dry"); and (4) assessment of the economic impact of IAS (Department for Environment Food and Rural Affairs et al. 2008).

Where are we now? What needs to be done?

Taking action is mandatory in order to avoid that loss of biodiversity, economic costs (Pimentel et al. 2001; Kettunen et al. 2009) and socio-economic damage could increase because of biological invasions. The total number of NIS has indeed increased by 76 % between 1970 and 2007 in Europe (Mediterranean marine, mammal and freshwater; Butchart et al. 2010). However, the difficulty to increase awareness has led to this increase. Given limited financial resources, species have to be ranked according to their overall impact for setting management priorities (McNeely 2001). However, it is important that applied ranks be not only based on economic interests. Species without or with low economic impact and invertebrates in general tend to draw less attention and hence receive less scientific focus. With this unbalanced distribution, lacking knowledge on potential threats will most likely result in actions being taken too late for any potential measure. Furthermore, the general understanding of invasive species biology has to go further than prevention, eradication and control (Engelmann et al 2000). Behavioural and ecological studies as well as predictive measures (considering ecological modelling, human-mediated vectors and ongoing climate change) can provide a lot of information about the future spreading of invasive species. Once a species is introduced and becomes invasive, early detection of further spreading is crucial for possible control measure. Modern molecular diagnostic marker (such as DNA barcoding) will be of great importance and a great advantage in the assessment and control (Trivedi et al 2015). Training local authorities in easily sampling methods can provide a lot of valuable information of IAS dispersion (Le Roux & Wieczorek 2008). For that account, scientific approaches such as the Aquainvad-ED Marie Skłodowska-Curie Innovative Training Network (funded by H2020-MSCA-ITN-2014-ETN-642197), which focuses on early detection, control and management of AIS by conducting eight PhD fellowships across Europe in a cooperative network involving academic and non-academic partners from a cross-sectorial programme of research, can be of great future value.

Impacts

For centuries, the spread of alien species facilitated by anthropogenic influence has broken biogeographic barriers through the creation of new pathways (Ricciardi 2003; Mooney & Cleland 2001). Biotas isolated for millions of years are affected along their evolutionary path by this biotic rearrangement. These evolutionary influences are manifold and can vary among niche displacement, hybridization, introgression, predation or even extinction. Nevertheless, not only indigenous species are being influenced. Alien species are also affected because of their interaction with indigenous species and the new environment (Mooney & Cleland 2001).

How to define impacts

Negative effects of invasive species have shown to significantly overweigh the benefits (McCarthy et al. 2006; Skelton et al. 2013), but several examples where alien species have proven to be beneficial do exist (Gherardi 2007). However, all of the reported positive effects an organism has on human society is valued from the anthropogenic perspective and can vary depending on emphasis. The introduction of the common carp by the Romans, which were harvested for human and as an alternative pet food, was beneficial for the Roman Empire, while monks facilitated the aquaculture of carp for the Lent in the 13th and 15th century (Fagan 2008). One further example is *Lasioglossum leucozonium*, a solitary sweat bee, introduced in North America a long time ago, which seemed to have found its own ecological niche, becoming an important pollinator of caneberries and other cultivated plants (Adamson 2011). The introduction of a NIS can have some beneficials, e.g. by restoring traditions proper to the cultural heritage of a country (e.g. crayfishing in Sweden and Finland) or by providing income for families in poorly developed areas (in Andalusia, Spain: Geiger et al. 2005), even if negative impacts on native ecosystems are reported for the aforementioned cases and, thus, a cost-benefit analyses should be undertaken.

Introductions were also beneficial as in the case for the introduction and use of new species to diversify agriculture and aquaculture e.g. astaciculture. Established crayfish farming in Britain and in Spain (Holdich 1999) affected the inner-European market as well as between European and extra-European countries (Ackefors 1999). The ways and means of how NIS affect indigenous species and ecosystems has been the focus of several studies in the last decades. Even though impacts are usually considered as the last stage of an invasion process (after species establishment and spread), invasive species can exhibit impacts at any point after their introduction (Ricciardi et al. 2013), but quantified impact assessments do only exist for a few chosen species (e.g. Capinha et al. 2013b; Roy et al. 2014). Potential impacts need to be quantified in order to be properly recorded. These impacts can affect Economy, Socio-Economical aspects and Ecosystem Services and are strongly dependent on the invading organisms and their surrounding environments.

Ecological impacts

NIS can directly affect indigenous species by competition, niche displacement, hybridisation predation or parasitism, potentially driving them extinct, or indirectly by changing the composition and structure of habitats. Being generally accepted as one of the leading causes of animal extinctions and biodiversity loss (Sala et al. 2000; Gurevitch & Padilla 2004a, b), impacts of AIS can even cause biotic homogenization (Clavero & Garciá-Berthou 2005), i.e. the presence of same NIS in several ecosystems associated with the decline of indigenous species. By altering the distribution of biota, a homogenisation will substantially facilitate the decline in diversity. An important aspect of this loss in diversity is the possibility of genetic pollution. With the possibility of hybridization and introgression between closely related species, genotypes are replaced and homogenized resulting in the pollution of gene pools strongly affecting the less abundant species (Meilink et al. 2015). One example is the spread of closely related species of the genus *Triturus*, which, when populations overlap, show the potential to hybridize, causing a decline in the genetic identity and health of these species (Rhymer & Simberloff 1996; Meilink et al. 2015). Beside this direct causality, another possibility to explain the loss of biodiversity is the indirect consequence of habitat modification by anthropogenic and IAS influence (Didham et al. 2005). Through habitat alteration and exploitation, ecosystems have become more accessible for invading species. Furthermore, the presence of an invasive species is a potential gateway for several successive introductions by lowering the ecosystems resistance to invasions (Ricciardi et al. 2013). NIS can change the functions of ecosystems: for example, the invasive grass *Bromus tectorum*, when dried, alters the fire regime. Several invasive species such as the Louisiana red swamp crayfish or the common carp can alter the ecosystem with their natural behaviour affecting abiotic factors of the environments (increase of water turbidity, decrease of oxygen and primary production, damages to bank stability, etc.) (Lougheed et al. 1998; Barbaresi et al. 2004; Scalici & Gherardi 2007). One of the rather infamous examples is the largest true freshwater fish in Europe, the wels catfish, *Silurus glanis*. Based on its high reproduction rate of approximately 30.000 eggs per kilogram of body weight, enormous size of up to 3-4 m and plastic feeding habit, it can cause the extinction of indigenous fish and especially threatened crayfish species in its introduction areas (Blanc 1997; Carol Bruguera 2007).

Economic impacts

Managing established populations of IAS can cause high economic costs (Pimentel et al. 2005). Although national approaches to quantify costs have been more anecdotal and vaguer (Pejchar & Mooney 2009), the high costs and enormous economic damage outgoing from invasive species is undeniable (Lovell & Stone 2005; Cecchinelli et al. 2012). The high estimated economical damage generated by invasive species (including costs of control measures) has been estimated to be higher than $138 billion per year just for the US (Pimentel et al. 2005) and exceeding €12 billion per year in Europe (Kettunen et al. 2009). This last amount might seem very low compared to general costs in the US, but it has to be considered that only a handful cost-efficiency analyses have been applied and that there have been only a few cross taxa estimations at the national level (which differ from the North American perspective). In Europe, most expenses generated by invasive species are due to management costs including eradication, control and environmental education programs targeting some particular natural areas (Vilà et al. 2009). Particularly, the Great Britain-wide costs of controlling invasive freshwater species were estimated to be approximately £26.5 million per year. However, the costs of control could aggregate to approximately £43.5 million per year if management efforts were undertaken at all invade locations (Oreska & Aldridge 2011).

Furthermore, an assessment of the economic impact of invasive non-indigenous species by the Department of Environment Food and Rural Affairs (2008) indicated annual costs of £1.7 billion to the British economy. Even if economic impacts are usually direct as a loss of production in agriculture or management costs, there are also losses of recreational and tourism revenues which can be considered as passive economic impacts. Since environmental damage, loss of biodiversity and loss of ecosystem services (the benefits provided to human society by natural ecosystems) are often not considered among production loss or management costs, the general economic costs are probably far beyond estimated values (Pimentel et al. 2005). The major position among economic costs falls to the plant industry related to accidental introductions of pest plant species that accompany seeds for agricultural/ornamental use. These can be a threat to young cattle (e.g. *Euphorbia esula*) or unpalatable (*Centaurea solstitialis*) (Pimentel et al. 2005). Additionally, the economic impact on tourism should not be underestimated. Imported fish species such as the bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*) in the Chicago Area Waterway System are listed as invasive and considered a threat to recreational fisheries in the Canadian Great Lake systems (Cudmore et al. 2011). Therefore, different species cause varying economic damage. For instance, invasive crayfish or the green crab have shown the ability to greatly damage agriculture and fisheries reducing annual production while also causing high regulatory costs (Lovell & Stone 2005; Cecchinelli et al. 2012; Lodge et al. 2012).

Other examples are the introduced frog species common coquí (*Eleutherodactylus coqui)* and American bullfrog (*Lithobates catesbeianus*) whose populations, once established, can reduce real estate values with their loud call (Sin & Radford 2007; Orchard 2011). The introduction of alien crayfish facilitated the spread of the crayfish plague, which subsequently diminished the abundance of indigenous European species and resulted in a loss of festivals to celebrate indigenous crayfish services (Lodge et al. 2012). Although the introduction of invasive species has shown to restore traditions proper to the cultural heritage of a country (e.g. crayfishing in Sweden and Finland), which were previously threatened due to the decline of crayfish abundance, the introduction of invasive species for a certain advantage implies generally negative consequences – a relation called Frankenstein effect. However, whenever the economic costs of an invasive species were quantified, the affected ecosystem services have been often partially not considered or completely neglected (Charles & Dukes 2007).

Ecosystem-services

Another often neglected aspect is the potential change to ecosystem characteristics caused by invasive species (Ehrenfeld 2010), including the economic impact of invasive species on ecosystem services (Pejchar & Mooney 2009). Ecosystem services can be defined as the benefits provided to human society by natural ecosystems. They can be divided into 1) supporting services, 2) provisioning services, 3) regulating services and 4) cultural services (Millennium Ecosystem Assessment 2005). While most NIS negatively affects ecosystems, the vulnerability of ecosystems may vary. The disruption of services can have socio-economic and cultural impacts as in the case of the invasive plant *Heracleum antagarrianum* (Tiley et al. 1996). Introduced carp populations can alter the habitat structure and ecosystem composition in a way that can change the region, making it unattractive for sport fishing while also decreasing the water quality. Furthermore, invasive species can be potential carriers for diseases such as the Chinese mitten crab (*Eriocheir sinensis*) potentially carrying the Asian lung fluke (Lerner & Heimowitz 2000) or illnesses such as malaria, typhus and yellow fever which are all carried by several species (Elton 2000). Additionally, invasive species used as a food source can become a problem, such as in the case of the crayfish *P. clarkii,* possibly causing severe problems due to its ability to accumulate toxins in its body (Tricarico et al. 2008; Lodge et al. 2012).

While the classification of impacts is mostly kept fairly general, only few invasive species tend to impact all five categories (Blackburn et al. 2014). Those that outstretch their impacts in several fields were depending on research question established as model organisms and used by scientists to understand impacts on the food web, environment, other species, economy and many more. While species with a broad spectrum of impacts and high associated economic costs tend to get broad attention by public and science, the ecological and economic impacts of the other invasive species are often overlooked.

Invasive plant species impacts are spreading undetected being heterogeneous and changing nutrient cycles, affecting other plant species and communities (Vilà et al. 2011) but, since non-indigenous plant and mammal species are often economically beneficial and have had a history of cultural integration, it is easy to understand why the general focus was more towards them.

Freshwater invasive species

Below we are going to present the cases of several chosen freshwater and marine invasive species, which will serve the AQUAINVAD-ed project as a model organism. They are some of the highest alert invasive aquatic species and the prevention of further spreading as well as their control should be a priority for authorities.

Zebra Mussel (*Dreissena polymorpha* Pallas, 1771)

The zebra mussel, *Dreissena polymorpha* is indigenous to Danube, Dniestr, Berezan, Southern Bug, Dniepr, Molochnaya, Don, Kuban, Kamchia, and Veleca river basins from where it started to spread throughout Europe during the nineteenth century (Son 2007). Zebra mussels are filter feeders that can attach to any stable substrate in the water column or benthos: rock, macrophytes, artificial surfaces (cement, steel, rope, etc.), crayfish, unionid clams, and each other, forming dense colonies called druses. Due to their temperature tolerance and their high spawning ability, zebra mussel spread in Europe and North America outnumbering the indigenous mussels and causing great environmental changes. The first zebra mussel record in the UK dates back to 1824. Since then, the species started spreading, but between 2000 and 2002, both its population size and impacts notably increased (Aldridge et al. 2004). However, it is not clear whether this increase is due to a real increase in the population of zebra mussel or to an increased awareness of its presence causing considerable concern (Aldridge et al. 2004).

Figure 1: Photo of a *Dreissena polymorpha* colony, taken by Matteo Rolla

Zebra mussel's ecological impacts are numerous. Since they are able to filter particles smaller than 1µm in diameter, all suspended material including bacteria, protozoan, zebra mussel veliger, and silt is filtered from the water column, even if they primarily feed on phytoplankton. The feeding of zebra mussel can change the phytoplankton community composition through differential grazing: some phytoplankton species are rejected or less digested by zebra mussel and stay trapped in the pseudofaeces so when the excretions are resuspended it can return to the pelagia and survive (Bastviken et al. 1998). Overall, they can significantly reduce the amount of phytoplankton biomass, augmenting water transparency (Holland 1993; Fahnenstiel et al. 1993). Zebra mussels compete with indigenous mussels where they are present. They compete for food and space, but the main threat is the extinction of indigenous unionids through epizootic colonization (Schloesser et al. 1996; Baker and Hornbach 1997). Zebra mussels impede valve functionality, causing shell deformity, smother siphons, impair movement, and deposit metabolic waste onto unionid clams. Ricciardi et al. (1995) found a strong relation between zebra mussel density in the wild and indigenous unionids mortality, elaborating a model to predict it.

The damage caused by zebra mussels is not only ecological, but also economical when this species colonizes human structures, causing problems to different human activities. They can colonize water supply, pipes of hydroelectric and nuclear power plants, public water supply plants, and industrial facilities. The small mussels can get into boats engine cooling systems causing overheating and damage. Navigational buoys can been sunk under the weight of attached zebra mussels and even deterioration of dock pilings increases when zebra mussels encrust them. Direct economic costs have resulted from the invasion of zebra mussels due to the maintenance and repair of power plants, industrial facilities, and other businesses, as well as research, monitoring, and control. In the US, a wide variety of estimations have been made regarding zebra mussel-related expenses, ranging from $92,000 per hydroelectric plant per year to $6.5 billion in total costs over 10 years (Lovell et al. 2006).

Louisiana red swamp crayfish (*Procambarus clarkii* Girard, 1852)

One model organism, which has been used to understand invasive biology, is the invasive red swamp crayfish (*Procambarus clarkii*), native to North America. It has been identified as one of the most invasive and dangerous species in Europe (Holdich et al. 2009; Gherardi 2011), for a variety of scientists in the last decades (Lodge et al. 1994; Nyström et al. 1996; Tricarico et al. 2008; Gherardi et al. 2011b). In 1859, infected North American crayfish species present in fish batches imported from North America into Italy exterminated all indigenous crayfish populations in Lombardy. After 1860, the export of indigenous crayfish species drastically sank by up to 90% in Scandinavia, Germany, Spain and Turkey, causing an enormous economic damage (Köksal 1988; Holdich 1999), and inducing the introduction of alien crayfish in Europe. The red swamp crayfish was first introduced in Spain in 1977 and then in Italy in 1989 where it became a highly invasive species (Gherardi et al. 1999; Aquiloni et al. 2010). It is one of the most introduced species worldwide for aquaculture purpose. This species is among the largest and longest living invertebrates (Nyström et al. 1996), feeding on benthic invertebrates, detritus, algae, hydrophytes (Whitledge & Rabeni 1997), fish and eggs (Lowery & Mendes 1977) and being the main prey for various species including birds, fishes and otter (Slater & Rayner 1993). While most other crayfish in its invaded area mainly belong to K-selected species with a slow growth rate, long life span and 2-3 years until maturity, *P. clarkii* is classified as an R-rated species found to carry up to 724 eggs (Chucholl & Pfeiffer 2010).

Figure 2: Picture of *Procambarus clarkii*, taken by Chris Lukhaup.

With its elevated growth rate of 50 g in 3-5 months and maturity with approximately 10 g, it reproduces faster than indigenous species, outcompeting or preying upon them (Gherardi 2011a). Experiments showed that *P. clarkii* is able to evict indigenous crayfish and fish species from shelters making them more susceptible for predation while simultaneously preying on eggs (Gherardi 2011b). Beyond this, *P. clarkii* diminished populations of indigenous crayfish in Europe (see e.g. Gil-Sanchez & Alba-Tercedor 2002; Gherardi 2011a) being a vector for the oomycete *Aphanomyces astaci*, the cause of the crayfish plague which decimated populations of *Astacus astacus* and *Astacus leptodactylus*.

P. clarkii is a strong and capable invader due to its ability to burrow and survive for long periods out of water, in brackish or deoxygenated water, enabling it to travel long distances even overland (Gherardi et al. 2000; Scalici et al. 2009). It tolerates elevated turbidity and wide ranges of water temperatures or salinity, while it is resistant to pollution and diseases (Angeler et al. 2001; Gherardi 2011a).

The burrows dug by *P. clarkii* are rarely constructed for permanent use, meaning that crayfish do not tend to return to previously dug holes. This burrowing activity of *P. clarkii* increases with the amount of fine sediments, causing extensive ecological damage to sandbank (Barbaresi et al. 2004), erosion of littoral zone sediments and reducing coastal protection from storms and sea level rise (Lodge et al. 2012). *P. clarkii* shows non-consumptive plant clipping and uprooting behaviour while choosing seedlings over older plants (Nyström & Strand 1996). Additionally, it is a selective feeder depending on the availability of food (Gherardi 2006). Young *P. clarkii* seem to prefer macro invertebrates to a herbivore or detrivore diet (Alcorlo et al. 2004; Banha & Anastácio 2011), but adult specimens are rather omnivorous (Correia 2005; Gherardi 2006) with organic plant detritus as the principal food source (Lorman & Magnuson 1978). Therefore, with establishment and increasing population density, *P. clarkii* is actively changing the water from a transparent to a turbid eutrophic state (Nyström et al 1996; Alcorlo et al. 2004) accompanied by a significant increase of dissolved inorganic nutrients, bioturbation and a general impoverishment of water quality (Angeler et al. 2001; Rodriguez et al. 2003). It becomes an integral part of the food web wherever *P. clarkii* establishes a population, posing a threat to human health when consumed due to its ability to survive in areas with contaminated water (Geiger et al. 2005) and accumulating higher concentrations of toxins in its organs (Tricarico et al. 2008). Due to its abundance, it became the main prey for Eurasian otter (*Lutra lutra*) in Spain (Adrian & Delibes 1987) and African clawless otter (*Aonyx capensis*) (Ogada et al. 2009). Toxic substances like arsenic or BMAA (ß-N-menthylamino-L-alanine) produced by cyanobacteria which are accumulated by crayfish can thereby enter the food chain and magnified along it (Geiger et al. 2005).

Amphibian populations are especially threatened. *P. clarkii* was found to prey upon larvae of up to 13 amphibian species (Cruz & Rebelo 2005), being more efficient than indigenous crayfish species in capturing larvae of e.g. the smooth newt (*Lissotriton vulgaris*) (Renai & Gherardi 2004). The presence of *P. clarkii* can therefore spoil valuable breeding ponds for several amphibian species (Nunes et al. 2010). Furthermore, the economic damage posed by this invasive crayfish cannot be underestimated. Crayfish caught in gillnets damage valuable fish (tilapia and largemouth bass) in quantities of nearly 30% of the catch and fish nets (De Moor 2002). Another major economic damage caused by *P. clarkii* is the destruction of valuable rice in china (Yue et al. 2010), Portugal (Anastácio et al. 2005) and Kenya (Rosenthal et al. 2005) causing economic losses exceeding millions of Euro.

It is questionable how other invasive species can facilitate or hinder the establishment of *P. clarkii* such as *Silurus glanis*. The presence of the invasive but naturalized mosquitofish *Gambusia holbrooki* or *P. clarkii* was found to facilitate the establishment of either one due to a mutual benefit (Anastácio et al. 2011). Finally, all exerted effects are strictly density dependent but even a small abundance of *P. clarkii* can reduce the biomass and species richness of macrophytes, invertebrates (Lodge et al. 1994) and rooted phyto-vegetation while increasing phytoplankton density (DeMoor 2002). Additionally, with increasing global climate, *P. clarkii* will most likely expand its range and spread even faster (Capinha et al. 2013). For these reasons, *P. clarkii* is listed among the 100 worst invasive species in Europe (DAISIE) and further spread needs to be contained whenever possible. Extrapolations of applied climate models showed that with rising global temperature, *P. clarkii*'s distribution in Europe would be favoured and the species would increase its invasive potency (Gherardi & Panov 2009) due to the ability to burrow and tolerate desiccation and low water quality (Gherardi 2006). However, many aspects regarding interaction and threats posed by invasive species are still unknown (Reynolds 2011).

Killer Shrimp (*Dikerogammarus villosus* Sowinsky, 1894)

The killer shrimp, *Dikerogammarus villosus*, is an amphipod indigenous to the Ponto-Caspian region that has recently invaded and spread throughout Western Europe (Tricarico et al. 2010). Killer shrimp inhabits fresh/brackish water, lakes, rivers, and canals in areas with low velocity currents. It can adapt to a wide variety of substrates as well as a wide range of temperature, salinity, and oxygen levels (Devin & Beisel 2006). Killer shrimps are omnivorous predators preying on many macroinvertebrates, including other gammarids, and are also able to collect detritus and to filter out suspended algae (Mayer et al. 2008). The name "killer" shrimp originates from its cannibalistic nature by occasionally eating nonspecific newborns and weak adults (Devin and Beisel 2006; Dick & Platvoet 2000; Dick et al. 2002; Platvoet et al. 2009). Moreover, killer shrimp have been observed to kill or injure potential prey without consuming it (Dick et al. 2002). Its populations have caused significant ecological disruption, including reduced biodiversity and local species extinctions. It started to spread in Western Europe in 1992 after the opening of the Danube-Main-Rhine canal (Germany) and reached UK in 2010. The arrival of killer shrimp in the UK may have implications for river water quality monitoring being the macroinvertebrate taxon, which contributes to biotic indices of water quality, possibly impacted by the invader (MacNeil et al. 2010).

Figure 3: Photo of *Dikerogammarus villosus*, taken by Jean-Françosi Cart

Due to its ecological traits, killer shrimps have strong impacts on the indigenous macroinvertebrate communities. Killer shrimp predation affects many taxa that have a high score in the River Invertebrates Classification Tool (RICT). A reduction in the number of high scoring families and little or no effect on low scoring families will result in both lowering of the Average Score per Taxon (ASPT) and a lowering in the number of scoring taxa in water of high and good quality. Additionally, the presence of *D. villosus* within the macroinvertebrate community may affect the RICT, as *D. villosus* individuals would score as Gammaridae under the RICT. Emerging evidence is that *D. villosus* has the capacity to withstand greater environmental extremes compared with indigenous gammarids (Devin & Beisel 2007). Furthermore, interactions between killer shrimp and indigenous gammarid species can result in displacement or local extinction of indigenous species, thereby reducing biodiversity (Dick & Platvoet 2000).

The socio-economic impact of this species on invaded areas of Western Europe is largely unknown. However, the ability of this species to consume eggs or juvenile stages of small fish creates a potential concern for fishery populations (Devin and Beisel 2006).

American Bullfrog (*Lithobates catesbeianus* Shaw, 1802)

It is the most commonly farmed amphibian (Garner et al. 2006) and therefore introduced worldwide as a food source in over 40 countries on four continents over the last century with at least 25 introductions in Europe. It is capable of establishing dense populations in a short period due to its adaptable, prolific, competitive loud and predatory nature (Moyle et al. 1973; Wu et al. 2005; Ficetola et al. 2007). Additionally, post-metamorphic stages are capable of long-distance dispersal (> 1200 m) and females possess the ability to produce 1000 – 25.000 eggs. Once established, a population can reach densities of up to > 780 adults per hectare (Adams & Pearl 2007). As a result, the American bullfrog is listed among one of the 100 worst alien invasive species in Europe (Orchard 2011).

Juvenile tadpoles readily become part of the food chain but have a comparably much higher survival rate than other amphibian species thanks to the much shorter time until metamorphosis (Cecil & Just 1979). Furthermore, this species shows the ability to digest bloom-forming algae, such as *Anabaena*, considerably affecting the nutrient cycle and primary production in freshwater ecosystems tackling the process of eutrophication (Pryor 2003). While research has focused mostly on juvenile interactions and the impact of the species on indigenous ranid frogs in terms of competition and predation (Kupferberg 1997, Lawler et al. 1999; Wang et al. 2007), the possibility of breeding interference interactions of adult specimens with indigenous species and the dispersal ability of female specimens has been mostly ignored (Pearl et al. 2005). Furthermore, the presence of *L. catesbeianus* can significantly increase indigenous species susceptibility of other predators (Kiesecker & Blaustein 1998) and decrease the abundance of all aquatic insects (Govindarajulu et al. 2006). Another interesting impact is the ability of adults to prey upon indigenous vertebrates and of its opportunistic ecological footprint (Jancowski & Orchard 2013). Many fish species avoid feeding on bullfrog tadpoles as being mostly unpalatable, passively increasing the feeding pressure on macroinvertebrates, possible predators for bullfrog larvae (Adams & Pearl 2007).

Figure 4: Picture of *Lithobates catesbeianus*, taken by Don White (don.white55; flickr)

Infectious diseases are increasingly recognized as key threats to indigenous species (Kilpatrick et al. 2010). The American bullfrog is a known carrier of Chytridiomycosis, causing *Batrachochytrium dendrobatidis* with implications of linked amphibian declines worldwide but being immune to most strains at the same time (Hanselmann et al. 2004; Gervasi et al. 2013; Eskew et al. 2015). At the same time, the spread of Chytridiomycosis is facilitated by the trade value of ranid species in the US economy. Between 2000 and 2005, 28 million individuals were imported into the US, showing an overall infection prevalence of 62 % and 8.5 % by *B. dendrobatidis* (Schloegel et al. 2009). The advancing habitat loss (Schloegel et al. 2009) combined with the rising threat of infectious diseases lead to 32.5% of amphibian species being threatened (Kilpatrick et al 2010). Especially those species that are critically endangered seem to suffer significant losses linked to *B. dendrobatidis* being the plausible reason for the extinction in 30 of the 113 species of the Harlequin toads *Atelopus* (Kilpatrick et al. 2010).

Chinese Mitten Crab (*Eriocheir sinensis* Edwards, 1854)

The Chinese mitten crab, *Eriocheir sinensis* is a catadromous species indigenous to the Far East, with a native distribution from the Province of Fukien, China ~26 N° northwards to the Korea Peninsula ~40 N°, from where it spread throughout Europe and into North America (Clark et al. 1998). Throughout its life, the Chinese mitten crab occupies different ecosystems depending on its life stage (Veilleux & de Lafontaine 2007), moving from freshwater habitats where it spends its juvenile years to saltwater habitats in order to reproduce (Rudnick et al. 2000). Adult crabs are found in fresh, brackish and salt waters, but oviparous females are normally found in greatest number in saltwater while larval stages are found in the open water of bays and estuaries (Rudnick et al. 2003; Veilleux & de Lafontaine 2007). Chinese mitten crab first records in Europe date back to 1912 in River Aller, Germany (Clark et al. 1998). From Germany, it has subsequently spread throughout Northern Europe. It was found in the UK in 1935 but became established in 1973 (Harold 1935; Clark et al. 1998). The study of *E. sinensis* spread in the UK suggests that when the population increases, exceeding the home range carrying capacity, the migration of partial populations to other upstream habitats becomes more probable. Now in the UK there is a concern that Chinese mitten crab will be spreading along the west coast and possibly further inland (Clark et al. 1998).

Figure 5: Photo of *Eriocheir sinensis*, taken by Martijn Winkelaar

Occupying different ecosystems, the damages caused by its presence can affect rivers, estuarine and saltwater ecosystems (Rudnick et al. 2003; Veilleux & de Lafontaine 2007). The ecological impacts are represented by: (1) predation: due to its opportunistic omnivore nature consuming aquatic plants, algae, detritus and a variety of macroinvertebrates (Gollasch 1999; Rudnick et al. 2003) while also preying on fish eggs (Veilleux & de Lafontaine 2007); (2) competition: indigenous crayfish species could be negatively affected by very abundant crab populations due to common freshwater habitats and diets (Veldhuizen & Stanish 1999; Rudnick et al. 2000; Veilleux & de Lafontaine 2007); and (3) biomass transfer from freshwater to estuaries: adult crabs migration out of freshwater systems to reproduce and die in estuaries indicates may impact the food web (Rudnick & Resh 2005).

Chinese mitten crab's economic impacts involve mainly the fish industry, since they can be the cause of profit losses for commercial fisheries and aquaculture facilities, feeding on the fish trapped in nets or on the farmed ones. Another relevant effect of *E. sinensis* presence is the erosion of dikes and riverbanks caused by its burrowing activities (Gollasch 2006). After juvenile crabs having migrated into channels and dikes, they dig burrows that provide a refuge and protection from desiccation on the banks between the high and low tide lines. The significant amount of sediment removed in areas with high densities of burrows can cause weakening of the bank, accelerate erosion and even cause banks to collapse (Rudnick et al. 2000).

Topmouth Gudgeon (*Pseudorasbora parva*, Temminck & Schlegel, 1846)

The topmouth gudgeon is a small cyprinid species indigenous to Japan, China, Korea and the Amur river basin. Today, it is widespread and locally abundant within favourable habitats throughout much of Europe and Russia (Perdices & Doadrio 1992; Wildekamp et al. 1997). Its life history is characterized by early maturity (sexually mature within 1 year); batch spawning, nest guarding and broad environmental tolerance limits. These traits enabled topmouth gudgeons to succeed in invading new water bodies (Ricciardi & Rasmussen 1998), facilitating its rapid invasion in European water bodies (Gozlan et al. 2005). This species is indeed now considered the most invasive fish species in Europe (Gozlan et al. 2010).

Figure 6: Photo of *Pseudorasbora parva*, taken by Michael J. Jung

Due to its ecological traits, topmouth gudgeons ecological impacts are yet visible after few years from its introduction. Britton et al. (2007) found that in a lake where the species was introduced in 2000, it rapidly established a breeding population that, by 2003, was the dominant species in size classes <70 mm and in 2004 it was the only species in the lake that produced young-of-the-year. *P. parva* can impede the recruitment of indigenous fish and become a potential threat for fisheries. *P. parva* can dominate fish communities and change the trophic web structure: a study comparing ponds with and without populations of *P. parva* shows how this invasive species overlaps with resident fishes resulted in depressed growth rates (and also production) and shifts in trophic position. For example, in the ponds with populations of *P. parva*, *Scardinius erythrophthalmus* has been observed to assimilate energy at a higher trophic level than in control ponds, probably by consuming *P. parva* (Britton et al. 2010).

A very important threat related to topmouth gudgeon is its potential ability to carry non-specific pathogens (Gozlan et al. 2005) and *Sphaerothecum destruens* that can cause diseases and mortalities in cultured North American Chinook salmon *Oncorhynchus tshawytscha* and Atlantic salmon *Salmo salar* (Andreou et al. 2011). For that reason, the spread of *P. parva* can lead to enormous damages in terms of indigenous biodiversity loss and economical costs for fish farming.

Rainbow Trout (*Oncorhynchus mykiss*, Walbaum, 1792)

Today, rainbow trout is the most widely introduced salmonid worldwide and one of the most widely introduced fish species in general (Crawford & Muir 2008). Since 1870, when the California Acclimatization Society conducted the first artificial propagation of rainbow trout from the San Francisco Bay area (Behnke 2002), this species has been introduced into at least 99 countries, with populations established in at least 53 of them (Gherardi 2010).

Figure 7: Photo of *Oncorhynchus mykiss*, taken by Anja Stettin

Rainbow trout introductions have strong impacts on indigenous salmonid species with problems related to red superimposition and competition for space and food (Scott & Irvine 2000; Seiler & Keeley 2009; Van Zwol et al. 2012) but also hybridization with conspecific species, leading to consequent loss of genetic diversity and integrity (Pearse et al. 2010; Simmons et al. 2010; Finger et al. 2011). Rainbow trout are also able to modify the indigenous macroinvertebrate community, changing invertebrate behaviour, drift and spatial distributions (McIntosh & Townsend 1994). They have also been observed to change the mean body size of macroinvertebrate populations through selective predation on the biggest specimens (Buria et al. 2007).

Due to its economic value as food resource and as valued game fish, rainbow trout are extensively farmed at the global level (Stankovic et al. 2015) and, as well as wild populations, trout farms can have a heavy impact on the surrounding wildlife. Trout raceway farms can modify the biodiversity in the outgoing stream, affecting the richness of Ephemeroptera, Plecoptera and Trichoptera by reducing the water quality (Loch et al. 1996). Trout cage farms tend to attract wild fish, changing also wild predator distribution (e.g. shags), since for them trout farms guarantee an easy access food resource that is constant during the year (Carss et al. 1993). The presence of wild predators often leads to conflicts with the fish industry because they are considered responsible of big economic loss and in the past years cormorants have become a highly politicised and unifying symbol of this problem (Buller 2008). The European Community is aware of the problem and in 2010 the European Commission has funded a Service Contract (No. 07-0307/2010/575579/SER/B3 Sustainable Management of Cormorant Populations, see http://ec.europa.eu/environment/nature/cormorants/Background-and-Activities.htm; last accessed January 2016) to **(1)** disseminate relevant information about cormorants through a Cormorant Platform, and **(2)** organise counts of cormorants in Europe during the breeding season and in winter.

Conclusion

The necessity for species-specific impact assessments is the first step in any attempt to prioritize management efforts. Moreover, prevailing studies on the impact of invasive species are short-term research. For example, 88 % of the 85 quantitative studies analyzed by Scalici & Gherardi (2007) monitoring the impacts of NICS had a duration of one year or less. Previous studies have shown an unequal distribution of scientific attention mostly after a species had already become invasive. Risk assessment and control measures for general pathways and vectors could have prevented economic and ecological damage in a variety of environments before AIS establishment. Therefore, it is necessary to 1) develop modelling tools for a broad spectrum of species to assess potential risks in the beginning of an invasion or even before; 2) quantify the impacts made by AIS to 3) better understand the effects AIS have on ecological, socio-economical and health aspects, and 4) develop existing control methods and novel approaches to provide successful future management of invasive species. Risk assessment and modelling of potential distribution of non-indigenous species is crucial to define those likely to cause harm in some areas, and then to have the possibility to better direct monitoring programs, early detection and control efforts on prioritized areas.

Hence, there is an urgent need for long-term studies, especially in multiple invaded areas with complex interactions among indigenous and non-indigenous species as a strong foundation for any further management or control measure.

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Chapter 1.2

Protocols to assess ecological and economic impacts of Aquatic Invasive Species.

Part of the AQUAINVAD-ed Project Deliverable 3.1

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Abstract

To completely observe and understand the impact of an alien species, impacts would have to be monitored from the introduction during its establishment up to a point where its presence allows its categorization as "invasive". Due to the difficulty in this application, most studies assess the impact of invasive species that are already present but most often lack important aspects or are designed as short time studies. Therefore, various protocols have been developed to assess ecological or economic impacts, but proper tools to assess cultural or social impacts are still afar. Additionally, such tools are rather species specific, but the influx of alien species due to globalization demands the design of protocols that comprise the impacts of more taxa based on standardized evaluations under the consideration of all potential impacts without being biased towards ecological impacts. In this review, outdated and presently used protocols are discussed and future needs are pointed out.

Introduction

When introduced, alien species can exert neutral or positive effects (Graves & Shapiro 2003; Schlaepfer et al. 2011) or become invasive, being very harmful for the recently colonised environment (McCarthy et al. 2006). Invasive alien species (IAS) impacts can vary in magnitude: they are considered one of the main causes of biodiversity loss (Sala et al. 2000; Gurevitch & Padilla 2004) and of biotic homogenization (Clavero & Garciá-Berthou 2005; Occhipinti-Ambrogi & Galil 2010), affecting native species by competition, niche displacement or hybridisation, and even causing their extinction (Figure 1). For these reasons, assessing and quantifying IAS ecological impacts (but also socio-economic and cultural ones) are crucial to prioritise species and promote effective policies of management and control (Clout & Williams 2009). However, this issue is complicated and controversial, because solid data on impacts are not always available or sometimes are only anecdotal. The quantification methods used can be different, depending on the choices of the researchers who conduct the investigation (Stiers et al. 2014), consequently making it more difficult to compare different studies that examine different IAS and measure their impacts at different ecological level or at different spatial and temporal scales (Hulme et al. 2013). Recently, Blackburn et al. (2014) proposed a new unified classification on the magnitude of their environmental impacts that could be integrated with existing practices and policies, allowing an easy comparison of the diverse impacts of IAS (see below).

Natural environments and therefore ecosystems provide a very broad range of services with fundamental significance to human well-being (Braat & De Groot 2012; De Groot et al. 2012). The loss of biodiversity and ecosystem degradation due to threats such as climate change, human overexploitation of natural resources and also the increasing spread of IAS continuously threaten the functioning of ecosystems and resilience to further invasions and self-sustainability (Pejchar & Mooney 2009; Gherardi et al. 2011; Bacher et al. 2017). IAS can affect and exploit not only their surrounding natural environments but also human well-being and activities, causing undeniable socio-economic impacts (Juliano & Lounibos 2005; Bacher et al. 2017). However, the socio-economic effect of IAS on the recipient ecosystem and its provided services remains less explored. Typically, socio-economic impacts are distinguishing into i) cultural, ii) economic and iii) social impacts, with the notion that impacts on health can be partially belonging to the socio-economic impacts (EPPO 2011; Ten Brink 2011). In addition to the impacts on water, land, air, and wildlife, also the impacts on health are considered as part of the biophysical impact realm. Classical examples of socio-economic impacts can be the different damages to food, fuel or fibre production or even more direct like negative effects on human health or tourism (Blackburn et al. 2014; Bacher et al. 2017; Figure 1).

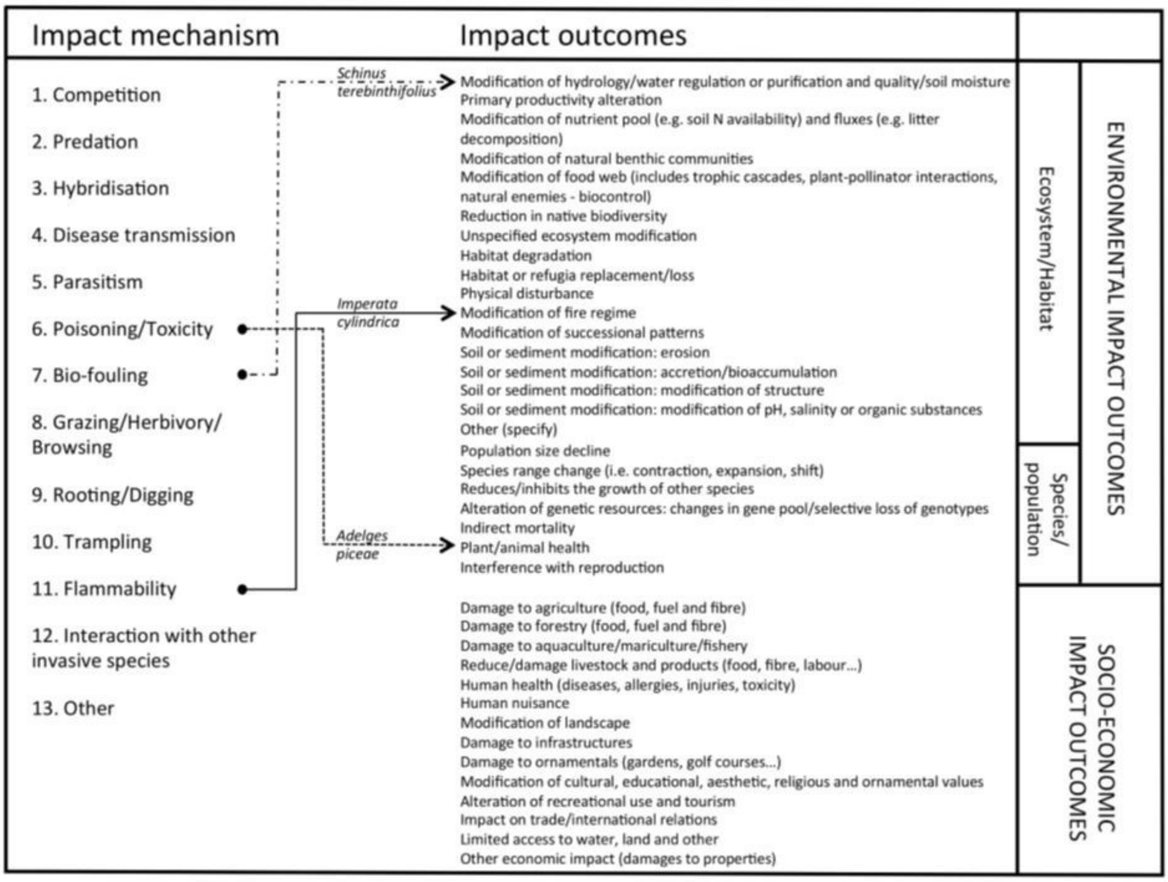

Figure 1: Impact scheme of the Global Invasive Species Database, implemented by the IUCN Species Survival Commission (SSC) Invasive Species Specialist Group (Blackburn at al. 2014).

Therefore, it will be one of the main obstacles and challenges for the future to find, establish and consequently apply comparable protocols to measure, assess and compare risks and damages associated to the impact of IAS. Here, we give a brief overview of classical approaches to assess impacts, followed by a short review on previously applied and novel protocols for these tasks.

Classical approaches to assess invasive species impacts

To better understand the potential impact of a newly arriving species studies are conducted to assess its biology (reproduction, behaviour, feeding habits, tolerance towards environmental factors) and its relationship with native species (e.g. predation, competition). Outcomes from these studies becomes increasingly important to provide crucial information that can be used to predict the potential invasiveness of a species, particularly in risk assessment processes. The major part of information on aquatic IAS impacts are provided by damages caused to some economic activities (e.g. fisheries, aquaculture; Katsanevakis et al. 2014), or by experiments conducted under laboratory conditions to study different aspects, such as survival and growth (Sáez-Royuela et al. 1995), feeding behaviour (MacIsaac et al. 1995; Horgan & Mills 2011), competition (e.g. Gherardi & Cioni 2004) or potential vectors for parasites (Gozlan et al. 2005). These kinds of studies provide useful information on the ecology of the invader, but they are usually confined to evaluate effects on a single or few species and should be complementary to the field research. The latter, despite being time demanding and context dependent (Kumschick et al. 2015), can provide a wider overview on the effects of the species in the invaded environment.

The selection of appropriate parameters should account for impacts at different organizational levels, such as individuals, populations, communities, and ecosystem functions (Kumschick et al. 2015). Most of these studies have involved comparisons of invaded and uninvaded reference sites, primarily at the fine resolution of plots and their restricted extent to infer the impacts of alien species on particular native species, on community structure (i.e. species diversity), and on ecosystem processes such as nutrient pools and fluxes (Kumschick et al. 2015). However, it is often very difficult to find contemporaneous similar but uninvaded reference sites to contrast with invaded sites (Kumschick et al. 2015). Moreover, most studies of the effects of IAS have been brief and lack a temporal context; 40% of recent studies did not even state the amount of time that had passed since the invasion, while ecologists need theory and empirical data to enable prediction, understanding and management of the acute and chronic effects of species invasions (Strayer et al. 2006). Therefore, without a general comprehension of a species invasiveness it will be difficult to assess species impact on environment as well as on socio-economically relevant ecosystem services.

A notable example of how a newly introduced species is able to influence local economy and culture is provided by the case of the European catfish *Silurus glanis,* introduced outside its native range into freshwater ecosystems of various European countries such as Italy (Nocita 2007; Nocita & Lenuzza 2016). At first, it was a sought-after target species for angler due to its enormous size, attracting anglers from all over the world to rivers with dense populations; then, it was considered invasive for its effects on local biodiversity and, consequently, control measures and attempts to eradicate it started (Nocita 2007; Nocita & Lenuzza 2016). However, today, almost 20 years after its introduction, various aspects of this species biology are still unknown (Copp et al. 2009a), making basic studies in regional ecosystems even more important as many attempts to eradicate it failed. Almost simultaneously introduced to the same rivers, the North

American channel catfish *Ictalurus punctatus* spread into European freshwater ecosystems, but, due to a longer lag phase, it was neglected by the general public. However, its establishment has affected not just the recipient environment, but also changed the behaviour of many anglers in Tuscany Region (Central Italy; Haubrock et al. 2018) for a second time: new abundant populations of *I. punctatus* have induced a switch in the preferred target species. Anglers are more and more targeting this species due to its easiness to catch compared to *S. glanis.* This change can be even observed in local fishing shops, which are selling specialized protocols for catching this fish and even entire fishing competitions focusing on this species (T. Busatto, pers. comm.).

Looking at the process of invasion itself, single invasions have to be differentiated from invasions of previously invaded ecosystems. The latter, in particular, are of increasing importance because many areas have already been invaded by several IAS. Interactions between IAS can determine the different success and magnitude of an invasion: IAS can indeed facilitate or impede the success of another IAS. Rolla et al. (2017) showed the importance of studying potential synergies between two notable invaders: in this study, it has been shown that killer shrimp (*Dikerogammarus villosus*) is able to chemically recognise and is attracted by the presence of zebra mussel (*Dreissena polymorpha*) in the water. Considering that zebra mussel can provide a suitable substratum for the survival of killer shrimp (Gergs & Rothhaupt 2008), this study has become a good example of how the presence of one IAS can potentially facilitate the spreading and the settling of a second one.

In the case of multiple invaded ecosystems, it is valuable to adopt an integrated approach, i.e. combining existing methods that can provide complementary information of the same situation, better describing it. For example, the combination of stomach content analysis, which provides a snapshot on interspecific relationships, with the stable isotope analysis, that is used to compare trophic niches among different regions, ecosystems, countries (Bearhop et al. 2004), is a highly recommended protocol. Stable isotope analysis is able to depict a longer framed view of relationships among species than the stomach content analysis. Haubrock et al. (2017), who indeed combined stomach content analysis with stable isotope analysis, revealed that the North American bullfrog *Lithobathes catesbeianus,* known for its heavy negative effects on native European amphibians (mainly through predation and competition), caused less impact when the invasive North American red swamp crayfish *Procambarus clarkii* was present in the same invaded pond. The availability of this abundant prey led to an almost entire shift in the diet of bullfrogs, which prefer to eat crayfish rather than the native common European frog *Pelophylax kl. esculentus*. Thus, this shift reduced the predation on European frogs and even decreased the competition with it, allowing the presence of both species in the same ecosystem and a rapid increase in the abundance of *L. catesbeianus* due to the presence of *P. clarkii* (Haubrock et al. 2017).

To understand these underlying changes in ecosystems, the investigation on single species should always be followed by studies on the entire species networks, analysis of trophic webs and feeding networks within the biome in a given area, to depict a more precise view on the overall situation. Overall, all this information has to be collected and combined for predicting or assessing impacts, using classical or more efficient novel protocols.

Protocols to predict/assess impact

The basis for every assessment has to be an in-depth knowledge on the ecology and behaviour of the IAS in both native and invaded ecosystems, as well as their impacts, considering also that they might change during or after a long phase of population growth and establishment or might vary among different invaded ecosystems. Anecdotal information is not allowed as they do not have a solid scientific basis, while thorough investigations on all important aspects related to the impacts and on the invaded ecosystem (invasibility included) should be conducted. Once retrieved these information, different approaches to assess the diverse impact of an IAS using specific protocols can be undertaken.

Classical protocols

In many European countries, IAS impacts are classified at a national level without standardized criteria e.g. Austria (Essl & Rabitsch 2004), Switzerland (Wittenberg et al. 2006), Liechtenstein (Staub 2006), Ireland (Stokes et al. 2006), Spain (Capdevila-Argüelles et al. 2006), and Ukraine (Protopopova et al. 2006). These classifications are mainly based on the spatial distribution and spread capacity of an IAS, and their negative impacts on biodiversity. The limit of this approach is the impossibility of comparing ecological impacts that have been measured with different criteria (e.g. impacts measured at a species-specific level with impacts measured at a community level). An additional issue concerning especially aquatic IAS impacts is that only data on a limited number of species is available, usually the ones with the heaviest economic impacts. While many publications are available on terrestrial species, especially plants (Kriváneк & Pysek 2006; Anastasiu & Negrean 2005; Weber et al. 2005; Andreu & Vilà 2010), the existing literature on freshwater species is less extensive, and on marine species is even more scanty, due to the difficulty to conduct field studies.

To overcome the different national approaches, several risk assessment protocols- some for more limited groups (e.g. EPPO), other for a wider spectrum of taxa (e.g. Harmonia)- have been developed to predict potential invasiveness of a species not already present in the area where the risk assessment is conducted, or to prioritize IAS already present in that area to provide management advices. These protocols do not assess the impacts but rank the species as low, medium or high impact species, and can be run if scientific information is available for that species.

Alien invasive fish species can serve as an example of this approach. Although exerting a generally lower economic impact than alien birds or mammals (Kumschick & Nentwig 2010; Nentwig et al. 2010), the assessment and regional comparison is of high value for future efforts as they have manifold negative impacts on invaded ecosystems (McKnight et al. 1995; Caraco et al. 1997; Crooks 1998; Simon et al. 2004; Caiola & De

Sostoa 2005; Gozlan et al. 2005; Hänfling et al. 2005; Alcaraz et al. 2008; Britton et al. 2010; Vila et al. 2010; Almeida et al. 2012) with economic costs up to annual losses of $5.4 billion in the USA (Pimentel et al. 2005).

Copp et al. (2005, 2009b) proposed a protocol for Great Britain based on eight topics (e.g. domestication, climate and distribution, invasive elsewhere) and 49 questions, the "Fish invasiveness scoring kit" (FISK). Conveniently, it applies a statistically appropriate threshold score for high-risk species, which makes it a useful and viable protocol to aid decision and policymakers in assessing and predicting potentially invasive fish species or to prioritize these already present depending on their impacts. FISK was also applied in other countries (e.g. Almeida et al. 2013), and a similar protocol was developed for freshwater invertebrates (Tricarico et al. 2010), while a novel protocol including all aquatic animal taxa (freshwater and marine) and climate change issue has recently been developed, AS-ISK (Copp et al. 2016). Branquart (2007) proposed a classification of IAS impacts on biodiversity and distribution in Belgium. His method applies to several taxa including freshwater fish and amphibians and assesses their impact basing on five criteria (spatial distribution, dispersal ability, occurrence in natural habitats, negative impacts on species and biodiversity, change of ecosystem functions). Miljoministeriet (2009) takes into account 32 aquatic IAS in Denmark, classifying their impacts according to feasibility of control. Gederaas et al. (2007) proposed to list invasive species in Norway (217 taxa, including freshwater species) using four criteria: negative effects on habitats or ecosystems, species, genetic diversity, vector for other species (e.g. parasites). A conservative additive model was developed to account for the Cumulative IMPacts of invasive ALien species (CIMPAL) on marine ecosystems (Katsanevakis et al. 2016). According to this model, cumulative impact scores are estimated on the basis of the distributions of invasive species and ecosystems, and both the reported magnitude of ecological impacts and the strength of such evidence.

Other approaches exist in Europe, for example habitat specific or taxa-specific trans-national risk assessment systems. In this context, risk assessment systems focusing on mammals (Nentwig et al. 2009), birds (Kumschick & Nentwig 2010) and plants (Brunel et al. 2010) need to be mentioned, while the only European habitat-specific assessment system for brackish and marine environments was tested in the Baltic Sea (Olenin et al. 2007).

Another approach is to adapt tested and consolidated risk assessment protocols developed elsewhere to the European context. Gassó et al. (2009) and Crosti et al. (2010) adapted the Australian Weed Risk Assessment to the classification of Mediterranean vascular plants. In a similar way, Krivánek and Pysek (2006) used the Australian Weed Risk and the scheme developed by Reichard and Hamilton (1997) for woody species in North America to assess the invasiveness of woody plant species in the Czech Republic. Indeed, even FISK is a by-product of the Australian Weed Risk Assessment.

It is generally known that IAS can affect human well-being in different ways other than the economic one, for example impacts on landscape, land use, aesthetics and cultural-historic value, loss of employment and general products and services such as water quality, animal grazing, hunting and fishing (Stiers et al. 2014). As these are of high value for humanity, it is becoming increasingly important to express the value of ecosystems in monetary units, mostly to raise awareness and attention among people and policy makers (Bacher et al. 2017). Although there have been various studies to evaluate such services provided by ecosystems on local scales (e.g. de Groot et al. 2012), these assessments remain difficult, vague and often anecdotal. Hence, it becomes important to estimate the impacts of IAS on such ecosystem-services.

To assess, systematically analyse and rank such socio-economic impacts, six steps are necessary: i) scoping: a preliminary analysis to identify and prioritize potential impacts of significance; ii) profiling baseline conditions: the gathering of information about the potentially affected environment and associated socio-economic components; iii) impact prediction: under the consideration of previous experiences and steps 1 and 2, trade-offs between adverse and beneficial impacts have to be analysed; iv) mitigation identification: following the identification of impacts, mitigate strategies, plans and programs to avoid, target or manage them have to be developed and applied; v) evaluation of significance: determine whether the proposed change is causing significant impacts on valuable social-economic components, and vi) application of mitigation and monitoring: with monitoring programs, the development has to be followed (Ramanathan 2001; Anand 2007; Petts 2009; Glasson et al. 2013). It is very much important to state that IAS do not tend to impact only the biophysical or the socio-economic realm. It is more reliable that IAS effect various ecosystem services simultaneously, and IAS themselves need to be assessed and prioritized according to it (Anand 2007; Gherardi et al 2011; Hawkins et al. 2015; Bacher et al. 2017). Landis' study (2004) on a conceptual model formulation for the Ecological Risk assessment for nonindigenous species can be considered as one of the earliest studies on this issue (see also Miller et al. 2010). It addresses the application of ecological risk assessments at the regional scale to the prediction of impacts due to IAS. Focusing mostly on models to assess risks, it also considers biodiversity impacts in the case of replacement of economically, culturally and socially important species through a decrease in habitats, biodiversity and change in population dynamics. In Australia, the Department of Primary Industries (2008) developed a prioritisation process based on potential risks in an Analytical Hierarchy Process (AHP), ranking alien weeds according to i) each one's invasiveness, ii) the previous and current distribution, and lastly iii) by determining the impacts of the plant on social, economic, and environmental values. A more elaborate approach was developed by Cowie et al. (2009), who assessed the importance of quarantine of alien non-marine snails and slugs for the US. In this preliminary risk assessment, species were ranked, based on a thorough literature review, according to 12 attributes from which seven are biological variabilities and five interactions with humans. Beside a general consideration of impacts on biodiversity, the author also considered economic impacts, more explicitly the potential economic damage and associated costs. A different approach, in the form of a "decision support scheme" for potential quarantine pests, was developed by the European and Mediterranean Plant Protection Organization (EPPO 2011) but can hardly be considered as an impact assessment approach.

Novel protocols

Novel methods have been developed with the aim of being transnational, more inclusive and adaptable to different taxa. Essl et al. (2011) produced GABLIS, a transnational and taxonomically universal risk assessment system, tested for invasive species in Germany and Austria. GABLIS uses five basic and six complementary criteria to assess invasive species impacts and assigns them to three groups: White List (no negative impact, non-invasive), Grey List (likely present, potentially invasive), and the Black List (negative impact confirmed, invasive). This methodology has been created with the aim of being applicable to all groups of organisms and it has been tested for fish, vascular plants, mammals, birds and macrozoobenthic species, confirming its effectiveness. Potentially GABLIS could be extended to a wider level to assess the impact of invasive species in Europe, analysing the data from the DAISIE project (www.europealiens.org) and the NOBANIS project (www.nobanis.org).

Blackburn et al. (2014) developed a standardised protocol to evaluate and compare the magnitudes of IAS different impacts, the so called “General Impact Scoring System” (GISS), which enables a simple classification of alien taxa according to their impact on invaded ecosystems (Nentwig et al. 2010). This is applicable at different levels of ecological complexity and different spatial and temporal scales. The peculiarities of GISS are that (i) it considers only environmental impacts; (ii) it highlights potential consequences on biotic and abiotic factors; (iii) it classify invasive species on the basis of evidence of their documented impacts in the regions where they have been introduced; (iv) it provides a consistent procedure for translating the broad range of impact types and measures into ranked levels of environmental impact; (v) it can be applied across taxa and at different taxonomical levels; (vi) it considers consequences and not likelihoods; (vii) it is based on the best available evidence, consequently a species can move up and down impact categories, and (viii) it can be applied to impacts assessed at a range of spatial scales, from global to national or regional. This protocol can be used to identify IAS that have impacts at different levels, facilitate comparisons of the level of impact from alien species among regions and taxa, and improve the ability of predicting potential future impacts of the target species. It can even get aligned with the mechanisms of impact identified in the International Union for Conservation of Nature (IUCN) Global Invasive Species Database ([GISD]; http://www.issg.org/database), and it can help the stakeholders to prioritise management actions. It uses five semi-quantitative scenarios to assign species according to different level of impacts (Minimal to Massive) while the “Level of confidence” is also considered (Figure 2).

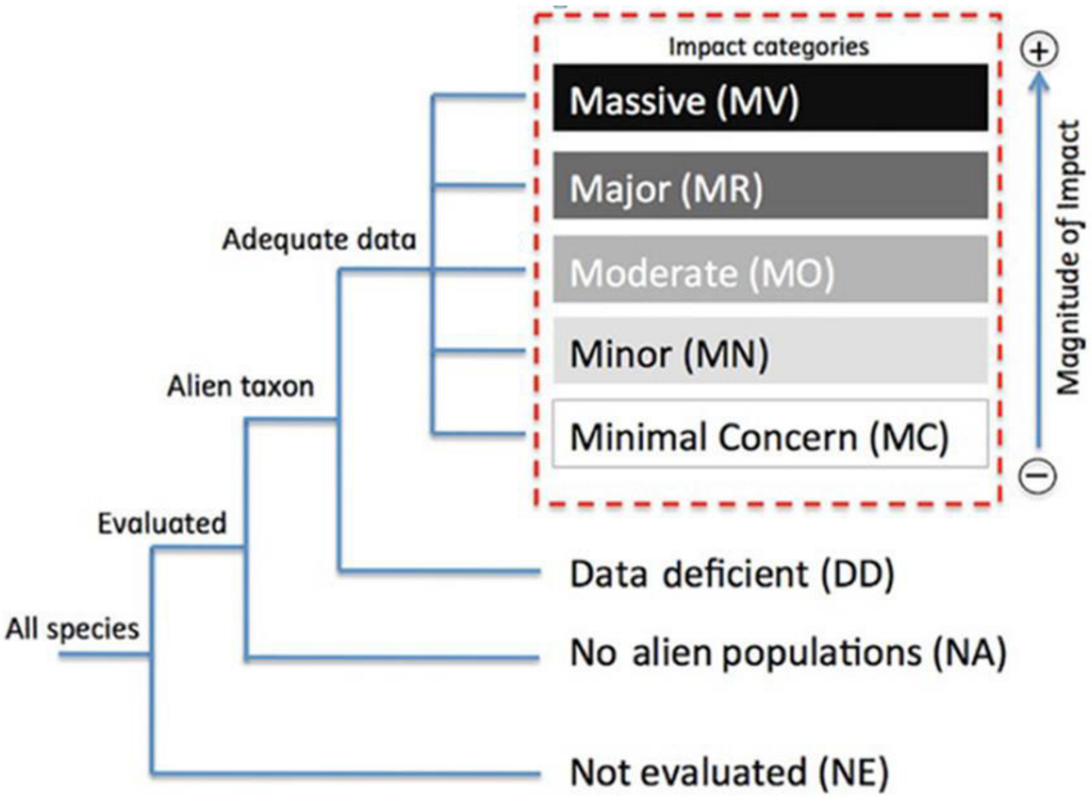

Figure 2: The different categories in the alien species impact scheme, and the relationship between them (Blackburn at al. 2014).

Therefore, and this is crucial, GISS cannot be considered as a predictive model. Nonetheless, it can be used to point out species with potentially high impacts that need to be evaluated in detail in a particular introduction context. Outgoing from species-specific protocols, GISS (Blackburn et al. 2014) can be seen as the first major protocol for a broader, analytical and applicable risk assessment of a wider spectrum of species. From then, considerable progress has been made in the last years to develop a unique protocol to quantify environmental impacts (e.g. Blackburn et al. 2014; Hawkins et al. 2015; Bacher et al. 2015; Kumschick et al. 2015).

A significant advance was the development of the "Environmental Impact Classification for Alien Taxa (EICAT)" (Hawkins et al. 2015). When applied consistently, the EICAT protocol can help at 1) comparing, ranking and prioritizing IAS according to impact, 2) comparing their impacts among regions, 3) facilitating predictions of future impacts, 4) aiding in prioritizing management actions, and 5) aiding at evaluating management methods. Species are therefore ranked into the five impact categories used in the Generic Impact Scoring System, which hence depend on the impacted biological organization (individual, population or community). Because of its transparent nature and simple application, EICAT aligns its mechanism with those identified by the International Union for Conservation of Nature (IUCN) Global Invasive Species Database (http://www.issg.org/database; Kumschick et al. 2012). The latest advance in the pursuit of establishing a protocol to assess the socio-economic impacts of IAS on ecosystem services is the “socio-economic impact classification of alien taxa (SEICAT)” protocol (Bacher et al. 2017). It differs from EICAT and other previous protocols as it only considers the impacts on human well-being by focusing on deleterious impacts and classifying IAS according to the best available evidence on these. As a mean of comparing EICAT and SEICAT, it should be states that the assessment units in EICAT are native species in local communities, while in SEICAT the assessment units are human activities. Hence, by assessing human activities, it does not weigh negative against positive impacts of IAS to estimate a “net” value, but rather highlights potential consequences. This is of considerable importance, as alien species may provide benefits for human well-being (Pienkowski et al. 2015), and therefore should be taken into consideration. More importantly, it can be combined with EICAT to additionally assess environmental to the socio-economic impacts of concern, enabling to assess the complete spectrum of deleterious impacts. Looking at the classification of impacts, the original scheme from GISS has been adopted, with a ranking starting at Data deficient species (DD), Minor Negative effects (MN), followed by Moderate Negative effects (MO), Major Negative effects (MR) and finally Massive Negative effects (MV) (Table 1). However, even if with high uncertainties and therefore a major lack of information, SEICAT is capable of successfully ranking species (Boscher et al. 2017).

Not only risk assessment protocols can be seen as “novel protocols” suitable for the assessment of IAS impacts. As their understanding increases, more questions will have to be answered. Studies on potential synergies between IAS have to be considered more thoroughly, especially as invaded ecosystems become prone to further invasions (Simberloff & Von Holle 1999). Hence, new methods, such as the investigation of strontium isotopes (Rundel et al. 2012) or modern genetic protocols (Schwartz et al. 2007), prove to be noteworthy for an increasing understanding of IAS, e.g. their biology, behaviour and the invasion process, concurrently showing the need for a further ongoing development of such protocols.

Table 1: Description of Socio-Economic Impact Classification of Alien Taxa (SEICAT) according to observed changes in peoples' activities (Bacher et al. 2017).

Minor	MN	AIS makes it difficult for people to participate in their normal activities Activities may suffer in at least one constituent of well-being Reductions of well-being Increased difficulty in accessing goods Disruption of social activities Induction of fear But no change in activity size is reported, i.e. the number of people participating in that activity remains the same
Moderate	MO	Effects on well-being leading to changes in activity size Fewer people participating in an activity, but the activity is still carried out Reductions in activity size can be due to various reasons, e.g. moving the activity to regions without the alien taxon or to other parts of the area less invaded by the alien taxon Partial abandonment of an activity without replacement by other activities; or switch to other activities while staying in the same area invaded by the alien taxon Spatial displacement, abandonment or switch of activities does not increase human well-being compared to levels before the alien taxon invaded the region
Major	MR	Appearance of an activity from all or part of the area invaded by the alien taxon Collapse of the specific social activity, switch to other activities, or abandonment of activity without replacement, or emigration from region Change is likely to be reversible within a decade after removal or control of the alien taxon "Local disappearance" does not necessarily imply the disappearance of activities from the entire region assessed, but refers to the typical spatial scale over which social communities in the region are characterised
Massive	MV	Local disappearance of an activity from all or part of the area invaded by the alien taxon Change is likely to be permanent and irreversible for at least a decade after removal of the alien taxon, due to fundamental structural changes of socio-economic community or environmental conditions ("regime shift")
Data deficient	DD	There is no information to classify the taxon with respect to its impact, or insufficient time has elapsed since introduction for impacts to have become apparent

Final considerations

According to Vilà et al. (2010), in Europe alien terrestrial vertebrates and freshwater organisms are shown to be of particular concern, with more than one-third of recorded species known to cause impacts. One reason for this is the preponderance of predatory or omnivorous taxa among alien vertebrates and aquatic invertebrates. Freshwater ecosystems are considered more vulnerable to introduced predators than are terrestrial and marine ecosystems, because native organisms generally have fewer defence mechanisms and greater naiveté toward novel predators (Cox & Lima 2006). The North Sea is the marine region with the highest number of alien species associated with ecological and economic impacts in Europe; this basin, together with smaller marine basins, such as the Baltic and Black Seas, harbour the highest proportions of species (Vilà et al. 2010). The most widespread detrimental aquatic organisms in Europe are crustaceans, such as the Chinese mitten crab (*Eriocheir sinensis*), and molluscs, such as the zebra mussel (*Dreissena polymorpha*) and the Pacific oyster (*Crassostrea gigas*).

Especially predatory aquatic alien species pose a substantial risk for recipient ecosystems and their flora and fauna. For example, looking at a major study on the ecological impact of alien invasive fish species by Van der Veer & Nentwig (2014), the greatest negative effects of alien fish species on the environment were associated with predation on other animals, followed by competition and disease transmission. Although the transmission of diseases might be assumed to contributed only to a minor part of a species invasiveness, it was estimated to be the fourth most important category, as aquatic species can carry a wide range of potential agents (McLoughlin & Graham 2007; Jutfelt et al. 2008; Grabner & El-Matbouli 2009). Interestingly, herbivory was generally a relatively less important impact category, assumed that it poses only a rather limited threat to the native aquatic vegetation. Nonetheless, various fish species are known (*C. auratus* / *C. gibelio* and *Ctenopharyngodon idella* in particular) or are assumed (*Ictalurus punctatus*), to have considerably negative influences on submerged vegetation, inducing strong bottom up effects (P. J. Haubrock, pers. comm.). Additionally, an aspect that is mostly not considered in any assessment is how the presence and associated diverse impacts of alien species may interact with potentially beneficial impacts of the same or other present alien species. Even in the most predictive assessments and social economic impact assessment, it has been impossible to estimate how IAS impact ecosystems that have been previously invaded by other alien species due to potential facilitation processes.

Human well-being is generally context- and especially culture-dependent and can therefore not be generalized from one ecosystem or one country to another and should not be solemnly assessed in terms of financial assets. Rather, socio-economic impact assessments should focus on the number of people affected and how massive their effects on human lives and well-being are (Diener & Seligman, 2004; Pejchar and Mooney 2009). Ecosystem services cannot be treated as limitless resources for humans, should rather be valued and hence be evaluated in its value to society, and more importantly the costs associated with their loss and degradation and the efforts to sustain them have to be accounted for (Costanza et al., 1997; Blignaut and Moolman, 2006; ten Brink et al. 2011). Most importantly, actions based on scientific studies need to be adapted into governmental policies. It therefore might appear strange that although aquatic IAS, especially fish that have caused huge global economic costs are still being introduced for aquaculture, as ornamental fish, or for sport fishing and fisheries (Gozlan 2008).

Conclusions

Future challenge is the comparable estimation of monetary costs associated with the socio-economic and cultural impacts of IAS. Nonetheless, science never rests, and new approaches, new methods and new ideas will be developed to evaluate ecosystem services, ecological and socio-economic impacts. These novel protocols like SEICAT have to be implemented into the work of stakeholders and policy makers, and generally and repeatedly applied to help protect the socio-economic value of ecosystems to human society. Such approaches need to be made for every potentially new invasive species as soon as enough reliable data are available (Haubrock et al. 2018). It will be necessary to start at the basis of every impact assessment: the understanding and analysis of an IAS biology and behaviour.

In conclusion, future works on the assessment of aquatic invasive species impacts should give priority to (1) taking into account IAS introduction history and potential synergies with other IAS already present in the area; (2) combining different scientific and evaluation methods; (3) using assessment protocols already existing (e.g. GISS, EICAT, SEICAT); and (4) collaborating with national and international partners to obtain consistent results and more data.

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CHAPTER 2

ASSESSING THE IMPACTS OF AQUATIC ALIEN SPECIES

Chapter 2.1

Burrowing activity of *Procambarus clarkii* on levees – analysing behaviour and burrow structure

Short Title: Burrowing on River Levees

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Abstract

The North American crayfish *Procambarus clarkii* is considered among the most invasive species in freshwater ecosystems, causing declines in habitat-changes as well as biodiversity. The burrowing behaviour and the possible impact of *P. clarkii* on levees have not yet been studied in depth. To assess shape, volume and structure of its burrows and the associated behaviour, experiments were conducted introducing two size-matched adult crayfish into a water tank containing an artificial levee and video-recording their behaviour for 96 hours under a natural day-night cycle. At the end of each replicate, casts of excavated burrows made with polyethylene foam were retrieved. Crayfish (n=40) dug 17 burrows, six of which having an enlarged terminal chamber. The average excavated levee volume of burrows was 1.9% (0.00528 m^3; 5.0256 l) ±0.86% of the total volume with a maximum of 4% (0.0109 m^3; 10.9 l) and the chambers (mean volume of 0.9 ±0.6 dm^3) contributed to up to 50% of the excavated volume. No significant difference between sexes was found for any observed behaviour. Our study demonstrated *P. clarkii* burrowing behaviour in an artificial setup simulating a levee structure and additionally showed how female and male *P. clarkii* are similar for burrowing activity. As a result, we quantify the potential pressure exerted by the red swamp crayfish on levees and lastly highlight the observation of cooperating burrowing behaviour of male and female individuals in this species.

Keywords: red swamp crayfish, impact assessment, invasive alien species, impact

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Introduction

As an effect of the advancing breakdown of biogeographic barriers, the introduction of alien invasive species is ranked among the main drivers of biodiversity loss (Sala et al. 2000; Clavero & García-Berthou 2006; Millennium Ecosystem Assessment 2005; Gherardi 2007; Copp et al. 2014a, b), and homogenization of ecosystems (Rahel 2002). Invasive alien species (IAS) have often caused irreversible damage (Gozlan 2008; Jackson et al. 2002, Keller et al. 2011), eliciting severe ecological, economic and social impacts (Gherardi et al. 2009; Mazza et al. 2014a). The lack of geographical barriers (Lodge 1993), the specifically high intrinsic dispersal ability of aquatic organisms (Ricciardi & Rasmussen 1999; Beisel 2001) and extensive human influence make freshwater ecosystems particularly prone to biological invasions (Havel et al. 2015). The North American crayfish *Procambarus clarkii* is considered a highly invasive species (Huner et al. 1999, 2002) that has been intentionally introduced (Holdich 1999; Souty Grosset et al. 2006, 2016) throughout the world in the 20th century (Lodge et al. 2012). The European invasion by *P. clarkii* began with an introduction to Spain in 1973 for aquaculture purposes (Souty-Grosset et al. 2016). The species has steadily spread across Europe, becoming abundant in Portugal, Spain, France and Italy. It was originally introduced to increase crayfish harvests (Souty Grosset et al. 2006, 2016), but it is currently traded also for ornamental purposes (Mazza et al. 2015).

Procambarus clarkii is a successful coloniser in Europe (Souty-Grosset et al. 2016), quickly establishing in new and different environments. It is listed among the 100 worst invasive species in Europe (DAISIE, 2011), and among the species of unional concern under the new EU Regulation 1143/2014 regarding alien invasive species. *Procambarus clarkii*, widespread in freshwater and even brackish ecosystems (Scalici et al. 2010; Souty Grosset et al. 2016), has been recently reported to also colonise cave ecosystems (Mazza et al. 2014b). It exhibits a wide range of impacts through predation, competition, bioaccumulation and increased eutrophication, and pathogen and disease transmission (Barbaresi & Gherardi 2000; Angeler et al. 2001; Mazza et al. 2014a; Souty-Grosset et al. 2016). The ability to exert heavy impacts, while being tolerant to diverse environmental conditions, makes the species able to completely transform habitats, thus giving *P. clarkii* the role of an ecosystem engineer (sensu Jones et al. 1997). The species is considered a tertiary to secondary burrower (Gherardi 2000; but see also Ilhéu et al., 2003), i.e. digging burrows to withstand environmental extremes periods (e.g. drought) and during reproductive periods (Huner et al. 1994; Gherardi & Barbaresi 2000; Gherardi 2006; Souty Grosset et al. 2014). It can thus overcome unfavourable conditions in the invaded habitat (Souty-Grosset et al. 2016). Its burrowing activity is known to vary according to soil particle size, water cycle and, as indicated by diverse studies on burrow behaviour, structure of land as well as to damage agricultural and natural systems, causing channel bank erosion (Figure 1), increase of water turbidity and fine sediments (Huner 1977; Anastacio and Marques, 1997; Holdich 1999; Rodriguez et al., 2003; Correia & Ferreira 1995; Ilhéu et al., 2003; Barbaresi et al. 2004; Orlandini et al. 2015), significant physical modification (Barbaresi et al. 2004), and reduction of plant density (Souty Grosset et al. 2014). Moreover, burrowing animals were identified as one of the main causes for levee failures (Chang & Lange 1967; Rudnick et al. 2000; Gribsholt et al. 2003; Serre et al.

2008; Hanson et al., 2010; Condicelli 2016; Consumi 2016) causing floods (Figure 1) and damage in excess of $500 million in Italy (Orlandini et al. 2015). In Italy, crayfish burrowing activity has already damaged about 30% of partially human shaped irrigation canals with huge costs for management authorities (Lodge et al., 2012).

Figure 1: A = Typical burrows produced by *Procambarus clarkii* on irrigation ditches; B = Due to the burrowing activity of *Procambarus clarkii* broken levee alongside the river Secchia (Emilia Romana, Italy.

Previous studies have looked at burrowing behavior of invasive crayfish outside of their native range (Correia & Ferreira 1995; Barbaresi et al. 2004; Souty-Grosset et al 2014; Kouba et al. 2016), but only a very few have directly quantified burrow morphology and related this directly to damage of river banks and/or levees (e.g. Guan 2010; Orlandini et al. 2015; Consumi 2016). Nonetheless, burrowing species have been hypothesized to negatively affect fisheries, aquaculture (Darryl 2001; Nates & Felder 2007) and physical structures (Jones et al 1997; Dorn & Volin 2008; Orlandini et al. 2015; Conicelli et al. 2016).

Previous studies suggest that behavior of crayfish in invaded habitats can differ from that in the native range. For example, the Signal Crayfish (*Pacifastacus leniusculus*) is considered to be non-burrowing in its native North American range but commonly burrows in riverbanks of invaded habitat in England (Guan 2010). Red Swamp Crayfish typically mate in open water prior to the commencement of burrowing (Huner & Barr 1991) but anecdotal information suggests that behavioural differences in burrowing behaviour exists between invasive and native populations, as mating between male and female *P. clarkii* usually occurs before females start to burrow (Huner & Barr 1991). Females hold eggs and rear juveniles in her burrow but the extent of male participation in the construction and defence of the burrow remains unexplored. Hence, the aim of the present work is to assess the burrowing activity of *P. clarkii*, analyse the structure of the constructed burrows and the behaviour displayed by male and female crayfish to identify possible differences between sexes.

Methods

Structure of burrows

In spring 2016, 80 specimens of *P. clarkii* were collected in local ponds in Northern Tuscany (Central Italy) using baited traps. Cephalothorax lengths (CL) were measured using a Vernier calliper (accuracy: 0.01 mm) and then labelled (numbers and letters) on the cephalothorax using a white marker. The specimens were maintained in plastic containers (12.5 x 22.5 x 13 cm; water level: 3 cm; weekly water change) for the entire duration of this study in the laboratory of the Department of Biology, University of Florence, under a natural light/dark cycle at room temperature (range: 17.1 – 25.7°C) and fed with carrots. Carrots are commonly used to feed *P. clarkii* under laboratory conditions as they are considered as an ideal food source due to the diet spectrum of mature *P. clarkii* and carotenoids sustaining its natural red colouration (Huner & Meyers 1979; Gherardi et al., 2013). Three days before the start of each experiment, specimens were moved in the same plastic containers to DICEA (Department of Civil and Environmental Engineering, University of Florence) laboratory. Mature adult specimens with cephalothorax length comprised between 36 and 46 mm (average length: 44.5 ± 3.1 mm) were selected, being the individuals of this size the most active (in terms of mobility) and frequent in natural environments (Gherardi et al. 2000). For each replicate, two couples were formed, composed of size-matched (maximum difference CL: 5-6%) male and female, and, after each replicate, were not used for further experiments. To minimize bias in the experiments, only individuals in good condition without mutilations were selected. From April to August 2016, ten independent replicates of the experiment were conducted, each in five-day cycle using artificial model levees simulating Italian irrigation canals built at laboratory of DICEA. The experimental soil granulometry (composed of 61% silt, 21.7% sand, 17% clay and 0.3% gravel) is particularly favourable to crayfish burrowing activity (Barbaresi et al. 2004) and is typically used by local Basin Authorities for the construction of levees in many areas colonized by this species in Tuscany. The scale of the constructed levee was a 1:1 copy (same size as levees in the field) of levees that can be frequently found along ditches, the most commonly invaded habitat by *P. clarkii*.

The levee was constructed in a static water tank (L: 300 cm; W: 96 cm; H: 110 cm) during three days prior to the five-day cycle of each experimental replicate. It was built by laying multiple soil layers (5-8) of approximately 10-12 cm height on top of each other with the first one being rectangular shaped (100 x 96 x 10 cm) and each consecutives layer decreasing in size. All layers, except the bottom one, were compacted using a common dynamic loading procedure whereby soil was regularly hit by a given weight of about 15 N from a height of about 20 cm. The first day and before laying soil layers, portions of hydro-expanding bentonite (waterstop) were attached to the glass sides and middle section of the tank bottom to prevent preferential water pathways along the contact surfaces. The completed levees had a trapezoidal shape over a wider rectangular basal layer, total height was 50 cm and length 100 cm (Figure 2)

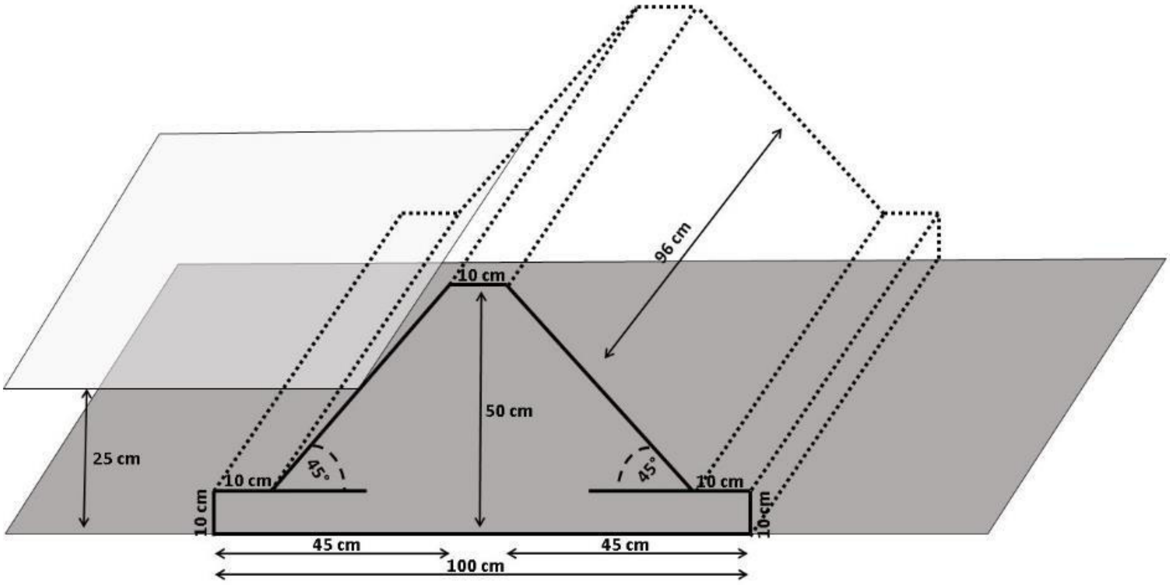

Figure 2: Model of the artificial levee used for the experiment at DICEA (Department of Civil and Environmental Engineering, University of Florence).

Before each replicate, water level (at room temperature) was set to 25 cm on one side to cover 50% of the levee's height and maintained constant during the experiment; this level gave the possibility to observe the burrowing behaviour of crayfish in the levee. A barrier was put on top of the levee to exclude movements of crayfish from the waterside to the empty side. On the first day of each replicate (after the three days to build the model), two pairs of size-matched crayfish were placed into the water and the experiment was video-recorded for 96 consecutive hours. During night-time, light was provided by a halogen lamp covered with a professional blue filter simulating "moon light", enabling observing animal movements without disturbance. Air and water temperature were recorded during all the experimental replicates and controlled as best as possible but were subject to fluctuations due to the different climatic conditions occurring during the study period. At the end of the replicate, crayfish were baited out of the burrows using cat food. Then, water was removed from the tank and from the burrows, using a syringe and a rubber pipe if needed. Polyethylene foam was inserted into the burrows while applying pressure on the entrance to ensure that the foam could expand inside the burrow. To be sure that every burrow was filled with foam, the levee was taken down carefully, injecting more foam in instances when empty burrows were uncovered. After the entire structure was taken down, the foam structure was place in the empty and cleaned tank to take pictures (Figure 3). The total excavated volume was measured by submerging the foam structure in a volumetric water bowl. The maximum length (longest tunnel) of each burrow, the maximum and minimum diameter of each tunnel at its beginning, middle and end section were also measured to generate a single mean diameter value per tunnel. The presence, diameter and volume of each chamber (enlarged structure which significantly differs from the tunnel; Gherardi 2000) as well as the presence of multiple openings were assessed.

Figure 3: Constructed model levee at DICEA (Department of Civil and Environmental Engineering, University of Florence) 48 hours after release of crayfish into the setup.

Behavioural parameters

The following parameters were recorded from the camera placed in a 90° angle over the levee-front and analysed for each individual: 1) latency in seconds (time until crayfish started burrowing after being placed into the setup); 2) time in seconds spent in different behaviours, such as "burrowing" (i.e. visible active digging into the soil on the external surface of the levee), "burrow protection" (i.e. standing directly in or above the burrow edge), "horizontal movement" (i.e. movement under the water level on top of the first rectangular layer of the levee), "outside movement" (i.e. movement outside of the water line), and "breathing" (i.e. the typical sideway position used in this species to breath air oxygen outside the water, see Holdich 1999). When animals were in the burrow, no activity could be recorded.

Statistical analyses

Data were tested for normality by applying the Shapiro-Wilk's test ($p > 0.05$), skewness and kurtosis z-values (-1.96 to +1.96) and displaying the data distribution using histograms, normal Q-Q plots and boxplots. Data on burrowing latency did not meet assumption of normality and were analysed in relation to sexes, using a Mann-Whitney-U test. Burrow latency, burrow length, excavated volume and air temperature were analysed with Spearman correlations. To test whether one sex started new burrows significantly more often than the other, chi square (χ^2) test was applied. Following the statistical approach by Gherardi et al. (2011), a two-way repeated measures multivariate analysis of variance (MANOVA; statistic: Wilk's Lambda Λ) was applied to all the behavioural parameters using sex and day/night as factors after assumptions of multivariate normality were controlled with quantile-quantile (Q-Q) plot and met. MANOVA was followed by univariate tests for between-subject's effects.

Using the data from all the experiments, time spent in each behavioural pattern was compared between day and night (Wilcoxon-signed-rank-test, statistic: W), and between sexes (Mann-Whitney-U-test, statistic: U). Moreover, behaviours of males and females were analysed separately between day and night (Wilcoxon-signed-rank-test, statistic: W). For replicate #5 no video data were obtained due to a technical error in the camera setup. The level of significance under which the null hypothesis was rejected is $\alpha = 0.05$. Text and figures give mean values ± standard errors (SE) or median values (+ 1° and 3° interquartile). SPSS®, Statistical Package for Social Science 13.0 for Windows was used for the statistical analyses.

Results

Structure of burrows

Overall, 17 burrows were constructed by 40 crayfish during the ten experiments. In seven replicates, levees contained two burrows with three openings (n=4) and two openings (n=3). In the remaining three replicates, levees had only one burrow with two openings and in two cases burrows with only one opening (Table 1). During ten replicates and out of 17 burrows, only five replicates contained chambers (n=6) which were constructed directly at the end of the entry tunnels with two having partially constructed tunnels outgoing from it (Figure 4).

Figure 4: Reconstructed model of the levee after the experiment, showing the position and the excavated volume of burrows.

Mean size of chambers (n=6) was 15.8 ± 5.2 (height) x 11.4 ± 2.5 (width) x 10.9 ± 1.3 (depth) cm and had a mean volume of 0.9 ± 0.6 dm^3. The average length of the excavated tunnels was 50.2 ± 32.4 cm (ranging from 7.0 to 123.6 cm) with a mean perimeter of tunnel sections of 17.9 ± 2.4 cm (ranging from 14.1 to 21.6 cm). Diameter of tunnels (n=17) was shown to be between 4.8 ± 0.9 cm and 6.0 ± 1.0 cm. The excavated volume on the total levee volume showed a mean of 1.9% ± 0.9% (0.00528 m^3; 5.0256 l) with 0.9% (0.0024 m^3; 0.24 l) being the smallest and 4.0% (0.011 m^3; 10.9 l) the largest percentage of excavation. In seven replicates, crayfish were found to occupy burrows in couples of one female and one male (Table 1). Additionally, in various replicates one pair of crayfish constructed a complex burrow while the other pair constructed a shorter and simpler structure (Table 1, 2).

Table 1: List of total amounts of burrows and entries constructed by crayfish during 10 replicates plus information of occupation of burrows by crayfish at the end of each replicate. In case of two numbers (e.g. 2/2) within a column, the numbers refer to the number of burrows.

Replicate	# Burrows	# Entries	Chamber present	# Crayfish per burrows	# Entries per burrow
1	1	2		1	1
2	2	2		2 / 2	1
3	2	3	x	2 / 1	1 / 2
4	2	3	x	2 / 2	1 / 2
5	1	1		2	1
6	1	1		1	1
7	2	3	x	2 / 0	1 / 2
8	2	3	x	2 / 2	1 / 2
9	2	2	x	2 / 1	1
10	2	2	x	1 / 1	1

Table 2: Description of excavated structures (chambers and tunnels) during the experiments performed.

Structure	n	Mean Length [cm]	Mean Perimeter [cm]	Mean Height [cm]	Mean Width [cm]	Mean Depth [cm]	Mean Volume [m³]
Chamber	6			15.8 ± 5.2	11.4 ± 2.5	10.9 ± 1.3	0.00098 ± 0.0006
Tunnel	17	50.2 ± 32.4	17.9 ± 2.4				0.0043 ± 0.0016
Total							0.00528 ± 0.0023

Behavioural parameters

In seven cases, the establishment of couples (i.e. both sexes starting burrowing together without fighting and protecting the burrow against other individuals) was observed after 5100 ± 1452 s. No correlation was found between latency time and burrow length (rs= 0.25, n= 7, p= 0.589), latency time and excavated volume (r_s= 0.452, n=7, p=0.260), burrow length and excavated volume (r_s= -0.43, n= 7, p= 0.337), air temperature and latency time (r_s= -0.71, n= 7, p= 0.071), temperature and burrow length (r_s= 0.19, n=7, p= 0.602). Overall, males and females started constructing burrows with the same frequency (χ2= 1.17; n= 13; p= 0.279; females: n= 8; males: n= 5), also in case of the first burrow (n= 4 for both sexes). Both sexes did not significantly differ for latency time (U= 15, n= 8, p= 0.06; male: 740 s, 486 – 1862 s; female: 3281 s, 1536 – 8036 s). The time spent in each behaviour (Figure 6) was found not to correlate with neither air nor water temperature (r_s= -0.38 – 0.30; n= 9, p always >0.05). The MANOVA revealed no statistical significance for interaction of factors (Λ= 0.11, df= 5.28, p= 0.99) and single factor (sex: Λ= 0.67, df= 5.28, p= 0.65; time: Λ= 1.39, df= 5.28, p= 0.28) for the considered behavioural parameters. The univariate post hoc test following the MANOVA showed only a difference in the burrowing activity for time, with crayfish spending more time during the day rather the night (F= 5.206, df= 1, p= 0.03; other parameters: F: 0.001-3.417, df= 1, p: 0.981-0.076). Comparisons of the total time spent by males and females in different behaviours at night or during the day showed an increased burrowing activity and horizontal movements during the day (Table 3). In contrast, no significant difference in the total amount of time spent in the different behaviours was observed between males and females (Table 3).

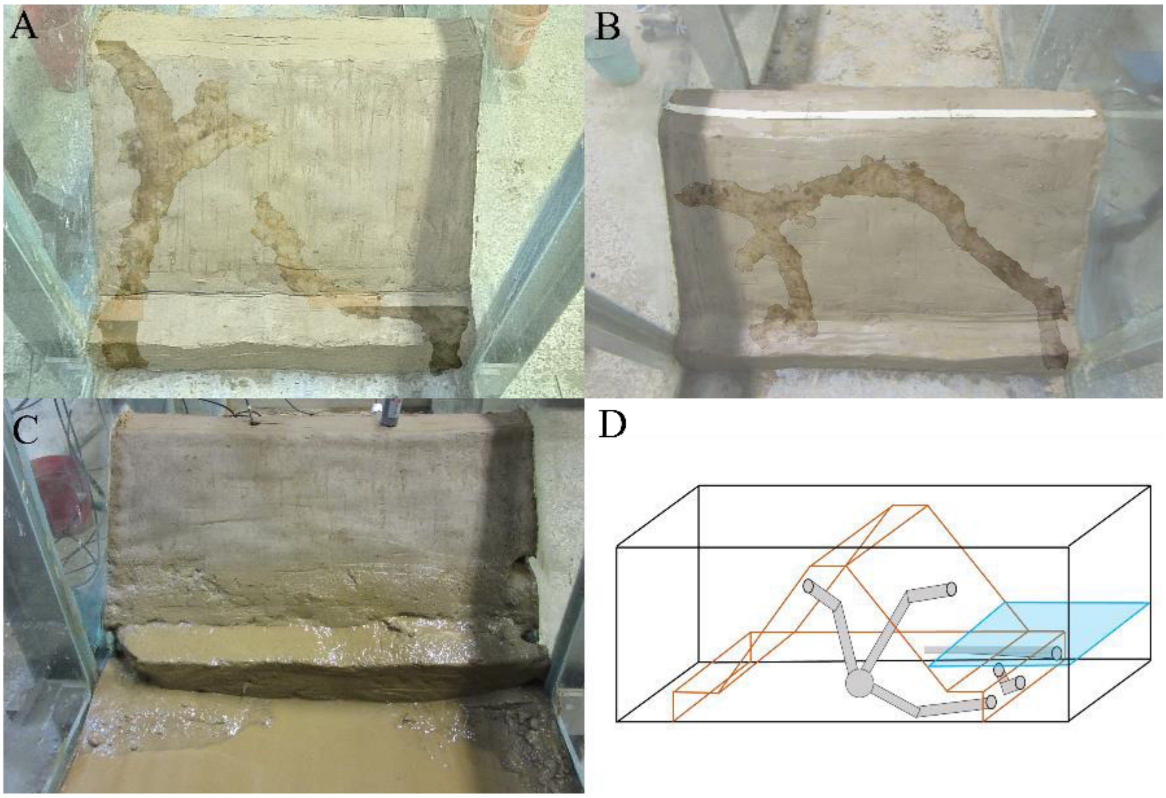

Figure 5: Reconstructed model of the levee after the experiment, showing the position and the excavated volume of burrows. A and B = observed burrows as positioned inside the levee; C = Frontal view of the model levee after water has been removed showing both types of typically constructed burrows: the straight into the levee dug burrows near the water line and the "u-shaped" burrows near the ground.; D = model of all differing types of burrow structures observed during the experiments.

Table 3: Comparisons between sex and day time, within sex and within day time for each behavioural parameter, using Mann-Whitney-U test [U] or Wilcoxon-signed-rank test [W]. Medians and 1°-3° interquartile are reported. Significant values are highlighted in bold.

Factor	Behaviour	Test value	n	p	Time (s)
Males vs. Females					
Day and Night	Burrowing	[U] = 151.5	36	0.743	0 (0 – 3464)
	Protection	[U] = 152	36	0.767	0 (0 – 621)
	Horizontal	[U] = 191	36	0.372	39 (0 – 227)
	Outside	[U] = 143	36	0.563	2199 (420 – 10052)
	Breathing	[U] = 202.5	36	0.203	55 (0 – 441)
Day	Burrowing	[U] = 40	18	1.000	3383 (243 – 11033)
	Protection	[U] = 37	18	0.796	0 (0 – 755)
	Horizontal	[U] = 33	18	0.546	100 (0 – 481)
	Outside	[U] = 38	18	0.863	3515 (1028 – 15961)
	Breathing	[U] = 31	18	0.436	150 (0 – 688)
Night	Burrowing	[U] = 36	18	0.730	0 (0 – 0)
	Protection	[U] = 39.5	18	0.931	0 (0 – 147)
	Horizontal	[U] = 34	18	0.605	0 (0 – 113)
	Outside	[U] = 33	18	0.546	1604 (36 – 5336)
	Breathing	[U] = 28	18	0.297	45 (0 – 332)
Day vs. Night					
Males and Females	Burrowing	[W] = 201.5	18	**0.001**	Day: 3383 (243 – 11033) Night: 0 (0 – 0)
	Protection	[W] = 301	18	**0.018**	Day: 0 (0 – 755) Night: 0 (0 – 147)
	Horizontal	[W] =272	18	**0.004**	Day: 100 (0 – 481) Night: 0 (0 – 113)
	Outside	[W] =283	18	0.124	Day: 3515 (1028– 15962) Night: 1604 (36 – 5336)
	Breathing	[W] =307.5	18	0.334	Day: 150 (0 – 688) Night: 45 (0 – 332)
Males	Burrowing	[W] = 52.5	9	**0.012**	Day: 3222 (228 – 21674) Night: 0 (0 – 0)
	Protection	[W] = 76.5	9	0.068	Day: 0 (0 – 758) Night: 0 (0 – 338)
	Horizontal	[W] = 72	9	**0.043**	Day: 18 (0 – 550) Night: 0 (0 – 112)
	Outside	[W] = 76	9	0.327	Day: 4205 (1575 – 11455) Night: 2296 (24 – 7259)
	Breathing	[W] = 80	9	0.499	Day: 55 (0 – 558) Night: 12 (0 – 172)
Females	Burrowing	[W] = 54	9	**0.018**	Day: 6009 (416 – 14519) Night: 0 (0 – 0)
	Protection	[W] = 79	9	0.109	Day: 0 (0 – 974) Night: 0 (0 – 294)
	Horizontal	[W] = 68	9	0.028	Day: 108 (31 – 490) Night: 0 (0 – 169)
	Outside	[W] = 73	9	0.214	Day: 2825 (473 – 16731) Night: 642 (198 – 8720)
	Breathing	[W] = 78	9	0.484	Day: 244 (22 – 764) Night: 268 (19 – 438)

Discussion

The results showed that crayfish promptly started digging and that both sexes are involved in burrowing activity. These data are thus important to increase the knowledge of *P. clarkii* biology and could help develop levee structures that are less vulnerable to the presence of *P. clarkii* under consideration of burrow structure (Kouba et al. 2016). In several countries, the damage to levees caused by crayfish like *P. clarkii's* burrowing activity is noteworthy, due to the increased vulnerability to floods (Chang & Lange 1967; Souty-Grosset et al. 2016; Consumi 2016) and leakage from irrigation canals (Fischar 2006; Orlandini et al. 2015). Here, the burrow structure of typically in Italy constructed levees (Orlandini et al. 2015; Condicelli 2016) is described, and the behaviour of *Procambarus clarkii* associated with the construction of burrows in an artificially environment is analysed (but see also Kouba et al. 2016).

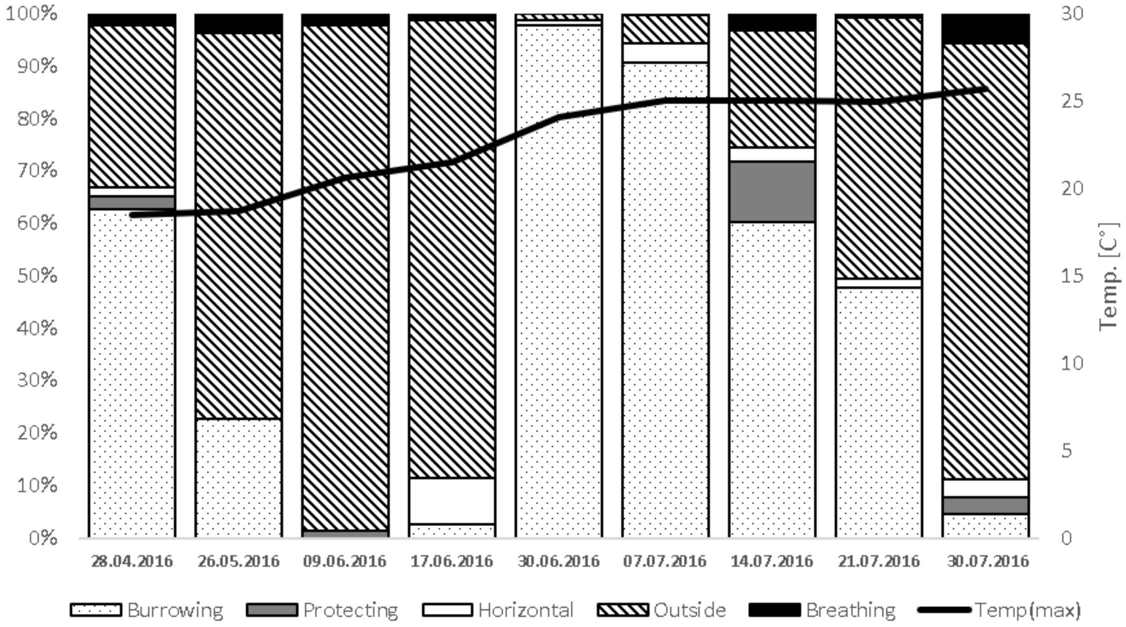

Figure 6: Total time spent in each behaviour (%) through the nine replicates. Left y-axis: % of time spent in each behaviour, right y-axis: mean air temperature measured during each experiment.

Structure of burrows

In the present study, two types of burrows were observed: 1) complex burrow entrances at the water line commencing straight into the levee (Figure 5) and in some cases with a central chamber, which volume can contribute to almost 50% to the overall excavation volume of the burrows, and 2) a considerable number of short burrows constructed u-shaped and placed near the ground in the first basal layer of the levee (Figure 5), as described by Holdich & Lowery (1988).

While some variation between experimental and natural conditions are possible, obtaining similar data from the field is most often impossible. However, due to the simulation of similar conditions (no vegetation, presence of water, 50-50 sex ratio, comparable densities), results presented in this study are likely to be expected under natural conditions. However, the overall low level of variation (only two types of burrows) was likely due to the constant water level (Holdich & Lowery 1988; Ilhéu et al. 2003) and the experimental setup, but is in agreement with previous studies that also reported complex and simple burrow structures (Correia & Ferreira 1995; Ilhéu 1996; Gherardi 2002) with similar burrow opening diameters (Souty-Grosset et al. 2014) and a burrow depth ranging from 0.28-0.58 m (Portugal: Correia & Ferreira 1995). In contrast to the in this study at 0-20cm below the water level horizontally constructed burrows, previously described burrows showed a simpler morphology, with usually one opening at a distance of 0-10 cm above the water surface enlarging in a tunnel with a terminal chamber, potentially with muddy plugs or a chimney reaching up over the burrow opening. These were generally occupied by two mature crayfish (Jaspers & Avault 1969; Correia & Ferreira 1995) as observed in ten out of 17 constructed burrows in the present study. From field observations conducted in July 2017, three out of seven burrows had a chamber (P. Haubrock, pers. comm.). In Europe, burrows can be inhabited by about 4.8 younger individuals, on average (Correia & Ferreira 1995), while in the native area by up to 50 individuals (Huner et al. 1984). We found that the ultrastructure of constructed chambers was overall consistent with previous described structures (Japsers & Avault 1996; Huner & Barr 1984; Correia & Ferreira 1995; Ilhéu 1996). On the contrary, Souty-Grosset et al. (2014) observed more complex burrowing activity in dry fishponds, with burrows having also mud plugs. Besides water level, morphology, slope and structure of levee could have influenced the type of burrows constructed in the present study.

Generally considered as a “secondary burrower” in its native area, this species spends most of its life outside of burrows, retreating into them for reproduction, and to avoid predation or dehydration. However, this species often seems to be considered as a “secondary to tertiary burrower” (depending on whether the author considers burrow usage or burrow structure; Hobs & Hart 1959; Holdich 1999; Ilhéu et al. 2013) in invaded area, due to its ability to shift its behaviour according to the different environments (Souty-Grosset et al. 2014). Hence, it is likely that burrowing behaviour, burrow structure and use of burrows differ among invaded areas due to differing particle size and water cycle (Ilhéu et al. 2013; Souty-Grosset et al. 2014) as well as location of burrow (ground vs. levee; Orlandini et al. 2015; Conicelli et al. 2016; Solari et al. 2016). Although the experimental setup was not suited to analyse the different burrow types under dry conditions (vertical & submerged burrows – see Gherardi et al. 2002), two different types of burrows were produced.

As stated by Barbaresi et al. (2004) and Correia & Ferreira (1995), apart from the presence of rocks that seemingly decrease the burrowing activity (Souty-Grosset et al. 2014), sediment composition, presence of vegetation and water availability affect the structure of constructed burrows. This becomes obvious, as i) shelter (i.e. rocks, structure) would provide additional cover and reduce the drive to burrow, ii) too small or large particles would increase the difficulty of burrow-construction but is considered as ideal in Italy (Barbaresi et al. 2004; Solari et al. 2016), and iii) vegetation (and the roots within the sediment) affect the porosity of the sediment (Ilhéu et al. 2003; Souty-Grosset et al. 2014;

Orlandini et al. 2015). Additionally, Kouba et al. (2016) showed that *P. clarkii* is capable of constructing different burrow structures in response to environmental changes, directly increasing its survivability. In the present study, burrow structures were generally either “u-shaped” (Gherardi 2000) or, likely due to the availability of constant water levels, similar to secondary burrower’s burrow morphologies but horizontally with more complexity (Figure 5), usually expected from “primary burrowers” (Hasiotis & Mitchell 1993, Holdich et al. 2002; Gherardi 2002). While the typical “u-shaped” burrow could be “tertiary”, providing suitable short time protection under low construction effort, the more complex “secondary burrow” morphologies may be advantageous (aeration, additional exit) and suitable for longer refuge. Nonetheless, considering the present experimental setup and the reproductive phase of this species in Italy overlapping with the time of the study, it is not clear why in various experiments only one pair of crayfish constructed a larger, more extensive burrow, while the other pair remained constructing the previously mentioned u-shaped burrow. While a lack of space is possible, interactions between couples as well as different responses to the experimental setup, (directly constructing a more permanent shelter vs. trying to get shelter fast) are probable.

The recorded burrowing activity led to a total amount of excavation up to 4 % of the levee volume (with a high percentage of the excavated volume contributed by the chambers), that can directly affect levee stability and alter the seepage process by shortening filtration pathways with consequent possible collapse of the levee (Orlandini et al. 2015; Solari et al. 2016). Since the constructed model levee was a reproduction of typically irrigation canal levees, a damage and threat to the stability of levees similar to that ones we observed in the laboratory can be hypothesized for levees in nature, especially in the case that burrows go through the entire levee with an entrance of the other side as observed in a preliminary run (pers. obs. Phillip Haubrock) and considering that the number of burrows increases over time (Chang & Lang 1967).

In order to avoid cannibalism and aggressive interactions, the density used in the experiment was low (four crayfish per m²). Moreover, according to the Catch Per Unit Effort values found in field studies (e.g. >20: Aquiloni et al. 2010; Cecchinelli et al. 2012) or densities used in semi-natural studies to quantify species impact ($8m^{-2}$: Gherardi & Acquistapace 2007), higher abundances of *P. clarkii* are possible, but detailed information on actual densities are currently only anecdotal. However, the general layout (shape and granulometry) simulated model levees in Italy, as they are usually not covered in vegetation and free of structure, offer low to no shelter in surface waters (Figure 1), reproducing the burrowing activity observed in invaded habitats. Because burrow density is stated to be independent from crayfish density (Barbaresi et al. 2004; Tricarico et al. 2010), heavy impacts at low densities as observed in this study, do not necessarily occur at low densities in real systems. However, while 4% of excavation by four individuals seems low, the estimated percentage relates to 96 h. With increasing time and potentially higher densities of *P. clarkii*, the probability of levee breaches as well as collapses increases (Orlandini et al. 2015; Conicelli 2016). Also, collapsing river banks are only one issue, while the creation of holes through irrigation ditches and related damage of the construction can lead to water leakage and shortage of water in irrigation canals for agriculture (Orlandini et al. 2015). Therefore, the excavation of 4% during 94 h can be considered as a high impact.

However, an intense immediately starting burrowing activity was always recorded in all the experiments by four crayfish, highlighting the considerable impact especially since *P. clarkii* tends to rebuild rather than reuse older burrows. Periodically changing of water level, due to the use of local waterways for irrigation and associated water cycle during summers, leads to a consistent annual fluctuation (water present between late autumn and spring, no water during summer) and might induce an increased digging activity with a high impact on levee stability in times of draught (Figure 1), especially due to the presence of chambers as observed in this study, but *ad hoc* experiments are necessary to solve this issue. On the contrary, the constant shallow water level used in the experiments likely results in several shorter and horizontally constructed burrows (Gherardi et al. 2002) that will have a delayed effect on levees. However, more detailed information on the quantitative breakpoint are unknown.

Behavioural parameters

Burrows were generally occupied by a couple composed by a male and a female as previously observed by Holdich & Lowery (1988). In the absence of shelter, both males and females began burrowing rapidly, usually within times < 60 s, potentially as there is no available shelter. Both sexes started digging, with similar latency time, and thus males do not initiate burrowing to induce mating. The observed data indicates a trend for a decrease in latency time under an increase in temperature, highlighting the more active pattern and the possible relation to the reproductive phase (Daniels et al. 1994). Indeed, in Tuscany species reproduction usually occurs between June and July when females need a burrow (Gherardi et al. 2002). It is interesting to note that the indicated mating behaviour differs from mating in the native range, where mating typically occurs in surface waters and females, that store sperm in their anulus ventralis, start digging a burrow in which they fertilize their eggs. In this study, mating was not observed outside the burrow, but rather a protective guarding of females by males who immediately approached advancing other *P. clarkii* while both were constructing the entry of the respective burrow (P. Haubrock, pers. obs.). The rapid pairing of *P. clarkii* in male-female couples and subsequent cooperative burrowing suggests several interesting hypotheses. For instance, it could be to induce mating but 1) *P. clarkii* typically mate in open water before burrowing, and 2) if it was to induce mating one would expect males would initiate burrowing, but this was not the case. Since mating was never actually observed, it could just be that the crayfish cooperated with burrowing to get underground (into shelter) more quickly as speculated for *Cambarus hartii* (Helms et al. 2013a). The evidence against this scenario is that *P. clarkii* always collaborated in teams of 1 female and 1 male, which suggest it was related to mating. Additionally, both sexes displayed burrow protection, e.g. guarding outside the burrow entrance from other crayfish, and sharing the burrowing itself, indicating that before the mating, both contribute to these tasks. Helms et al. (2013a) found, that in artificial burrowing setups, multiple individuals of *C. hartii* initially shared burrows as a mean to get underground, but, nonetheless, burrowing activity was not shared equally.

Overall, no differences were observed between males and females (Gherardi et al. 2002), and both sexes alternatively worked at the same burrow, equally participating to burrowing activity. Kouba et al. (2016) inserted *P. clarkii* in a situation of drought and forced to construct typical vertical burrows. In this study, it was found that burrows constructed by both male and female *P. clarkii* differed from previous studies, in that they are constructing no plugs and burrows were lateral rather than vertical. In our study a more visible burrowing activity on the levee was observed during the day. However, it is not possible to affirm that crayfish always dig more during the day, because the activity inside the burrows was not visible, even if this is in part indirectly supported by the less frequent horizontal movements observed during the night and the observation of freshly produced tunnels after nights.

Conclusion

Our study showed the extent of burrowing behaviour of the invasive *P. clarkii* in an artificial setup and, in contrast to previous studies (e.g. Huner et al. 1984 and Huner 2002 for an overall summary), revealed how similar female and male crayfish are for burrowing activity. Considering the used experimental procedure, the observed excavation values of maximum 4 % are likely an underestimation, especially because in the field a higher abundance of crayfish is present, and the number of burrows increases over time. Moreover, levees are generally not vegetated and affected by other environmental pressures (e.g. loss of stability and integrity due to scarce maintenance). Our results indicate, how burrowing activity (considering the extent of lateral burrows as well as crayfish reconstructing rather than reutilizing burrows) can possibly reduce the levee stability, underlining the pressure exerted by *P. clarkii* in invaded habitats. Additionally, *P. clarkii* is considered a warm water species, but its distribution in Europe and high tolerance towards low temperatures suggest its overwintering and reproduction in colder areas for which the observed burrows structures are an advantage.

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Chapter 2.2

The North American channel catfish, *Ictalurus punctatus*, in Europe: a neglected but potentially invasive freshwater fish species?

Short title: Alien channel catfish in Europe

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Abstract

Several non-native species introduced to Europe have received little or no scientific study and thus in-depth assessments of their potential invasiveness is lacking despite evidence from elsewhere of, e.g. ecological damage, prolific reproduction, tolerances to a wide range of conditions, opportunistic feeder, at least partial 'predator release', and disease transmission. One such species is the North American channel catfish *Ictalurus punctatus*, which has been introduced to several locations in Europe and Asia. To highlight the species' potential impacts on invaded communities in Europe, the Environmental Impact Classification of Alien Taxa (EICAT) was applied using evidence of impacts from the species' introduced range in North America, with scores generated for both North America and Europe. The EICAT outcome scores were "Major" for both introduced ranges, but with a high and medium levels of confidence for North America and Europe, respectively. These results highlight the urgent need to carry out in-depth studies and update data on current presence in Europe to understand better its invasive potential so as to inform management decisions on the appropriate control or eradication measures for invaded water bodies.

Keywords: alien species, review, impact, management, assessment

Submitted to: Aquatic Invasions

Introduction

In recent decades, the multiple impacts of the well-known and highly invasive alien fish species, such as topmouth gudgon *Pseudorasbora parva* (Temminck and Schlegel, 1846), European catfish *Silurus glanis* (Linneaus, 1758) or goldfish *Carassius auratus* (Bloch, 1782), have been the focus of an intensive scientific research in Europe (see e.g. Copp et al. 2009; Luscová et al. 2010). On the contrary, several other alien species have established remarkable and widespread populations, receiving, however, scanty or no attention by scientists and managers. One such species is the North American channel catfish *Ictalurus punctatus* (Rafinesque, 1818), which is commercially important aquaculture and sport angling species in its native and introduced ranges in the U.S.A. (Brown 1942; Appelget and Smith 1951; Boyd et al. 2000; Rezk et al. 2003; Tucker and Hargreaves 2004; Simmons et al. 2006), where the species' impacts on invaded areas have received some study (e.g. Carlander 1969; Minckley 1973; Poe and Rieman 1988; Townsend and Winterbourn 1992; Jackson and Badame 2002; Adams 2007). The invasion biology and impacts of *I. punctatus* in the species' introduced ranges outside of North America remain largely unstudied.

Although it is among the top 27 species produced in European aquaculture (Savini et al. 2010) and seemingly widespread (Welcomme et al. 1988), it is one of the least studied species in Europe (especially in terms of its potential invasiveness), most likely due to a shorter invasion history and lower "species profile" than other notable invaders such as *S. glanis* (Copp et al. 2009). This almost complete lack of data on its behaviour, ecology and potential impacts (but see Elvira and Almodóvar 2001; Banha et al. 2017; Haubrock et al. 2017, 2018) has led to a neglect by policy makers and conservation efforts, resulting in a slow response time and eventually favouring a spread through European freshwater ecosystems (Maguire 2004; Crooks 2005). However, studying its introduction history is important to enable implementation of management and risk assessment strategies. Here, for the first time, we reviewed all the relevant available information (environmental biology, biological and life-history traits) on this species to provide a comprehensive overview on the existing knowledge and gaps to be addressed in future works. Hence, the overall aim was to retrieve data on its impacts in order to evaluate the potential invasiveness of this species in Europe.

Methods

For the present review of the biological traits of *I. punctatus* related to the species' potential invasiveness, literature searches were undertaken via www.google.com and www.google.scholar.com using various search terms (invasive, alien, impact, aquaculture, disease, pathogens, control, management) in combination with the species common and scientific names. A total of 511 articles was retrieved, and of these, those containing valuable information relevant to this study are presented. Additionally, online databases (www.fishbase.org, www.cabi.org, www.gbif.org) were used to find useful information and publications including grey literature. Lastly, various ichthyologists, zoologists and experts from several European countries were consulted for their expertise on *I. punctatus*' introduction in their respective country. Although this literature search and thus the here presented and summarized results provided a considerable amount of information for North America, information for Europe and other geographical regions was limited. Hence, information was ranked in their reliability between 'low' (one reference), 'medium' (multiple references or single plus personal reference) and 'high' (multiple recent references). However, it is likely that some articles were not found due to possible restrictions associated by keyword searches. Using this collected information on species introduction history and impacts, the Environmental Impact Classification of Alien Taxa (EICAT; Blackburn et al. 2014) was applied to evaluate the magnitude of its impacts for the invaded range in North America and comparably, in Europe.

Results

Taxonomic status: Kingdom Animalia; Phylum Chordata; Subphylum Vertebrata; Superclass Osteichthyes; Class Actinopterygii; Subclass Neopterygii; Infraclass Teleostei; Superorder Ostariophysi; Order Siluriformes; Family Ictaluridae; Genus *Ictalurus*; Species *punctatus.* Diagnostic description (adapted from Pool 2007): upper jaw extending over the lower jaw, deeply-forked caudal fin, dark points on greyish – golden skin, lacking scales, eight barbels, rounded anal fin with 24–29 rays, fleshy and long adipose fin separated from the caudal fin. *I. punctatus* is considered as a benthic ecomorphotype due to its dorso-ventrally compressed body shape, characteristic for species living in fast flowing currents. (Bower and Piller 2015). Furthermore, it exhibits high morphological variability in its punctuation and head as well as anal fin shape (Filipe Ribeiro, Phillip Haubrock, pers. comm.).

Growth and maturation

Although little is known about the size structure and growth characteristics among the different environments, back-calculations of fish lengths show that it takes 4–6 years to reach 300 mm total length (TL) on average in its distribution in North America (Holland and Peters 1992; Hubert 1999). However, Bates et al, (2001) investigated a population in Lake Maurepas, Louisiana, where all specimens matured at <170 mm and <2 years, which is, compared to other populations in Louisiana (>500 mm and 3–4 years), considerably low.

Under optimal conditions, *I. punctatus* can reach adult life stages in few years, showing a rapid egg, hatching and juvenile development, although displaying a diminishing growth rate between age groups (Holland and Peters 1992). While the optimal growing temperature of adults is comprised between 26 and 29°C (Shrable et al. 1969; Cheetham et al. 1976), with temperatures below 21°C decreasing and below 18°C completely stopping its growth (McCammon and LaFaunce 1961; Macklin and Soule 1964; Andrews and Stickney 1972; Stickney and Andrews 1972; Starostka and Nelson 1974).

The actual growth (total length per age) differs among populations (Table 1) but is generally affected by various factors (Shephard and Jackson 2006, 2009). Indeed, Hubert (1999) identified significant differences for growth rates between mature *I. punctatus* from different habitat types (rivers, ponds, or lakes) and high regional variability, while the collected data suggested a higher growth rate in in Southern regions. Moreover, he identified certain habitat features (inter- and intraspecific competition, water velocity, high biological productivity, length of growing season, instream cover, abundance of pools and gravel, shallow areas, etc.) to affect growth rates apart from density (Shephard and Jackson 2009). Additionally, Rypel (2011) concluded that not specific climatic metrics positively affect *I. punctatus* growth, but rather lentic than lotic conditions which affect the nutrient stored in sediments, as *I. punctatus* is known to be illiophagus (Shephard and Jackson 2006). Additionally, *I. punctatus* exhibits differing growth rates among sexes with males growing faster than females when diet is not a limiting factor (Albaugh 1969).

Lastly, the life span is, indeed, about 6–10 years on average, but even up to 14- and 28-years old individuals have been recorded (Tyus and Nikirk 1990; Bouska et al. 2011). According to its broad distribution, age of maturity is very variable (Bates et al. 2001). In its native range, maturity is reached in specimens of ≈305 mm TL (Appelget and Smith 1951) in approximately 2–5 years or later (DeRoth 1965) (Table 1). In Southern regions of North America, where growing seasons are significantly longer, individuals mature at a smaller total length and in five years or earlier, while in Northern regions they mature within six or seven years for males and females, respectively, or even later (Davis and Posey 1958; Scott and Crossman 1973; Starostka and Nelson 1974; McMahon and Terrell 1982). In North America, the species can reach a maximum total length (TL) of 915 mm and mass of 13 kg (Pool 2007; Smith 1974).

Table 1: Information gathered from all populations used in this study and from the investigated population in the Arno river; A_M = Age at maturity; L_M = Length at maturity; n = Number of sampled specimens; GSI = mean monthly Gonadosomatic-index; K = Fulton conditioning factor; na = data not available; Sources: 1= present study; 2 = Matzusaki et al. 2011; 3 = Scott and Crossman 1973; 4 = Elroy et al. 1990; 5 = Ackerman 1965; 6 = Steeby et al. 1991; 7 = De Roth (1965); 8 = Hesse et al. 1980; 9 = Mahoney 1982; 10 = Starostka and Nelson 1975; 11 = Elrod 1974; 12 = Bouska et al. 2011; 13 = Appleget and Smith 1951; 14 = Barnickol and Starrett 1951; 15 = Shephard and Jackson 2006; 16 = Hesse et al. 1980; 17 = Hancock 1969; 18 = Hall and Jenkins 1952; 19 = Blank 2012; 20 = Holland and Peters 1992; 21 = Shipmann 1977; 22 = McClellen 1954; 23 = Michaletz et al. 2009.

Location	Ecosystem	Mean length at age (cm)										A_M	L_M	n	GSI (%)	Sex ratio	Source
		0	1	2	3	4	5	6	7	8	9						
North America																	
Quebec, CA	St. Lawrence River		10	17	20.5	24	26	28.5	30.5	32	33.8	na	na	na	na	na	3
Louisiana, US	Lake Maurepas		16.6	26.2	32.3	42.4	47.7	na	na	na	na	na	na	na	na	na	4
Iowa, US	Des Moines River		na	24.9	29.7	34.8	39.9	46	53.5	56.6	63.2	4	27.2	2222	na	na	3; 5
Mississippi, US	Aquculture pond	Na, instead information on growth and length weight relationship										na	na	8885	na	na	6
Michigan, US	Lake Erie		6.3	16.6	22.6	26.8	29.8	32.8	36.2	na	na	4	30.5 (m) - 27.9 (f)	2158	na	na	3; 7
Missouri, US	Missouri River	na										5	31.8	506	36-62	1:1	8; 9
South Dakota, US	Lake Oahe		3.3	11.3	19.3	26.6	33.3	39	43.9	48	51.5	8	54.3	1470	na	1:1	10
South Dakota, US	Lake Sharpe		na	na	24.4	28.4	30	34.6	37.2	39.3	43.3	8	34.7	52695	na	na	11
Missouri, US	Francis Case Reservoir		20.5	24.5	31.2	34.3	37.5	40.2	42.6	44.4	47	na	na-	606	na	na	12
Missouri, US	Lewis Clark Reservoir		15.7	21.8	27.5	35.8	41.9	47.6	53.1	56.2	59.1	na	na	580	na	na	12
Iowa, US	Mississippi River		na	24.9	29.7	34.7	39.9	45.8	53.2	56.6	63.3	5	38	535	na	1:1.2	13
Missouri, US	Nemaha River		17.6	21.2	25.1	29.3	33.1	37.6	42.3	48.2	46.9	5	21.9	56028	na	na	9
Missouri, US	Acquaculture pond		11.2	14.7	15.8	16.9	18.0	18.9				na	na	na	na	na	23
Mississippi, US	Mississippi River	na										4	30.5	180	na	na	14
Mississippi, US	8 river systems**		65	127	20.5	27.5	34.0	39.6		na		4.1	3.8	712	na	na	15
Mississippi, US	Sunflower river***		10.7	19.3	28.0	36.7	42.6	50.1		na		2.8	na	63	na	na	15
Mississippi, US	Upper Pearl		6.5	12.2	18.2	24.0	29.2	32.8		na		na	na	97	na	na	15
Mississippi, US	Pascagoula		6.6	13.4	20.9	29.4	33.0	35.3		na		na	na	97	na	na	15
Mississippi, US	Buttahatchee		6.1	11.4	17.7	24.4	31.0	35.9		na		na	na	97	na	na	15
Mississippi, US	Middle Pearl		6.8	12.8	19.9	26.2	32.9	39.4		na		na	na	58	na	na	15

Mississippi, US	Chichasawhay	6.3	13.1	21.3	29.2	35.9	42.4		na		na	na	102	na	na	15
Mississippi, US	Yalobusha	6.5	12.9	20.9	28.9	36.1	43.1		na		na	na	92	na	na	15
Mississippi, US	Noxubee	5.9	11.6	18.5	26.2	35.1	42.5		na		na	na	103	na	na	15
Mississippi, US	Big Black	7.3	14.2	23.0	31.8	39.1	45.0		na		na	na	66	na	na	15
Missouri, US	Niobrara River					Na					6	28.9	154	30-80	1.2:1	9; 16
Kentucky, US	Kentucky Lake	10.6	20.4	27.3	33.2	37.9	41	43.9	47.5	49.4	na	na	1298	na	na	17
Oklahoma, US	Lakes	12.7	20.3	28.4	34.3	39.4	44.4	47	52.7	58.8	na	na	4054	na	na	18
Oklahoma, US	Rivers	6.9	14	20.1	26.2	32.5	39.9	45.1	54.6	61,2	na	na	1265	na	na	18
Nebraska, US	River Platte	na	na	24.4	31.5	37.4	40.9	44.7	49.2	53	na	na	2979	na	na	19
Nebraska, US	River Platte	8.4	14.7	20	24.9	29	34.6	37.3	41.9	45.5	4	24.9	4502;	na	na	20
Utah, US	Willard Bay Reservoir					na					4	33 (m) - 35 (f)	na	na	na	21
Texas, US	Ponds					na					1.5	30.5	na	na	na	22

* combination of specimens older than 5 years

** mean values of 8 rivers

*** differing from other Mississippi rivers for its small area (553km^2), exceptionally high agricultural land use (0.71%) and very low discharge (16.5m^3/s).

Reproduction, fertility and ontogeny

Spawning temperatures in *I. punctatus* are broadly variable, comprised between 21 and 29.5°C with the spawning occurring in late spring or early summer (Appleget and Smith 1951; Katz 1954; McClellan 1954; Smith 1974; McMahon and Terrell 1982; Minnesota Department of Natural Resources 1988) or in captivity after the accumulation of 57–172 degree-days (Pawiroredjo et al. 2008). Shephard and Jackson (2005) identified a positive correlation between sexual maturity and length of growing season as well as watershed soil fertility. Additionally, the species is claimed to mate annually once in monogamous pairs (Tatarenkov et al. 2006) and to have a high fecundity: in its native range, mature females spawn once a year producing up to 3000–4000 eggs per pound (≈ 6700–8800 per kg) of body weight, when temperatures are suitable, producing up to 20.000 eggs in females of the native range between April and July. Additionally, Boehler and Baker (2013) observed the resulting larval drift at the end of June and the beginning of July. The development of gonads in immature *I. punctatus* show seasonal patterns, leading to successful spawning in about 10% of age 2 fish while in age 3 fish 60% spawn successfully (Davis 2009).

Variations in temperatures and conditions can result in more than one reproductive cycle (Appleget and Smith 1951; Toole 1951; McMahon and Terrell 1982). According to the availability of spawning sites (such as rubble, stones, holes, covered areas), males dig the nest for the female. During mating, male and female catfish align with each other in opposite directions, and the female wrapping its tail around the head of the male. Then, the male stimulates the female by “shivering”, who then releases the eggs. Males fertilize the eggs and in the following chase the female away (although McKaye et al. (1994) observed that females remain close) and remain to protect the up to 8000 laid eggs, fanning oxygenated water towards them for 5–10 days (Brown 1942; Appleget and Smith 1951; Mayhew 1987; Pool 2007). Freshly laid eggs have a diameter of 3.2 mm and adhere in one large mass (Saksena et al. 1961). The developing eggs, which require water temperatures between 15.5 and 29.5°C, hatch in six days at 25°C or 10 days at 15.6°C (Toole 1951). The hatched fry will remain in a school close under parental protection for 5-10 days before being left on their own (Appleget and Smith 1951).

Native distribution and habitat use

Ictalurus punctatus is believed to be native to the Gulf States and Mississippi Valley, from Canada to Mexico (Wellborn 1988), but the exact native range is uncertain (Etnier and Stanes 2001). Being a desired sport fishing species, its presence now outstretches across North America with populations being greatly sustained by increased annual stockings of even small impoundments, blurring the line of the native and introduced range (Scott and Crossman 1973; Tucker and Hargreaves 2004; Leonard et al. 2010) (Figure 1). *Ictalurus punctatus* shows a preference for environments with low turbidity, slow currents (Holland and Peters 1992) and well-structured, muddy or sandy bottoms (Buck and Cross 1951; Finnell 1954; Marzolf 1957; Becker 1983).

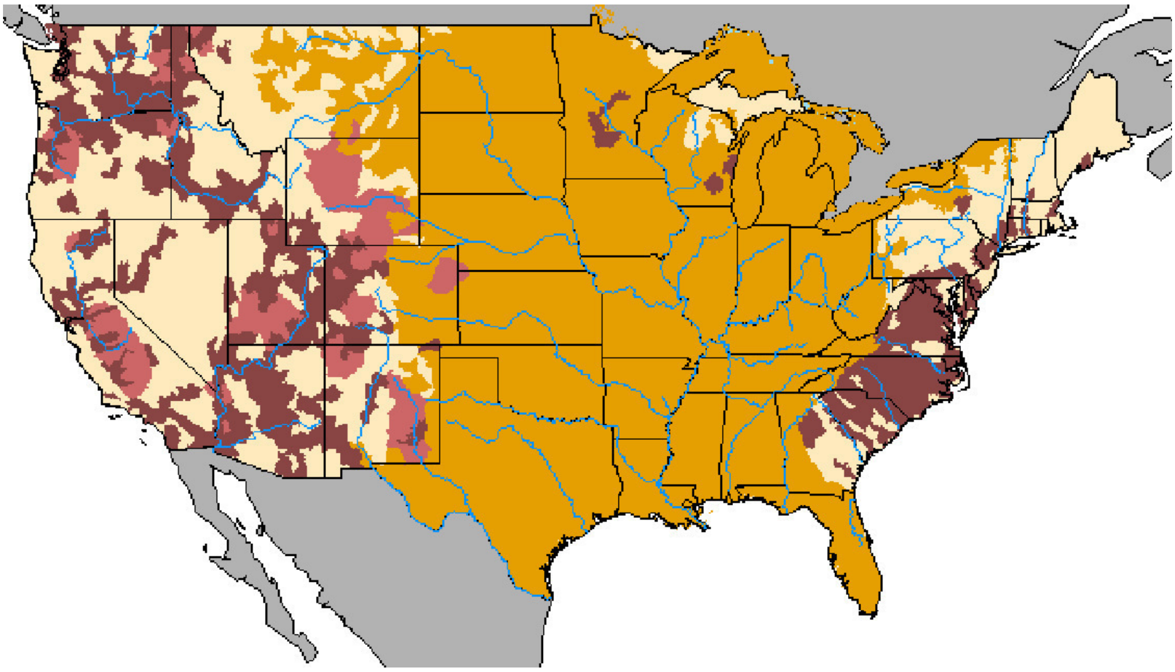

Figure 1: USA distribution of *Ictalurus punctatus*. Brown area: native distribution; purple: non–native range. (USGS https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=2341). Use of map copy permitted as per USGS Information Policies and Instructions: www.usgs.gov/information–policies–and–instructions/crediting–usgs)

It is able to thrive in most freshwater habitats due to its wide tolerance to e.g salinity (Bringold et al. 2005) and temperature (Mohseni et al. 2003), including fast flowing currents, creeks, swamps or reservoirs (Etnier and Starnes 2001), with some populations reported for brackish waters with up to 11.400.000 ppt (11.4 ppm) salinity (Perry 1968; Scott and Crossman 1973; Wellborn 1988; Meisenheimer 1988; Etnier and Starnes 2001; Ross 2001). During spring, adult fish migrate upstream to find suitable prey and spawning habitat, while in fall, they return to deeper pools following potential prey or to sustain during the cold winter months (Pellet et al. 1998). During the warmer period of the vegetative season, juvenile *I. punctatus* were observed to prefer reed zones (Endo et al. 2015; Shin-Ichiro Matsuzaki, David Keller pers. comm.), while adult individuals feed throughout the aquatic environment (Shin-Ichiro Matsuzaki, pers. comm.). However, the species' habitat use is strictly linked to feeding activity, thus differing between adult and immature individuals and according to ecosystem and species community (Brown 1942; Becker 1983; Gerhardt and Hubert 1991). In addition, adults are usually inactive during wintertime when temperatures drop below 15.5°C (Appleget and Smith 1951; Gerhardt and Hubert 1991).

Introduction history

In North America, *I. punctatus* is mainly distributed by anglers for the creation of fisheries and unattended spread due to a lack of management (Michaletz and Dillard 1999; Chizinski et al. 2005). Although *I. punctatus* is mainly produced for aquaculture in the US, Canada and Russia, China and Brazil have recently established *I. punctatus* farms (FAO

Fishery statistics, 2002; Pool 2007). Furthermore, it has been intentionally introduced in several countries for restocking, aquaculture, angling, ornamental or research purposes with further ongoing, accidental and intentional introductions having occurred (see Table 2 for the complete list of countries).

After its first introduction to Europe in the 19th century for angling and aquaculture, it has been recorded in at least 22 EU-countries, being considered as established in nine (Welcomme 1988; Elvira and Almodóvar 2001; Copp et al. 2005; Olenin et al. 2008, CABI 2016; Table 2 with special focus on Europe; Figures 2, 3). However, as many information is outdated due to the age of the published data or not based on a high confidence, the presented information on *I. punctatus* distribution were ranked according to their reliability into “low”, “medium” and “high”.

Table 2: Introduction history of the North American ictalurid, channel catfish *Ictalurus punctatus*, and reliability of information. Adapted from CABI (2016) and Froese and Pauly (2013) and modified with further information.

Country	Year of	Pathway	Status	Reliabilit	References	personal
Caribbean						
Puerto Rico	1938	angling	established	high	Erdman 1984; Welcomme 1988; Burgess and Franz 1989; Neal et al. 2009	
Dominican Republic	1954–55	aquaculture	unknown	high	Welcomme 1988; Chakalall 1993; Kairo et al. 2003	
Honduras	1960s	aquaculture	unknown	high	Matamoros 2009	
Panama	1981	aquaculture	unknown	medium	Welcomme 1988	
Mexico	1933 and 1978	aquaculture	established	high	Contreras and Escalante 1984; FAO 1997; Zambrano and Macias–Garcia 2000	
Cuba	1979 and 1984	aquaculture	unknown	low	Welcomme1988	
Costa Rica	1976	aquaculture	unknown	high	Cam 2011	
South America						
Brazil	1971 and 1980	aquaculture	unknown	high	Welcomme 1988; Piedras 1990; Cruz et al. 2012, Virtule et al. 2009	
Chile	1995	aquaculture	established	high	Pérez et al. 2003; Iriarte et al. 2005	
Paraguay	1971	unknown	established	high	Perez et al 2003; Froese and Pauly 2013	
Venezuela	1971	unknown	unknown	high	Nava 2007	
Africa						
Nigeria	1970 and 1976	aquaculture	unknown	low	Welcomme 1988	
Egypt	1982	aquaculture	not established	low	Welcomme 1988	
Côte d'Ivoire	unknown	aquaculture	unknown	low	Welcomme 1988, Froese and Pauly 2004	

Asia					
Japan	1971	aquaculture	established	high	Welcomme 1988; Chiba et al. 1989; Japan Ministry of Environment 2005, Matsuzaki et al. 2011; Katano et al. 2015
Republic of Korea	1972	aquaculture	unknown	low	Welcomme 1988; Jang et al. 2002
Philippines	1974	aquaculture, aquarium	not established	medium	Juliano et al. 1989; ASAP 1996; FAO 1997; Guerrero 2014
Taiwan	1975–76	aquaculture	not established	medium	Welcomme 1988; Liao and Lia 1989
China	1984	aquaculture, accidental	unknown	high	Tan and Tong 1989; FAO 1997; Ma et al. 2003; Gu et al. 2018
Indonesia	1986	experimental research	not established	medium	Eidman 1989
India	1985–89	aquaculture	unknown	high	Csavas 1995; Molar and Walker 1998; Kumar 2000; Singh and Lakra 2011
Thailand	1989	aquaculture	not established	low	Csavas 1995; Vidthayanon 2005
Turkey	1989	aquaculture, angling	not established	high	Innal and Erk'akan 2006; Cowx and Nunn 2008; Olenin et al. 2008
Uzbekistan	unknown	unknown	established	high	Salikhov and Kamilov 1995; Bartley 2006
Russia	unknown	aquaculture escaped into wild	established	high	Bogutskaya and Naseka 2002; DAISIE 2013
Armenia	unknown	aquaculture	established	high	Gabrielyan 2001
Malaysia	unknown	aquaculture	unknown	high	Freshwater Fisheries Research Centre Malaysia (FFRC) 2001
Pakistan	2003	aquaculture	unknown	high	Rab et al. 2007
Pacific islands					
Hawaii	1953	aquaculture	established	high	Brock 1960; Maciolek 1984; Welcomme 1988
Guam	1966	aquaculture	not established	medium	Welcomme 1988; Eldredge 1994
French	1966	unknown	unknown	low	Eldredge 1994; Bartley 2006

Polynesia						
Europe						
UK	1968	angling, aquarium	uncertain	high	Wheeler and Maaitland 1972; Lever 1977; Welcomme 1988; Kirk et al. 2002; Copp et al. 2007; Copp et al. 2010	pers. comm. Gordon Copp
Serbia	1971	aquaculture	not established	low	Welcomme 1988; Olenin et al. 2008	
Montenegro	1971	aquaculture	not established	low	Welcomme 1988; Olenin et al. 2008	pers. comm. Danilo Mrdak
Cyprus	1975	aquaculture, angling	established	high	Welcomme 1988; Dill 1993; Elvira 2001; Petrou and Zaptis 2016	pers. comm. Annamaria Nocita
Italy	1976	aquaculture, angling	established	high	Amori et al. 1993; Bianco and Ketmaier 2001; Copp et al. 2005; Ligas 2007; Castaldelli et al. 2013	pers comm. Annamaria Nocita
Slovakia	1985	aquaculture	not established	high	Welcomme 1988, Kosco et al. 2004 ; Olenin et al. 2008	pers. comm. Katharína Jakubčinová
Czech Republic	1985	aquaculture, angling	established	high	Welcomme 1988; Olenin et al. 2008; Lusk et al. 2010; Musil et al. 2010; Lusk et al. 2011;	pers. comm. Tomas Juza
Belgium	1968 and 1984	aquaculture	established	high	Holcík and 1991; FAO 1997; Vrielynck et al 2003; Verreycken et al. 2009	pers. comm. Robbert Striekworld
Bulgaria	1975	aquaculture, angling	established	high	Uzunova and Zlatanova 2007; Olenin et al., 2008; Hadjinikolova et al. 2010	pers. comm. Eliza Uzunova, Teodora Trichova
Romania	1978 and 1991	aquaculture	not established	high	FAO 1997; Gavriloaie and Chiamera 2005; Telcean and Cupsa 2009; DAISIE 2013	
Estonia	2002	unknown	unknown	???	NOBANIS 2005	
Poland	–	–	–	medium	Grabowska et al. 2010; Semenchenko et al. 2011	pers. comm. Balogh Csilla
Hungary	1975 ongoing	aquaculture	established	high	Holcík 1991; FAO 1997; Harka and Salil 2004; Olenin et al. 2008	
Lithuania	1975 ongoing	aquaculture	unknown	???	NOBANIS 2005; Olenin et al. 2008; DAISIE 2013	
Ukraine	1935	aquaculture	established	high	Welcomme 1988; Holcík 1991; Grinjevskiy et al. 2001; FAO 2006; Alexandrov et al. 2007; DAISIE 2013	pers. comm. Filipe Ribeiro
Belarus	1979	aquaculture	established	medium	Shumak and Mischenko 1989; Olenin et al. 2008; Semenchenko et al. 2009; Mastitsky et al. 2010	

Germany	unknown	aquaculture	not established	high	Hilge 1980; Elvira 2001; Wiesner et al. 2010; Fishbase 2016	pers. comm. Phillip Haubrock
Netherlands	unknown	angling	not establishes	low	Cowx and Nunn 2008	pers. comm. Robbert Striekworld
France	unknown	aquaculture	not established	medium	Welcomme 1988; Holcík 1991	pers. comm. Julien Cucherousset
Slovenia	–	–	–	low	Povz and Sumer 2005	
Spain	1995	aquaculture	established	high	Welcomme 1988; Elvira and Almodóvar 2001; Doadrio 2002	pers. comm. Filipe Ribeiro
Portugal	unknown	unknown	established	high	Banha et al. 2017	pers. comm. Pedro Anastácio; Filipe Ribeiro
Greece	2000	aquaculture	unknown	high	Economou et al. 2007; Zenetos et al. 2009; Froese and Pauly 2013	pers. comm. George Hotos
Austria	–	aquarium	not present	low		pers. comm. Thomas Friedrich
Croatia	–	–	not present	high		pers. comm. Tomislav Treer

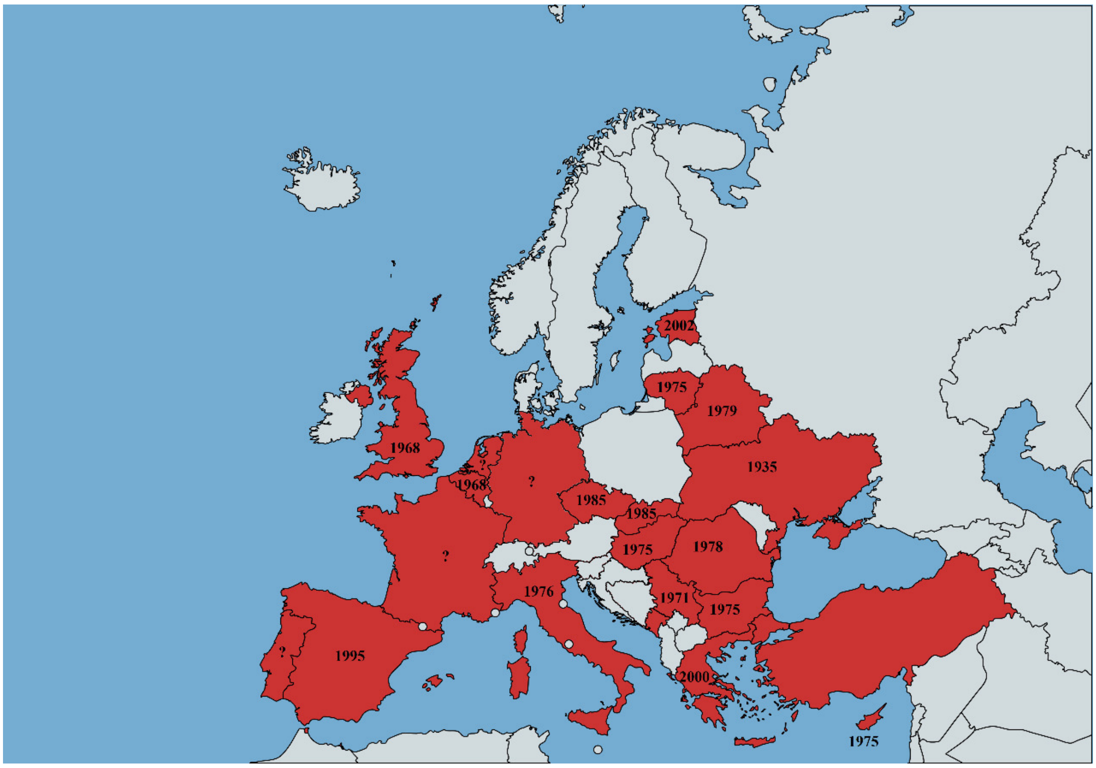

Figure 2: Map of Europe indicating countries (red) and year when *I. punctatus* has been introduced. Map generated by, and reproduced with permission of, https://mapchart.net/europe.html

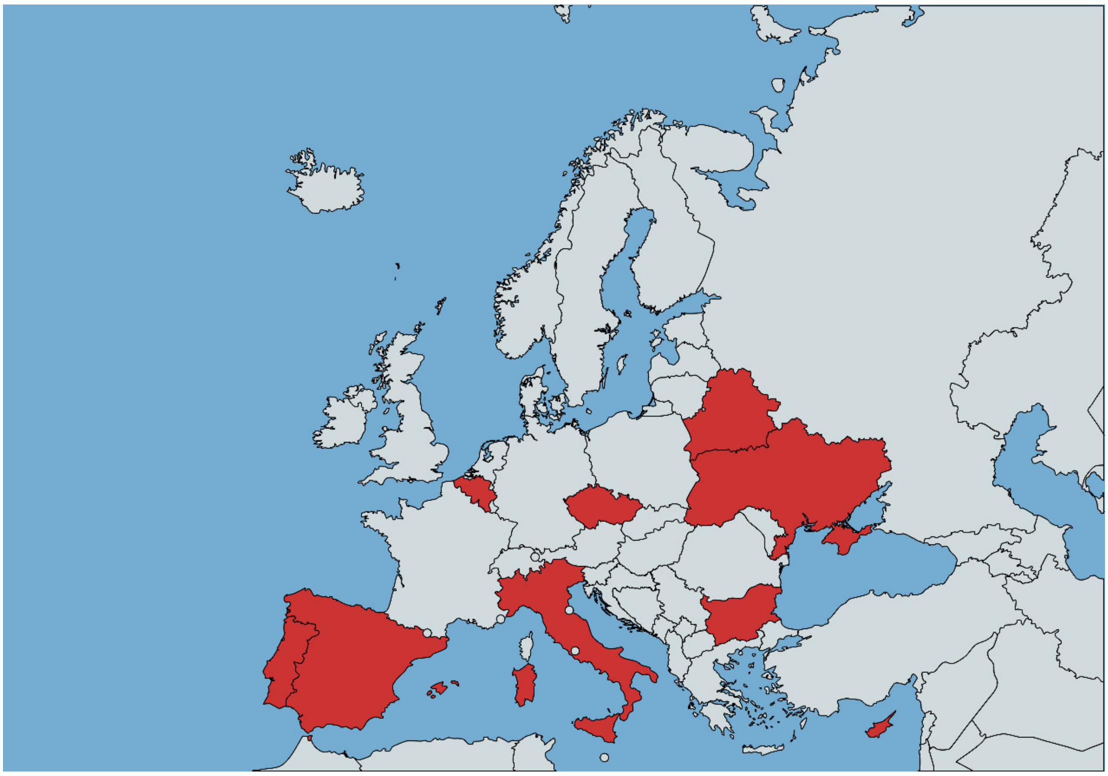

Figure 3: Map of Europe indicating countries where provided evidence for the establishment of wild *I. punctatus* populations exist. Map generated by, and reproduced with permission of, https://mapchart.net/europe.html

Invasiveness

Ictalurus punctatus has been described as one of the fastest expanding species (Tucker and Hargreaves 2004; Olden and Poff 2005). It possesses characteristics (such as high annual survival rates; Blank et al. 2017) suitable for introductions and the ability to establish outside its native range. Moreover, its invasiveness is bolstered as it is popular among anglers and being easily produced in aquaculture. However, it has been shown to exhibit a variety of negative impacts in the Western US (Hawkings and Nesler 1991) in the introduced range, especially on endangered species, as well as on fisheries and angling. Considering the data retrieved from North America, and the possibility of accidental escapes during natural events such as floods (Robinette and Knight 1981; Townsend and Winterbourn 1992; Orsi and Agostinho 1999; Zanata et al. 2016), the introduction of *I. punctatus* into New Zealand has been evaluated as highly risky for at least one native and other species of conservation concern, and it has not been allowed. Recorded impact mechanisms are manifold and include i) predation, ii) competition, iii) hybridization, iv) disease transmission, and v) modification of ecosystem services. Besides ecological impacts, also socio-economic impacts are considered.

In Europe, risk assessments were made by applying the Fish Invasiveness Scoring Kit (FISK) as well as the Aquatic Species Invasiveness Screening Kit (AS-ISK) with varying results, but generally highlighting the potential for *I. punctatus* to exert impacts on the recipient ecosystem (Table 3 & 4; Almeida et al. 2013; Puntila et al. 2013; Simonovic et al. 2013; Perdikaris et al. 2016; Tarkan et al. 2018)

Table 3: Results from *Ictalurus punctatus* risk assessments made for EU-countries using the Fish Invasiveness Scoring Kit (FISK); MH = moderately high; VH = very high; ML = moderately low.

Region / Country	**Crit**	**Invasiveness / Protection status**	**Score**				**Outcome**	**CF**				**Ref**
			Mean	**Min**	**Max**	**SE**		**Mean**	**Min**	**Max**	**SE**	
Greece	2	Invasive / Not evaluated	22.3	19.0	25.5	2.7	MH	0.83	0.80	0.86	0.03	Perdikaris et al. 2016
Bulgaria	na	Invasive / Not evaluated	10.0				MH	0.79				Simonovic et al. 2016
Southern Finland	na	Invasive / Not evaluated	11.0				M	0.79				Puntila et al. 2013
Iberian Peninsula	2	Invasive / Not evaluated	31.0	26.0	38.0	3.6	VH	0.81	0.71	0.96	0.08	Almeida et al. 2013

Table 4: Results from *Ictalurus punctatus* risk assessments made for Turkey using the Aquatic Species Invasiveness Screening Kit (AS-ISK); H = high.

Region / Country	**Crit**	**Status**	**AS-ISK**		**Outcome**		**CF**		**Ref**
			BRA	**CCA**	**FISK**	**BRA**	**FISK**	**BRA**	
Turkey	AQN	Invasive / Least Concern	31.0	41.0	H	H	0.68	0.68	Tarkan et al. 2017

Predation

Ictalurus punctatus is opportunistic omnivorous with a wide range of food items, being capable of exploiting new sources (see e.g. Heard 1958; Bailey et al. 1945; Joyce and Chapman 1987; Hawkins and Nesler 1991) and finding potential prey through their amino-acid sensitive taste buds. This ability gave them the nickname "swimming tongue" (Joyce and Chapman 1987; Caprio et al. 1993). Usually, adult feeding takes place between nightfall and dawn. Juveniles are active during the entire day, although previous studies observed a complete stop in feeding activity at water temperatures below 15.5°C (Brown 1942; Becker 1983; Gerhardt and Hubert 1991). Additionally, they are considered to be fairly effective visual predators despite being primarily nocturnal foragers.

Beside algae and detritus, insects and other invertebrates found in reed areas make up the majority of the smaller sized specimens' diet (TL < 102 mm) (Bailey and Harrison 1948; Walburg 1975; Endo et al. 2015). Bigger sized individuals change to a wider diet, feeding also on larger insects such as mayflies (adult and larvae), crayfish, molluscs, nymphs, snakes, birds, spiders and plants (Menzel 1945; Bailey and Harrison 1948; Miller 1966; Starostka and Nelson 1974; Becker 1983; Wellborn 1988; Dagel et al. 2010; Endo et al. 2015). Predation on fish (especially on *Anguilla* spp.) and terrestrial preys (earthworms, crickets, mice and rats) is highly dependable on availability, but, when available, can constitute the majority of the diet (Bailey and Harrison 1948; Robinette and Knight 1981). Particularly crayfish, a consistent food source (≈ 25 %) for juveniles and adults, are considered vulnerable to its predation (Poe and Rieman 1988; Townsend and Winterbourne 1992). This was confirmed in mesocosm experiments carried out by Adams (2007) in the US, leading to hypothesize that the spread of *I. punctatus* might be responsible for the decline of native crayfish. Furthermore, fish, amphibians in general (see e.g. Sartorius and Rosen 2000), and other benthic animals are heavily predated by *I. punctatus*, with a consequent decline in their abundance (Menzel 1945; Matsuzaki et al. 2011; Hill et al. 2015). This is bolstered by this species slow growth, favouring a demographic structure biased towards younger age groups (Holland and Peters 1992).

As individuals increase in size, a preference for predation on fish becomes prevalent, identified to threaten several species in North America (McGovern and Olney 1988; Hanzawa 2004; Arayama 2010; Matsuzakiet al. 2011). Boersma et al. (2006) observed, that gut contents of *I. punctatus* showed 50–100 % consumption of juvenile salmon, posing a considerable pressure on salmon populations. Even cannibalism is a common phenomenon in populations of *I. punctatus* (Unprasert et al. 1999). Predation and consumption of bigger fish occurs occasionally, as well as the consumption of dead fish: it has been observed that *I. punctatus* can prey on adult specimens of common carp *Cyprinus carpio* (Linnaeus, 1758), as revealed by the presence of their scales in *I. punctatus* stomach content (Phillip Haubrock, pers. obs.). Moreover, Reebs (2002) highlighted that in confined aquaculture systems the largest individuals of *I. punctatus* monopolized the demand feeders during late afternoon and early night, resulting in smaller individuals awaiting until late night or early morning.

Another aspect, indirectly related to predation, is the presence of spines on lateral and dorsal fins. These are highly modified anatomical parts of fins, which can harm or kill predators as an anti-predator adaptation serving as a pure defensive purpose (Bosher et al. 2005). Additionally, pectoral spines are responsible for the stridulatory "distress-call" sound production through binding and spine locking (Fine et al. 1997; Bosher et al. 2005). While the juvenile and smaller life stages have somewhat softer spines and are therefore rapidly consumed by predators, adult become unpalatable. It has hence been repeatedly reported that predators choke on them due to these hardened pectoral and ventral spines (McAda 1983; Pimental et al. 1985). Moreover, in contrast to its native range, *I. punctatus* has barely any native predators in Europe except diving beetle *Dytiscus sp*. for the juvenile stages (CABI 2016). Invasive alien species that are natural predators, such as largemouth bass *Micropterus salmoides* (Lacépède, 1802), have been introduced to Europe, but there is no data on their effective predation on *I. punctatus*. Other alien species like *Silurus glanis*, that are believed to be potential predators for *I. punctatus*, have been observed to avoid predation on it (Thomas Bussatto, pers. obs.)

Competition

Species with wide feeding habits can overlap with native species (e.g. trout species), leading to significant impacts and severe threats for native species (Tyus and Nikirk 1990; Townsend and Winterbourne 1992). As populations of *I. punctatus* rapidly grow (Hawkins and Nesler 1995), they have been observed to compete with several native species in North America (Mitzner 1989; Hawkins and Nesler 1991; Nesler 1995). Individuals become increasingly piscivorous (Hubert 1999) and can compete with other top predators such as largemouth bass for resources (Hackney 1975). Competition can also occur when *I. punctatus* occupies the same habitat of native species, particularly during periods of limited resource availability (Wick et al. 1985; Tyus and Karp 1989; Nesler, 1995). Additionally, a recent study by Leonard et al. (2010) suggests that largemouth bass growth and suitable conditions are lowest if stocking rates of *I. punctatus* were highest, thus implying that with higher abundance of adult fish competition might increase proportionally. Thus, it is likely that longer vegetative growing seasons in the introduced range, affecting annual growth, could lead to an earlier maturation and, thus, increasing capability to compete with other predators (Durham et al. 2005; Shephard and Jackson 2006).

Hybridization and disease transmission

In its introduced North American range, *I. punctatus* has been observed to hybridize with the endangered congeneric *Ictalurus pricei* (Rutter, 1896) and *I. lupus* (Girard, 1858), threatening their populations where distributions overlap (Kelsch and Hendricks 1990; Sublette et al. 1990; Kelsch and Jensen 1997). Furthermore, *I. punctatus* females and male blue catfish *I. furcatus* (Valenciennes, 1840) can hybridize, producing faster growing (Sublette et al. 1990) and disease resistant offspring with a better food conversion ratio, tolerant to low oxygen (1.0 mg^{l-1}) and crowding (Masser and Dunham 1998; Dunham and Brummett 1999; Tucker 2000; Dunham et al. 2008; Arias et al. 2012). As hybrids are fertile, back crossing with parental populations can represent a threat of genetic pollution accompanied by competition among hybrids and parental populations. Usually, hybridisation between *I. punctatus* and *I. furcatus* or other species of Ictaluridae is uncommon in the wild due to differences in reproductive cycles, different preferences for spawning environments, and size differences (*I. punctatus* being generally smaller). However, conditions may differ in closed or introduced environments. *I. punctatus* (female) x *I. furcatus* (male) (C x B) hybrids, which can produce large numbers of second-generation (F_2) hybrids (Dunham and Masser 2012), are widely established in aquaculture. Additionally, the existence of hybrids in regions where *I. punctatus* and *I. furcatus* are introduced (e.g. Italy) could pose an increased threat to the communities and the environment for the aforementioned characteristics they have (Dunham and Masser 2012).

Ictalurid catfishes are well-known carrier of several potentially infectious parasite, viral or bacterial agents (Terhune et al. 2002; FishBase 2007; Pool 2007). However, no impacts were reported in the North American invaded range, while potential transmission to other native/alien species has been reported in Europe. One recent discovered pathogen is the Myxosporidian *Sphaerospora* species (e.g. *S. ictaluri*), a so called “blood protozoan”, which can be considered as the presporogonic stage of sphaerospores. It was described from infected *I. punctatus* in farm ponds in Italy (Molnár and Baska 1992), likely affecting cyprinid fish and causing “carp-dropsy complex”. This disease is characterized by dropsy and exophthalmos (Lom et al.1983; Dykova et al. 1986; Rukyani 1990). Another frequent disease transmitted by *I. punctatus* is the channel catfish virus (CCV) (Ictalurid herpesvirus 1), a member of the Alloherpesviridae family causing significant economic losses in catfish aquaculture (Hedrick et al. 2003). Although *S. glanis* fingerlings have been resistant to infections by the channel catfish virus in the lab (Plumb et al. 1985), no information is available about potential implications regarding other diseases affecting different live-stages of other species. Additionally, Ictaluridae are well-known carriers of several parasitic helminths, several being potentially host switching nematodes (Pérez-Ponce de Leon and Choudhury 2002; Choudhury and Perryman 2003), and *Flavobacterium columnare*, a pathogen known to cause columnaris disease, a serious condition affecting numerous freshwater fish species all over the world (Decostere 2000; Welker et al 2005).

Ecosystem services and socio-economic impacts

The presence of an invasive alien species can damage the services provided by the ecosystem (provisioning, regulating and maintenance, cultural: Haines-Young and Potschin 2013). For *I. punctatus*, it is likely that provisioning services are affected through direct predation on valued food items (as for the abovementioned Japanese lake). As excessive consumption of algae, detritus and aquatic plants by immature *I. punctatus* has been observed (e.g. Menzel 1945; Braun and Phelps 2016), *I. punctatus* can cause a decrease in water quality and change the primary production as well as the entire trophic chain, with bottom-up cascade effects, thus modifying provisioning, regulating and maintenance services. Additionally, cultural services are likely affected: angling and associated tourism can make up an important percentage of the regional income and they can be damaged by the feeding habits of the species (Aylward et al. 2005). On the other side, it is hence possible that economic impacts could be positive, drawing in tourism and that anglers increasingly enthusiastic about the fishing opportunities opening up with the abundance and presence of *I. punctatus* (P. Haubrock, pers. comm.). A study by Matsuzaki et al. (2011) showed that in a Japanese lake the presence of invasive *I. punctatus* changed the native community composition and negatively influenced commercially important species. This led to a reduction in the provision of ecosystem goods, showing the great impact of *I. punctatus* also under suboptimal climatic conditions. In North American invaded range, economic losses are also reported for failed conservation actions on native amphibian and fish species that cannot re-establish because of the heavy predation by *I. punctatus* (Marsh and Brooks 1989; Hawkins and Nesler 1991; Rosen et al. 1995; Nelson et al. 1995; Lentsch et al. 1996; Tyus and Saunders 1996, 2000; Hughes and Herlihy 2012). Nonetheless, these impacts as well as a cost-benefit analysis have yet not been assessed and quantified.

Although being a desired target species for angling and aquaculture activities, *I. punctatus* was reported to prey upon other commercial sport and fishery species (Menzel 1945; McGovern and Olney 1988), causing a serious economic impact to commercial fisheries (Hanson et al. 2004; Hanzawa 2004; Hanzawa and Arayama 2007; Arayama 2010; Matsuzaki et al. 2012), specially to angling (Tyus and Nikirk 1990; Townsend and Winterbourn 1992; Leonard et al. 2010). Finally, spines are venomous and can inflict a burn similar to that of wasps without however being a significant threat for human well-being (Birkhead 1967).

Environmental Impact Classification of Alien Taxa EICAT

As emerging from the literature analysis, most of the information for the impacts of *I. punctatus* is coming from the introduced range in North America, while for Europe data are really scanty. It is thus crucial to assess its potential invasiveness in European freshwaters to undertake consequent management actions. Here, we applied the classification system developed by Blackburn et al. (2014), the Environmental Impact Classification of Alien Taxa (EICAT), based on generic impact scoring system (GISS; Nentwig et al. 2010) and modified to align it to the impact scheme of IUCN (Kumschick et al. 2012). It evaluates the magnitude of species environmental impacts and uses five semi-quantitative scenarios (ranked 1-5) to assign species to different level of impacts, from Minimal (species is unlikely to have caused deleterious impacts on the native biota or abiotic environment) to Massive (species leads to the replacement and local extinction of native species, and produces irreversible changes in the structure of communities and the abiotic or biotic composition of ecosystems). The scheme measures the impact of species as environmental impact in twelve classes of impact mechanisms (Table 5, 6). Each of these mechanisms is associated with one of a sequential series of five impact scenarios, describing increasing levels of impact exerted by species by that mechanism. It is based on scientific information available in peer-reviewed publications. For each impact mechanism, level of confidence is also considered: high confidence level is usually related to published scientific data on the study area, medium to some direct observational evidence to support the assessment, but with some information inferred from the literature coming from other invaded areas, and low to only inferred data used as supporting evidence. Species are assigned to impact category on the basis of the largest impact value recorded, while final confidence is considered as confidence of impact category with maximum score.

Table 5: Outcomes of the Environmental Impact Classification of Alien Taxa (EICAT; Blackburn et al. 2014) applied to the channel catfish *Ictalurus punctatus* in the introduced range in North America.

North America			
Ecological impacts	**Impact**	**Uncertainty**	**Explanation**
Competition	4	1	Competition for food, site for reproduction showed in field and laboratory experiments
Predation	4	1	Stomach content analyses and laboratory experiments with different prey items
Hybridisation	4	1	It can hybridize with other native Ictaluridae
Transmission diseases to native	1	3	No data reported
Parasitism	1	3	Nematodes are reported in the species but transmission to other species has not been documented so far
Poisoning/toxicity	1	1	Spines are toxic and a defence mechanism, but no data on heavy effects on native species
Bio–fouling	1	1	No impacts reported
Herbivory	3	2	It is opportunistic and can eat algae and small aquatic plants
Physical, chemical, structural	1	2	No impacts reported
Interaction	1	3	No data reported
Final score	***4***		
Category	***Major (MU)***		
Level of confidence	***3 (High)***		

EICAT is a simple, objective and transparent assessment process that can facilitate comparisons of the level of impact by alien taxa among regions and taxonomic groups, facilitate predictions of potential future impacts of taxa in the target region and elsewhere and aid in prioritisation of management actions. EICAT was used to evaluate the magnitude of impacts of catfish in North America by using available scientific data and in Europe by evaluating existing literature on other areas and personal observations. The outcomes revealed that in North America the species can be classified under the level Major impacts with low uncertainty (Table 5), while in Europe under the level Major with a medium uncertainty (Table 6). So, the species can be considered invasive in Europe, but with a lower certainty as there are no published data on the European situation, and impacts were inferred from literature related to North American invaded areas and, for some impact mechanisms (e.g. predation), from direct observations (e.g. stomach content analysis, Haubrock, pers. comm.).

Table 6: Outcomes of the Environmental Impact Classification of Alien Taxa (EICAT; Blackburn et al. 2014) applied to the channel catfish *Ictalurus punctatus* in the introduced range in Europe.

	Europe		
Ecological impacts	**Impact**	**Uncertainty**	**Explanation**
Competition	3	3	No data of competition with native species. It can only be hypothesized from data
Predation	4	2	Stomach content analysis revealed high predation and opportunistic behaviour
Hybridisation	1	2	No hybridization reported
Transmission diseases to native	1	2	No documented diseases transmitted to native species, but possibly to *Silurus glanis*
Parasitism	1	3	No impacts reported
Poisoning/toxicity	1	2	Spines are toxic and adefence mechanism, but no data on heavy effects on native species
Bio–fouling	1	1	No impacts reported
Herbivory	3	2	It is opportunistic and can eat algae and small aquatic plants.
Physical, chemical, structural	2	3	Only hypothesis
Interaction	2	3	It can transmit diseases to other alien fishes, it can predate American crayfish; it can hybridize with other alien Ictaluridae
Final score	***4***		
Category	***Major (MU)***		
Level of confidence	***2 (Medium)***		

Using the first developed version of GISS for environmental and economic impacts, van der Veer and Nentwig (2015) found a medium risk score for the species, while Copp et al. (2009) classified *I. punctatus* as potential pest for UK with the Freshwater Fish Invasiveness Scoring Kit (FISK). All these results highlight the urgent need of more research on the species in Europe to retrieve data in order to better support its invasiveness and promote some management plans.

Management options

According to Pool (2007), there is very little effort to control populations of *I. punctatus* in introduced ranges, and, especially in the Pacific Northwest region, where this species has been present for over a hundred years, there is no specifically designed programme targeting its populations (Davis 2003). Chemical measures (Sodium sulfite, Guthion®, TFM (3-trifluoromethyl-4-nitrophenol) and Bayluscide®) could effectively remove Ictalurids, but they are highly tolerant to such chemical substances. Thus, high concentrations of these chemicals are required, and they potentially would harm endangered species, which occupy the same or neighbouring habitats (Dawson and Kolar 2003). Furthermore, substances like Antimycin which are toxic for scaled fish, have shown to less affect *I. punctatus* (Clearwater et al. 2008). Mechanical removal is the most used technique for removal of invasive alien fish. Even if long-term fishing campaigns decrease

I. punctatus' abundance, depending by the context, they can fail to totally remove this species from environment and can be very expensive in terms of time, manpower, and money. However, responses of *I. punctatus* to electrical pulse rates and voltage amplitudes have been investigated and efficient electrofishing protocols exist (Ellis 1975; McInerny and Cross 2005). This technique has been used for longer than a decade in Lake Kasumigaura, the second largest natural lake in Japan, where Ibaraki Prefecture initiated an *I. punctatus* removal project in 2005 with the support of local anglers, which had to remove *I. punctatus* when caught as a by-product of stationary nets (Matsuzaki et al. 2011). Thus, the population appeared to have decreased (Craig 2015), and it is likely that population will gradually decline if removal of the population will be increasing (Matsuzaki et al. 2011). Since chemical and mechanical measures are small-scale control options, for well-established and widely distributed populations, the only realistic options could be biological and autocidal control measures, which have barely been used with fish species in general (Brooks et al. 2000). Santos et al. (2009) assessed the potential of the native characid, *Brycon orbignyanus* (Valenciennes, 1850), to help controlling some invasive alien fish currently present in the Paraná River basin through microcosm experiments. Since *B. orbignyanus* showed preference for two invasive species (*I. punctatus* and *Cichla piquiti*: Kullander and Ferreira, 2006), restoring its populations was recommended to limit the spread of *I. punctatus* which is currently not widespread.

Discussion

The present review has revealed many aspects of *I. punctatus* environmental biology, especially pertaining to the species' invasiveness, have received little or no study. These gaps in knowledge become more prominent when focused on populations in Europe, as only a handful studies focused on the invasiveness and impact of this species (see e.g. Haubrock et al. 2017). Moreover, data on the distribution revealed that many information on its establishment success are purely anecdotal and not presenting a high confidence level. However, *I. punctatus*'s current distribution in the Mediterranean is most likely linked to angling (Banha et al. 2017), as Mediterranean countries usually produce higher quantities of marine fish species rather than freshwater species, resulting in anglers being considered as the main vector for its introduction and successive spread. In contrast, populations in eastern Europe highlight this different usage of *I. punctatus*, as most introductions are linked to aquaculture escapes. Indeed, Savini et al. (2010) listed *I. punctatus* among the top 27 animal species introduced to Europe for aquaculture and related activities.

Data retrieved from invaded areas of North American show that *I. punctatus'* threat for invaded environments is undeniable. Its invasive capability is increased by its migratory activity during fall and spring, looking for suitable spawning sites or prey after increased river flow (Duncan and Meyers 1978; Becker 1983; Dames et al. 1989; Hanzawa and Arayama 2007), and by the average equal sex distribution to a slightly higher percentage of males among mature fish (Appelget and Smith 1951; Devlin and Nagahama 2002). Additionally, its opportunistic feeding habit, its broad adaptation to several different environments and the venomous spines are an advantage in invaded areas when competing with the native fauna for food or shelter.

Previously in Köppen-Geiger climate type countries applied risk assessments for *I. punctatus* (FISK, Table 3; AS-ISK, Table 4) underline the potential of *I. punctatus* to exert high impacts. Considering the results presented by EICAT for the European continent, a major impact with high uncertainties underline the importance of future studies. Thus, for Europe, the species resulted to be potentially invasive, even if up to now no published data are available. Among the impact mechanisms the species could exert in Europe, predation on benthic species, insects, plants and macroinvertebrates would especially pose a high ecological pressure and potentially considerable environmental modification to European freshwater habitats, likely reducing native biodiversity. Particularly, with the high densities tending towards young age groups, behavioural interferences may result in decreasing abundances of native fish. In a study by Jenkins and Burkhead (1994), the extinction of *Percopsis omiscomaycus* (Walbaum, 1792) populations was explained as the result of *I. punctatus* predation. However, beside native fishes, other several already endangered species may be threatened by its presence.

In Japan, Endo et al. (2015) showed that *I. punctatus* expressed a preference towards native crayfish over the invasive alien crayfish *Procambarus clarkii* (Girard, 1852), as this species, already overlapping its native distribution, is probably adapted to predation by *I. punctatus*. A good example of a potentially affected species is the already threatened native trout species *Salmo trutta* (morpha *fario* Linnaeus, 1758) in low species rich ecosystems in Europe, already endangered by the introduction of the American rainbow trout *Oncorhynchus mykiss* (Lee et al. 1980; Rinne and Minckley 1985; Page and Burr 1991; Blinn et al. 1993; Thibault and Dodsen 2013). Jenkins and Burkhead (1994) have shown that cold water adapted *I. punctatus*, and especially young *I. punctatus* that feed on aquatic plants, outcompete trout and perch for food and shelter. Thus, low species rich European ecosystems could offer catfish new opportunities to dominate entire ecosystems.

As spawning can be inhibited if suitable nesting cover is unavailable (Marzolf 1957), it is particularly interesting to investigate whether European freshwater systems can offer suitable nesting cover and whether these overlap with native species. Furthermore, climatic differences can affect growth, spawning, and metabolism and eventually lead to unforeseeable changes in behavioural patterns altering its invasiveness (Wellborn 1988). Studies on the invasive alien sun perch *Lepomis gibbosus* (Linnaeus, 1758) have shown that, according to life-history traits (mortality rate, plasticity and reproductive strategy), maturity is reached earlier in Europe (Cucherousset et al. 2009). Furthermore, Europe acts as a “melting pot” for invasive alien species, as several species are already present. The additional impact caused by the hybridization between *I. punctatus* and *I. furcatus*, both already present in Europe, cannot be discarded.

Another very interesting aspect is the complete lack of natural predators since generally only young fish are consumed by predatory insects or birds (e.g. cormorants, herons and pelicans). Most of the species that naturally predate on *I. punctatus* are not present in Europe or, if present, considered invasive alien. The grey heron *Ardea cinerea* (Linnaeus, 1758) could be a potential European predator (Glahn et al. 1995a, b; King et al. 1995; Wywialowski 1999; Glahn et al. 2000). However, potential predation by European or alien species has yet not been assessed. At last, the human exploitation rate through fishing activity, being lower than in the native region, has been neglected (Leonard et al. 2010), implying that no pressure is exerted on this species. Finally, the alteration of the environment through human activities could potentially increase this species invasiveness (Olden et al. 2005; Cucherousset et al. 2009, 2012) or impede it, as an establishment of *I. punctatus* was unsuccessful in Turkish waters (Innal 2012).

In conclusion, in view of the presence of *I. punctatus* in freshwater habitats of 22 European, the potential impacts of this species require urgent study so as to inform the risk assessment of this species for the new EU Regulation on the Management of Alien Invasive Species (European Union 2014). The high physiological tolerance and fecundity accompanied by parental care make this species a good candidate for future successful invasions throughout European waterbodies (Marchetti et al. 2004). By natural or human mediated spread, urban areas with rivers already disturbed and invaded by other species are prone to introductions and subsequently facilitate its population growth. With only few scientific attentions on advancing spread of this potentially invasive species, heavy impacts to European freshwater systems could be caused and lead to an irreversible situation, as control or eradication methods do currently not exist.

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Chapter 2.3

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Research Article CORRECTED PROOF

The diet of the alien channel catfish *Ictalurus punctatus* in the River Arno (Central Italy)

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Abstract

The North American channel catfish *Ictalurus punctatus* has been widely introduced to Europe, but no in-depth studies on its ecology and potential impacts in the introduced European range have been carried out. In 2016, 248 specimens of *I. punctatus* were collected from the Arno river, Florence (Central Italy), and analysed for their length, weight, size, sex, and stomach contents to assess their diet. Specimens < 30 cm total length (TL) represented the majority of the sampled population. Detritus and phytoplankton dominated the diet, while in larger fish (≥ 30 cm TL) two invasive alien species, the topmouth gudgeon *Pseudorasbora parva* and the red swamp crayfish *Procambarus clarkii*, were dominant prey items. Diet composition of *I. punctatus* significantly varied among size classes, but not between sexes. The results indicate an opportunistic but gape size limited feeding behaviour, suggesting an intra-specific competition avoidance mechanism.

Key words: fisheries, freshwater, impact, feeding habit

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Introduction

Europe has been a centre of globalization for the past two centuries making invasive species an increasing concern (Schulz and Della Vedova 2014). Increasing numbers of alien species introductions highlight the need for assessments to determine those that potentially pose invasive threats (Dukes and Mooney 1999; Walther et al. 2009; Vilà et al. 2010; Cucherousset et al. 2017). Managers and policy makers must thus allocate efforts to minimize the threat of more species becoming invasive (Keller et al. 2011). Scientific efforts should therefore focus not only on alien species that have already been classified as invasive, but also on those that have yet to be assessed. One example in this respect is the multiple cases of alien catfish species negatively impacting recipient European freshwater ecosystems (Benejam et al. 2007; Leunda et al. 2008).

The channel catfish, *Ictalurus punctatus* Rafinesque, 1818 (Siluriformes: Ictaluridae), is native to North America where it is extensively cultured (Appelget and Smith 1951; Etnier and Starnes 2001; Olenin et al. 2008), widely distributed and provides both a major sport and food species (Tucker and Hargreaves 2004; Leonard et al. 2010). It inhabits a wide variety of ecosystem types and is highly tolerant of extreme environmental conditions (Dunham and Masser 2012). It also shows an opportunistic feeding habit and high fecundity (Appleget and Smith 1951; Toole 1951; McMahon and Terrell 1982). In North America, several studies on its life history, growth, and reproduction as well as on its ecology and behaviour show predictable differences in diet among juveniles and adults (see e.g. Townsend and Winterbourn 1992; Adams 2007). In its native area, it is known for its opportunistic diet, negatively impacting populations of amphibians (Rosen et al. 1995) and threatened endemic fish species (Marsh and Douglas 1997).

Despite introduction and consequent establishment in 22 European countries (Welcomme 1988; Elvira and Almodóvar 2001; Copp et al. 2005), information and data on these introductions are scanty, and no in-depth studies focusing on *I. punctatus'* potential impacts in Europe have been conducted to support the initial literature (Welcomme 1988; Elvira and Almodóvar 2001; Copp et al. 2005; García-Berthou et al. 2005). In Italy, this catfish was first reported in 1986 in the river Oglio in the Northern part of the country (Gandolfi et al. 1991), while in the province of Florence in Central Italy, the area in which this study was conducted, the species was first recorded a decade later in 1998 (Nocita 2001). The species is currently considered established in Italy (Nocita and Zerunian 2007); however, studies until now only report its presence, without any information on population structure and possible impacts (Welcomme 1988; Tyus and Saunders 1996).

This Italian study is the first investigation of *I. punctatus*' diet in a European waterbody and includes size class, fish sex, habitat and seasonality in the assessment of this invasive catfish species.

Materials and Methods

Sampling

Specimens of *Ictalurus punctatus* were collected in the inner-city section of the Arno river, Florence, Central Italy. The Arno is characterised by an irregular flow rate, a length of 241 km, a drainage basin of approximately 8.200 km² and a mean annual discharge of about 110 m³/s. It is considered the second most important freshwater river in Central Italy (Bartolomei et al. 2006), and it is inhabited by several alien species such as the European catfish *Silurus glanis* (Linnaeus, 1758), the pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758), the topmouth gudgeon *Pseudorasbora parva* (Temminck and Schlegel, 1846) and the red swamp crayfish *Procambarus clarkii* (Girard, 1852), while native species are considered rare in the studied ecosystem (see Nocita and Zerunian (2007) and Nocita (2007) for detailed species and fish community information). Sampling was conducted two to three times per week at the same inner-city site (43º45′49.9″N; 11º18′04.2″E) from March to November 2016, the estimated active period for Ictalurus. To catch fish, standard fishing rods (2.20–3.90 m), monofilament line (0.22–0.36 mm) and size 2–26 fishing hooks baited with a variety of baits (maggots, worms, and freshly cut liver or baitfish) were placed on the river bottom and mid-water. Captured fish were immediately laid on ice, and then transported to the Department of Biology and Natural History Museum's laboratory, "La Specola" in Florence.

Morphometry and population structure

Total lengths (TL, mm) were taken, wet weights (g) were measured, and sex of all specimens were determined using pelvic fin morphology, as female *I. punctatus* have two openings, whereas male fish develop just one (the anus) in addition to a small protruding fleshy flap (genital papillae) (Norton et al. 1976). We attempted to assess the maturity of each individual by conventional methods. For males, maturity was confirmed by the presence of white milt within the gonads. In the case of females, ovary maturity was determined based on i) one or more eggs containing yolk and ii) red capillary arteries as part on the circulatory system around the outside of the ovaries (De Silva 1973; Infa et al. 2015) as well as oocyte development, i.e. analysing if gonads demonstrate the capacity to ripen (Gordon Copp, pers. comm.).

During this assessment, however, maturity could not be determined for every specimen in our study due to the absence of gonads in some large (> 50 cm TL) specimens. For this reason, we instead chose size classes as a proxy for maturity. We set the "maturity" threshold at 30 cm (corresponding to maturity thresholds set in Appleget and Smith 1951; DeRoth 1965; Perry and Carver 1973). This threshold was chosen because in this survey we found no mature gonads [as defined by De Silva (1973) and Infa et al. (2015)] in fish < 30 cm total length. Size ranges of 7–29.9 cm TL were therefore designated as "immature" (n = 175) and 30–52 cm as "mature" individuals (n = 73).

To initially characterise the population, the length-weight relationship was plotted on a graph. As the data were not normally distributed, the distribution of length and weight of sampled specimens was compared between males and females using a non-parametric Mann-Whitney U-test to characterise population and enable comparisons with other populations. For the same purpose, frequencies of males/females and "maturity" stage ("mature"/'immature') were compared using a chi-squared test to estimate if they significantly differ in numbers. The Fulton condition factor (K), generally used to compare populations, was applied using the formula $K = 100 * W / L^3$ (Nash et al. 2006) to better visualize length-weight relationship of this species outside its native region and to be available as a reference for future studies on *I. punctatus* outside its native area.

Dietary analysis

In the laboratory, stomachs were removed from the fish, and their content analysed (Zacharia and Abdurahiman 1974). Prey items were identified to the lowest possible taxa with a standard stereo-microscope and standard fish and invertebrate identification keys. Fragmented prey items were considered part of a whole organism and counted as such. Only number of occurrences of prey items was recorded. A comparison of prey composition was made for fish sex, maturity and sampling season. The nine months of sampling in 2016 were merged into three groups (spring: March–May; summer: June–August; autumn: September–November), resulting in a distribution of individuals among spring (n = 42), summer (n = 131) and autumn (n = 75; note no fish were caught in November). Diet was analysed as frequency of occurrence (F% = 100 * Ai / N) where Ai was the number of fish containing prey item i and N the total number of fish analysed (excluding those with empty stomachs; Zacharia and Abdurahiman 1974). The diet breadth of the "mature" and "immature" sub-population was estimated, also for all seasons, based on Levin's index formula (Whittaker et al. 1973): $B_i = \frac{1}{\sum p_i^2}$ where Bi is the standardized index of diet breadth for subpopulation i and ∑pi2 the sum of all squared proportions of individuals found with prey item i in their stomach, estimated by dividing the number of individuals containing prey item i by the total number of individuals sampled (Krebs 1998). To determine whether sample size was sufficient to describe the diet of non-native channel catfish in the Arno river, the cumulative number of prey taxa was plotted against the cumulative number of examined stomachs while analysed stomachs were randomized ten times (Ferry and Cailliet 1996; Ferry et al. 1997). Cumulative curves were considered to be asymptotic if ten previous values of the total number of prey taxa were within ± 0.5 of the range of the asymptotic number of prey, indicating the minimum sample size required to describe the diet (Cailliet et al. 1986; Cortés 1997; Huveneers et al. 2007). Because specimens yielded multiple prey taxa, prey composition was analysed using multivariate statistical methods. Fish without any stomach content (n = 16) were excluded from the dataset and the analysis, resulting in 166 stomach contents (spring = 32; summer = 76; autumn = 58) from "immature" and 66 (spring = 7; summer = 48; autumn = 11) from "mature" specimens. A bivariate non-parametric Spearman-correlation was conducted between the total sum of different prey items found in the stomach and the total length of individual fish.

A presence/absence matrix was built including stomach content records from each sampled specimen and a Permutational Analysis of Variance (PERMANOVA; 3 orthogonal fixed factors: “maturity” [“immature”, “mature”] (threshold TL = 29.9 cm), “sex” [male, female] and “season” [spring, summer, autumn]; sums of squares: type III, partial; permutation of residuals under a reduced model) was used to test if the diet of the studied population differed according to the total length of specimens, the sex of individuals, the season or a combination of factors. A post-hoc test (pair-wise test) was included to identify differences between pairs of levels for each factor/factor interaction found significant by the PERMANOVA main test. Additionally, a Canonical Analysis of Principal Coordinates (CAP) for factors whose levels were found to be significantly different was applied, thus identifying the variables (i.e. prey items) contributing more consistently in differentiating the levels. Spearman correlations for each variable with CAP1 axis, the only one found informative in differentiating “mature”/”immature” specimens and the three seasons considered, are reported. PERMANOVA and CAP were performed using PRIMER v. 6 (Clarke 1993). For all tests, the level of significance under which the null hypothesis was rejected is $\alpha = 0.05$ and values are reported as median and interquartiles (i.e. the first and third quartile).

Results

Morphometry and population structure

Overall, 248 specimens of *I. punctatus* (females = 135, males = 113) ranging from 7 to 52 cm TL (Md = 23 cm, Q1–Q3 = 17–31 cm) were analysed (Figure 1). Males and females differed in TL (Mann-Whitney U-test, U = 4.395; n = 248; $p < 0.001$; females: Md = 27 cm, Q1–Q3 = 19–35 cm; males: Md = 20.8 cm, Q1–Q3 = 16–26.5 cm) and weight (Mann-Whitney U-test, U = 4.607; n = 248; $p < 0.001$; females: Md = 198 g, Q1–Q3 = 89–420 g; males: Md = 91 g, Q1–Q3 = 59–181.5 g) with females being longer and heavier. No significant difference for the overall number of sampled males and females was found (males = 45.5%; females = 54.5%; chi-square test, $\chi 2 = 0.9777$; n = 248; $p > 0.5$). The size of captured specimens was strongly biased towards “immature” specimens under 30 cm, (“immature” = 70.6%; “mature” = 29.4%; chi-square test, $\chi 2 = 21.9$; n = 248; $p < 0.05$). The Fulton condition factor for the entire population was estimated between 0.07 and 5.27 (overall: Md = 1.174, Q1–Q3 = 0.936–1.424; females: Md = 1.123, Q1–Q3 = 0.904–1.326; males: Md = 1.256, Q1–Q3 = 0.981–1.640) and showed the highest values in March (Md = 1.526, Q1–Q3 = 1.157–1.814) and the lowest in in September (Md = 0.887, Q1–Q3 = 0.717–1.370) (Figure 2).

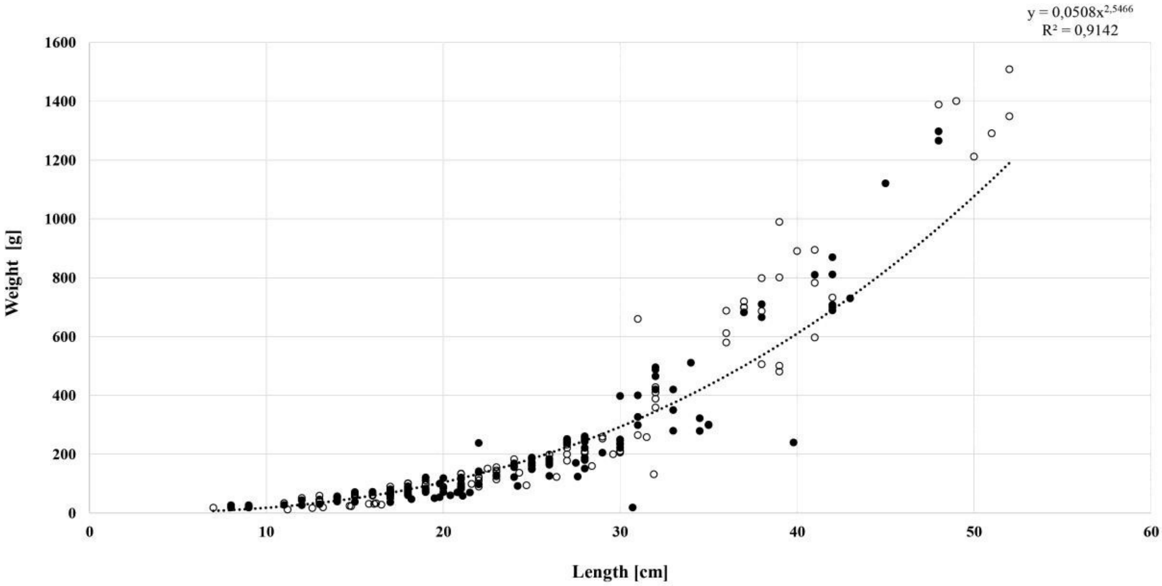


Figure 1: Length-weight relationship for the overall sampled population of channel catfish, differentiating female (n = 135, open circles) and male (n = 113, filled circles) specimens.

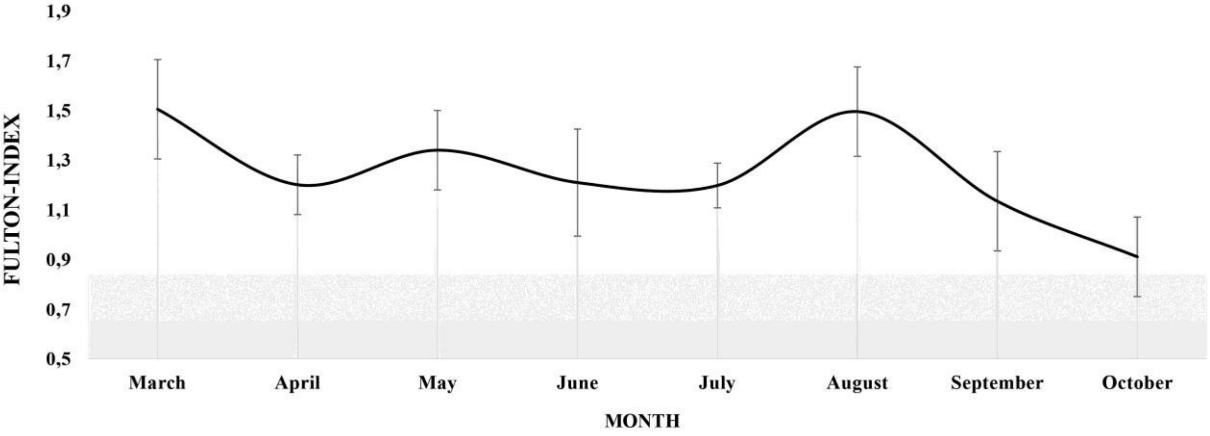


Figure 2: Development of the length-weight relationship in form of the estimated monthly Fulton-Index (mean±SD) over the activity period of caught specimens.

Dietary analysis

Overall, 16 invertebrate and fish prey taxa were identified (Table 1). The plotted cumulative prey curve for total number of prey taxa reached the asymptote after 74 stomachs (Figure 3). A significant correlation was found between the total number of individual prey items in catfish stomachs and fish size (TL) (rs = −0.129; n = 232; p < 0.043), showing that larger fish generally had fewer prey items in their stomach. On the other hand, the diet breadth for "immature" (B‘immature’ = 10.8) and for "mature" fishes (B‘mature’ = 10.7) was almost identical. Seasonally, the diet breadth for "mature" and "immature" catfish was similar in spring (B‘matureXspring’ = 9.4; B‘immatureXspring’ = 9.0) and summer (B‘matureXsummer’ = 8.6; B‘immatureXsummer’ = 8.1), while immature fish expressed a higher diet breadth in autumn (B‘matureXautumn’ = 5.3;

B‘immatureXautumn’ = 9.7). The most common prey items found in stomachs was detritus, followed by algae and plants. Generally, detritus, algae and plants were found to occur in higher percentages in “immature” rather than in “mature” specimens (Table 1). The most common animal prey found in “immature” fish were the topmouth gudgeon *Pseudorasbora parva* and Coleoptera, while in “mature” fish *P. parva* and the red swamp crayfish *Procambarus clarkii* were most common. Also, a high frequency of white “crumbs” with the consistency of soap products, visually and gustatorily detected as detergent (P. Haubrock, pers. obs.), occurred in the stomach contents of “immature” fish. Overall, *P. parva*, Coleoptera and *P. clarkii* were major contributors to the diet of *I. punctatus* (Table 1).

Table 1: Frequency of occurrence (%) for observed prey items according to season in “immature” specimens (n = 166), “mature” specimens (n = 66) and overall sampled specimens (n = 232).

Prey	**Total**	**“Immature”**				**“Mature”**			
	n=232	**Overall n=166**	**spring n=32**	**summer n=76**	**autumn n=58**	**Overall n=66**	**spring n=7**	**summer n = 48**	**autumn n = 11**
Fish									
Pseudorasbora parva	29.7	19.0	56.3	11.8	8.6	56.0	14.3	58.3	81.8
Unid. fish larvae	8.2	8.4	6.3	9.2	8.6	7.5	28.6	6.3	0.0
Cyprinus carpio	6.0	1.8	3.1	1.3	3.4	16.4	28.6	6.3	27.3
Lepomis gibbosus	4.7	1.8	6.3	0.0	1.7	11.9	0.0	14.6	0.0
Gobio gobio	0.4	0.0	0.0	0.0	0.0	1.5	0.0	2.1	0.0
Crustacean									
Procambarus clarkii	17.6	10.2	15.6	11.8	5.2	35.8	28.6	39.6	9.1
Palaemon sp.	10.3	12.0	21.9	10.5	10.3	6.0	0.0	4.2	9.1
Dikerogammarus villosus	6.9	8.4	12.5	3.9	10.3	3.0	0.0	2.1	0.0
Amphibian									
Tadpoles	1.7	0.6	0.0	1.3	0.0	4.5	0.0	6.3	0.0
Molluscs									
Sinanodonta woodiana	0.4	0.6	0.0	0.0	1.7	0.0	0.0	0.0	0.0
Radix auricularia	0.4	0.6	0.0	0.0	1.7	0.0	0.0	0.0	0.0
Insects									
Coleoptera	18.5	22.9	15.6	17.1	34.5	7.5	14.3	6.3	0.0
Unid. insect larvae	10.3	13.9	3.1	14.5	19.0	1.5	0.0	2.1	0.0
Heteroptera	8.6	10.8	15.6	10.5	8.6	3.0	0.0	2.1	9.1
Diptera	5.6	6.6	0.0	3.9	13.8	1.5	14.3	0.0	0.0
Hymenoptera	5.6	6.0	6.3	6.6	5.2	4.5	0.0	6.3	0.0
Fragments of insects	3.9	5.4	3.1	9.2	1.7	0.0	0.0	0.0	0.0
Odonata	3.4	3.0	6.3	3.9	0.0	4.5	0.0	4.2	9.1
Dermaptera	0.9	0.6	0.0	0.0	1.7	1.5	0.0	2.1	0.0
Other									
Detritus	55.8	66.9	59.4	63.2	75.9	28.4	42.9	22.9	36.4
Algae	48.5	58.4	53.1	75.0	37.9	23.9	42.9	18.8	36.4
Phytoplancton and terrestrial plants	28.8	32.5	50.0	22.4	37.9	19.4	14.3	20.8	9.1
Detergent	25.3	31.3	6.3	7.9	6.9	10.4	14.3	10.4	9.1
Plantseeds	7.3	9.0	18.8	22.4	50.0	3.0	0.0	4.2	0.0

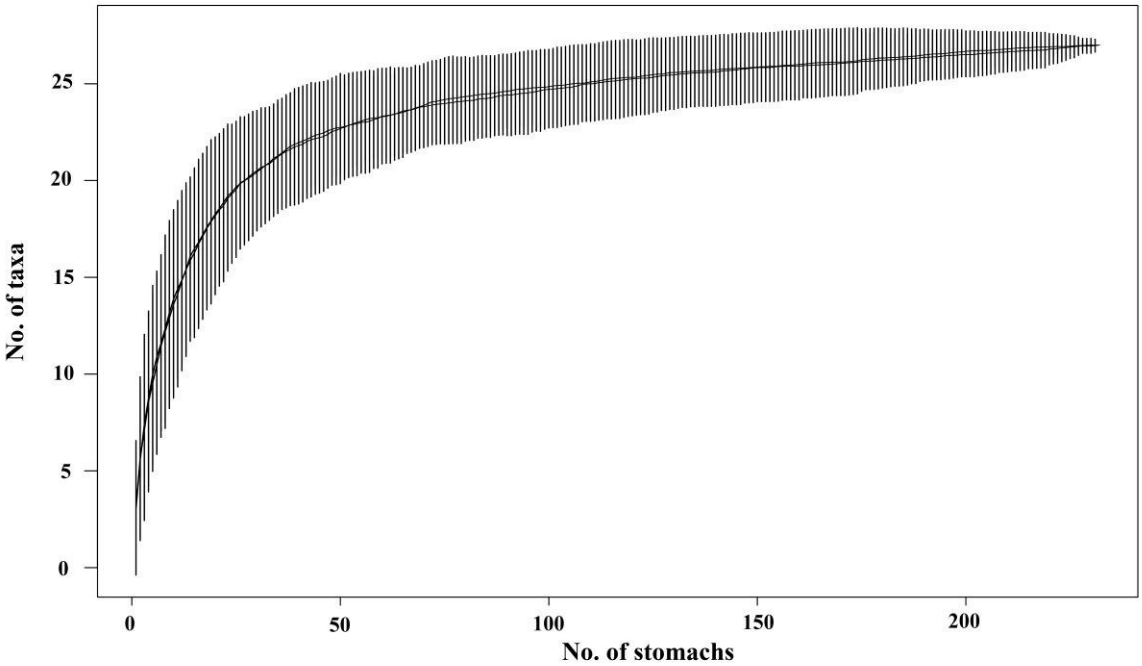


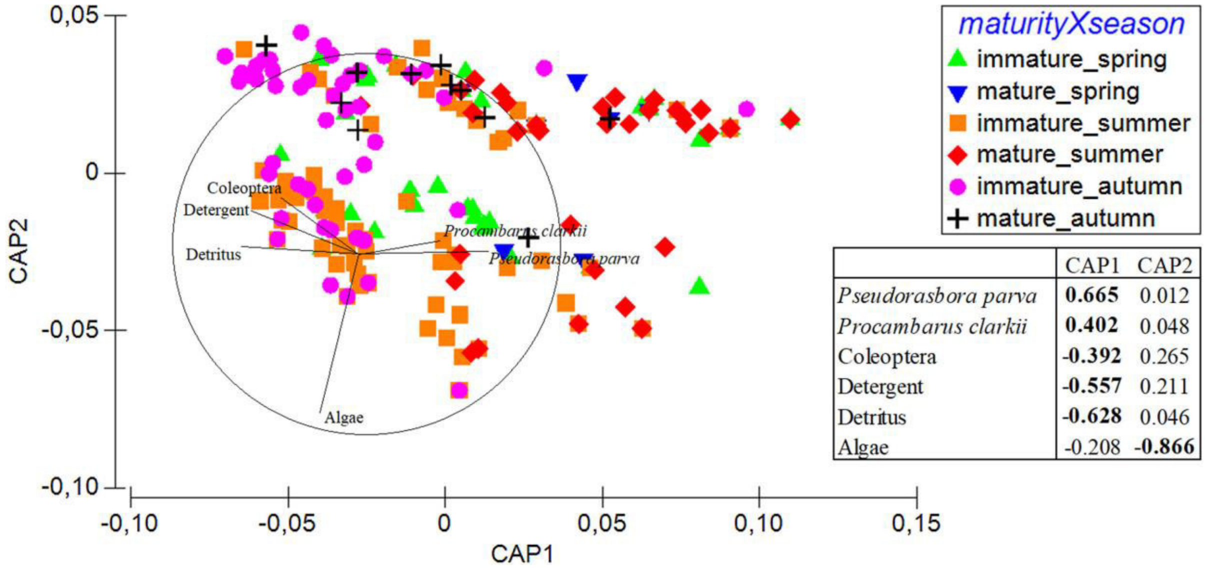
Figure 3: Cumulative prey curve displaying prey taxa per stomach for *Ictalurus punctatus* collected in Arno river in Florence, Central Italy (n = 232). Error bars represent standard deviations.

The PERMANOVA main test confirmed differences for the interaction between the factors “maturity” and “season” (Table 2), with no inter-sex differences (i.e. no differences in item composition between males and females). In particular, post-hoc tests revealed that in “immature” fish, the diet differed significantly according to season, while a significant difference for “mature” fish occurred only between summer and autumn (Table 3). Moreover, focusing on each season level, significant differences in diet (i.e. item composition) of “immature” and “mature” fish were found in summer and autumn, but not in spring (Table 3).

Table 2: Results of PERMANOVA on stomach content of collected specimens. The value of F statistic and its probability values P (after Monte Carlo correction) are shown together with degrees of freedom (df) and mean squares (MS). Significant differences are marked with (*).

Source	**df**	**MS**	**Pseudo-F**	**P(perm)**
Season	2	13001	5.1548	0.001*
Sex	1	3595.4	1.4255	0.221
Maturity	1	19933	7.9032	0.001*
Season x sex	2	3326.5	1.3189	0.211
Season x Maturity	2	5423.1	2.1502	0.012*
Sex x Maturity	1	5399.2	2.1407	0.068
Season x Sex x Maturity	2	1992.9	0.79017	0.647
Res	220	2522.2		
Total	231			

The CAP1 and 2 axes (squared canonical correlation of δ12=0.484 and δ22=0.183, respectively), with an overall mis-classification error of 48.28% (i.e. reallocation of each sample to the right level of the factor), only partially separated the groups of specimens identified by the combination of factor “maturity” and “season” (Figure 4). Correlation of variables’ scores on the CAP1 axis revealed detritus (rs = −0.63), detergent (rs = −0.56) and Coleoptera (rs = −0.39) as major variables in characterizing “immature” specimen diet (negative sector of CAP1 axis), especially in summer and autumn, while *P. parva* (rs = 0.66) and *P. clarkii* (rs = 0.40) highly characterized “mature” specimen diet in summer and spring (positive sector of CAP1 axis). Correlations with CAP1 and the other variables ranged from −0.23 to 0.14, therefore cannot clearly characterize identified groups, and are thus not reported in the text (Supplementary material Table S1). CAP2 axis was not considered for further analysis, due to its low power in discriminating groups and inconsistent correlations with variables, despite a unique high inverse correlation with algae (rs = −0.86), that characterised specimens from the negative sector of the CAP2 axis (Figure 4).



	CAP1	CAP2
Pseudorasbora parva	**0.665**	0.012
Procambarus clarkii	**0.402**	0.048
Coleoptera	**-0.392**	0.265
Detergent	**-0.557**	0.211
Detritus	**-0.628**	0.046
Algae	-0.208	**-0.866**

Figure 4: Two-dimensional scatter plot of the first and second principal coordinates axis (after resemblance matrix with Sørensen distance on presence/absence matrix of data, n samples = 232, n variables = 26) for “maturityXseason”, a combination of factor “maturity” (“immature”, “mature”) and “season” (spring, summer, autumn). Vectors of the linear correlations between individual variables are superimposed on the graph (only those with Spearman correlation index >0.4 are shown) and are listed in the lower right of the figure.

Table 3: Results of pairwise comparison (PERMANOVA) after significant differences founded in the interaction between TL and season on stomach content of collected specimens. The value of t and its probability values P (after Monte Carlo correction) are shown. Significant differences are marked with (*).

Source	t	P(perm)
Within level “immature” of factor “TL”		
……spring, summer	2.4849	0.001*
……spring, autumn	2.9868	0.001*
……summer, autumn	2.7633	0.001*
Within level “mature” of factor “TL”		
……spring, summer	0.66594	0.751
……spring, autumn	1.2633	0.171
……summer, autumn	2.1626	0.001*
Within level “spring” of factor “season”		
……“immature vs. mature”	0.68573	0.439
Within level “summer” of factor “season”		
……“immature vs. mature”	4.388	0.001*
Within level “autumn” of factor “season”		
……“immature vs. mature”	1.634	0.031*

Discussion

This study showed an imbalanced *I. punctatus* population structure in the inner-city section of the Arno river in Florence, with more “immature” individuals representing a young population present, suggesting stable reproduction and a well-established population, similar to observations from native populations in North America (Holland and Peters 1992). Also, females were significantly longer and heavier than males, matching the sexual dimorphism described in native populations (Wang 1986). The Fulton condition index for both sexes was higher compared to specimens analysed in other North American studies (see e.g. 0.62–0.73, Holland and Peters 1992; 0.77, Mesa and Rose 2015). This could be due to increased linear growth (without a simultaneous rapid weight growth) or a fundamentally different growth pattern in the Arno, an introduced habitat, possibly due to environmental factors (availability of prey, longer activity time, temperature, etc.). A link between growth and water temperature has been also suggested for *I. punctatus* in Japan (Endo et al. 2017) and was observed in non-native populations of the black bullhead *Ameiurus melas* (Copp et al. 2016). Nonetheless, the Fulton index has also been identified as an indicator for migratory behaviour (Gillanders et al. 2015). Because channel catfish are known to reduce their feeding activity in relation to falling ambient temperatures, the observed low Fulton index values in September may indicate a potential fall migration for adult and sub-adult catfish (Pellett et al. 1998), while low values between April and July but also in September could relate to periods of breeding (Peters et al. 1992). An adaptation towards secondary reproductive phases has been described for several alien fish species (Copp and Fox 2007), but never from North American Siluriformes in Europe.

The stomach content analysis showed a wide and opportunistic feeding habit of channel catfish, without any differences between sexes, but did reveal a difference between lifestages. With increasing size, the total sum of stomach contents decreased, likely due to changing prey composition. “Mature” fish expressed a more piscivorous diet typical for this species (Hubert 1999). Detritus with algae and Coleoptera represented the most frequent non-animal and animal portion of the diet respectively in "immature" fish, confirming that they forage close to the river bank in reed areas, where algae are present (Endo et al. 2015). In "mature" fish, detritus and algae were less frequent, while the invasive species, *P. clarkii* and *P. parva*, were major dietary contributors. As the crayfish *P. clarkii* is benthic, whereas *P. parva* is found occupying the middle and upper water layers (Gozlan et al. 2010; Čech and Čech 2011), the mature catfish in the Arno site are likely to be predators both on the benthos and throughout the water column as previously recorded for *I. punctatus* in other areas (Heard 1958; Poe and Rieman 1988; Townsend and Winterbourn 1992; Matsuzaki et al. 2011).

Dietary variation according to age and size classes are commonly related to idealized energy intake, especially as the diets of fish are partitioned by life history to lower potential intra-specific competition (Flecker 1999; Couture and Pyle 2015). This may be especially true for opportunistic channel catfish (Busbee 1968), which exhibit varying predation according to predator size, higher mobility, and predation in open water by adult specimens and also by availability of prey (Robinette and Knight 1981; Boersma et al. 2006; Matsuzaki et al. 2011). In our case, dietary differences and proposed implications for the frequented habitat are indeed potentially linked to gape-limited predation (Nowlin et al. 2006; Johnson et al. 2008; Slaughter and Jacobson 2008). *Pseudorasbora parva* occurred in both mature and "immature" specimens, although with low frequency in the smaller catfish. This could be explained both by optimal foraging behaviour in *I. punctatus* with their capacity to predate small sized or ailing fish (~ 3 cm), and by the developmental threshold for gape size to consume *P. parva*, suggesting an intra-specific competition avoidance mechanism.

It is likely that the observed diet change in "mature" specimens would not be observable without the presence of *P. clarkii* and *P. parva*, both being highly invasive and frequently abundant species (Gherardi and Acquistapace 2007; Nocita and Zerunian 2007; Britton et al. 2010). The applied CAP analysis underlines the importance of *P. clarkii* and *P. parva* in the diet of *I. punctatus*, as both these prey items characterize the diet of "mature" specimens while also contributing to the diet of "immature" catfish. The significance of *P. clarkii* and *P. parva* for "mature" specimens was apparent especially in spring and summer, the activity peak of these two species, although they were less frequent in the diet of "immature" individuals during summer and autumn. Especially in summer, populations of *P. parva* and *P. clarkii* can reach high densities in the Arno river, although considerably lower than observed in standing water bodies (Correia and Ferreira 1995; Gozlan et al. 2010; Gherardi and Acquistapace 2007). Therefore, it is no surprise that both prey items were frequently consumed by "mature" catfish in summer, and in the case of *P. parva* also in autumn. These results are furthermore consistent with studies from Lake Kitaura, Japan, where it was shown that invasive channel catfish exerted heavy predation on *P. clarkii* and *P. parva* opposed to available native prey (Endo et al. 2015). The highest frequency of *P. parva* in "immature" *I. punctatus* was observed in spring, coinciding with the lowest frequency of this prey items in the diet of "mature" fish. This can possibly be explained by spring habitat use shift in *P. parva*, which at this time typically searches for suitable spawning substrate such as plants or structure close to the river banks, i.e. the parts of the river, where most of the other prey items of "immature" *I. punctatus* occur. *Procambarus clarkii* was less frequently found in the diet of both "mature" and "immature" catfish caught in autumn, which is possibly linked to a changing habitat use of *P. clarkii* in autumn due to rising water levels (Correia and Ferreira 1995).

The only season, in which PERMANOVA detected similar diet for "mature" and "immature" *I. punctatus* was spring, which is likely based on the high occurrences of detritus and algae in both subpopulations as revealed by the post-hoc PERMANOVA. Although fish generally tend to prey on the most available and easy to obtain as well as energetically valuable prey (Vanni 1987; Gill 2003), it is unlikely that these similar diets of "immature" and "mature" fish during spring can be based solely on the availability of prey items. More likely, it can be assumed that higher levels of water and oxygen (respectively increasing water temperature) in spring lead to higher activities of all catfish and, thus, lower selectivity in all catfish. Additionally, "mature" catfish are likely to increase their energy intake after decreased rates of physiological processes due to colder temperatures during the winter and in preparation for the reproductive season (Kim and Lovell 1995), potentially explaining the high occurrences of algae and detritus, as both items are present in high quantities and are easily accessible. Although regular consumption of algae with potentially positive physiological effects on weight gain has been observed in other studies of this species (Lilyestrom et al. 1987; Tyus and Nikirk 1990; Guerry et al. 2009; Li et al. 2009), it is possible that catfish consume algae and detritus because of the lack of other, more energetically valuable prey, or in their search for any potential energy source prior to spawning. Seasonally, the flow regime changes from high water level and strong current in winter towards low water level and almost stagnation in summer, between river weirs; while increased water temperature leads to increasing catfish activity and the abundance of potential prey.

Our study furthermore shows an unusual high frequency of detergent in summer and autumn, likely remains of water pollution or filter attempts by sewage disposal facilities (Annamaria Nocita, pers. comm.) undermining the dominant benthic feeding. Detergent in stomach contents could generally be described as "accidental ingestion" that could potentially also occur in other fish species feeding on the river bottom (e.g. the common carp *Cyprinius carpio*). Nonetheless, the ingestion of detergent, as observed by Mahajan and Singh (1973), leads to pronounced "spitting" and decreasing appetite, resulting in fish starving rather than feeding on detergent treated prey items (Gupta et al. 1983). However, commercially available soap has been successfully used to catch *I. punctatus* in its native range and in the Arno river (P. Haubrock, pers. comm.) but has not been described for any species so far, raising the question of whether *I. punctatus* willingly ingests detergents found on the river bottom and how it might be affected by it. The occurrence of detergent in the diet of catfish clearly needs further investigation.

In conclusion, channel catfish and especially the "mature" fish in this study frequently feed on invasive alien species during summer and autumn in this highly-invaded environment, which is seemingly a common behaviour in alien fish species (Copp et al. 2009). Interestingly, these prey species are also major prey items of other predators such as the Wels catfish Silurus glanis (Carol-Bruguera 2007; Copp et al. 2009). The wide-ranging feeding habit, with "immature" specimens feeding on algae and detritus and "mature" specimens feeding on higher invertebrates (and to some degree on highly abundant *P. clarkii* and *P. parva*), potentially lowers intra- and inter-specific competition between co-occurring species and other life stages (Tyus and Nikirk 1990; Townsend and Winterbourn 1992; Copp et al. 2004; Simberloff and Von Holle 1999). Therefore, it would be interesting to investigate the relationship between *I. punctatus* and competitors such as *S. glanis* in respect to possible overlaps in feeding niches, especially considering observed declines in *S. glanis* populations after introductions of *I. punctatus* (Annamaria Nocita, pers. comm.). Additional studies including more data from different European ecosystems and a more detailed assessment of reproduction are needed. Finally, potential relationships with other alien species and thus varying feeding dynamics and the occupied ecological role in invaded communities should be analysed, to gain a better understanding of this species potential impacts on recipient ecosystems.

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Supplement 1: List of variables (n=26) used for PERMANOVA tests, along with the correlation (Spearman rank correlation r_s) with CAP1 and CAP2 axis of the Canonical Analysis of Principal Coordinates performed.

PREY	CAP1	CAP2
Fish		
Pseudorasbora parva	0.66456	0.01213
Unid. fish larvae	-0.00411	-0.12922
Cyprinus carpio	0.13731	-0.02595
Lepomis gibbosus	0.08647	0.19368
Gobio gobio	0.07614	0.05944
Crustacean		
Procambarus clarkii	0.40221	0.04851
Paleomonetes antennarius	0.00845	0.13759
Dikerogammarus villosus	-0.10137	0.14835
Amphibian		
Tadpoles	0.11769	-0.09642
Molluscs		
Radix auricularia	-0.04667	-0.01130
Insects		
Coleoptera	-0.39183	0.26529
Unid. insect larvae	-0.23058	0.14055
Heteroptera	-0.17177	0.09792
Diptera	-0.18775	0.16043
Hymenoptera	-0.09166	0.06703
Fragments of insects	0.00450	-0.14350
Odonata	0.05821	-0.08113
Dermaptera	-0.03063	0.12532
Sinanodonta woodiana	-0.02997	-0.04863
Other		
Detritus	-0.62772	0.04554
Algae	-0.20841	-0.86559
Aquatic and terrestrial plants	-0.15118	0.18044
Detergent	-0.55671	0.21055
Plant seeds	-0.04712	0.06861

Chapter 2.4

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SHORT COMMUNICATION

Opportunistic alien catfish: unexpected findings in the diet of alien *Ictalurus punctatus* from Central Italy

Short title: Plastic diet of alien catfish

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Abstract

Alien fish species are known for their negative impacts on native species and ability to shape the recipient community. One such case is the North American Channel Catfish *Ictalurus punctatus* in the Arno river, Central Italy. During river bank reconstructions, specimens of this species (n=40) were collected and the stomach content analysed. As a result, we highlight the opportunistic feeding behaviour of this alien species rapidly responding to new available resources and additionally, the threat to native species.

Keywords: *Ictalurus punctatus*; feeding, opportunistic, impact

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Introduction

Dietary analyses of invasive species are a common technique for invasion biologists and have revealed many information about the biology and associated impact of IAS (Kolar and Lodge, 2002; Feyrer et al., 2003). Especially opportunistic fish species are known for their generally plastic diet and adaptability (Copp et al., 2009; García-Berthou, 2002; Tyus and Nikirk,1990), often showing high variability among populations, years or even seasons (Panek and Weis, 2013; Purdom et al., 2015).

Catfish, by nature, are known to be opportunistic scavenger (Tucker and Hargreaves, 2004; Andrews et al., 1977). In the river Arno in Florence, Italy, the abundant alien North American Channel catfish *Ictalurus punctatus* has become a highly abundant species in terms of biomass and numbers (Nocita 2007). In the native habitat, it has been observed to show seasonally variable and generally opportunistic predation (Busbee 1969; Ploeg and Tucker 1994), but information about this species in Italy are rare or only anecdotal (Ligas 2007; Haubrock et al. 2017, Haubrock et al. 2018a, b). In a highly anthropogenically affected ecosystem like the Arno river, aquatic alien species are dominating in terms of numbers and biomass (Nocita and Lenuzza, 2016), exerting interactions within the aquatic ecosystem and potentially with semi-aquatic organisms (Gelwick and McIntyre 2017).

During mechanical restructuring works by the local Arno River Basin Authority on the vegetated zone alongside the Arno river within the inner-city section of Florence (Italy), plant material and masses of gravel were discarded into the river (Figure 1). These zones, apart from the function as a natural barrier, are characterized by large energy, nutrient, and biotic flow due to the proximity to aquatic (Cummins 1974; Sedel et al. 1974) and terrestrial ecosystems (Odum 1978). Eliminating these zones likely has negative effects on both, aquatic and terrestrial ecosystem (Odum 1978), while the discarding of cut plant material and gravel into the river might have unforeseeable impacts on the aquatic ecosystem.

Figure 1: North riverbank of the Arno river after mechanical removal of vegetation.

To investigate how the abundant catfish species would respond to such external impact on the aquatic ecosystem, a representative variety of *Ictalurus punctatus* was sampled and their diet was analysed. Such studies can help increase our understanding of alien fish behaviour in general and moreover present much needed information about the feeding behaviour of alien catfish.

Materials and Methods

Sampling

Fish were captured during the time of the constructions April - June 2018) in the inner-city section of the river Arno (43°45'49.9"N, 11°18'04.2"E). To catch *Ictalurus punctatus*, standard fishing rods (2.20 – 3.90 m), 0.22 – 0.36 mm monofilament line and size 2 - 26 fishing hooks baited with a variety of baits (maggots, worms, and freshly cut liver or bait-fish) were placed on the bottom and in the middle water. Caught fish were placed in ice before being transported to the laboratory of the Department of Biology and the Natural History Museum “La Specola” in Florence.

Data and dietary analyses

For all samples, total length (TL), weight (g) and sex were measured (Norton et al. 1976). Prey items in the stomach of fish were identified to the lowest possible taxa using a standard stereo-microscope. Fragmented prey items were considered part of a whole organism and counted as such. Only numbers of prey items were recorded. Consumed prey items were analysed as frequency of occurrence (% = 100 x A^I x N^{-I}) where A^I was the number of fish preying on species I, N the total number of fish analysed (excluding those with empty stomachs) and number of food items (N%) following the formula $N^i = \frac{N_i}{\sum_{i1}^{Q} N_i}$, where N_i is the number of food category i.

Results

During the time of the study, both riverbanks were cleaned of vegetation and to some amount discarded into the river, resulting into an increased nutrient entry (Figure 1) and thus, to a strong increase in turbidity. Collected specimens (21 male, 19 female) ranged from 13.5 to 64 cm (33.8 ± 2.2cm) and weighed between 20 and 3500 g (538.5 ± 106.3g). Dietary results are listed in Table 1.

Table 1: Dietary analysis results for sampled *Ictalurus punctatus* (n=40) from the Arno river

Prey	**N%**	**F%**
Plants	15,1	45
Crustacean	33,3	50
Aq. Molluscs	3,2	5
Hirudinea	0,6	2,5
Aq. Insects	4,4	12,5
Terr. Insects	29,6	42,5
Small Cyprinidae	8,2	25
Large Cyprinidae	0,6	2,5
Fish larvae	1,9	7,5
Small mammals	0,6	2,5
Small birds	1,9	7,5
Testudines	0,6	2,50

Discussion

Sampling predatory and especially opportunistic fish species to analyse diets and potentially diet shifts can give important information about imbalanced trophic interactions or other disturbances (Kolar and Lodge 2002; Syväranta et al. 2010). Additionally, such analyses in the case of alien species can reveal important information about behaviour or the studied species' impact (Copp et al. 2009; Moris and Atkins 2009). In this studies' case, plant material (aquatic and terrestrial), crustaceans (*Procambarus clarkii, Dikerogammarus villosus*) and terrestrial insects were the most dominantly consumed prey, while other prey categories were present in lower percentages (Table 1). Nonetheless, the high percentages of terrestrial insects were based on items such as Scarabaeidae (larvae of which can be found in the soil) and other Coleoptera (e.g. Coccinellidae and Curculionidae), items that are usually found in vegetated areas. As well as having found multiple juvenile birds identified as Columbidae and mammals (*Rattus norvegicus*) indicates an opportunistic feeding behaviour, likely intensified due to the reconstruction works on the river banks and thus, availability of prey. Indeed, comparing these observations with the analysed diets from native populations showed an increased consumption of terrestrial prey items. Apart from these high quantities of terrestrial prey items, the observed diet spectrum was similar to that identified from native populations (Bailey and Harrison 1948; Tyus and Nikirk 1990).

Lastly, having also found the remains of an alien pond slider (*Trachemys scripta*) in the stomach of *I. punctatus* is the first documented occurrence of alien Ictaluridae feeding on Testudines outside their native range and at least to our knowledge, the overall first documentation of this behaviour, underlining the opportunistic behaviour and thus, potentially negative impact of this species on recipient species communities (Cady and Joly 2003; Fritz et al. 2005; Strayer 2010). Aside from mechanical removal of the vegetated zone having negative effects for both, aquatic and terrestrial ecosystem as well as their inhabitants, it can be concluded that *Ictalurus punctatus* exerts an opportunistic feeding behaviour when given the opportunity due to additional prey.

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Chapter 2.5

The invasive potential and ecology of alien channel catfish *Ictalurus punctatus* in the Arno River, Italy

Short title: Channel Catfish in Italy

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Abstract

This study examined the growth, reproduction and invasiveness of channel catfish *Ictalurus punctatus,* native to North America, in the Arno River in Central Italy, where the species has been introduced about 2 decades ago. The multiscale approach was used to understand how channel catfish behave in the invaded ecosystems and how it impacts the native community and ecosystem. Our stomach contents analysis revealed a broad diet spectrum of this species, ranging from invertebrates, algae, and other fish species. Cannibalism on young was also detected. Overall, both benthic and pelagic preys were found, and also items of terrestrial origin, indicating that the channel catfish has a plastic feeding strategy and strongly rely also on bottom organic deposits. The Arno River presents ideal growth conditions for juvenile Ictaluridae. The estimated age at maturity was 3 years and reproduction seemed to occur at least twice a year, indicating an ongoing adaptation to Tuscan environments and a potentially invasive behaviour. This study presents the first complex work on this alien species' biology in Europe, raising the question if *I. punctatus* should be considered as an invasive threat to native European species and recipient ecosystems. Our findings clearly highlight the need to avoid this species expansion in European countries.

Key Words: reproduction, diet, population structure, population structure, impact assessment, alien species

In prep

Introduction

Freshwater ecosystems have been the target of frequent introductions by fish species (Oberdorff et al. 2002, Copp et al. 2005; Garcia-Berthou et al. 2005). Especially alien fish species are known to affect the structure and stability of native communities (Huxel et al. 2002; Strayer 2010). Their establishment process and the successive population growth rely on biological traits (high fecundity, asexual modes of reproduction, ability to colonize disturbed habitats, rapid population growth, etc.) to overcome selective biotic and abiotic barriers (Rejmánek and Richardson 1996; Kolar and Lodge 2001a, b; Ricciardi & Cohen 2007; Strayer 2010; Blackburn et al. 2011), traits generally common in invasive species (Cook 1990; Keller et al. 2007). These abilities of alien species function as a selective regime promoting higher frequencies of nuisance species in freshwater systems (Ricciardi 2006). Overall, factors and variables affecting the success of alien species, such as life history traits or environmental characteristics like human use have been previously studied (Alcaraz et al. 2005; Olden et al. 2006). Additionally, the presence of naïve recipient communities that have no native ecological vicariant or counterpart to the newly arriving alien species can result in less predation and competition for alien invasive species (AIS) and could enhance the alien invasive species' invasiveness (Ricciardi & Atkinson 2004; Cox & Lima 2006; Hill & Kotanen 2009).

In many cases, the arrival of another alien species can be highly visible, e.g. in the case of the Asian hornet, *Vespa velutina* (Cini et al. 2018) or, most often in the case of aquatic invasions, go silently without any visible affects until years have passed. Urban areas and anthropized freshwater ecosystems are often target of such invisible but consecutive invasions with complex interactions among species (Riley et al. 2005) while serving as source for further ongoing spread (Lodge 1993; Ricciardi & Rasmussen 1999; Beisel 2001). In Tuscany, allodiversity is very prominent, making up more than 3% (48 species) of freshwater fauna, with the Arno River basin presenting the highest amount of non-indigenous species (NIS) (Nocita 2007; Tricarico et al. 2008). Due to the high anthropogenic influence, the Arno River's environment, biodiversity and species composition were highly affected. Nocita (2007) and Nocita et al. (2010) showed the presence of several invasive species with diverse invasion histories and low abundances and even the loss of several native species within the inner Florence city section. Such exerted ecological impacts, that can be defined as a measurable change of variables in an ecosystem over time, strongly increase during first stages of the establishment according to population growth, eventually reaching a plateau phase according to demography or predator-prey dynamics, adaptation or interactions among species and lastly, depending on varying geographic scales (Ricciardi 2003; Strayer et al. 2006). An invasive species can exhibit ecological impacts at any point after its introduction, even before the establishment of an abundant population (Ricciardi et al. 2013), contrasting the general perception of only exhibiting significant effects (e.g. pests) in rich abundances (Hassan & Ricciardi 2014).

Such an exemplary invasion is the case of the North American channel catfish *Ictalurus punctatus* (Rafinesque 1818) which has been introduced to the Italian peninsula for aquaculture purposes in 1976. It was first observed in the wild in the river Oglio in

Northern Italy in 1986 (Gandolfi & Zerunian, 1987). Nowadays being present in the pet trade, its introduction to further freshwater ecosystems was likely due to the activity of recreational angling of Italian fishing associations (pers. comm. Giuseppe Castaldelli). Although information on this species in Europe are scarce and mostly anecdotal (see e.g. Ligas 2008, Castaldelli et al. 2013), records for Tuscany and in regard to the Arno River basin indicate, that it was first introduced to the province of Pisa and was subsequently spread up-river (Nocita, 2002), now dominating entire river stretches (pers. obs. Annamaria Nocita). Although the information on *I. punctatus* in its native region (Southern Canada, USA, Northern Mexico – Naylor et al. 2001) and its impact in North America are plenty (Bailey et al. 1945, Hawkins & Nesler 1991, Olden & Poff 2005), its presence, impact and potential threat to ecosystems and provided ecosystem services have yet remained unstudied in Europe (but see Haubrock et al. 2018a for a detailed summary). However, those studies available indicate an ongoing spread and the potential for high impacts (Banha et al. 2017; Castaldelli et al. 2013).

Especially the Arno River in Tuscany is environmentally and anthropogenically highly altered, a factor greatly facilitating fish introductions (Moyle & Light 1996a, b) and more so for *I. punctatus* (Hansen 1971), but especially the modification and fragmentation has affected present, native and alien, ichthyofauna, while facilitating invasions (Marchetti et al. 2004). Especially alien fish species can adapt to non-native environments in diverse ways. Such adaptations can be changes in reproductive patterns, different activity times or even morphological changes. While these are dependent on a variety of factors, it is likely that they are species specific. With the growing investments in monitoring and controlling invasive species, such information on biological adaptations of alien species are becoming increasingly important, likely improving the effectiveness of management efforts.

As *I. punctatus* represents an alien species not entirely unknown to European freshwater ecosystems but new to assessments and scientific approaches, this work presents a first but multidisciplinary attempt to approach the biology and ecology as well as invasiveness of *I. punctauts* in a highly invaded European freshwater ecosystem. The critical lack of information is likely due to the known risks and danger this species poses in other introduced ranges outside of Europe (Townsend & Winterbourn 1992, Haubrock et al. 2018a). In this context, data on various aspects of the *I. punctatus* population in the Arno River in its Florentine tract was collected and analysed to lay the ground work for further studies, aiming at understanding the impact this species has had and could have on the recipient environment in the future.

As a result, the reported information should contribute to the data needed for an assessment of invasiveness, thus, enabling this work to contribute to the potential categorization of I. punctatus as “invasive”.

Materials and Methods

Study area

The Arno River (Tuscany, Central Italy) is the second largest and most important freshwater river in the Central Italy. It is characterized by high water levels during winter and spring and progressively decreasing water levels in summer (Nocita 2002). Also, as a result of high-water consumption in summer, water flow can reach almost stagnation, which is particularly evident for fragmentation by consecutive dams in the inner-city tracts of e.g. the city of Florence. The anthropogenic influence (straightening of watercourse, overfishing, water pollution, eutrophication, etc.) has taken its toll on the river, rendering native species near extinction or even locally extinct (Nocita 2002). Additionally, the combination of human activities has favoured the annually re-growing dominance of *Myriophyllum spicatum*, common reed *Phragmites australis* and pondweed *Potamogeton nodosus*.

Sampling

Sampling was conducted on the far East (43°45'47.39"N; 11°18'23.27"E) and far West (43°45'56.30"N; 11°16'6.39"E) part of the longest non-segregated inner-city section of the Arno River in Florence, between March and October 2017, using local fisherman and standard angling techniques used by recreational anglers (i.e. baited size 16 to 22 hooks throughout the water column). Immediately after capture, fish were euthanized and out on ice to be transported to the laboratory of the Department of Biology c/o the Natural History Museum “La Specola” in Florence for sample preparation.

Data analysis

Total length (TL, mm) and net weight (g) were measured and sex was determined using the morphological difference between the pelvic fins, as female *I. punctatus* have two openings, whereas male fish develop just one (the anus) and a small extremely noticeable fleshy flap (genital papilla) (Norton et al., 1976). The mean length of males and females was compared with a t-test.

The age of specimens was estimated by counting annuli in pectoral spines according to Buckmeier et al. (2002). 60 specimens from all length classes were selected, spines extracted and boiled clean; then, spines were included into epoxidic resin and, once dry, the uppermost proximal piece of the shaft was cut out, mounted on a glass slide and skimmed off to 200-300 µm to be analysed under a standard microscope (25x magnification or more). Interpreting annuli can be difficult (Marzolf 1955; Turner 1982; Crumpton et al. 1987) because i) with increasing age, the expanding central lumen erodes earlier annuli with the outcome of underestimated ages in older fish (Mayhew 1969), ii) a too basal cut could lead to fewer visible annuli (Nash & Irwin 1999) and iii) outer annuli in older slow growing fish can be indistinguishable (Lai et al. 1996; Kocovsky & Carline 2000). Age determinations were validated using secondary and tertiary reading from two indepent readers and cross checked. If results varied among readers, a third reader was involved. Hence, back-calculations of age groups on the studied population were accomplished by following the approach of Francis (1990) and Carlander (1981). As fish size effects fish weight, examination of this relationship was done with a logarithmic transformation and back calculation using a linear regression model. This relation was compared among populations by testing for homogeneity of slopes in an Analysis of Covariance (ANCOVA; Dhillon & Fox 2004). To compare the weight gained with increased length among populations, weight was set as the dependent variable and length as covariate. To compare the annual length growth among populations from the native and introduced range (Table 1), length was chosen as the dependent variable and age as covariate. Replicates were pooled for all growth comparisons after it was determined that there were no significant differences between any within-run replicates (ANCOVA, $p<0.05$ in all cases). To analyse the fecundity and reproductive behaviour, gonads were extracted from mature specimens and weighed to the nearest 0.01g. Statistical analyses were performed in R (R Core Team 2013).

Reproductive pattern

The Fulton's condition factor ($K = 100 * W / L^3$; Froese 2006; Nash et al., 2006) was applied for all age groups. Additionally, the Gonado-Somatic Index (GSI) was calculated using the formula: GSI = (gonad weight / body weight) x 100 (Gutiérrez-Estrada et al. 2000). While the Fulton's condition factor K is usually employed as a measurement for fish's health, it can be used as an indicator of reproductive or migratory phases. Hence, both factors were tested for correlation and thus, can be used as an indicator of reproductive behaviour (Roff 1983; Lamas & Godinho 1996) when plotted against the month and monthly mean air temperature.

Maturity and prediction of invasiveness

To analyse the age of maturity, gonads were extracted from all samples. The level of maturity was determined using the morphological characteristics according to Infa et al. (2015) and De Silva (1973), whereas gonads were divided into mature and immature. The mean age at maturity (A_M) was calculated using a formula from DeMaster (1978) based on the proportion of mature females within each age class, whereas the total length at maturity was calculated using an adapted version of the DeMaster (1978) formula using 10 mm size-classes (Trippel & Harvey 1987) as adapted by Fox (1994):

$$a = \sum_{x=0}^{w} (x)[f(x) - f(x-1)]$$

where a is the mean age of maturity, x the age in years, f(x) the proportion of fish mature at age x and w the maximum age in the sample (Tarkan et al. 2016). Based on the close linkage between age and length at maturity (Fox 1994) allows the use of the estimated mean age at maturity for a population to be plotted against the mean juvenile growth (TL at the respective age), proposed as an identifiable physiological transition phase between non-native and invasive populations (Copp & Fox 2007). However, this approach requires that assumed invasive species populations mature earlier or reach the typical reproductive size earlier than native populations and might not be the case in all non-native species. Furthermore, by plotting the juvenile growth (mean TL of maturity, age when most fish were mature) against the estimated age at maturity, non-typical behaviour (i.e. earlier maturation) in contrast to native populations can be identified. For this purpose, the available literature on the age and mean TL at maturity of *I. punctatus* was reviewed and compared to the studied population in the Arno River. Both, TL at maturity and TL at age 3 were tested for correlation with age at maturity using Spearman-correlation analyses.

Diet analysis

From all sampled specimens, stomach contents were extracted and stored in 96% ethanol until further analysis. Prey items were identified to the lowest possible taxa with the use of a standard microscope. Fragmented prey items were taken to be parts of a whole organism and counted as such, while only numbers of prey items were recorded, and prey compositions were inferred according to maturity. The amount of sampled stomachs was considered as sufficient for analysis based on a previous assessment (Haubrock et al. 2018b). To analyse the frequency of consumption of different prey items, diet was analysed as frequency of occurrence with the formula $F\% = 100 \times A^{I} \times N^{-I}$ where A^{I} was the number of fish preying on species I, N the total number of fish analysed (excluding those with empty stomachs). To summarize months according to seasons, sampling months were merged (spring: March–May, summer: June–August, autumn: September–November). Although 2016 (Haubrock et al. 2018b) and 2017 were very different, i.e. very hot and dry climate in summer and autumn 2016 leading to low water levels and stagnating river sections, diets were combined to enlarge the dataset, hence, willingly neglecting variability between the years as the focus laid on seasonal differences and variability between immature and mature specimens. The overall diet composition was analysed using multivariate statistics. For this purpose, a qualitative presence/absence matrix was built from overall stomach contents. A Permutational Analysis of Variance (PERMANOVA; 2 orthogonal fixed factors: ‘TL’ [immature, mature] & ‘season’ [spring, summer, autumn]; sums of squares: type III, partial; permutation of residuals under a reduced model) was applied using the software PRIMER v. 6 (Clarke, 1993) to identify differences between levels of each factors and their interaction. Additionally, to enlarge the data set, data from 2016 (Haubrock et al. 2018b) was included to analyse variability among years. Lastly, an indicator species analysis (R package “labdsv” for ordination and multivariate analysis for ecology; Roberts & Roberts 2016) was performed to evaluate the indicator values (i.e. characterization of factors; fidelity and relative abundance) of diet items for the same two fixed factors ‘maturity’ and ‘season’ and groups from their interaction ‘maturityXseason’. This indicator species analysis finds items, that are significantly concentrated into specific classes.

Results

Sampling

Overall, 427 specimens (male: 152 (35.6 %); female: 275 (64.4 %)) of *I. punctatus* were collected. These ranged from 8.8 to 59.3 cm of total length (FL: 7.5 – 53 cm; STL: 7 – 48.2 cm) and weighed between 6 and 2100 g.

Length-Weight relationship

From 78 extracted and analysed spines, seven age classes were identified (0+, n=3; 1+, n=12; 2+, n=16; 3+, n=14; 4+, n=7; 5+, n= 5; 6+, n=1, 7+, n=2), from which age 6+ and 7+ were combined and listed as “>5+” due to not being sufficient to assign length boundaries. These age classes were then applied to the entire dataset, revealing a high tendency towards fish of younger age classes with age 2+ fishes being the dominant age class and median age of the sampled population. Male and female fish did not differ for mean total length (T = 0.663; df = 427; p = 0.508).

To compare the length-weight ratio of populations and the growth over the age classes, the weight of specimens was plotted against the total length (Figure 1) and the mean total length against the respective age (Figure 2) to be comparable to openly accessible data from other populations (Table 1). While fish under ~30 cm expressed a higher weight to length ratio than most other populations, fish above ~30 cm showed a consistently lower weight. In contrast, fish from Lake Kasumigaura (Japan) were observed to have the highest weight to length ratio but did not reach the same sizes (Figure 1, 2). Additionally, the growth was significantly different among populations (T = 3.6 – 13.5; df = 722 – 1228; $p < 0.05$) and the applied ANCOVA highlighted that populations were significantly different for annual length growth (F= 5.88, $p<0.05$) as well as weight per total length (F= 482.34, $p<0.05$) Furthermore, the post-hoc test indicated similar growth rates among the Arno and Missouri Reservoirs populations ($p> 0.5$) as well as high variability among native populations (Table 2; Supplement 1).

Hence, the mean total length across age-classes for several populations revealed that the alien population in Italy exerts a comparably greater growth, while the alien population in Lake Kasumigaura was generally slower, although comparable with other North American populations until age 4 (Figure 1, 2; Table 1).

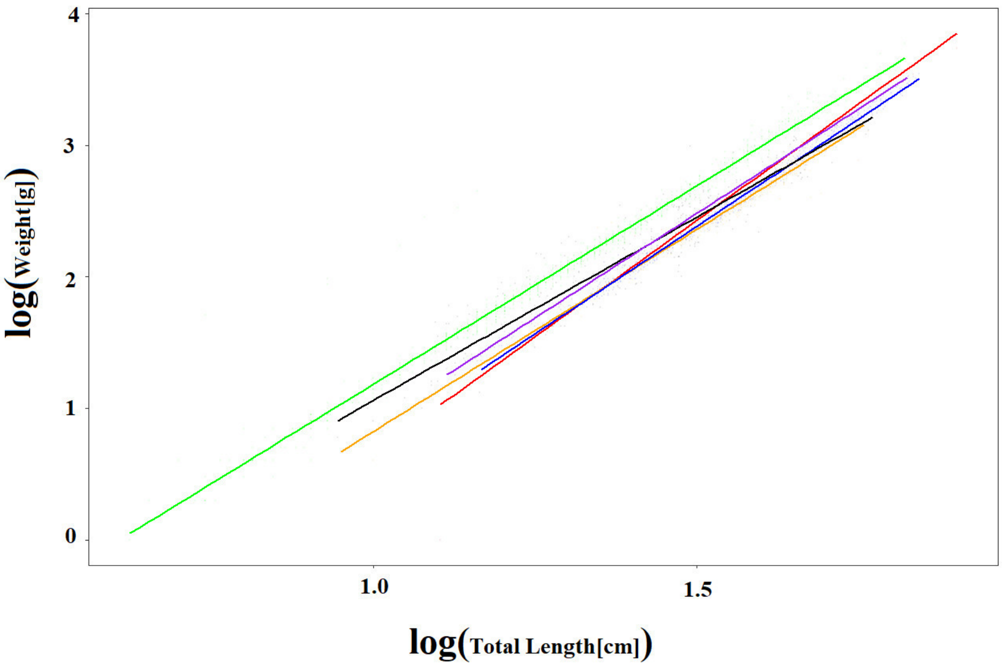

Figure 1: Weight according to length of six populations of *I. punctatus*; black =Arno River, Italy (n = 427; y = 2.69x - 1.563; r² = 0.900); red = Oklahoma (n = 2891; y=2.79x-1.630; r² = 0.983), Finnell 1954; orange = Francis Case Reservoir, Missouri (n= 606; y=3.06x–2.241; r² = 0.965), Bouska et al. 2011; blue = Lewis Clark Reservoir, Missouri (n = 580; y=3.17x–2.510; r² = 0.992), Bouska et al. 2011; purple = aquaculture, Mississippi (n= 54; y=3.17x–2.274; r°= 0.999) Steeby et al. 1991; green = Lake Kasumigaura, Japan (n = 823; y=3.01x–1.630; r² = 0.974), Matzusaki et al. 2011.

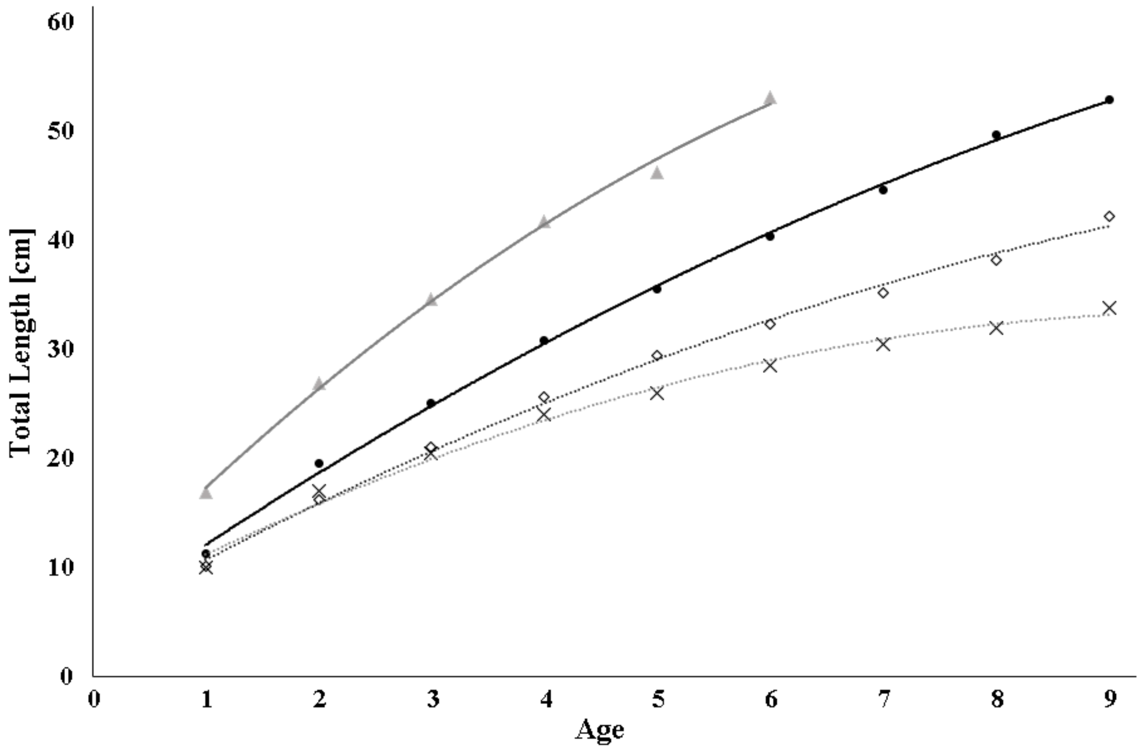

Figure 2: Mean length across different age classes for native US populations and non-native population of *I. punctatus*. Black circle (filled) = mean length at age for native US populations; grey triangle = Arno River, Italy; black circle (empty) = Quebec, Canada; black cross = Lake Kasumigaura, Japan.

Table 1: Information gathered from all populations used in this study and from the investigated population in the Arno river; A_M = Age at maturity; L_M = Length at maturity; n = number of sampled specimens; GSI = mean monthly Gonadosomatic-index; K = Fulton conditioning factor; na = data not available; Sources: 1= present study; 2 = Matzusaki et al. 2011; 3 = Scott & Crossman 1973; 4 = Elroy et al. 1990; 5 = Ackerman 1965; 6 = Steeby et al. 1991; 7 = De Roth 1965; 8 = Hesse et al. 1980; 9 = Mahoney 1982; 10 = Starostka & Nelson 1975; 11 = Elrod 1974; 12 = Bouska et al. 2011; 13 = Appleget & Smith 1951; 14 = Barnickol and Starrett 1951; 15 = Shephard and Jackson 2006; 16 = Hesse et al. 1980; 17 = Hancock 1969; 18 = Hall & Jenkins 1952; 19 = Blank 2012; 20 = Holland and Peters 1992; 21 = Shipmann 1977; 22 = McClellen 1954; 23 = Michaletz et al. 2009. Adapted from Haubrock et al. (2018a).

Location	**Ecosystem**	**Mean total length at age (cm)**										**A_M**	**L_M**	**n**	**GSI (%)**	**Sex ratio**	**Source**
		0	1	2	3	4	5	6	7	8	9						
	Europe																
Italy	River Arno	11.5 ± 0.4	16.9 ± 0.3	26.9 ± 0.3	34.6 ± 0.2	41.7 ± 0.4	46.2 ± 0.2	53.1 ± 0.5*	na	na	na	2.83 (f) –2.95 (m)	31.2 (f) – 31.8 (m	427	3-57	1.8:1	1
	Asia																
Japan	Lake Kasumigaura		10.1	16.2	21	25.6	29.4	32.3	35.2	38.1	42.2	5	33.2	937	na	na	2
	North America																
Quebec, CA	St. Lawrence River		10	17	20.5	24	26	28.5	30.5	32	33.8	na	na	na	na	na	3
Louisiana, US	Lake Maurepas		16.6	26.2	32.3	42.4	47.7	na	na	na	na	na	na	na	na	na	4
Iowa, US	Des Moines River		na	24.9	29.7	34.8	39.9	46	53.5	56.6	63.2	4	27.2	2222	na	na	3; 5
Mississippi, US	Aquculture pond			Na, instead information on growth and length weight relationship								na	na	8885	na	na	6
Michigan, US	Lake Erie		6.3	16.6	22.6	26.8	29.8	32.8	36.2	na	na	4	30.5 (m) - 27.9 (f)	2158	na	na	3; 7
Missouri, US	Missouri River	na										5	31.8	506	36-62	1:1	8; 9
South Dakota, US	Lake Oahe		3.3	11.3	19.3	26.6	33.3	39	43.9	48	51.5	8	54.3	1470	na	1:1	10
South Dakota, US	Lake Sharpe		na	na	24.4	28.4	30	34.6	37.2	39.3	43.3	8	34.7	52695	na	na	11
Missouri, US	Francis Case Reservoir		20.5	24.5	31.2	34.3	37.5	40.2	42.6	44.4	47	na	na-	606	na	na	12
Missouri, US	Lewis Clark Reservoir		15.7	21.8	27.5	35.8	41.9	47.6	53.1	56.2	59.1	na	na	580	na	na	12
Iowa, US	Mississippi River		na	24.9	29.7	34.7	39.9	45.8	53.2	56.6	63.3	5	38	535	na	1:1.2	13
Missouri, US	Nemaha River		17.6	21.2	25.1	29.3	33.1	37.6	42.3	48.2	46.9	5	21.9	56028	na	na	9
Missouri, US	Acquaculture pond		11.2	14.7	15.8	16.9	18.0	18.9				na	na	na	na	na	23
Mississippi, US	Mississippi River	na										4	30.5	180	na	na	14
Mississippi, US	8 river systems**		65	127	20.5	27.5	34.0	39.6		na		4.1	3.8	712	na	na	15
Mississippi, US	Sunflower river***		10.7	19.3	28.0	36.7	42.6	50.1		na		2.8	na	63	na	na	15

Mississippi, US	Upper Pearl	6.5	12.2	18.2	24.0	29.2	32.8	na			na	na	97	na	na	15
Mississippi, US	Pascagoula	6.6	13.4	20.9	29.4	33.0	35.3	na			na	na	97	na	na	15
Mississippi, US	Buttahatchee	6.1	11.4	17.7	24.4	31.0	35.9	na			na	na	97	na	na	15
Mississippi, US	Middle Pearl	6.8	12.8	19.9	26.2	32.9	39.4	na			na	na	58	na	na	15
Mississippi, US	Chichasawhay	6.3	13.1	21.3	29.2	35.9	42.4	na			na	na	102	na	na	15
Mississippi, US	Yalobusha	6.5	12.9	20.9	28.9	36.1	43.1	na			na	na	92	na	na	15
Mississippi, US	Noxubee	5.9	11.6	18.5	26.2	35.1	42.5	na			na	na	103	na	na	15
Mississippi, US	Big Black	7.3	14.2	23.0	31.8	39.1	45.0	na			na	na	66	na	na	15
Missouri, US	Niobrara River	Na									6	28.9	154	30-80	1.2:1	9; 16
Kentucky, US	Kentucky Lake	10.6	20.4	27.3	33.2	37.9	41	43.9	47.5	49.4	na	na	1298	na	na	17
Oklahoma, US	Lakes	12.7	20.3	28.4	34.3	39.4	44.4	47	52.7	58.8	na	na	4054	na	na	18
Oklahoma, US	Rivers	6.9	14	20.1	26.2	32.5	39.9	45.1	54.6	61,2	na	na	1265	na	na	18
Nebraska, US	River Platte	na	na	24.4	31.5	37.4	40.9	44.7	49.2	53	na	na	2979	na	na	19
Nebraska, US	River Platte	8.4	14.7	20	24.9	29	34.6	37.3	41.9	45.5	4	24.9	4502;	na	na	20
Utah, US	Willard Bay Reservoir	na									4	33 (m) - 35 (f)	na	na	na	21
Texas, US	Ponds	na									1.5	30.5	na	na	na	22

* combination of specimens older than 5 years
** mean values of 8 rivers
*** differing from other Mississippi rivers for its small area (553km²), exceptionally high agricultural land use (0.71%) and very low discharge (16.5m³/s).

Table 2: Tests of Between-Subjects Effects for the One-Way ANCOVA testing for a) significant differences for dependent factor ‘weight’ among ‘populations’ under the covariate ‘total length’ and b) significant differences for dependent factor ‘length’ among ‘populations’ under the covariate ‘age’.

a) Dependent Variable: Weight

Source	Typ III Sum of Squares	df	Mean Square	F	P(perm)	Partial Eta-Squared
Corrected Model	856.026[a]	6	142.671	11064.987	.000	.962
Constant Term	132.004	1	132.004	10237.719	.000	.796
Length	741.349	1	741.349	57496.020	.000	.956
Population	31.096	5	6.219	482.337	.000	.479
Error	33.795	2621	.013			
Total	15410.382	2628				
Corrected Total	889.821	2627				

a. R^2 = ,962 (corr. R^2 = ,962)

b) Dependent Variable: Length

Source	Typ III Sum of Squares	df	Mean Square	F	P(perm)	Partial Eta-Squared
Corrected Model	871639,249[a]	12	72636,604	11,019	,000	,691
Constant Term	76152,052	1	76152,052	11,553	,001	,164
Age	445234,430	1	445234,430	67,545	,000	,534
Population	426404,819	11	38764,074	5,881	,000	,523
Error	388910,404	59	6591,702			
Total	5247567,000	72				
Corrected Total	1260549,653	71				

a. R^2 = ,691 (corr. R^2 = ,629)

Reproductive pattern

The calculated Fulton index and variability steadily decreased with age. The applied Kruskal-Wallis test revealed statistically significant differences between age classes (H^2=17,155 df=6 p=0,009) and the applied Mann-Witney U post-hoc test for pairing of age classes highlighted that age classes 0+ and +1 group together while also all age classes except age 1+ fish were similar (Table 3).

The analysed gonads of sampled fish varied strongly (GSI: 0.21 (October) – 0.58 (May)) with most analysed individuals being already spent. Moreover, the estimated monthly Fulton Index correlated with the monthly GSI values ($r_S = 0.831$; n = 8; p = 0.011). Plotting mean Fulton Index and GSI over the sampling months revealed a strong annual variation. The Fulton Index rose from March to April, followed by low values in May and June and again an increase in July and August before succumbing towards September. In contrast, plotting GSI values showed, that March, May and July / August were the months with the highest values. Fulton and GSI varied opposed to each other in the beginning of the year, then smoothing up during summer and being synchronic in autumn. The measured air temperature, which directly relates to the water temperature and thus the activity of present species was highest between June and August with lowest values in April and October (Figure 3).

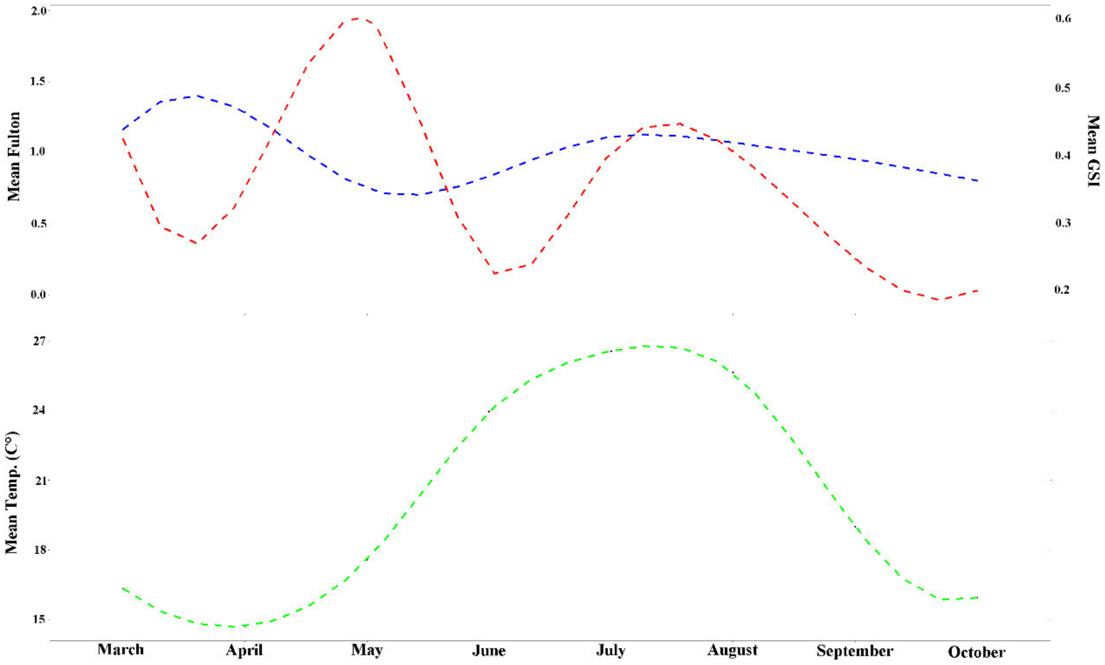

Figure 3: Monthly calculated mean Gonadosomatic-Index (GSI) and Fulton Index (K) and measured air temperature (°C) over the study period; Blue: Fulton Index; red: GSI; green: mean temperature measurements.

Table 3: Summary of age classes and percentage of occurring gonads among age classes as well as mean Fulton index for the respective age groups and grouping identified by the applied Kruskal-Wallis test are indicated by letters a and b

Age	n	% with mature Gonads	% with immature Gonads	Fulton-Index	Group
0+	12	0	0	1.07 ± 0.15	a, b
1+	70	3.5	2.9	1.26 ± 0.07	b
2+	170	7.1	4.7	0.96 ± 0.03	a
3+	70	25.6	8.6	0.88 ± 0.03	a
4+	33	30.3	6.1	0.92 ± 0.04	a
5+	35	34.3	2.9	0.76 ± 0.05	a
6&older	37	37.8	0	0.86 ± 0.54	a

Maturity and prediction of invasiveness

Ictalurus punctatus expresses a high variability in the age at maturation (Le Cren 1951; Trippel 1995; Richard et al. 1986). The youngest individual with immature but potentially ripening gonads measured 14.2 cm and 42 g. The age at maturity for *I. punctatus* of populations in the native area ranged between 1.5 and 8 years. In Italy, the total length at maturity for the Italian population was estimated as 20.3 cm in females and 26.3 cm in males by using the DeMaster (1978) formula. The age at maturity was calculated to be 2.83 (mean TL = 31.2 cm) for males and respectively 2.95 (mean TL = 31.8) for females. Specimens from the population in Japan matured with approximately 5 years of age in both sexes and average total length of 33 (females) to 36 cm (males) at the respective age (pers. comm. Shin-Ichiro Matsuzaki) (Table 1). Following this determination of age at maturity, immatures (n = 263; 61.6 %) overweighed mature fish (n = 164; 38.4 %). However, almost 12% of age 2 fish and > 30% of fish from older age classes were observed to have mature or potentially maturing gonads. Furthermore, although the least squares relationship between variables was low, juvenile growth and A_M strongly correlated ($r_s = -0.823$, $n = 9$, $p = 0.006$; Figure 4), while A_M and mean TL at maturity did not ($r_s = 0.352$, $n = 14$, $p = 0.217$; Figure 5), indicating that juvenile growth, thus the length at age 3, is a better fit for the determination of the age at which fish mature than the estimated length at maturity.

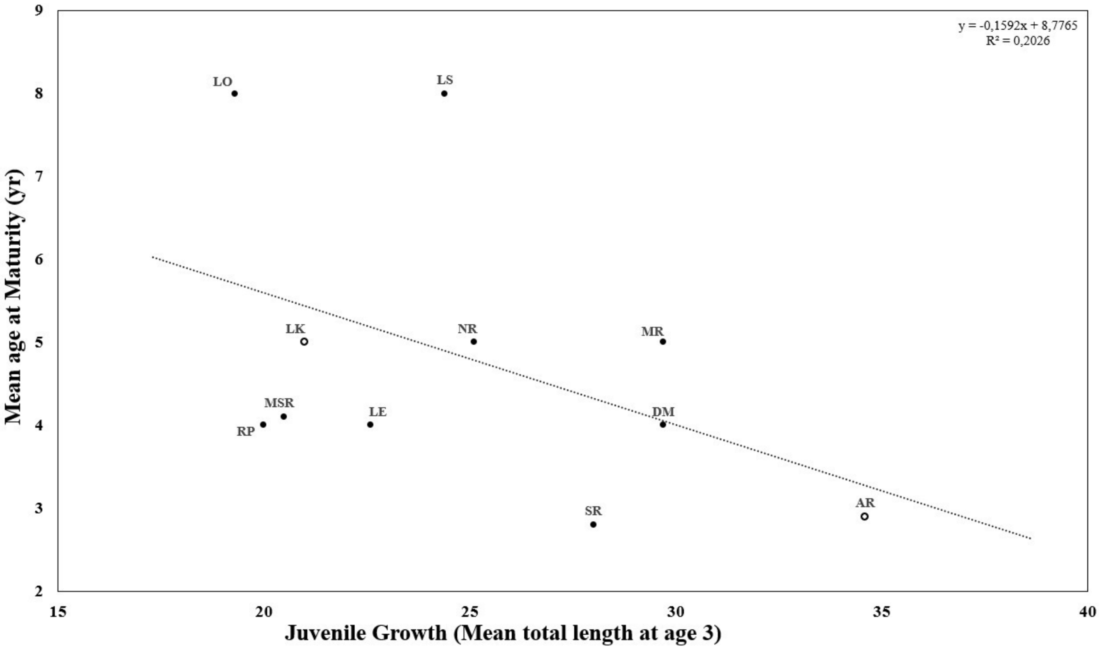

Figure 4: Relationship between mean total length at age 3 and mean age at maturity in channel catfish populations; native populations (black circle): LO = Lake Oahe, South Dakota, Starostka & Nelson 1974; MR = Mississippi River, Iowa, Appleget & Smith 1951; LS = Lake Sharpe, South Dakota, Elrod 1974; NR = Nemaha River, Missouri, Hesse et al. 1982; RP = River Platte, Nebraska, Holland & Peters 1992; SR = Sunflower River, Mississippi, Shephard and Jackson 2006; DM = Des Moines River, Iowa, Ackerman 1965; MSR = Mississippi River, Mississippi, Steeby et al. 1991; LE = Lake Erie, Michigan, De Roth 1965; alien populations (hollow circle): AR = Arno River, Italy, present study; LK = Lake Kasumigaura, Japan, Matzusaki et al. 2011.

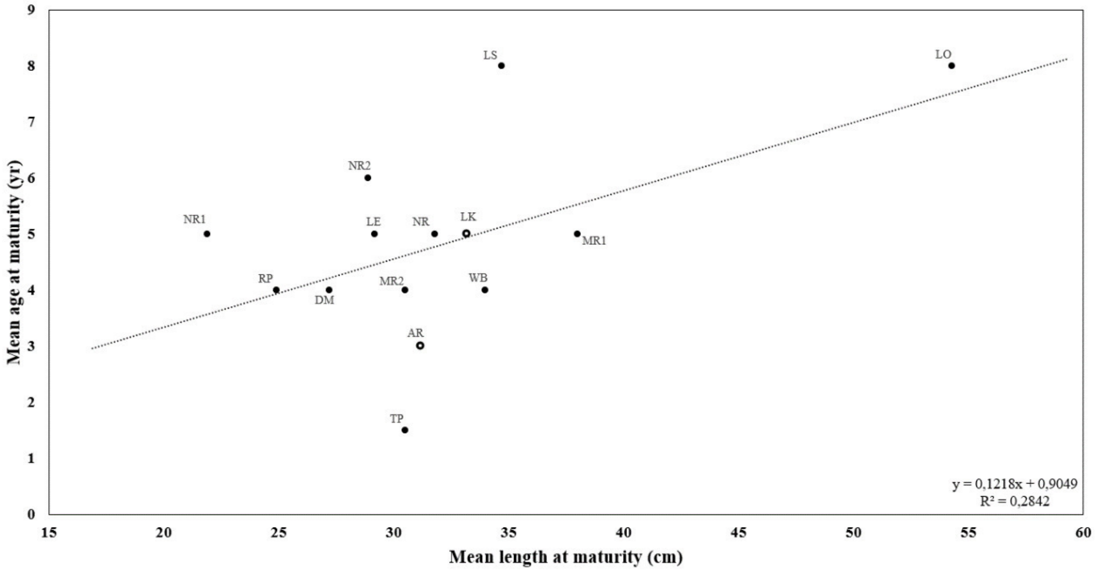

Figure 5: Comparison of different ages at maturity (A_M) according to mean total length (TL) at maturity. Native populations (black circle): LO = Lake Oahe, South Dakota, Starostka & Nelson 1974; MR1 = Mississippi River, Iowa, Appleget & Smith 1951; LS = Lake Sharpe, South Dakota, Elrod 1974; NR = Little Nemaha river, Missouri, Mahoney 1982; NR1 = Niobara River; Missouri, Mahoney 1982; NR2 = Nemaha River; Missouri; Hesse et al. 1982;RP = River Platte, Nebraska, Holland & Peters 1992; TP = Ponds, Texas, McClellan 1954; DM = Des Moines River, Iowa, Ackerman 1965; MR2 = Mississippi River, Mississippi, Steeby et al. 1991; LE = Lake Erie, Michigan, De Roth 1965; WB = Willard Bay Reservoir, Utah, Shipman 1977; alien populations (hollow circles): AR = Arno River, Italy, present study; LK = Lake Kasumigaura, Japan, Matzusaki et al. 2011.

Diet analysis

The limit between immature and mature fish was set to 34.6 cm according to the high correlation between juvenile growth (TL at age 3) and A_M. The analysis of stomach contents showed, that insects were the most frequently consumed prey, directly followed by detritus, algae and plants. Crustacean, fish and molluscs played a secondary role in the overall diet of fish. While fish, crustaceans and molluscs were mostly present in spring and summer, they were lower in autumn and generally more dominant in mature fish. In general, crustaceans were over the different seasons not as frequent in the diet, while insects contributed to about 50% of the diet, molluscs to 5-8%. From all consumed animals, Coleoptera and small cyprinids (*Pseudorasbora parva* and *Alburnus alburnus*) were dominating, followed by *Palaemon* sp. and *Procambarus clarkii*. While the frequency of consumed prey items such as Coleoptera increased over the seasons, aside from detritus which increased, non-animal items decreased but overall made up about 85%. Young *I. punctatus* were found in a few mature catfish stomachs, indicating that cannibalism is present (Table 4).

Table 4: Diet analysis for sampled specimens in 2017 (excluding those fish with empty stomachs; n=51) estimated for sampled seasons and life-stage (i.e. immature specimens < 34.6 cm; mature specimens > 34.6 cm TL) and overall. Numbers indicate frequencies of occurrence [F%].

Prey	**SPRING n=194**	**SUMMER n=132**	**AUTUM n=50**	**IMMATURE n=229**	**MATURE n=147**	**OVERALL n=376**
Fish	18.04	18.18	8	3.49	37.41	16.76
unidentified larvae	4.12	5.30	2	0.87	9.52	4.26
Ictalurus punctatus	1.03	0.00	0	0.00	1.36	0.53
Pseudorasbora parva (<4cm)	10.82	9.09	6	1.75	21.77	9.57
Pseudorasbora parva (>4cm)	3.61	3.79	4	0.87	8.16	3.72
Alburnus alburnus	4.64	1.52	0	0.87	6.12	2.93
Barbus barbus	2.06	3.79	2	0.00	6.80	2.66
small cyprinids	1.03	0.00	0	0.00	1.36	0.53
Lepomis gibbosus	0.00	0.76	0	0.00	0.68	0.27
Gobio gobio	0.00	0.00	0	0.00	0.00	0.00
Padogobius sp.	0.52	0.00	0	0.00	0.68	0.27
Cyprinus carpio	2.06	3.79	2	0.87	5.44	2.66
Crustaceans	22.16	25.00	16	12.66	37.41	22.34
unid. fragments	4.64	3.79	6	2.62	7.48	4.52
Procambarus clarkii	7.22	7.58	2	1.75	14.29	6.65
Palaemon sp.	6.70	10.61	6	2.62	16.33	7.98
Dikerogammarus villosus	6.70	4.55	2	6.11	4.08	5.32
Insects	48.45	57.58	52	58.95	41.50	52.13
Arthropods	0.52	3.03	2	1.75	1.36	1.60
unid. fragments	20.10	40.15	38	36.24	19.05	29.52
Diptera (larvae)	4.64	2.27	6	3.49	4.76	3.99
Diptera (pupae)	3.09	0.00	0	1.31	2.04	1.60
Diptera (imago)	3.61	0.76	4	3.49	1.36	2.66
Orthoptera (Caelifera)	0.00	0.00	0	0.00	0.00	0.00
Orthoptera (Ensifera)	1.03	0.00	0	0.00	1.36	0.53
Plecoptera (larvae)	0.00	0.00	2	0.44	0.00	0.27
Dryopidae (larvae)	1.55	0.76	0	1.31	0.68	1.06
Dryopidae (matures)	0.52	0.00	0	0.00	0.68	0.27
Coleoptera	10.82	19.70	18	16.16	12.93	14.89
Pentatomidae	3.09	0.00	0	1.75	1.36	1.60
Apidae	3.09	0.76	0	1.75	2.04	1.86
Odonata	1.03	0.00	0	0.87	0.00	0.53
Curculionidae	0.52	0.00	0	0.00	0.68	0.27
Dermaptera	2.58	0.00	0	1.31	1.36	1.33
Heteroptera	1.55	5.30	0	3.49	1.36	2.66
Hemiptera	2.06	3.79	0	2.62	2.04	2.39
Hymenoptera	4.64	3.03	0	3.06	4.08	3.46
Formicidae	0.00	0.00	2	0.44	0.00	0.27
Trichoptera (larvae)	4.64	0.76	0	2.62	2.72	2.66
Trichoptera (imago)	0.00	0.76	0	0.00	0.68	0.27
Zygoptera	0.52	0.00	0	0.00	0.68	0.27
Coccinellidae	0.52	0.00	0	0.00	0.68	0.27
Molluscs	8.76	5.30	6	3.93	12.24	7.18
unid. fragments	6.19	5.30	6	3.49	9.52	5.85
Gastropoda	0.00	0.76	0	0.00	0.68	0.27

Radix auriculata	1.03	0.00	0	0.00	1.36	0.53
Sinanodonta woodiana	1.55	0.00	0	0.44	1.36	0.80
Non-animal items	87.11	93.94	82	91.70	84.35	88.83
Detritus	41.24	53.03	56	51.09	41.50	47.34
Algae	54.12	34.09	28	46.72	38.78	43.62
Terr. plants	35.57	21.97	10	26.64	28.57	27.39
Detergent	6.70	11.36	6	9.61	6.12	8.24
Stones	3.09	2.27	0	1.31	4.08	2.39

Combined with data for 2016 (see Haubrock et al. 2018b), a total of 251 specimens for spring, 293 for summer and 158 for autumn, 203 mature and 420 immature specimens were obtained. Differences in different levels for each of the three factors has been suggested by the explorative analysis of data in a multivariate environment. The PERMANOVA main test (Table 5) confirmed supposed differences for the interaction between factor ‘maturity’ and ‘season’.

Table 5: PERMANOVA test results using dietary data of 2016 and 2017 excluding fish with empty stomachs.

Source	df	SS	MS	Pseudo-F	P (perm)	Uniqueperms	P (MC)
SEASON	2	37262	18631	6.5487	0.001	999	0.001
MATURITY	1	32763	32763	11.516	0.001	999	0.001
SEASON x MATURITY	2	17319	8659.6	3.0438	0.004	999	0.002
RESIDUALS	588	1.6728E6	2845				
Total	593	1.7758E6					

In particular, post-hoc tests revealed that the diet of immature and mature fish does not differ in autumn, while a significant difference for ‘maturity’ was pointed out for summer and autumn (Table 6). Moreover, focusing on maturity revealed, that mature fish eat differently according to seasonality (i.e. item composition), immature fish were found to show no different feeding spectrum in spring and summer, while both seasons differed to autumn. The applied PERMANOVA on combined dataset from 2016 and 2017, confirmed the analysis of diet frequencies based on 2017, but investigating the wider picture, revealed that mature and immature fish diets do not differ in autumn, likely linked to the lower availability of prey.

The applied indicator species analysis determined for factor ‘maturity’, indicated that matures were defined by 7 components from which 5 were fish. In contrast, immatures had less characterizing diet items. Seasons were calculated to be characterized by 6 items, algae in plants in spring, *A. alburnus* in summer and detritus, detergent and terrestrial insects in autumn. On the clustered level of ‘maturityXseason’, matures were better defined that immatures, showing that while molluscs where characteristic in spring, matures fed on fish (*A. alburnus, P. parva*, small fish) in summer and consumed *P. clarkii* in autumn (Table 7; Supplement 2).

Table 6: PERMANOVA based post-hoc test results of 2016 and 2017 excluding fish with empty stomachs.

Factor Season	Groups	t	P (perm)	Unique perms	P (MC)
Spring	Immature / Mature	1.9373	0.004	999	0.008
Summer	Immature / Mature	4.0984	0.001	998	0.001
Autumn	Immature / Mature	1.4065	0.126	999	0.119
Factor Maturity	**Groups**	**t**	**P (perm)**	**Unique perms**	**P (MC)**
Immature	Spring/Summer	1.178	0.223	999	0.261
	Spring / Autumn	2.3224	0.001	999	0.002
	Summer / Autumn	2.091	0.003	998	0.005
Mature	Spring / Summer	2.6231	0.001	999	0.001
	Spring / Autumn	2.1803	0.001	999	0.001
	Summer / Autumn	1.6805	0.02	999	0.029

Table 7: Results of the indicator species analysis for factor ‘maturity’, ‘season’ and ‘MaturityXSeason’.

Level	Item	Cluster	Indicator value	Probability	Sum of prob	Sum of Indicator value	Sum of sig. Indicator values	Number of sig. Indicators
Maturity	*P. parva*	Matures	0.1842	0.001	1.032	2.19	1.83	12
	P. clarkii	Matures	0.1778	0.001				
	A. Alburnus	Matures	0.1395	0.001				
	Small fish	Matures	0.1118	0.001				
	Palemon sp.	Matures	0.0789	0.016				
	Molluscs	Matures	0.0685	0.002				
	C. carpio	Matures	0.0635	0.002				
	Detritus	Immatures	0.3188	0.005				
	Algae	Immatures	0.2863	0.009				
	Terr. Insects	Immatures	0.1644	0.039				
	Detergent	Immatures	0.1194	0.013				
	Aquatic insects	Immatures	0.1172	0.022				
Season	Algae	Spring	0.2239	0.002	2.42	1.47	0.96	6
	Plants	Spring	0.1536	0.017				
	A. alburnus	Summer	0.0850	0.025				
	Detritus	Autumn	0.2309	0.008				
	Detergent	Autumn	0.1389	0.001				
	Terr. insects	Autumn	0.1285	0.027				
Maturity X Season	Molluscs	MatureXSpring	0.0603	0.012	2.024	1.21	0.7	7
	A. alburnus	MatureXSummer	0.1283	0.001				
	P. parva	MatureXSummer	0.1118	0.001				
	Small fish	MatureXSummer	0.0650	0.023				
	P. clarkii	MatureXAutumn	0.0950	0.007				
	Algae	ImmatureXSummer	0.1320	0.047				
	Detergent	ImmatureXAutumn	0.1095	0.002				

Discussion

I. punctatus has established dense and reproducing populations outside its native area, specifically in the Arno River in Central Italy. The results furthermore provide evidence that the presence of this alien species puts pressure on the recipient species community, potentially rendering native species at risk as previously indicated by Nocita (2002), who observed more indigenous fish species previous to the introduction of catfish.

In the overall sampling, females overweighed males with a ratio of almost 2:1, which is unusual for this species (see Table 1). However, immatures were more abundantly present in the sampling than matures, being in accordance with observations from native areas and can be seen as characteristic for this species (Appelget & Smith 1951). Especially the dominance of pre-mature age groups, furthermost fish of age 2, are typical for this species (Appleget & Smith 1951; Gerhardt & Hubert 1991; Holland & Peters 1992). Moreover, the distribution of age groups showed, that while age 2 fish are the most abundant age class and the median age of the sampled population, the abundance of younger and older fishes decreased rapidly. While the size of used hooks potentially affects the abundance of caught young fish, this is not necessarily true for the gape size limited but highly opportunistic *I. punctatus* (Busbee 1968; Froese 2006; Johnson et al. 2008). Nonetheless, using angling to catch specimens potentially affects the observed population structure comparable when using other single techniques (Walker et al. 1990), although observed population structure resembled native populations in especially anthropogenically altered habitats (Appleget & Smith 1951; Hansen 1971; Gerhardt & Hubert 1991; Holland & Peters 1992). Hence, it is likelier, that the gape size influences the potentially consumed prey, contributing to the difference between adult and juveniles, rather than the effective sample. Nonetheless, these results indicate a good reproductive state of the studied population, that has a high recruitment, as shown previously in the same ecosystem (Haubrock et al 2018b).

Length-Weight relationship and growth

Comparing weight-length relationships among populations showed, that the Arno population changes its behaviour after a total length of ~ 30 cm (Figure 3, 4): while the specimens below 30 cm TL were generally heavier than native populations at equal lengths, longer specimens were generally in the lower weight range. This difference can be linked to various potential reasons such as more available food sources for immature fish, as it has been previously been stated that juvenile Ictaluridae tend to remain close to the reed zone, generally a habitat offering abundant suitable prey items, whereas older and larger fish tend to use the entire water column and deeper areas (Matzusaki et al. 2011; Endo et al. 2015). Also, different feeding behaviours among immature and mature fish could be related to lessen the pressure (competition; i.e. predatory), as the observed change occurs peculiarly close to the age at maturity and length of age at maturity estimated by the analysis of pectoral spines. Therefore, it is likely that the life stages behave different due to the ability to consume larger prey items with increasing gape size. Especially the alien population of Lake Kasumigaura showed a huge difference to native populations, as specimens in Japan tend to express a lower weight to length ratio while not reaching lengths over 45 cm in general (pers. comm. Shin-Ichiro Matsuzaki). While such differences in diets might be likely, the origin of this difference is supposedly based in the different ambient temperature and climate, as Lake Kasumigaura is highly eutrophic, potentially providing a suitable habitat to grow rapidly (pers. obs. Shin-Ichiro Matsuzaki; Hubert 1999; Rypel 2011). Another particular aspect when comparing weight-length relationships are the two reservoir populations from Missouri, which both behave similar, but also show a relative high longevity compared to other populations (Bouska et al. 2011). Thus, as the invasion of the Arno by *I. punctatus* is still progressing and potentially not on a plateau phase, it is likely that this might be a similar case.

Although the presence of *I. punctatus* in the Arno was signalled about 20 years ago, different sections of the river have been invaded subsequently at later points. As such, *I. punctatus* was first observed in the inner-city stretch of the river Arno in 2004 (pers. obs. Annamaria Nocita). With the age distribution in mind, older specimens >5 years are generally not as abundant as younger fish especially in channelized rivers (Hansen 1971), for what reason it is not a surprise that only 3 fish from which spines were analysed were of age 6 or 7. Even if this could be a consequence of the difficulty of catching larger specimens, it could also indicate that the life span in the Arno River is shorter, i.e. the mortality is higher than in native areas; same consideration could be valid also for the introduced population in Japan (Figure 3, 4, 7). Another possible explanation for this observation could be the competition with the also present European catfish *Silurus glanis* and/or intraspecific competition, as this species can reach very high densities (Hansen 1971; Shephard & Jackson 2006, 2009; Haubrock et al. 2018c). However, after plotting the length against the estimated age and comparing plots to other populations indicated, that the population in the sampled stretch of the Arno River is likely not as old. Indeed, as growth-rates in catfish are highest in younger ages and generally higher in early years after the introduction as well as considering that older catfish mostly increase in weight, underlines the possibility, that older fish were sampled, but grouped as age 6 or 7 fish due to the lack of analysed spines of older individuals (Buckmeier 2002). Nonetheless, plotting the mean total length against the respective ages for native and alien populations listed in table 1 showed a considerably higher length-growth compared to native populations which could be an adapted trait characteristic for the alien population and relate to nutrient rich sediments as *I. punctatus* is detrivorous (Shephard and Jackson 2006). Indeed, Hubert (1999) identified significant differences for growth rates and thus, linked lengths and ages at maturity, between *I. punctatus* in indifferent habitat types (rivers, ponds, or lakes), while the collected data suggested a higher growth rate in in southern regions. Moreover, he identified certain habitat features (inter- and intraspecific competition, water velocity, high biological productivity, length of growing season, instream cover, abundance of pools and gravel, shallow areas, etc.) to affect growth rates. Additionally, Rypel (2011) identified that not specific climatic metrics positively affected *I. punctatus* growth, but rather lentic than lotic conditions.

Reproductive pattern

The Fulton Index showed higher values and more variability in younger age classes. Although it is generally considered as an indicator of a fish's or population's health, it can be used as an indicator for migrations (Gillanders et al. 2015; Mozsár et a. 2015). This agrees with the known behaviour of mature *I. punctatus* migrate upstream during spring for reproduction and to deeper pools in autumn. This idea of a reduction in K determined by the reproductive biology, is coherent with the observed GSI trend, that was opposite to that of K, as gonads development implies an energetic cost, while in autumn both trends were similar, likely due to less food availability. Furthermore, the GSI of the population showed, that maturation and thus reproductive activity does indeed start as early as in age 2 fish (although few age-1 fish expressed fully developed or potentially ripening gonads). Lastly, the annual pattern of GSI (Figure 3) indicated that there are two peaks of reproduction, one in spring and one in summer, while in native areas reproduction occurs once annually closely after the beginning of the activity phase (Holland & Peters 1992).

Plotting both, monthly means of estimated Fulton Index and GSI over the mean temperature, indicated adaptative behaviour in the reproductive biology of *I. punctatus*. While the Fulton Index varied strongly with the highest values in March and April, July and August, and a last peak in September, the GSI behaved opposed to it, less variable, but with low values in March and April, June and another low in October. Interestingly, the highest variation of both indices occurred previously to July, the month with the highest measured mean air temperature while afterwards with decreasing temperatures, curves behaved almost in synchrony and both, GSI and Fulton Index once more increase with an increase of temperature in September. Moreover, the high correlation between GSI and Fulton can be interpreted as the nature of the linkage between them, as the growth of gonads has a generally high energy demand. These results indicate multiple occurrences of reproductive activity, partly corroborated by the spine annuli indicating spawning near the end of the year, it also highlights how behaviour of this species is influenced by ambient temperatures and subsequently differs from expressed behaviour in the native area of this species.

Maturity and prediction of invasiveness

The estimated mean age at maturity appeared somewhat nonsensical, being based on the assumption of fish being mature in age classes after the first one with >15%, as males mature usually before females and many individuals were already spent. Additionally, information on mean total length at maturity and age at maturity for native populations are mostly anecdotal, as means of their estimation were not available and their origin and aim most often aquaculture based. It can be assumed, that values from previously published literature are generally values less accurate, as they were not calculated using the DeMaster formula and thus, might not be the "true" mean values. Moreover, it is likely that at some point, males and females were spent and thus, no gonads could be found, instead of individuals studied not being mature. However, males tend to grow faster and mature earlier when diet is abundant (Albaugh 1969).

Thus, comparing the age at maturity in regard to mean TL at maturity among populations also revealed a high variability. The factors underlying this variability are likely related to the different growth rates (diet and especially ambient factors, Hansen 1971; Shephard and Jackson 2009; Hubenova et al. 2014), but overall, non-native populations were more similar in their mean length at maturity than native populations but differed in their age at maturity. Nonetheless, as Lake Kasumigaura is colder than the Arno River throughout the year and presents a different ecotype (lake vs. river), it takes *I. punctatus* longer to reach the respective length at maturity, possibly due to a lesser nutrient availability (Rypel 2011). The Arno population had an age at maturity lower than all the native populations except one, but available data from alien populations, with only the Japanese lake and Arno River, were not sufficient to apply the model by Copp & Fox (2007) to predict invasiveness, so more studies from different non-native areas are needed.

Diet analysis

In regard to the diet, it was observed that the diet spectrum became less diverse towards the end of the year. Additionally, mature fish were shown to be more piscivorous while immature fish remained rather opportunistic with a wider diet spectrum. Indeed, crustaceans and fish were more abundant in mature fish, while non-animal items and insects were more frequent in immature fish, indicating also that immature fish tend to predate mostly near the reed zone, while matures feed throughout the water column. This feeding zone partitioning could be used to avoid intra-specific competition, as well as cannibalism by older catfish. The applied PERMANOVA and Indicator species analysis, thus using a combining dataset from 2016 and 2017, confirmed the analysis of diet frequencies based on 2017, but investigating the wider picture, revealed that mature and immature fish diets do not significantly differ in autumn, likely linked to the lower availability of prey. Moreover, they are characterized by different diets, showing a higher piscivory in mature than in immature fish linked to the availability of prey (i.e. crayfish for mature fish in autumn).

Conclusion

Ictalurus punctatus has been considered as an alien species in Europe, its biological traits (reproduction, growth, diet, etc.) affect the recipient ecosystem and its inhabitants. Considering the rather short introduction history and angler associated pathway (Haubrock et al. 2018a) of this species paired with its opportunistic feeding, it can be assumed that *I. punctatus* poses a direct threat for the already threatened native species such as like *Padogobius* sp., while potentially predating or facilitating other alien species, e.g. reducing the competition or creating new ecological niches. For these reasons, a classification as invasive should be considered for this species.

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Supplement 1: Pair-wise hoc-test for the applied one-way ANCOVA; 1 = Italy; 2 = Francis-Case; 3 = Lewis-Clark; 4 = Mississippi; 5 = Japan; 6 = Oklahoma;

Dependent Variable: 'Length*Weight'

(I) Population	(J) Population	Mean Diff. (I-J)	SE	Sig.[b]	95% Confidence interval for Dif[b]	
					Lower	Upper
1	2	-,638*	,053	,000	-,741	-,535
	3	-,589*	,052	,000	-,690	-,487
	4	-,667*	,127	,000	-,916	-,419
	5	,400*	,046	,000	,309	,490
	6	-1,036*	,194	,000	-1,417	-,655
2	1	,638*	,053	,000	,535	,741
	3	,049	,056	,379	-,061	,159
	4	-,030	,129	,818	-,282	,223
	5	1,037*	,051	,000	,937	1,137
	6	-,398*	,196	,042	-,781	-,015
3	1	,589*	,052	,000	,487	,690
	2	-,049	,056	,379	-,159	,061
	4	-,079	,128	,538	-,330	,172
	5	,988*	,050	,000	,890	1,086
	6	-,447*	,195	,022	-,830	-,064
4	1	,667*	,127	,000	,419	,916
	2	,030	,129	,818	-,223	,282
	3	,079	,128	,538	-,172	,330
	5	1,067*	,126	,000	,820	1,314
	6	-,368	,227	,105	-,814	,077
5	1	-,400*	,046	,000	-,490	-,309
	2	-1,037*	,051	,000	-1,137	-,937
	3	-,988*	,050	,000	-1,086	-,890
	4	-1,067*	,126	,000	-1,314	-,820
	6	-1,435*	,194	,000	-1,816	-1,055
6	1	1,036*	,194	,000	,655	1,417
	2	,398*	,196	,042	,015	,781
	3	,447*	,195	,022	,064	,830
	4	,368	,227	,105	-,077	,814
	5	1,435*	,194	,000	1,055	1,816

Based on the estimated marginal funds

*. The mean difference is significant at a level of 0.5

b. Multiple comparison comparison: least significant difference (does not match any adjustments).

Supplement 2: Results from the indicator species analysis (R package "labdsv"; Roberts & Roberts 2016) to evaluate indicator values of diet items for the two fixed factors 'maturity' and 'season' and groups from their interaction 'maturityXseason'.

MaturityXSeason	**Mature X Spring**	**Mature X Summer**	**Mature X Autumn**	**Immature X Spring**	**Immature X Summer**	**Immature X Autumn**
Small fish		0.07				
P. parva	0.05	0.11				
A. alburnus		0.13				
P. clarkii		0.08	0.09			
OtherArthropods					0.05	0.07
Ter. Insects					0.05	0.09
Molluscs	0.06					
Detritus		0.06	0.12	0.09	0.10	0.12
Algae	0.11			0.13	0.13	
Plants	0.06		0.08	0.09		0.05
Detergent						0.11

Season	**Spring**	**Summer**	**Autumn**
P. parva	0.07	0.08	
A. alburnus		0.09	
P. clarkii		0.06	
OtherArthropods	0.06	0.09	0.12
Aqu. insects	0.05		0.07
Ter. insects	0.07	0.06	0.13
Detritus	0.13	0.16	0.23
Algae	0.22	0.16	0.06
Plants	0.15	0.05	0.12
Detergent			0.14

Maturity	**Mature**	**Immature**
Small fish	0.11	
P. parva	0.18	
A. alburnus	0.14	
C. carpio	0.06	
P. clarkii	0.18	
OtherArthorpods	0.09	0.15
Paleomonethessp.	0.08	
Aqu. insects		0.12
Ter. insects	0.07	0.16
Molluscs	0.07	
Detritus	0.17	0.32
Algae	0.15	0.29
Plants	0.14	0.16
Detergent		0.12

CHAPTER 3

INVASIVE SPECIES INTERACTIONS

Chapter 3.1

INVASIVESNET
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Management in Practice

Control and eradication efforts of aquatic alien fish species in Lake Caicedo Yuso-Arreo

Short title: Alien fish removal from Lake Arreo

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Abstract

Lake Caicedo Yuso-Arreo (Spain) supports dense populations of alien fish species, namely common carp (*Cyprinus carpio*), largemouth bass (*Micropterus salmoides*), pumpkinseed (*Lepomis gibbosus*), and the red swamp crayfish (*Procambarus clarkii*). These introductions have caused a reduced transparency, decreasing submerged vegetation and population decline of native tench (*Tinca tinca*). Alien species were caught and removed for 76 days in 2014 and 2015 with the objectives of eradicating carp and reducing largemouth bass and pumpkinseed populations under 20% of 2014 observed density and biomass. Only two individuals of tench were captured, underlining its scarcity. A total of 27,077 individuals (1089 kg) of alien species were removed, but eradication was not achieved for any species. The largest sizes of carp and largemouth bass almost disappeared, while in 2015 an increase in the abundance of large recruits was observed. A hydroacoustic fish stock assessment was carried out at the end of the action in 2015 to obtain data on fish density and biomass in open water as a mean to compare results to measurements from 2010. Although the density of fish increased from 2010 to 2015, biomass fell drastically. In 2017, the alien species community was controlled again, revealing a substantial recovery from previous control efforts, a change in the size distribution towards smaller specimens and interactions among North American species. Additionally, no tench could be identified and the species is considered locally extinct. Thus, the results from this study suggest the need for ongoing control efforts with increased electrofishing and implementation of alternative control methods such as the introduction of biological control agents to achieve the ecological status improvement and environmental restoration goals.

Key words: Fish removal, boat electrofishing, gillnets, hydroacoustics, fish management, ecological status, wetland restoration

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Introduction

Freshwater ecosystems, especially in industrialised countries, have been the target of frequent introductions, especially by fish species (Oberdorff et al. 2002; Copp et al. 2005; García-Berthou 2007). Lake Caicedo Yuso-Arreo, henceforth referred to as Lake Arreo, is a peculiar ecosystem in the Iberian context, as it is one of the eight Iberian lakes on a saline chimney (González-Mozo et al. 2000; Camacho et al. 2009). In addition, it is the only lake in Basque country (González-Mozo et al. 2000). In the 19th century, its fish community was composed of trout (*Salmo trutta* Linnaeus, 1758), European eel (*Anguilla anguilla* Linnaeus, 1758), and tench (*Tinca tinca* Linnaeus, 1758) (Madoz 1845). At the beginning of the 21st century however, trout was already absent and the alien largemouth bass (*Micropterus salmoides* Lacépède, 1802) and pumpkinseed (*Lepomis gibbosus* Linnaeus, 1758) were detected in surveys under the Water Framework Directive ecological status monitoring network (Vasco 2004). The fish census carried out in 2010 (Monteoliva et al. 2011b) also detected common carp (*Cyprinus carpio* Linnaeus, 1758) and quantified dense populations of invasive *M. salmoides* and *L. gibbosus*. The densities of these species were significantly higher than that of tench, the only species that could be considered native in the absence of precise data on the historical composition of the community. This species tends to be considered native in most of Europe (Freyhof and Kottelat 2008), although its native distribution in Spain is uncertain (Doadrio et al. 2011).

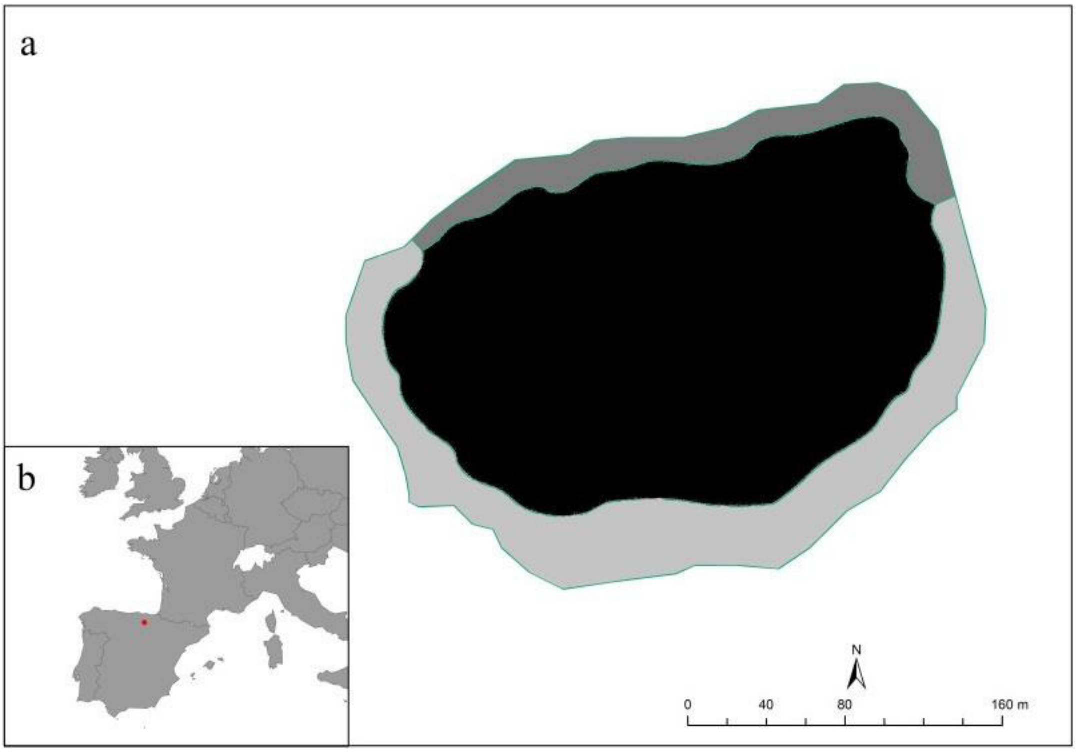


Figure 1: Distribution of fish capture zones (black: pelagic zone; dark grey: slope zone; light grey: helophytes zone) of lake Caicedo Yuso-Arreo (a) and location in Europe (b).

C. carpio has been introduced to more than 100 countries worldwide as a result of human action (Balon 1995; Badiou et al. 2011). Several negative impacts on recipient ecosystems have been reported for this species, such as an increase in sedimentation, erosion and increasing dissolved nutrients in the water column and thus, an increased phytoplankton growth; a disturbance of the structure of the planktonic community; decreased abundance of submerged macrophytes, macrozooplankton and benthic invertebrates; decreased diversity and abundance of native fish; competition with aquatic birds for food resources, and others (Lougheed et al. 1998; Chumchal et al. 2005; Scheffer 1998). Hysteretic state changes resulting from modulation of the resources available for other species (Badiou et al. 2011) were also reported.

The centrarchid species *M. salmoides* and *L. gibbosus* are native to North America and have been frequently introduced to reservoirs specifically for sport fishing (Doadrio 2001; Naspleda et al. 2012). In the Iberian Peninsula, they occupy lentic habitats and slow stretches of large rivers, where they reach high densities. Especially *L. gibbosus* is known for its omnivorous diet and negative effect on water clarity as well as being a common prey for *M. salmoides* (Angeler et al. 2002), which is a voracious predator of invertebrates, amphibians, fishes, and even of micromammals and reptilians when reaching larger sizes (Hodgson and Hansen 2005; Shelton et al. 2008; Wasserman et al. 2011; Magoro 2014).

The presence of invasive fish species puts pressure on Lake Arreo, as they are potentially exerting negative impacts on the recipient species community and in the case of *C. carpio* facilitate the eutrophication process (Lougheed et al. 1998; Miller and Crowl 2006). Therefore, a proposal was made to implement corrective measures based on the desired removal of alien species (not only fishes but also the red swamp crayfish (*Procambarus clarkii* Girard, 1852) in the framework of the LIFE TREMEDAL project (LIFE11 NAT/ES/ 000707). Considering the characteristics of the lake, an integrated approach of electrofishing and gillnet techniques was selected for removal of alien species. The specific objectives of removal were: (1) recovery of tench population; (2) common carp eradication; (3) removal of up to 80% of alien fish populations.

Hence, this work investigates if previously defined objectives for Lake Arreo are achievable by applied manual removal techniques (electrofishing and gillnetting) and furthermore, aside from highlighting conducted monitoring and control tasks, presents information about changes within population structures of present fish species two years later. Lastly, this article provides complementary data about feeding activity as well as reproductive status of removed invasive fish, investigating species interactions and exploring potential tasks for the future.

Materials and Methods

Study site

Lake Arreo is located in Basque Country (north of Spain, see Figure 1), 655 m above sea level. It is part of the Ebro river basin and covers about 136 ha. Its morphology is simple, with only one basin located on the north side with a maximum depth of 24 m in an area with a steep slope associated with an ENE-WSW positioned fault (Martínez-Torres et al. 1992). The south and west areas are shallow and feature a low slope, allowing the development of a dense helophytic margin dominated by *Phragmites australis* and *Cladium mariscus*. As a result, three different habitats for fish can be distinguished (Figure 1): the pelagic habitat (limited to the superficial zone by hypolimnetic anoxia); the shallow slope habitat (abundant submerged dead tree trunks; without helophytes); and the shallow flat habitat (helophytes constitute a dense refuge for fish). Water transparency was measured using a Secchi disk at the beginning of each session and a multiparametric probe (Hydrolab DS5) was used to measure physico-chemical parameters (water conductivity; water temperature). Meteorological data from the nearest station were gathered for each day on the site (www.ogimet.es).

Goals and objectives

The main aim of conducted control efforts in Lake Arreo was the recovery of the previously present tench population. To achieve this objective and a stable recovery of the previously invaded ecosystem, a complete eradication of the for resources competing common carp (Adámek et al. 2003) was intended. Because of the high densities of other also present alien fish species (pumpkinseed, largemouth bass) that are likely exerting a negative impact on the ecosystem and thus, lowering the chances of recovering tench (Lorenzoni et al. 2002), their populations should be decimated by 80% to eventually lower their densities.

Fishing techniques

In 2014, 15 days between June and September were devoted to fishing as part of five surveys (called ARR14_01 to ARR14_05), and 61 days between May and August as part of 17 surveys (called ARR15_01 to ARR15_17) in 2015. Multi-mesh gillnets (trammel-, benthic- and pelagic nets) were deployed overnight in the pelagic habitat for about 12h. Gillnets conformed to "EN14757:2005: Sampling of fish with multi-mesh gillnets", with 5 to 50 mm mesh sizes and four additional mesh sizes (70–135 mm) for large fishes (Šmejkal et al. 2015). Electrofishing in shallow habitats was conducted from a boat, using a 13-kW electric power equipment (Hans Grassl EL65II GI). The electric output was a continuous pulse current of 300 V with 80–100 Hz, generating an electric current of approximately 9 A (ranging from 7 to 11 depending on conductivity, depth and helophyte density). Electrofishing paths were positioned with a GPS in order to standardize the effort with fishing duration and were conducted separately for both kind of shallow habitats (helophytes and slope), navigating around the lake parallel to the shore. For every fish, fork length (in cm) and weight (in g) were recorded. Alien fishes were euthanized using an anaesthetic (Eugenol) overdose and taken out of the lake. Tench individuals were excluded from anaesthesia and kept in clean water before being released back into the lake. Capture related variables (gillnets: hours in the water, size and mesh size of gillnets; electrofishing: hours fished, distance covered with the boat) were standardized and the Catch per unit of effort (CPUE) using the numbers of caught fish, and the Biomass per unit of effort (BPUE) using the total mass of collected fish were calculated for every species. A regression model was applied to identify relationships between species' specific CPUE values and abiotic variables. Population sizes were estimated, assuming that the CPUE serves as a surrogate for density and correlates with the abundance of a species, by plotting the standardized CPUE (x-axis) against the accumulative total catch (y-axis) for each sampling and extrapolating a regression line that, once reaching the x-axis indicates the total population size (Leslie and Davis 1939).

Open water census methodology

A hydro-acoustic survey was carried out using a scientific split-beam echo sounder (Biosononics-DTX) synchronized to a *Differential Global Positioning System* (DGPS), generating a digital bathymetric model and detecting fish and their acoustic target strength by means of their size (Monteoliva and Schneider 2005). Both were combined with direct sampling (capture) data to generate density and biomass estimates in open non-shallow waters. For echograms registering in vertical position during the mobile echo sounding survey, the basic equation of Love (1977), adjusted for the transducer frequency, was applied to the single echo detections (SED). In shallow waters (less than 5 m depth) the transducer was oriented in horizontal position and echo integration was used instead of SED analysis. In this case, the median size of the fish catches was used to calculate the backscattering cross section and to estimate the fish density and biomass in each analysis segment, using the regression proposed by Kubecka et al. (2009). Global estimates were compared to 2010 data (Monteoliva et al. 2011a), a similar survey prior to fish removal campaigns.

Evaluation of efforts

To enable and observe a potential recovery of the tench population as well as to evaluate the effectiveness of the control and eradication efforts in 2014 and 2015, the status (CPUE, BPUE) of present fish populations was assessed again in 2017 (ARR17_01, 02 and 03) using the same electrofishing approach used in previous years. Specimens were measured, and the sex was determined. The reproductive status of randomly chosen individuals of alien fish species were examined, and gonadal weight was measured to 0.01 g (Jadever SNUG 300). The Gonadosomatic Index (GSI) was calculated as the percentage of gonads per total body weight (Devlaming et al. 1982). The stomach contents of mature but randomly chosen fish species were examined using a stereomicroscope and found items were identified to the lowest possible taxa. Additionally, fish diets were analysed as frequency of occurrence ($F\% = 100 \times A_i \times N_-$) where A_I was the number of fish containing prey item i and N the total number of fish analysed (excluding those specimens with empty stomachs). These observed data were used to evaluate potential niche overlaps by calculating Pianka's index (Pianka 1974) using the R-package "EcoSymR" (Gotelli et al. 2015).

Additionally, data of the from 2013 and onwards sampled *P. clarkii* was assessed (Asensio 2015) and, as an integral part of the invasive species community, sampled again in 2017 using 6 funnel traps along the hydrophyte zone overnight for 12 hours.

Table 1. Summary of used gillnets incl. mesh size and Catch per Unit Effort (CPUE). Only gillnets that caught fish are listed.

Year	Survey	Date	overall catch	net types	Mesh size	CPUE
2014	1	02.06.2014	125	3x Trammel	2 × 1.5; 3 × 1.5; 5 × 1.5	0.39–0.59
	2	16.06.2014	45	2x Trammel	5 × 1.5	0.26–0.14
	3	06.08.2014	22	Pelagic	16 × 6	0.16
2015	2	12.05.2015	3	Pelagic	16 × 6	0.17–0.20
	7	15.06.2015	2			
	9	29.06.2015	1			
	10	06.07.2015	63			
	11	14.07.2015	1			
	12	20.07.2015	27			
	13	27.07.2015	26			
	14	05.08.2015	8			
	15	10.08.2015	2			
	17	24.08.2015	12			
	Total		337			

Results

The lake remained stratified during the entire process of removal, with an average epilimnetic conductivity of 1,139 µS/cm in 2014 and 946 µS/cm in 2015. The available habitat for fish was limited to the epilimnion due to the lack of oxygen in deeper strata. Surveys were defined as the unit of effort for data analysis. The total effort for gillnets was 76.3 units (45 m^2, 12 h) and 25,625 m for electrofishing in 2014, whereas in 2015 the effort was 88.9 units for gillnets and 122,000 m for electrofishing.

The total catch of individual fish in 2014 and 2015 using electrofishing amounted to 6,063 specimens in the slope zone and 20,679 in the helophyte zone. The use of different gillnet types with different mesh sizes resulted in the catch of 337 fish from the deep zone (Table 1). Gillnets proved to be ineffective in removing high quantities of fish in the pelagic zone. Therefore, and because boat electrofishing was unable to sample in the epipelagic zone, only pelagic nets were used in 2015. The total biomass taken out of the lake was 1,089 kg. The percentage of total biomass showed the following distribution: *C. carpio* 45.3% (due to the 46 large sized mature individuals), *L. gibbosus* 37.3% (due to abundance), *M. salmoides* (17.4%) and *T. tinca* (0.05%). The total number of caught specimens showed a different distribution. *L. gibbosus* was the most abundant species (73.2%), followed by *M. salmoides* (23.6%), *C. carpio* (3.2%) and finally two individuals of *T. tinca* (0.01% of the total).

The standardized CPUE values varied between days and surveys. For *C. carpio*, CPUE values (Figure 2a) dropped quickly in all habitats after effort accumulation, remaining at very low levels. Catches of carp were scarce in the deep zone, and the average size was generally higher in the slope zone than in the helophytes zone. However, from June 2015 onwards, a marked increase in *L. gibbosus* catches (Figure 2b) was observed, especially in the helophytes zone. After that date, CPUE and BPUE dropped progressively with increasing removal. *M. salmoides* CPUE (Figure 2c) experienced a high increase from July 2015 on, but BPUE did not. The annual number of *Procambarus clarkii* caught increased from 2013 (2,250 individuals, 16 traps, 72 hours, CPUE: 1.953) to 47,450 individuals in 2014 (50–54 traps, 2,136 hours, CPUE: 0.444) and to 70,500 in 2015 (50–70 traps, 86,400 hours, CPUE: 0.014) (Asensio 2015) but CPUE decreased strongly. The applied regression model revealed that *C. carpio* catches related significantly to conductivity and air temperature (inversely). *L. gibbosus* catches related significantly to turbidity and *M. salmoides* catches to water temperature and conductivity (Table 2).

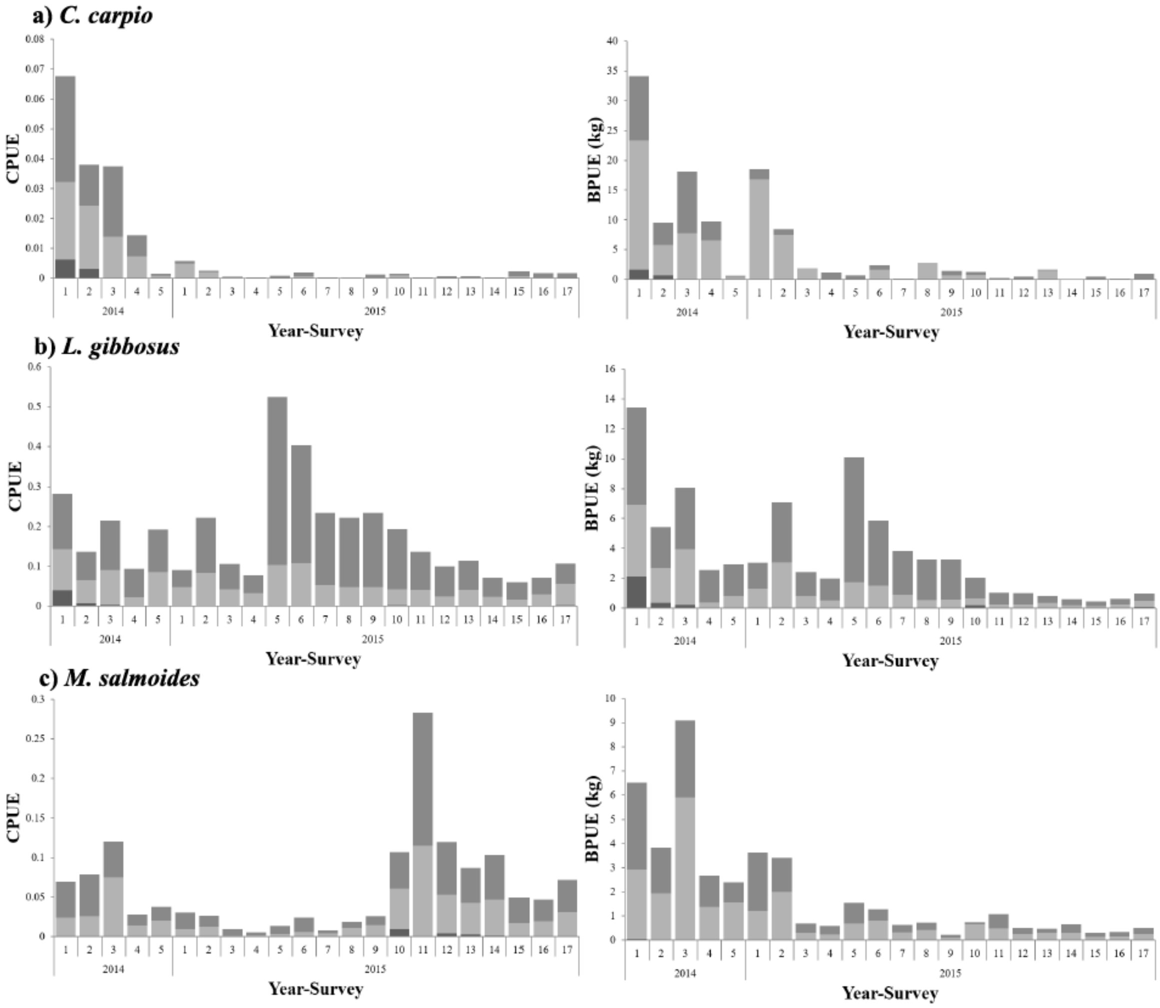


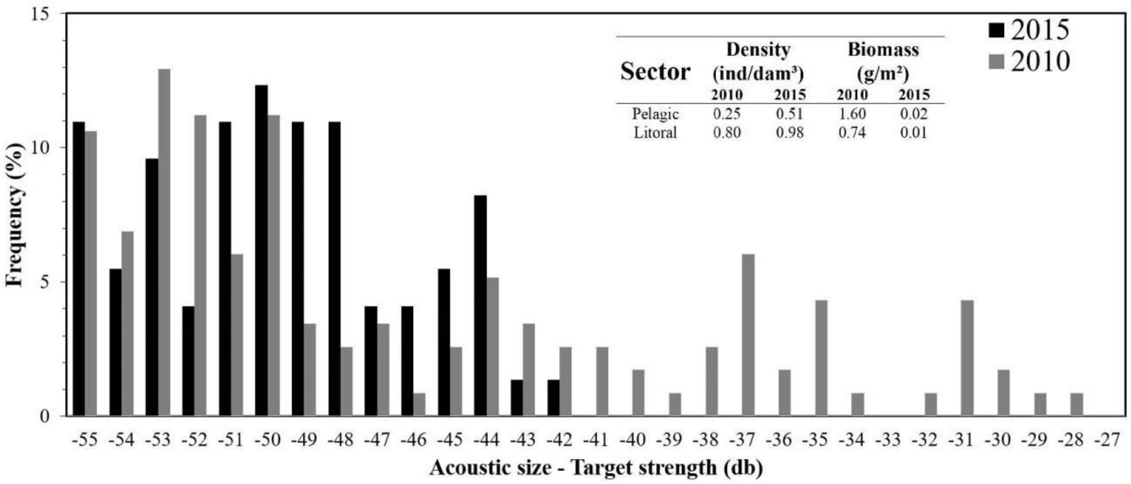
Figure 2: Development of Catch per Unit Effort (CPUE) and Biomass per Unit Effort (BPUE) in 2014 and 2015 surveys. Black: pelagic habitat; dark grey: slope habitat, light grey: helophytes habitat. Numbers on the x-axis indicate the survey for 2014 and 2015.

Open water census methology

Comparison of hydroacoustic estimates in 2010 and after control efforts in 2015 revealed major changes of partial fish community in Lake Arreo, likely induced by removal efforts. Density increased considerably in the pelagic sector and moderately in the littoral, whereas biomass fell drastically in both sectors. Frequency distribution of acoustic sizes (target strength) changed also (Figure 3) from 2010, prior to removal, to 2015, showing the disappearance the largest size fishes.

Table 2: Multiple stepwise regression analysis of Catch per Unit Effort (CPUE) and environmental variables. ns, $p>0.05$;*,$p<0.05$; **, $p < 0.01$.

Dependent variable	Adjusted R^2	F (ANOVA)	Selected independent variables	Coefficient	Standard deviation	Student t
CPUE *C. carpio*	0.54	45.00**	Intersection	0.000	0.078	–
			Conductivity	0.707	0.079	8.97**
			Mean air temperature	−0.188	0.079	−2.39*
CPUE *L. gibbosus*	0.14	13.42**	Intersection	0.000	0.107	–
			SD transparency	0.394	0.108	3.66**
CPUE *M. salmoides*	0.22	11.29**	Intersection	0.000	0.102	–
			Water temperature	0.402	0.103	3.91**
			Conductivity	0.261	0.103	2.53*
CPUE	0.03	3.12^{ns}	Intersection	0.000	0.114	–
Total CPUE	0.12	4.45**	Intersection	0.000	0.108	–
			Water temperature	0.223	0.110	2.02*
			Conductivity	0.281	0.117	2.40*
			SD transparency	0.346	0.118	2.92**



Sector	Density (ind/dam³)		Biomass (g/m²)	
	2010	2015	2010	2015
Pelagic	0.25	0.51	1.60	0.02
Litoral	0.80	0.98	0.74	0.01

Figure 3: Frequencies of acoustic sizes (target strength) in lake Caicedo Yuso-Arreo from 2010 and 2015. Table: Hydroacoustic density and biomass estimations for respective years.

Population shifts and evaluation of efforts

Initial population sizes were calculated under the assumption of CPUE being an indicator of population density, and the results for each year of removal are listed in Table 3. The measured turbidity showed an increase during the removal efforts in 2015 (3.0 to 1.9 m) and decrease in 2017 (1.6 to 2.3 m). Results of length and weight shifts of removed species for all years are presented in Figure 4, showing an increase in *M. salmoides* recruits from 2014 to 2015 and a balanced distribution among immature and mature individuals in 2017. From 2014 to 2015, the carp population remained similar in length and weight distribution with an overall decimation of the population, while the few individuals caught in 2017 were all mature. The removal of *L. gibbosus* in 2014 resulted in an increase of recruits in 2015. In 2017, the population stabilized in between the two distributions of recruits and mature specimens from 2015.

In 2017, the effort for electrofishing accumulated to 15,500 m. Overall, 11 individuals of *C. carpio* (20.8 kg), 3,250 specimens of *L. gibbosus* (97.7 kg) and 499 individuals of *M. salmoides* (83 kg) were sampled, making up a total of 3,760 fish (201.5 kg). 47 crayfish weighing 1,310 g in total were caught with a CPUE of 0.653. No individuals of *Tinca tinca* could be observed. Stomach contents of *C. carpio* was identified with detritus being the dominant component (F%: 45%). *L. gibbosus* diet consisted mostly of Coleoptera (F%: 45.7), Orthoptera (F%: 14.3), *P. clarkii* (F%: 14.3) and smaller frequencies of aquatic invertebrates and diverse insects. The diet of *M. salmoides* was less diverse, with a high abundance of *P. clarkii* (F%: 82.1), followed by Araneae (F%: 21.4), *L. gibbosus* (F%: 17.9) and Odonata (F%: 14.2) (see Table 4). The niche (diet) overlap for the two predatory Centrarchidae was estimated as 0.2352 (Pianka's index) for *L. gibbosus* and *M. salmoides* and therefore generally low (< 0.4). Spawning individuals were observed for every alien species from May onwards. Reproductive investment varied among species (Figure 5), with average GSI values of 4.3% for *C. carpio*, 8.7% for *L. gibbosus* and 3.5% for *M. salmoides*.

Table 3: Estimation of annual population sizes for targeted species based on Catch per Unit Effort (CPUE) and accumulated annual catchments.

Species	Year	Pop. size	R^2
L. gibbosus	2014	66671.1	$R^2 = 0.4162$
	2015	52062	$R^2 = 0.1151$
	2017	23449.2	$R^2 = 0.9614$
C. carpio	2014	797.9	$R^2 = 0.9034$
	2015	45.1	$R^2 = 0.5284$
	2017	10.9	$R^2 = 0.9865$
M. salmoides	2014	2303.9	$R^2 = 0.6315$
	2015	1384.9	$R^2 = 0.2317$
	2017	525.7	$R^2 = 0.8884$

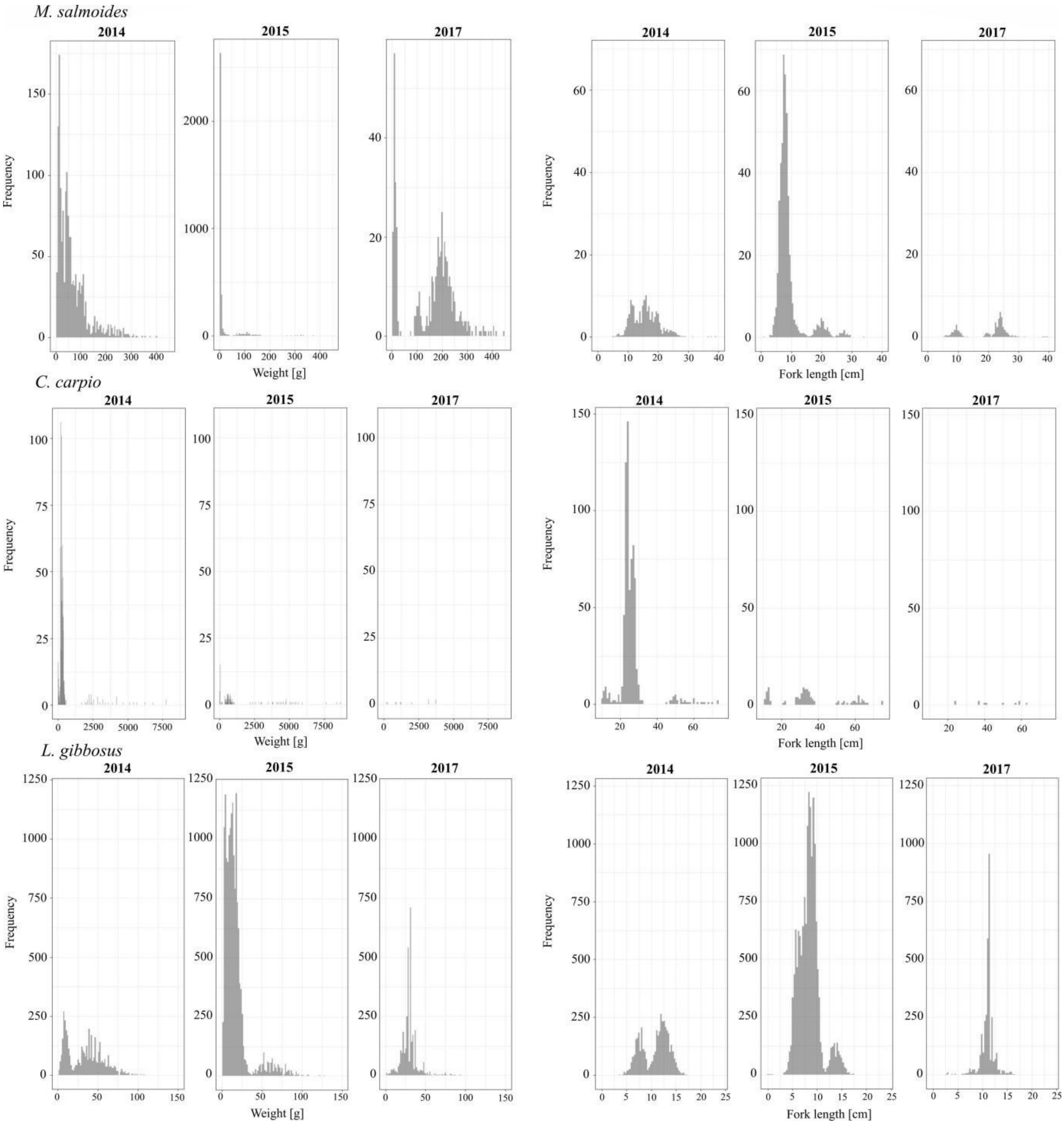


Figure 4: Changes within the population in regard to fork length and weight distribution of removed fish over the years 2014, 2015 and 2017.

Discussion

The eradication of alien fish populations has usually been accomplished with biocides (such as rotenone; see e.g. Britton et al. 2011), which are strongly restricted in Europe and which are normally used only in relatively small and enclosed water bodies (European Commission 2014). In the case of Lake Arreo, it could affect the rare tench if used. Hence, physical removal (Britton et al. 2011) by e.g. commercial harvesting, manual removal as well as gillnetting have been shown to positively minimize alien fish populations (Gliwicz and Rowan 1984; Bahls 1992; Knapp and Matthews 1998; Colvin et al. 2012), although need to be chosen carefully (Nuñez et al. 2012). In the late 20th century, new theories about management of natural resources focused on preserving indigenous biodiversity, reducing the stocking with potentially invasive species, and biologists began to focus on restoration and renaturalization (Parker et al. 2001). In more recent works, electro-fishing was used in combination with gill netting (Parks Canada 2016), resulting in a rapid recovery of extirpated zooplankton species (Schindler and Parker 2002; Sarnelle & Knapp 2004). These successful case studies lasted three or four years at least, but targeted lakes were higher and usually shallower with simpler fish assemblages (usually with only one non-native species) than that in Lake Arreo.

Effectiveness of applied techniques

In the case of Lake Arreo, gillnets were identified as less effective than electrofishing. Indeed, boat electrofishing proved to successfully remove fish from the littoral area. However, it was not suitable for gathering especially small-sized fish swimming in between dense helophytes.

Table 4: Identified dietary components for *Lepomis gibbosus* (N = 38), *Micropterus salmoides* (N = 38) and *Cyprinus carpio* (N = 11). Results are displayed as frequency of occurrences [F%] in percentages.

Component	*Lepomis gibbosus*	*Micropterus salmoides*	*Cyprinus carpio*
P. clarkii	14.29	82.14	0
L. gibbosus	0	17.86	0
Odonata	0	14.29	0
Mollusks	2.86	0	0
Hymenoptera	20	3.57	0
Heteroptera	11.43	0	0
Snails	0	0	0
Araneae	2.86	21.43	0
Diptera	5.71	0	0
Odonata	5.71	0	0
Orthoptera	14.29	0	0
Coleoptera	45.71	0	0
Formicidae	2.86	0	0
unid. Insects	51.43	0	0
detritus	42.86	3.57	100
fisheggs	8.57	0	0
plant material	5.71	0	0

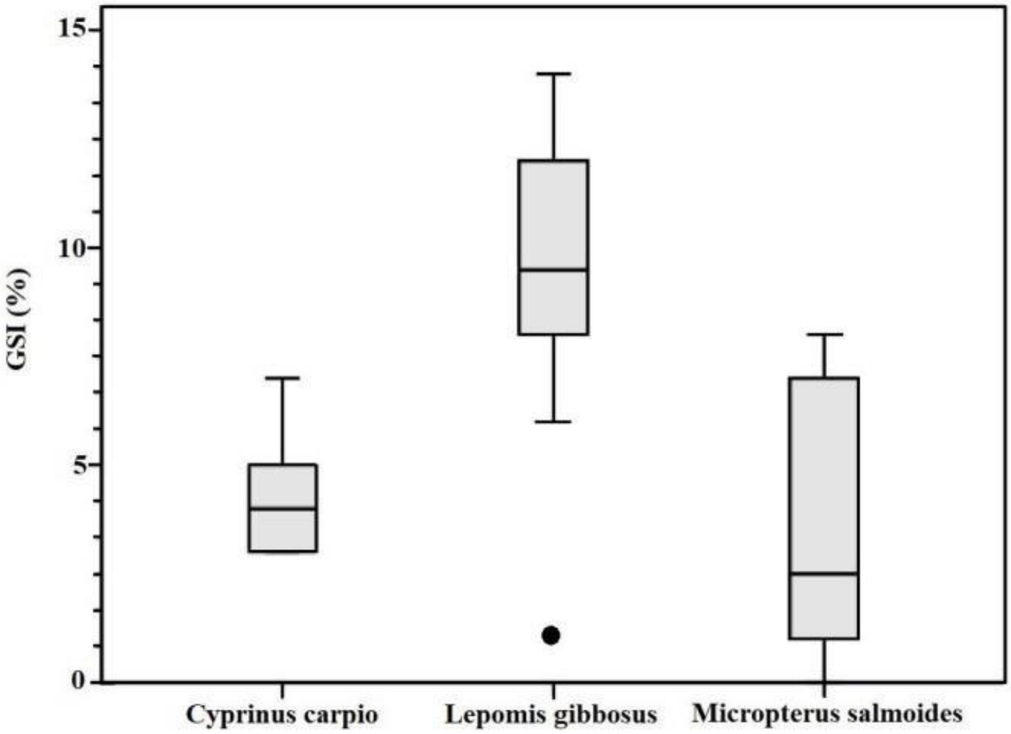


Figure 5. Gonadosomatic Index, i.e. percental weight of gonads for *Cyprinus carpio* (N = 9), *Lepomis gibbosus* (N = 26) and *Micropterus salmoides* (N = 22) caught in Lake Caicedo Yuso-Arreo.

The total effort applied on Lake Arreo was not enough to eradicate present carp, but the aimed reduction in abundances of populations to low levels was achieved. Recruitment and environmental variables (meteorological factors, water surface temperature, conductivity and transparency) were considered as the causes for variations in CPUE because while most of the effort was made in 2015, field work in 2014 had likely started after the onset of the reproductive season. Furthermore, the density-dependent survival in the immature phase of fish is a key factor in regulating fish populations (Rothschild 1986). Density-dependent growth due to competition for food in immature fishes has been described for many species (e. g. Beverton and Holt 1957; Rochet 1998; Post et al. 1999; Jenkins et al. 1999) and is suggested as a common and important mechanism in the regulation of fish populations (Lorenzen and Enberg 2002). Hence it is likely, that mature individuals present in the lake in between the end of removal efforts in 2014 and the beginning of the 2015 removal period probably had high reproductive potentials, and successive population reductions liberated more resources for immatures, thus allowing them to maintain high growth and increased survival rates.

In this context, the CPUE of *M salmoides* was observed to remain consistent, but the BPUE decreased. This was due to the presence of increasingly small sized individuals captured by that time, frequently seen in dense shoals. For *L. gibbosus* and *C. carpio*, a significant decrease in CPUE and BPUE was observed.

Classic depletion models (Leslie and Davis 1939) expect the consecutive observed CPUE to fall proportional when plotted against the accumulated catches, but in Lake Arreo, the CPUE tended to stabilize at low levels. The assumed constant catchability and zero natural mortality combined with the recruitment or migration make estimations of initial population sizes difficult, but it can be assumed that populations of all species successfully decreased. The observed CPUE stabilization can hence be due to several reasons: 1) a prolonged removal period (translating into recruitment); 2) inability of fishing techniques to affect fishes hidden in the helophytes; 3) lack of information about migration patterns between refuge and fishable zones; and 4) inconsistent catchability due to changing environmental factors.

Overall, the reduction of non-native fish populations was very high, especially in terms of biomass. BPUE (considered a predictor for total biomass) indicated a reduction of 98% from the beginning to the end of the management effort, whereas CPUE (predictor of density) reduced by 81%. Thus, it can be highlighted that the initial aims were achieved except for the eradication of carp. Because of the successful removal of large parts of the non-indigenous fish community, a positive effect on transparency and submerged macrophytes was expected for the next years. Especially the reduction of carp biomass (> 75% of total biomass) benefits aquatic vegetation (Bajer et al. 2009) and water clarity (Schrage and Downing 2004) and likely has immediate, long-lasting effects such as a clear-water phase in spring allowing for hydrophytes to recover (Lammens 2001).

Restoration of tench population

Tinca tinca population density in 2015 was very low, as only two individuals were caught. Thus, it can be assumed that the present population faces a high risk of extinction, but its recovery could be supported by the reduced pressure of non-native species. Additionally, this species typically occurs in low-density populations, has resistance to low oxygen levels and an omnivorous diet (Doadrio et al. 2011), factors that should contribute to maintain a viable population in the Lake Arreo. Nonetheless, having caught no individual of *T. tinca* in 2017 points towards a probable extinction of this species in Lake Arreo. Therefore, population reinforcement by stocking from viable populations would be another considerable measure.

Procambarus clarkii

Because the control of *P. clarkii* has been shown to be a long and difficult process, the continuous application of trapping to control the abundance of *P. clarkii* cannot be considered as effective and thus, other techniques need to be explored. Although *P. clarkii* was not directly sampled as part of the last control and eradication effort, an action for evaluation of its eradication was carried out from 2013 to 2015 (Asensio 2015). The presence of this species is of integral importance as this species is a common food source of *M. salmoides* and *L. gibbosus*, two species with which it shares a common co-evolution and life-history. Additionally, it is known for its devastating effects on recipient ecosystems (see e.g. Gherardi and Acquistapace 2007). Placing the traps for crayfish was practiced the same way in all the years by positioning them on the muddy ground in the shallow zone between patches of hygrophytes. The capture and CPUE values decreased between 2013 and 2014, but not during 2015, although the mean size of individuals was lower. These results hint towards a successful decimation (~ 72%) of the population to a certain threshold, below which it was impossible to progress by applying only one technique, for which reason other potential control techniques should be examined. In addition, it was concluded that baiting and placing the traps closer to the surrounding marshland might result in higher CPUE rates.

The overall turbidity showed a strong increase after high values in the beginning of 2015 followed by decreasing values in 2017, likely linked to the lack of control efforts in 2016 and a successive recovery of present alien species. It should be noted that the presence of *P. clarkii* and fish such as *C. carpio* has often been linked to an increased turbidity (Rodríguez et al. 2003). Nonetheless, it is not possible to determine a causality between observed turbidity and presence nor successful decimation of *P. clarkii* and *C. carpio* in Lake Arreo. Additionally, the presence of *P. clarkii* has been linked to facilitating establishment and increased population growth of other aquatic invasive species, for what reason the abundance of *P. clarkii* for the control and thus eradication of *M. salmoides* and *L. gibbosus* should be reduced.

Population shifts and evaluation of efforts

In 2017, the decreasing catchability of *L. gibbosus* specimens (day 1: 2,284 individuals; day 2: 590; day 3: 376) and *M. salmoides* specimens (day 1: 281; day 2: 131; day 3: 87) is in consensus with catches from 2014 and 2015, but also revealed the recovery of both species' populations. Considering that a reduction of *L. gibbosus* and *M. salmoides* populations was achieved, it is not surprising, that the length and weight distribution of alien fish in Lake Arreo differed from the previous years. Increased density but decreased biomass between 2010 and 2015, most likely affected these species' reproductions, making immature survival after 2015 the key factor for the recovery of populations in 2017 and the future years. Having caught, in 2017, only 11 carp (27–64 cm; 302–3,750 g) from which eight had fully developed gonads, showed the effectiveness of previous efforts, but also signified that the population of carp was on the verge of recovering. Thus, having achieved neither the population recovery of tench, nor the aimed eradication of carp, has major implications for the future. For once, because the applied efforts of carp were effective but not sufficient, control and management should be continued. With the observed effectiveness of electrofishing, it is likely that future efforts to eradicate carp will also affect other alien fish species, further reducing their populations. In the case of *P. clarkii*, due to its long invasion history and threat to European freshwater environments, several potential control techniques have been assessed in the past (Gherardi et al. 2011; Lodge et al. 2012; Souty-Grosset et al. 2016) and could be considered. Especially approaches like the introduction of biological control agents (Aquiloni et al. 2010) or increased electrofishing and funnel trapping after having trimmed back the abundant hydrophyte zone, should be explored.

Conclusion

It is likely that dense populations of *L. gibbosus* and *P. clarkii* are nurturing the population of *M. salmoides* and as the abundance of crayfish adds to the diet of *L. gibbosus*, enables both species of predatory fish to recover and generate high abundances and biomasses as observed in 2017. The very low diet overlap combined with the observed diet make it likely that the three present North American species are forming a highly linked trophic web with low competition, leaving the bottom feeding carp, the only species that can be considered as almost eradicated, outside. Nonetheless, with no individuals of *T. tinca* caught in 2017, it can be assumed that a population recovery of this species was not achieved, while the invasive species have been successfully reduced.

Positive effects of removal of non-indigenous invasive species such as lower population densities of invasive species can be enhanced in a near future by additional fishing effort (mainly by electrofishing). These works should be undertaken before the onset of the reproductive season to avoid or reduce density-dependent effects. This combination of electrical techniques and trapping of crayfish is postulated as an optimal strategy for the control and/or eradication of highly abundant invasive fish populations, as a key contribution to the recovery of the lake's ecological status, but additional methods such as the introduction of a biological control agent (e.g. the European eel, *Anguilla anguilla*; see Aquiloni et al. 2010) and the cutting back of zones that serve as a refuge for targeted species (e.g. the hydrophyte zone) should also be considered. Lastly, the interactions among species with a common origin, as in the case of Lake Arreo *L. gibbosus*, *M. salmoides* and *P. clarkii,* need to be investigated more thoroughly and especially a highly prolific invader such a *P. clarkii* needs to be controlled among invasive fish.

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Chapter 3.2

Predicting the effects of reintroducing a native predator (European eel, *Anguilla anguilla*) into an invasive freshwater community using a multidisciplinary approach.

Short title: Predicting bio control agent effectivity

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Abstract

Multiple introductions of alien species can lead to the formation of new and diverse communities with diverse interactions, particularly in isolated freshwater ecosystems. In Lake Arréo, located in Basque Country, Northern Spain, the introduction of several North American species (largemouth bass *Micropterus salmoides*, pumpkinseed *Lepomis gibbosus* and the red swamp crayfish *Procambarus clarkii*) and the Asian carp *Cyprinus carpio* has resulted in a unique community composed mainly by alien species. Previous efforts to eradicate these species by intensive fishing/trapping have failed. Reintroduction of native predator could represent an alternative control method, with an added biodiversity value. The reintroduction of the European eel *Anguilla anguilla* could lead to an increased predation on crayfish as shown by previous studies, but also affect the abundance of juvenile fish of *L. gibbosus* and *M. salmoides*. To investigate the current situation of Lake Arreo, Stable Isotope Analyses were conducted using 15 muscle tissue samples per each fish and crayfish species while stomach contents of the same species were analysed. Additionally, samples from the common reed *Phragmites australis*, the lowest food source available for fish and crayfish species, were collected and used as baseline for the isotope analysis. To investigate the usefulness of stable isotopes to predict the effects of species reintroductions on present species communities, available stable isotope and diet data from *A. anguilla* in a German freshwater lake with a similar species composition were retrieved and included in the Arreo community analysis. While results from both, dietary and stable isotope analyses, indicate high interactions among alien species with *P. clarkii* having a central position, the modelled reintroduction of *A. anguilla* shows to possibly affect recruits of alien fish species as well as an increased feeding of *M. salmoides* on reintroduced eels.

Key words: Stable Isotope Analysis, biocontrol, management, invasive species, eradication, European eel

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Introduction

There is no saturation in the global accumulation of alien species and this has important implications for local biodiversity (Seebens et al. 2017): introductions of species and their subsequent dispersal can indeed change the structure and composition of entire communities (Huxel et al. 2002; Strayer 2010), especially in freshwater ecosystems, often invaded by many alien species. Moreover, simultaneously present alien species tend to occupy different trophic levels (Gamradt and Kats 1996; Kiesecker and Blaustein 1998) and interact across trophic levels, leading to increased and often unforeseeable effects due to the prevalence among predators (Peckersky & MacIntosh 1998; Pringle & Hamazaki 1998; Sih et al. 1998). The complex interactions among alien species within the same ecosystem are not well understood (Parker et al. 1999; Huxel et al. 2002; Strayer 2010; Bissattini and Haubrock et al. 2018): as described in the “invasional meltdown hypothesis” (Simberloff & Von Holle 1999) they can favour the chance of a species establishment success and increase the impact on the recipient environment (Simberloff & Von Holle 1999; Simberloff 2006) while in certain cases lowering the impact of predatory invasive alien species on native species (Soluk and Collins 1988a, b; Soluk 1993; Rosenheim 1998; Bissattini and Haubrock et al. 2018).

Lake Arréo in Basque Country (Northern Spain) provides an interesting example of a multiple invaded freshwater ecosystem. The dominant species (*Micropterus salmoides* LACÉPÈDE 1802; *Lepomis gibbosus* LINNAEUS1758; *Cyprinus carpio* LINNAEUS 1758 and *Procambarus clarkii* GIRARD 1852) are all considered invasive. They dominate community biomass and change its composition and structure, negatively affecting the remaining native flora and fauna and thus the lake’s status as a protected area (see e.g. Costantini et al. 2018).

From 2014 to 2017, the lake Arreo has been monitored, and these four invasive alien species (IAS) have been controlled: their abundance was reduced, but eradication was not achieved (Haubrock et al. 2018). The reintroduction of a native predator, the European eel *Anguilla anguilla*, which was once present in the lake, has proven to be an efficient biocontrol for population of the red swamp crayfish *Procambarus clarkii* (Aquiloni et al. 2010, Musseau et al. 2015). The introduction of predators has major impacts on trophic structures within and across trophic levels (Codron et al. 2018; Costatini et al. 2018). While effects of such reintroduction might be negative for present IAS, positive effects may occur when the activity of the reintroduced predator provides a stable food source for other species present (Cordon et al. 2018).

In general, European eels are considered to be night-active predators (Tesch 1999) that hunt close to the bottom rather than in the open water column (Barak & Mason 1992) but are highly opportunistic (Lammens et al. 1985; Schulze et al. 2004). Nonetheless, being a second-order carnivorous species, it can shift its diet and feeding strategy according to the available potentially seasonally varying resources presented by the surrounding ecosystem (Bouchereau et al. 2006). This, considering also its ability to survive in saline environments such as Lake Arreo (Skadhauge 1969), makes *A. anguilla* an ideal candidate for biocontrol in Lake Arreo. But since the interaction between a reintroduced predator and omnivorous IAS such as *P. clarkii*, and hence successive effects, are unpredictable, being able to provide a prediction is of increasing interest.

Stable Isotopes Analysis (SIA) of carbon and nitrogen provide long-term information on species, enabling to have a trophic snapshot of a community (Boecklen et al. 2011; Layman et al. 2012; Middleburg 2014), estimate trophic levels (Post 2002), and finely quantify ecological niches (Newsome et al. 2007). Hence, SIA proved to be a useful tool in investigating IAS (Vander Zanden et al. 1999a, b; Balzani et al. 2016). While carbon signatures identify the major energy sources, nitrogen signatures relate to the trophic position within a food web (Layman et al. 2012). This relationship relies on predictable changes in the isotopic values from prey to consumer, being enriched of 1‰ for C and 2.5-5‰ for N between consecutive trophic levels (Post 2002; Vanderklift & Ponsard 2003).

Combined with the analysis of dietary contents (DA), which provide a direct short temporal insight into the feeding habit of a species, relationships among species can be investigated (Bissattini and Haubrock et al. 2018; Meeuwig & Peacock 2017). By means of SIA and DA, the trophic web and interactions of lake Arreo were assessed. The use of overlapping isotopic niches has previously been taken into consideration when predicting consequences at the community level following the introduction of a new species (Vander Zanden et al. 1999; Gorokhova et al. 2004; Maguire & Grey 2006). Thus, isotope and dietary data of *A. anguilla* retrieved from different ecosystems were used to model and predict the effect of this species reintroduction and the potential consequences for other species, helping us predict the potential effectiveness of biocontrol agents for the management of IAS and the response of native species. Moreover, considering the diet preferences of *A. anguilla*, we hypothesize that the applied mixing models will predict the predation of reintroduced *A. anguilla* on the abundant crayfish *P. clarkii*.

Materials and Methods

Study area

Lake Arréo (42°46′42″N -2°59′28″E; surface area: 6 ha; max. depth: 24 m) in Basque Country, Spain, is a “Nature 2000 Network – Special Areas of Conservation (ZEC)” (ES2110007) habitat and the largest most peculiar and natural continental lake system among the scarce lakes of the Iberian Peninsula. Being fed by hypersaline water from the diapiric substratum (adding a high ecological value), there are small areas with crusts of salt allowing the existence of plant communities untypical for the area (with a high scientific value). Concerning the Basque Catalog of Endangered Species, two present plant species are listed as endangered (*Berula erecta; Utricularia australis*), one other as vulnerable (*Puccinellia fasciculata*) and two others as near threatened (*Cistus crispus; Juncus acutus*). Nutrient-drag and fertilizers used around the ecosystem reduced peripheral vegetation, the introduction of alien species has led to a poor ecological state, as determined by the Monitoring Network of the Ecological State of Wetlands of the autonomous community of the Basque Country (CAPV). The lake state has thus worsened in recent years. Lake Arreo is frequently visited by the common pochard *Aythya ferina*, the common reed bunting *Emberiza schoeniclus*, the common moorhen *Gallinula chloropus*, the common snipe *Gallinago gallinago* and migrating birds. Additionally, 27 families of invertebrates and macroinvertebrates were identified from 2010 to 2012 but no information on their current status has been assessed since. The native crayfish *Austrapotamobius pallipes* is considered to have disappeared as a result of the introduction of the North American crayfish *Procambarus clarkii*. Moreover, the conservation of the in Lake Arreo endemic aquatic beetle *Gyrinus paykulli* is listed in the management plan of the "Lago de Caicedo Yuso y Arreo (ES2110007)" as desirable. While the common carp (*C. carpio*), the largemouth bass (*M. salmoides*) and pumpkinseed (*L. gibbosus*) are the most dominant species in terms of abundance and biomass, barbel (*Barbus graellsi*), tench (*Tinca tinca*), European eel (*A. anguilla*) and *Carassius auratus* are considered extinct (Haubrock et al. 2018).

Field work

In September 2017, as part of the annual monitoring and control effort, 15 specimens of *M. salmoides* and *L. gibbosus*, 11 specimens of *C. carpio* as well as 10 specimens of *P. clarkii* from all size classes were collected under the consideration of an even spatial distribution across the lake ecosystem (Wilson et al. 2010). Additionally, five samples of the dominant introduced aquatic plant, the common reed *Phragmites australis* were collected to complement the primary consumer (*P. clarkii*) as primary producer (Vander Zanden & Rasmussen 1996; Vander Zanden & Vadeboncoeur 2002). Other macroinvertebrates were not sampled as they were not found during sampling. Samples were immediately put on ice before being transported to the laboratory for further processing.

Laboratory procedure

Total length (TL) of fish and carapace total length (CTL) of crayfish were measured with a calliper (accuracy: 0.01 mm) and body mass (BM) was measured using an electronic balance (accuracy: 0.01 g). From randomly chosen individuals of each species, muscle tissue for SIA was extracted. Muscle tissue was cleaned from fat, skin, scales, bones and carapace (for crayfish), and for plants, a 5-10 cm leaf was cut off. Samples were placed into separated glass trays, dried for 48 h in an oven at 60 °C and afterwards grinded into fine powder with an agate mortar and pestle. For each sample of fish and crayfish, two replicates of each 0.20-0.30 mg (respectively 1.00 – 1.10 mg for plants) were weighed on a Mettler Toledo AG245 microscale and enclosed into a tin capsule to be combusted with a FlashEA 1112 elemental analyser. Samples were subsequently analysed by a Thermo Finnigan Delta Plus Advantagean isotope ratio mass spectrometer at the Istituto di Geologia Ambientale e Geoingegneria of the National Research Council (CNR) in Montelibretti, Rome (Italy). Isotope compositions were expressed as ‰ with the δ notation (based on $\delta^{13}C$ or $\delta^{15}N = ((Rsample/Rstandard) - 1) \times 1000$, where R is $^{13}C:^{12}C$ or $^{15}N:^{14}N$ ratios). Results were referred to Vienna Pee Dee Belemnite for carbon and to atmospheric N_2 for nitrogen. Arithmetic mean ± 1 SE was calculated for each species. The trophic positions of all species (TPc) were calculated by applying the equation TPc= (($\delta^{15}Nc$ -

$\delta^{15}N_{base}$)/ΔN) +λ, where δ^{15}Nc is the mean δ^{15}N of the consumer, δ15Nbase is the mean δ^{15}N of primary consumers, ΔN is the standard enrichment of 3.4 ‰ between trophic levels and λ is the basal trophic level (=1 for plants in our case) (Post 2002; Bissattini and Haubrock et al. 2018; Britton et al. 2018).

Statistical analyses

DA of IAS in Lake Arreo were previously performed (Haubrock et al. 2018; supplement 1) but were used to compare a potential diet overlap with the results presented by SIA. To estimate and quantify intra-specific and community niche width, Layman metrics (Layman et al. 2007) were calculated with the R-package SIAR (Stable Isotope Analysis in R; Parnell et al. 2010). Additionally, the corrected standard ellipse areas (SEAc) for all species and community were calculated (Jackson et al. 2011). The application of dual plot graphs for δ^{15}N versus δ^{13}C of consumer tissues and food sources enabled the determination of probable prey sources and combinations of prey contributing to the diet of predators (Phillips & Gregg 2003). Thus, to investigate how sampled species contribute to the isotopic signatures of each other, Stable Isotope Mixing Models (SIMMs) were applied using the R-package SIMMr (Parnell et al. 2013). SIMMs were conducted for *M. salmoides* present in Lake Arreo and potential prey items. Results are presented as the average percent values with the possible range percentage for each prey item. A Canonical Analysis of Principal Coordinates (CAP) for both factors, i.e. δ^{15}N and δ^{13}C, was applied to investigate which contributed more consistently in differentiating species. Spearman correlations for each variable with CAP1 axis, the only one found informative in differentiating the species' niches are reported. Additionally, a PERMANOVA and post-hoc tests were used to identify significant differences in δ^{15}N and δ^{13}C among species and to eventually determine niche overlap between species.

Predicting the effect of reintroducing the European eel Anguilla anguilla

The probable impact of reintroducing *A. anguilla* as a biocontrol agent for the invasive species in Lake Arreo was predicted using the previously described application of mixing models with the inclusion of isotope data of *A. anguilla*. Additionally, the corrected standard ellipse areas (SEAc; considering 40% of central data points) as an indicator of niche overlap and the corresponding 95% prediction ellipses (SEAb) for all considered species, indicating effects due to common resource usage, were calculated. With these, the degree of isotopic niche overlap, which lays between 0, i.e. no overlapping, and 1, completely overlapping, can be estimated and subsequently used as "a quantitative measure of dietary similarity among populations" (Jackson et al. 2012). Calculations were computed using the R package SIBER (Jackson et al. 2011). Therefore, δ^{15}N and δ^{13}C values of IAS in Lake Arreo were standardized with the mean of the primary producer *P. australis*, and δ^{15}N and δ^{13}C isotope values of *A. anguilla* (from Dörner et al. 2009) were standardized with the combined means of the two present primary producers in Großer Vätersee (Germany), a species community containing native counterparts with similar ecological roles to the alien species present in Lake Arreo, to make communities comparable (Taylor et al. 2017). In the Großer Vätersee, piscivorous eels ranged from 31.5

to 77 cm and 17.8 % stomachs contained only fish (overall diet: 40% perch, 34% roach, 17% crayfish, 8% insects, 2% gastropods). Results were complemented with a short review of dietary analysis of *A. anguilla* (Costa et al. 1992; Dörner et al. 2003, 2009; Bouchereau et al. 2006) and discussed.

Results

Lake Arreo community structure

IAS in lake Arreo occupied all trophic levels (Table 1), with *M. salmoides* being the top predator, *L. gibbosus* predator, *C. carpio* occupying an intermediate position between primary consumer and predator and *P. clarkii* being the single primary consumer.

Table 1: Trophic position (TP) of species sampled in Lake Arreo.

Species	TP
Micropterus salmoides	3.0
Lepomis gibbosus	2.5
Cyprinus carpio	2.1
Procambarus clarkii	1.3
Phragmites australis	1.0

The dietary data from Haubrock et al. (2018) showed a relatively low diet overlap (< 0.4) and diet-based niche overlap between species (0.18 overall, 0.21 between *L. gibbosus* and *M. salmoides*) and was subsequently linked to a rather enclosed trophic web and low competition within the North American species community in Lake Arreo. Additionally, common carp *C. carpio* was mostly excluded from these interactions. While *M. salmoides* was shown to have a rather specialized diet, targeting only *L. gibbosus* and *P. clarkii*, *L. gibbosus* showed a wider diet feeding mainly on crayfish but also including a variety of insects (Figure 1; insects not included).

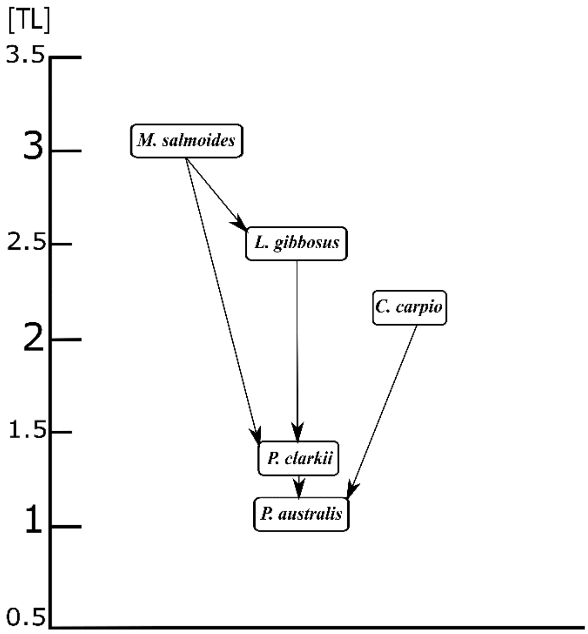


Figure 1: Trophic level (TL) and feeding interactions displaying the trophic web of alien species in lake Arreo. The y-axis indicates the trophic level of species.

Based on the assumption of an enrichment of $\delta^{15}N$ by 3.4 ‰ for each trophic level (Post 2002), Layman's metrics of the entire species community showed a wide range of nitrogen (NR=8. 62), indicating that the community spans over approximately two to three trophic levels. *P. clarkii* showed the widest isotope ranges for NR and carbon range (CR =4.81 and 8.31, respectively). *P. clarkii* also expressed the highest convex hull (TA=16.29; the smallest polygon containing all data points) and SEAc (corrected standard ellipse area) with 10.17, indicating a high trophic plasticity. Its CR values revealed that this is especially due to a plasticity in the carbon source use, making this species a potential omnivore rather than primary consumer and herbivore. *C. carpio* and *L. gibbosus* had similar Layman's metrics values, with the only difference being in NR, which was considerably higher for *L. gibbosus* (Table 2). Among fish species, *M. salmoides* expressed the lowest values for each metric. Moreover, SIMMs (Figure 2) of *M. salmoides* showed it feeds mainly on *L. gibbosus*, less on crayfish (Figure 2), this high feeding specialization being confirmed by the low values of each Layman metrics.

Table 2: Estimated Layman's metrics and Stable Isotope results for $\delta^{15}N$ and $\delta^{13}C$ of alien species in Lake Arreo. NR = $\delta^{15}N$ range, CR = $\delta^{13}C$ range, TA = convex hull area, CD = mean distance to centroid, MNND = mean nearest neighbour distance, SDNND = standard deviation of the nearest neighbour distance, SEAc = Standard Ellipse Area.

Group	***Layman metrics and Stable Isotope Analysis results***								
	Mean $\delta^{15}N$	**Mean $\delta^{13}C$**	**NR**	**CR**	**TA**	**CD**	**MNND**	**SDNND**	**SEAc**
Cyprinus carpio	12.2	-33.9	2.8	3.8	5.4	1.0	0.6	0.5	2.5
Lepomis gibbosus	13.5	-32.2	3.8	3.4	4.9	1.2	0.5	0.4	2.2
Micropterus salmoides	15.2	-30.8	2.2	2.3	2.6	0.9	0.3	0.2	2.3
Procambarus clarkii	9.4	-28.2	4.8	9.6	16.3	3.2	0.8	0.7	10.2
Phragmites australis	8.4	-28.6	1.0	1.4	0.1	0.6	0.3	0.2	0.1
Community	na	na	8.6	12.3	53.4	3.0	0.4	0.4	17.1

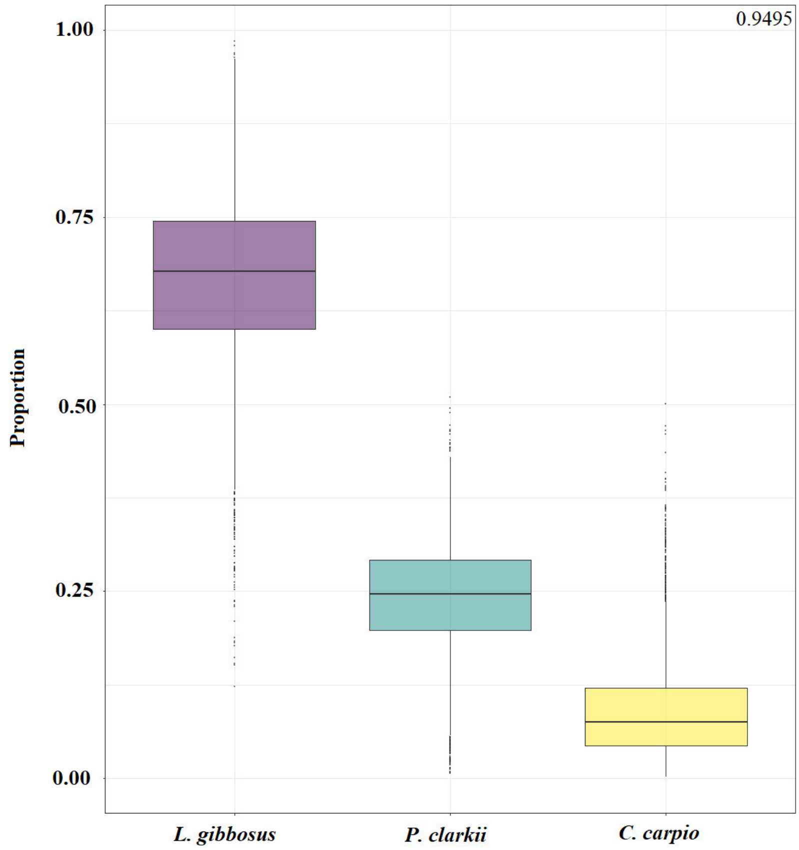


Figure 2: Results of Stable Isotope Mixing Models for *Micropterus salmoides*: the statistical probability of the applied model is reported in the top right corner (y-axis; mean plus standard deviation of prey items in x-axis) contribution to trophic level.

The applied PERMANOVA (Table 3) and post-hoc test (Supplement. 2) as well as CAP (Figure 3) analysis of isotope data highlighted that all species are clearly distinct and separated from each other for carbon and nitrogen levels ($F_{3,52}$= 41.78, p = 0.001), confirming the results of trophic position estimations.

Table 3: PERMANOVA main test for factor 'species'

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
sp	3	502.55	167.52	41.777	0.001	999	0.001
Res	52	208.51	4.0098				
Total	55	711.06					

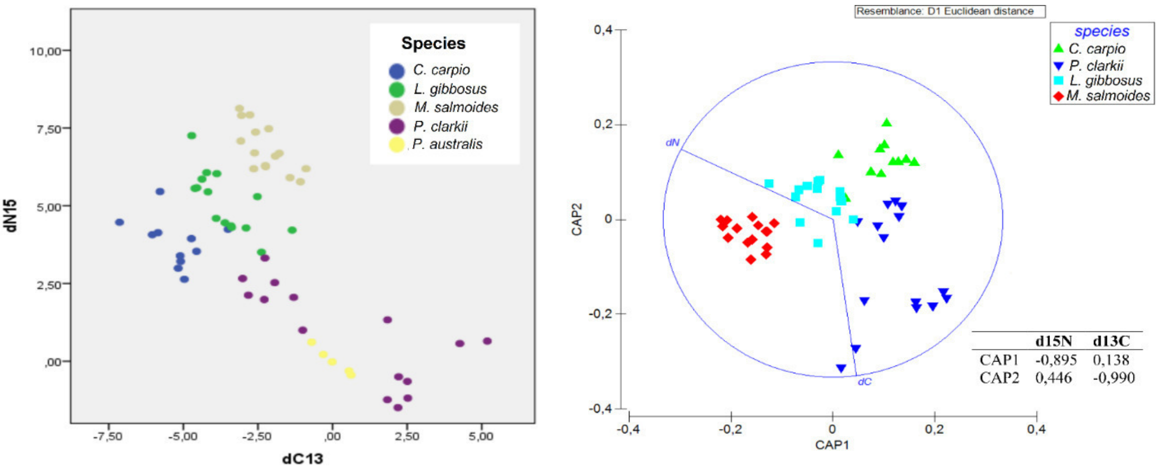


Figure 3: Stable Isotope distribution of sampled species (left) and visualization of CAP analysis (right) with the Spearman correlation of each isotope and the respective CAP axis (bottom right).

Predicting the effect of introducing the European eel Anguilla anguilla

Having a trophic level of 2.8, *A. anguilla* was positioned between *L. gibbosus* and *M. salmoides* but showed a high overlap in the distribution of δ^{15}N and δ^{13}C with *L. gibbosus* (Figure 4).

The review of dietary data and feeding habits showed a mixed diet between macrozoobenthic species in all size class of *A. anguilla* and also fish with an increase of predator size (according to abundance of potential prey items) (Figure 4). Layman's metrics for European eels showed a broad isotopic range similar to those of other fishes. TA (7.5) was considerably greater than other species present in the lake, although SEAc value, which is generally less affected by extreme samples and thus outliers, was comparable with other species. The standard deviation of the nearest neighbour distance (SDNND) showed little inter-individual variability in this species. Metrics for the community including *A. anguilla* showed very little variation, with a slight increase in TA.

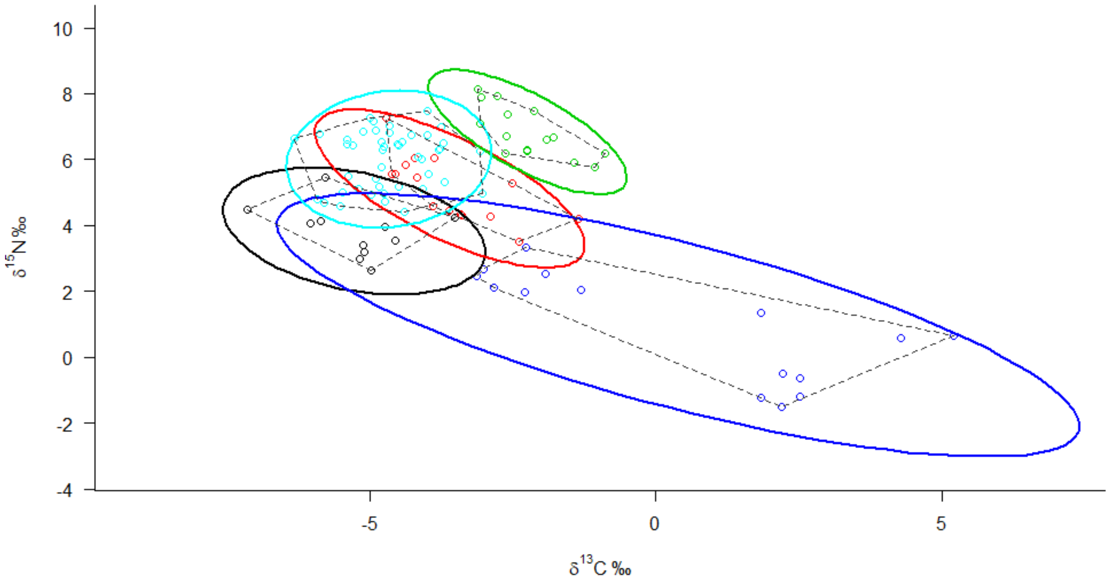


Figure 4: Distribution of δ^{15}N and δ^{13}C and ellipse area (95%) for Lake Arreo IAS *Micropterus salmoides* (green), *Lepomis gibbosus* (light blue), *Cyprinus carpio* (black), *Procambarus clarkii* (dark blue) and the potentially reintroduced biocontrol agent *Anguilla anguilla* (red).

The estimated standard ellipse area revealed no considerable overlap between species except for *L. gibbosus* and *A. anguilla* (0.4). However, under consideration of 95% of data points, overlap among species increased, indicating high values all IAS in Lake Arreo and potentially with *A. anguilla* (Table 4). Applying SIMMs on combined IAS in Lake Arreo for *M. salmoides* and *A. anguilla* from Germany indicated that *M. salmoides* might mainly feeds on *A. anguilla* and then secondarily on other species (Figure 5, 6). Additionally, results indicated an exclusively piscivorous diet of *A. anguilla* although crayfish are present in the German lake, thus indicating a very low probability of predation on crayfish (Figure 6).

Table 4: Overlap of the corrected Standard Ellipse Area (SEAc = 40%) and all data enclosing ellipse area (SEAb = 95%) for all IAS present in Lake Arreo and the potentially reintroduced biocontrol agent *Anguilla anguilla*.

X	***Cyprinus carpio*** (SEAc / SEAb)	***Lepomis gibbosus*** (SEAc / SEAb)	***Micropterus salmoides*** (SEAc / SEAb)	***Procambarus clarkii*** (SEAc / SEAb)	***Anguilla anguilla*** (SEAc / SEAb)
C. carpio	x	1.301043e-18 0.583	0 0.659	0 0.223	1.431147e-17 0.644
L. gibbosus		x	8.673617e-18 0.645	0 0.195	0.4067617 0.752
M. salmoides			x	5.155355e-17 0.110	0 0.404
P. clarkii				x	0 0.868
A. anguilla					x

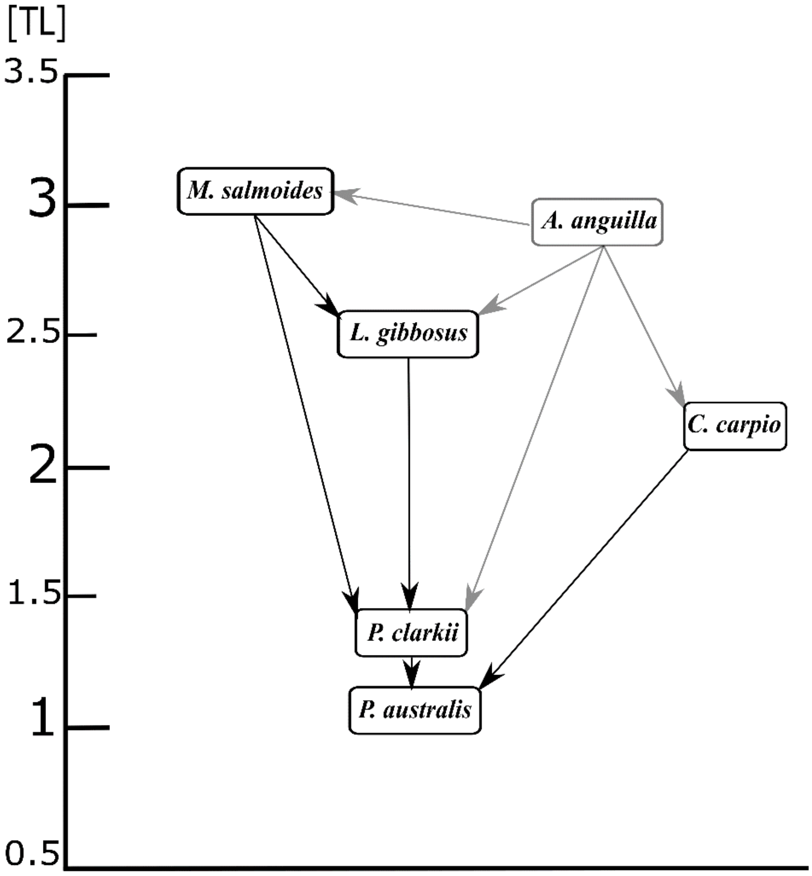


Figure 5: Likely feeding habits of *Anguilla anguilla* in lake Arreo. Grey lines and values indicate predation according to reviewed literature. The y-axis indicates the trophic level of species.

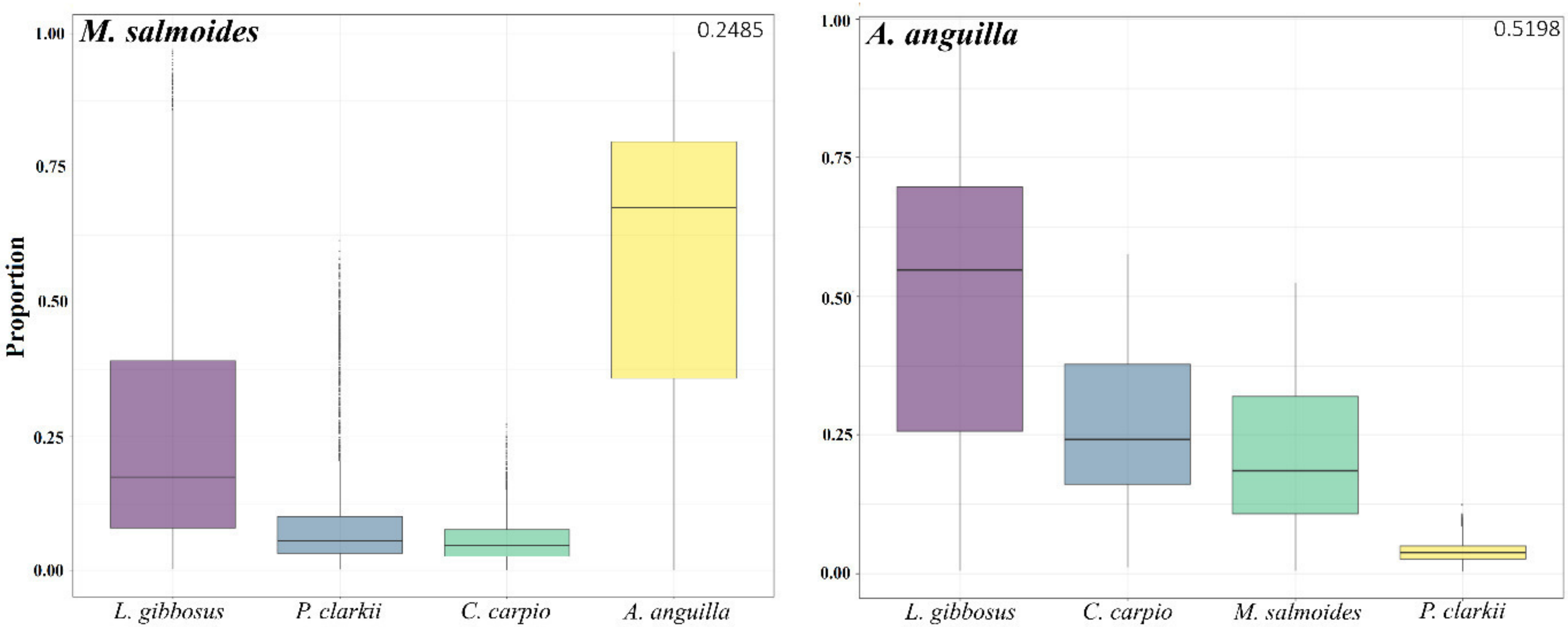
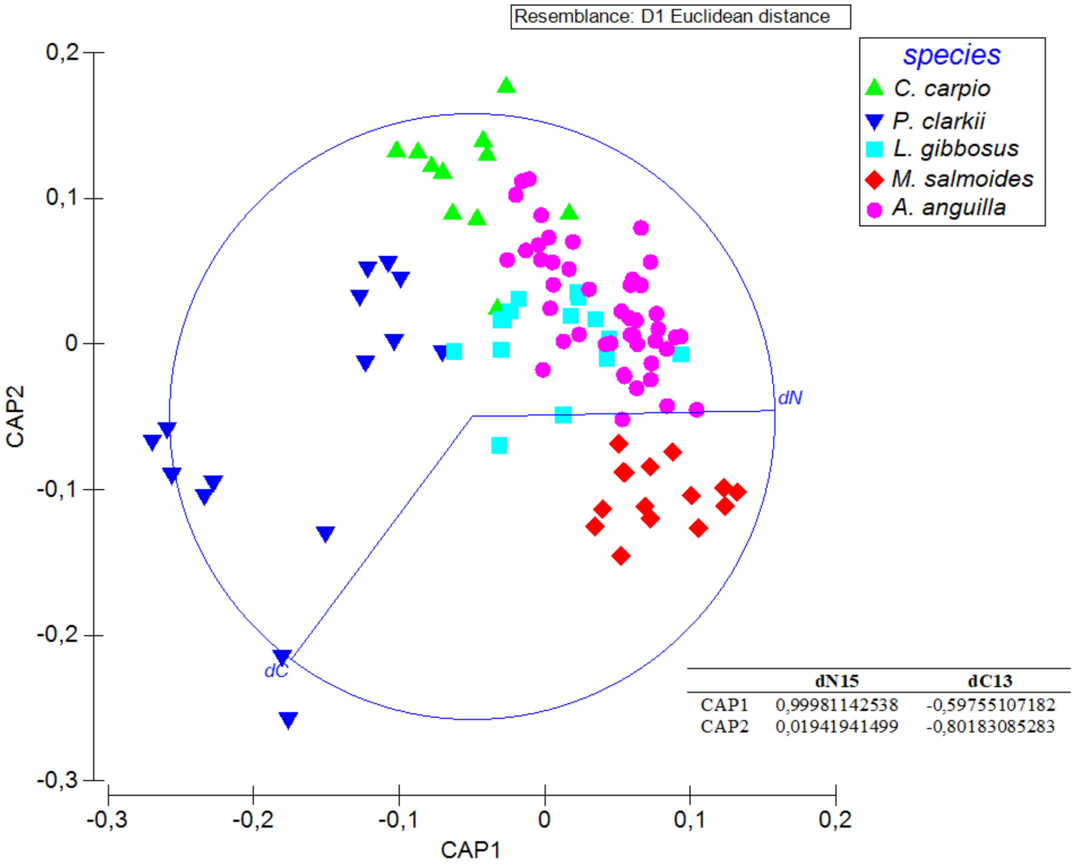


Figure 6: SIMMs for *Micropterus salmoides* (a) and *Anguilla anguilla* (b) by combining data from lake Arreo and lake Großer Vätersee (Dörner et al. 2009). The number on the top right corner of each diagram indicates the statistical probability of each result presenting the proportion (y-axis; medians and quartiles of prey items).

PERMANOVA as well as post-hoc tests (Supplement 3) and CAP analysis (Figure 7) on the combined and individually standardized datasets confirmed that all species remain distinct and separated for both carbon and nitrogen levels (Pair wise test: p = 0.001). Cross validation showed that *A. anguilla* overlaps with all fish species, which agrees with the PERMANOVA main test and left-one-out allocation explained 66.67% of correct reallocation.



	dN15	dC13
CAP1	0,99981142538	-0,59755107182
CAP2	0,01941941499	-0,80183085283

Figure 7: CAP analysis for IAS of lake Arreo and *Anguilla anguilla*. Table in lower-left corner indicates the correlation of Stable Isotopes with CAP1 and CAP2 axis.

Discussion

Studying communities of IAS is challenging due to the lack of information on species interactions and potential adaptations to the invaded ecosystem. However, these interactions can lower the impact on native species or facilitate it, but, to our knowledge, this is the first attempt of using Stable Isotopes to predict potential effects of a predator reintroduction as a biocontrol agent.

Our study in Lake Arreo revealed that IAS were highly interconnected through feeding activities, but their effect on native species could not be directly assessed due to their lack in the sampling. However, the native species abundance has been observed to steadily decrease in the last years, which has been hypothesized to be linked to the presence of IAS (pers. comm. Jose Augusto Monteoliva). Moreover, the novelty of the applied approach showed a potentially high predation on *L. gibbosus* by *A. anguilla*, but also on *A. anguilla* by *M. salmoides*, highlighting how already present predators might respond to the presence of this reintroduced species.

Lake Arreo community structure

Isotope values of sampled fish for $\delta^{15}N$ in Lake Arreo were high, likely due to the already high value of the primary producer *P. australis*, a species that is known to extract nitrates from the surrounding ecosystem and accumulating in its tissue (Wigand et al. 2007). The food chain is short, including two-three trophic levels. This simple community shows no niche overlap among species, that are distinct, especially for their N isotope values. Moreover, species are highly linked, with both *M. salmoides* and *L. gibbosus* relying on the abundance of *P. clarkii* as food source (aside from *P. australis* as the primary producer) in terms of dietary and SIMMs results. Carp stomach content was composed only by detritus, confirming the benthic feeding habit of this species. Nonetheless, its N isotopic signature placed carp in an intermediate position between primary consumers and predator, which could be explained by either high content of consumed detritus due to the eutrophic state of Lake Arreo or a considerable proportion of crayfish in the diet of carp, as *C. carpio* is known to actively prey on highly abundant macrozoobenthos (Britton et al. 2007; Anton-Pardo et al. 2014), i.e. small crayfish in our study site. Hence, SIMMs results supported the observation of Anton-Pardo et al. (2014) and furthermore confirm the highly interconnected community of North American species that simultaneously show low niche overlap, as previously observed by Haubrock et al. (2018) based on dietary analyses. Lastly, the observed interactions between IAS in Arreo Lake highlight that the origin of alien species eventually affects the arising interactions.

Predicting the effect of reintroducing the European eel Anguilla anguilla

European eels rely on macrozoobenthos and fish (Moriarty 1972, 1973; De Nie 1987) while being able to control the abundance of age 0 fish through intense predation (Radke & Eckmann 1996; Dörner & Benndorf 2003). In a study by Lammens et al. (1985), the low abundance of chironomid larvae resulted in a diet shift of eels over 40 cm towards smelts (*Osmeruseper lanus*). In the Mediterranean Ingril lagoon, France, fish constituted to only 1.01-3%, while *Gammarus gr. locusta* (24-48%) and Chironomidae (12.01-24%) overweighed. Nonetheless, in February fish (44%) and decapods (44%) overweighed, implying an overall mostly benthic predation. However, Costa et al. (1992) determined that fish are always secondary but significant food item, fluctuating due to availability and habitat.

The increasing number of isotope studies on a wide variety of species in an even wider range of ecosystems and community compositions allow the prediction of IAS impacts (France 1995; Vander Zanden et al. 1999) as well as the effectiveness of a species reintroduction as a biological control agent (MacNabb et al. 2001; Kraiss δ Kullen 2008). Although the potential outputs would be inevitably imprecise due to the various differences (i.e. origin of isotope values, different primary producers and soils, species number, abundance and composition of species and geographical and climatic variation), they could provide a valuable tool to forecast how species interactions could affect further introductions (Britton et al. 2010). Indeed, the use of stable isotope data from different areas has a relevant importance and manifold applications, as it was recently pointed out by Pauli et al. (2017), who called for a centralized database for such data in order to explore further applications. Moreover, potential results can refer to a system in equilibrium, given that used data originate from presumably stable communities. However, this is not always true, because at the beginning of an introduction relationships among species will vary depending on various factors (population growth, interactions, presence of other species, behavioural plasticity) from those predicted. This way of approaching IAS introductions could approximate the outcome in terms of ecological niches when using isotope data from the most comparable system available and dietary and behavioural studies. Such approach could be also integrated in invasive species risk assessment models.

Benndorf (1995) reports that eels were successfully used with other species of piscivorous predators such as *Esox lucius* to decrease the abundance of smaller planktivorous species, underlying that that introduced biomanipulators would need to cover all age classes to show a significant effect on the target community. Nonetheless, such studies (including Dörner et al. 2003) did not sufficiently assess the effectiveness of these introduced species and predation on non-target species. Isotope data on *A. anguilla* are rare, due to the protected status of this species, and concern different ecosystems. Dörner et al. (2009) investigated the trophic position and diet of European eels from two lakes in Germany. Due to the obvious climatic differences between Lake Arreo and the “Große Vätersee”, extracted information have to be supplemented with behavioural data from climatic more comparable ecosystems. Although dietary information on *A. anguilla* are only few, especially for Northern Spain, the available information agrees that, although fish appear to be of secondary importance, piscivorous feeding activity can dominate seasonally due to prey abundances (Lammens et al. 1985; Costa et al. 1992). However, predation of potentially present native macroinvertebrates cannot be excluded from noteworthy side effects. Therefore, results presented by the applied mixing models, predicting an intense feeding of *A. anguilla* on *L. gibbosus* (a small sized Perciformes), is consistent with the observed predation on perch in the Großer Vätersee (Dörner et al. 2009), where although less frequent consumed than chironomid larvae, fish majorly contributed to the biomass of analysed diets. Moreover, crayfish were also predated in a considerable amount, which was not the case in the for Lake Arreo estimated SIMM but is in accordance with the in 2017 observed higher abundance of fish than crayfish (Haubrock et al. 2018). In addition to this potential predation on *L. gibbosus*, the estimated SEAc indicated an overlap between *A. anguilla* and *L. gibbosus*, likely indicated a similar use of resources and prey (e.g. insects and small sized *P. clarkii*). However, the SEAb overlap underlined that introduced eels would affect all IAS in Lake Arreo. Additionally, the SEAc values estimated for *A. anguilla* showed a lower value in this reintroduction scenario, indicating that the total community niche space would be more compact, i.e. showing less niche space for present species, after the reintroduction of *A. anguilla*, likely due to the niche occupied by European eels falling in the already defined community cloud (trophic redundancy, Layman et al. 2007).

Concerning the diet of eels, the difference in carbon signatures should be considered. Dörner et al. (2009) stated that the difference in benthic reliance, i.e. carbon deriving from the benthic pathway, was about 45 %. This result would indeed indicate that eels serve as a link between benthic and pelagic food webs when availability of insects is low, meaning that, when the abundance of macrozoobenthos is low, eels may shift to a more piscivorous diet (i.e. piscivory is controlled by availability of insects), as in the case of Lake Arreo.

Similarly, the American eel (*Anguilla rostrata*) is known to predate on eggs in nests of *M. salmoides* and *L. gibbosus* plus early life stages. Therefore, the European eel could be a likely competitor for Centrarchidae (depending on niche overlap; Schiphouwer et al. 2017). On the other side, a possible predation of IAS and aquatic birds on *A. anguilla* has to be considered possible. The applied SIMMs estimated a potential high predation of *M. salmoides* on eels. Indeed, *Micropterus* sp. are known to occupy wide and variable trophic niches (Costantini et al. 2018) as well as to eat small sized *A. rostrata*, when available (McCord 2005).

Moreover, Lake Arreo provides a good fishery with high abundances of prey (small sized *L. gibbosus*, *M. salmoides* and *P. clarkii*) and a suitable habitat in the shallow hygrophyte areas for eels. The Lake is also considered as highly saline, a factor that likely contributes to the success of *A. anguilla* as a predator. Nonetheless, problems might arise if the mature specimens cannot migrate for reproduction: in this case, individuals showed a decrease in weight and fat content but stayed alive for up to 10 years (Westin 2002). However, Lake Arreo's inlet provides the opportunity to migrate during the late fall and winter period (pers. comm. Alberto Criado), but a return of individuals is not certain. Another possibility to assess the potential impact of a predator on a species community, native or alien, would be analysis of comparative functional responses (Dick et al. 2013). Such studies are usually conducted in model systems by investigating the feeding response on differing prey densities. Furthermore, SIA have been considered to be an interesting and potentially valuable add for these studies (Dick et al. 2013; Dick et al. 2014). For Lake Arreo and the assessment of reintroducing a biocontrol agent, conducting a functional response study, even if only on a theoretical model, could be noteworthy, as the effect of reintroducing a bio-manipulator would differ according to its propagule pressure as well as the present species densities.

Conclusion

Reintroducing eels to decrease the abundance of IAS as a complementary technique to electrofishing under an integrated pest management approach is an interesting and potentially promising approach (Aquiloni et al. 2010). An integrated approach can provide good indications of potential effects of eels on the IAS of Lake Arreo. The reintroduction of *A. anguilla* could affect at least the population of *P. clarkii* or *L. gibbosus*, but a more accurate prediction is currently impossible as outcomes highly depend on the abundance of potential prey, feeding behaviour of eels and lastly the arising interactions after the reintroduction of *A. anguilla*. The here tested approach needs to be validated using data collected during sampling pre- and post to a species reintroduction.

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Supplement 1. Identified dietary components for *L. gibbosus* (N = 38), *M. salmoides* (N = 38) and *C. carpio* (N = 11). Results are displayed as frequency of occurrences [F%] in percentages. Data taken from Haubrock et al. 2018.

Component	*Lepomis gibbosus*	*Micropterus salmoides*	*Cyrpinus carpio*
P. clarkii	14.29	82.14	0
L. gibbosus	0	17.86	0
Odonata	0	14.29	0
Mollusks	2.86	0	0
Hymenoptera	20	3.57	0
Heteroptera	11.43	0	0
Snails	0	0	0
Araneae	2.86	21.43	0
Diptera	5.71	0	0
Odonata	5.71	0	0
Orthoptera	14.29	0	0
Coleoptera	45.71	0	0
Formicidae	2.86	0	0
unid. Insects	51.43	0	0
detritus	42.86	3.57	100
fish eggs	8.57	0	0
plant material	5.71	0	0

Supplement 2. Post hoc comparison (pair-wise tests) after PERMANOVA.

Groups	t	P(perm)	perms	P(MC)
C. carpio, P. clarkii	6.0509	0.001	997	0.001
C. carpio, L. gibbosus	4.0555	0.001	999	0.001
C. carpio, M. salmoides	9.5748	0.001	998	0.001
P. clarkii, L. gibbosus	6.2421	0.001	998	0.001
P. clarkii, M. salmoides	7.1935	0.001	999	0.001
L. gibbosus, M. salmoides	4.9049	0.001	999	0.001

Supplement 3: PERMANOVA main test result for factor SPECIES using all species including data of *Anguilla anguilla* from the Großer Vätersee.

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
sp	4	700.34	175.08	63.76	0.001	998	0.001
Res	96	263.61	2.76				
Total	100	963.95					

Chapter 3.3

Trophic structure of an IAS-dominated pond community: insights from combined stomach content and stable isotope analyses

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Abstract

Freshwater ecosystems are theatre of spectacular invasions and are dominated by multiple alien species belonging to different trophic levels. However, contrarily to the "invasional meltdown theory", the combined effects of alien predators on native prey are often lower than expected because of interactions among invaders. Here, stomach content and stable isotope analyses were used to investigate a freshwater system (Latium, Central Italy) dominated by invasive alien species in order to understand the effects of the interactions among invaders on the structure, functioning and diversity of the community. Both analyses provided similar results highlighting the presence of up to four alien species at each trophic level with the exception of top consumers represented by native snakes. Trophic interactions seem to be well established with both alien and native species, the latter being highly dependent on invaders. Alien species occurring in keystone positions, at both the basal and the consumer level, seem to not strongly influence the structure of the whole community, especially when interacting with each other in a predator/prey relationship. Our research suggests that it is crucial to increase the knowledge on the dynamics of multiply invaded communities to better understand how they interact directly and indirectly.

Keywords: $\delta^{15}N$, $\delta^{13}C$, feeding ecology, food web, interactions, species facilitation

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Introduction

We are currently witnessing an age of biological invasions, with novel species being incorporated into local communities not only by dispersal, but also by intentional or accidental introductions (Seebens et al., 2017). Invasive alien species (IAS) are known to affect the distribution and abundance of organisms across multiple trophic levels, influencing the structure and stability of invaded communities (Huxel, McCann, & Polis, 2002; Strayer, 2010). To date, a robust framework to understand the interactions among invaders and their combined impacts on invaded communities has yet to be developed (Strayer, 2010). Most studies have been focused on the effects of one invader at a time in tightly linked food chains with distinct trophic levels (Polis & Strong, 1996; Rosenheim, 1998).

Freshwater ecosystems are particularly vulnerable to biological invasions due to the strong link of humans to water and the intrinsic dispersal ability of freshwater compared to terrestrial species (Tricarico, Junqueira, & Dudgeon 2016). Thus, freshwater communities are often dominated by multiple species belonging to different trophic levels (Gamradt & Kats, 1996; Kiesecker & Blaustein, 1998), whose impacts can be very severe and complex due to a combination of direct and indirect effects (Peckarsky & McIntosh, 1998), prevalence of omnivory (Pringle & Hamazaki, 1998), and interactions among predators (Sih, Englund, & Wooster 1998).

Based on the "invasional meltdown theory", positive interactions among invaders can increase the likelihood of establishment and the magnitude of their ecological impact on native species (Simberloff & Von Holle, 1999; Simberloff, 2006). However, several studies revealed that most often the combined effects of alien predators on native prey are lower than the expected sum of individual effects, due to interactions among IAS (Soluk, 1993; Rosenheim, 1998).

Therefore, to understand the effects of multiple predator invasions, both the invader trophic position and their direct or indirect interactions within the community should be considered (Nyström, Svensson, Lardner, Brönmark, & Granéli, 2001). Stable isotope analysis (SIA) has been proved to be a useful tool in evaluating the potential diet overlap and feeding competition between native and IAS and estimating their impact on local communities (Vander Zanden & Rasmussen, 1999; Balzani et al., 2016). Contrarily to stomach content analysis, SIA provides relatively long-term and time-mediated information on trophic resources consumed and assimilated by consumers, and on their trophic behaviour (Gherardi, Acquistapace, & Santini 2004; Huckembeck et al., 2014; Middelburg, 2014). It is based on predictable changes in nitrogen and carbon isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) between consumers and their food sources (Post, 2002): $\delta^{15}N$ indicate trophic position within a food web, while $\delta^{13}C$ identify major energy sources.

To date, there is a lack of detailed studies about the structural change of aquatic communities dominated by invaders, except for Lake Naivasha in Kenya (Gherardi et al., 2011). Moreover, little information is also available on the organization of food webs of ponds and puddles (Dalu, Weyl, Froneman, & Wasserman 2016; Holgerson, Post, &

Skelly 2016), since most isotopic studies in freshwaters have been carried out in lakes and streams (Post, 2002).

Here, we analysed a freshwater system dominated by IAS to understand the interactions among invaders and their effects on the structure, functioning and diversity of the community. We combined stomach contents and SIA to: i) assess the diet of species, ii) assign each species to a specific trophic level, iii) characterize the niche width of species, and iv) quantify niche overlap in potentially competing species. This integrative approach provided an in-depth view of the community structure in terms of trophic interactions, helping to improve our ability to predict the effect of invaders on native communities and to suggest effective management for IAS.

Material and Methods

Study area

Field work was carried out in Monterotondo Scalo, a wetland area located 20 km from Rome (Central Italy; 42°03’52.91’’N; 12°35’07.83’’E; 18 m a.s.l). The study area (surface: 9 ha; perimeter: 1 km) is composed by four semi-natural ponds (surface: 0.03-2 ha; depth: 1-1.8 m) originating from an abandoned clay quarry filled with waste material and meteoric water. The vegetation mainly consists of *Phragmites australis, Typha latifolia* and hygrophilous forests with *Alnus glutinosa*, *Populus alba* and *Salix alba.* The pond system hosts few native species, the aquatic snakes *Natrix natrix* and *N. tessellata* and the green frogs *Pelophylax esculentus* and *P. lessonae*. These two latter species were treated as a single species (*P*. kl. *esculentus*) as they are impossible to distinguish in the field, being morphologically almost identical (Vignoli, Luiselli, & Bologna 2007, 2009). The remaining fauna is composed by invaders: six Eurasian fish species (the goldfish *Carassius auratus*, the carp *Cyprinus carpio*, the chub *Squalius squalus*, the bream *Abramis brama*, the roach *Rutilus rutilus* and the stone moroko *Pseudorasbora parva*) (Kottelat & Freyof, 2007) and four North American species (the black bullhead catfish *Ameiurus melas*, the pumpkinseed *Lepomis gibbosus*, the American bullfrog *Lithobates catesbeianus* and the red swamp crayfish *Procambarus clarkii*).

Fieldwork and stomach content analysis

Sampling was performed during the summer of 2016. Fish were collected using electrofishing, while crayfish, snakes and frogs were collected by hand, dip-netting and with baited traps. Invertebrates (Coleoptera, Isopoda, Diplopoda and Gastropoda) were collected using pitfall traps placed along the pond shoreline. Basal organic resources (i.e. leaf litter materials) were not sampled extensively, but rather focused on the contributions of primary consumers to predator diets (Vander Zanden & Rasmussen, 1999).

To obtain stomach contents, crayfish and fish were dissected whereas amphibians were flushed (Solé, Beckmann, Pelz, Kwet, & Engels, 2005) and marked with the toe-clipping method (Phillot et al., 2007). Snakes were marked by clipping ventral scales

(Winne, Willson, Andrews, & Reed 2006) and recently-ingested prey were collected by forced regurgitation (Fitch, 1987). Snout-vent length (SVL) of frogs, total length (TL) of fish and snakes, and carapace total length (CTL) of crayfish were measured with a calliper (accuracy: 0.01 mm). Prey composition of each stomach was estimated by counting the number of prey (Callegari-Jacques, 2006). Niche width (B) was estimated using the formula

$$B_i = \frac{\left[\left(\frac{1}{\sum_{i=1}^{n} p_i^2}\right) - 1\right]}{(n-1)}$$

where Bi is the standardized index of niche breadth; P_{ij} the proportion of food category j in the diet of species I and n the number of food categories ingested by species i (Levins 1968; Hurlbert 1978). Trophic niche width was considered low (0 - 0.39), intermediate (0.4 - 0.6) or high (0.61 - 1) (Grossman, 1986).

Stable isotope analysis

Muscle from fish and crayfish, scale clips from snakes, and skin from clipped frog toes were taken for the analyses, whereas invertebrates were processed as whole individuals. Samples from tissues were preserved at -20 °C, freeze-dried at -60°C for 24–48 h (FD-10 Freezing Dryer), and then homogenized into a fine powder by a mortar and pestle. From each sample, 0.25 mg of powder were weighed on a Mettler Toledo AG245 microscale, packed in 53.5 mm tin capsules, and combusted with an elemental analyzer (FlashEA 1112) connected to an isotope ratio mass spectrometer (Thermo Finnigan Delta Plus Advantage) at the Institute of Agro-Envinronmental and Forest Biology of the National Research Council in Rome (CNR). Two replicates for each sample were measured. Carbon and nitrogen isotope compositions were expressed in standard delta notations ($\delta^{13}C$ or $\delta^{15}N$ with units of ‰) according to the equation $\delta^{13}C$ or $\delta^{15}N = ((R_{sample}/R_{standard}) - 1) \times 1000$, where R is ^{13}C:^{12}C or ^{15}N:^{14}N ratios. Results were referred to Vienna Pee Dee Belemnite for carbon and to atmospheric N_2 for nitrogen. To describe the $\delta^{13}C$ and $\delta^{15}N$ values for all samples, the arithmetic mean ± 1 SD was used. Trophic position was calculated using the following equation: $TP_{cf} = ((\delta^{15}N_{cf} - \delta^{15}N_{baseline})/3.4)+2$ where TP_{cf} is the trophic position, $\delta^{15}N_{cf}$ is the isotopic ratio of samples, $\delta^{15}N_{baseline}$ is the isotopic ratio of primary consumers (Cabana & Rasmussen, 1996). Metrics proposed by Layman, Arrington, Montaña, and Post (2007) were calculated to quantify intra-specific and community niche width and variability: $\delta^{15}N$(NR) and $\delta^{13}C$(CR) ranges, total convex hull area enclosing all individuals (TA), mean Euclidean distance of each individual from the centroid of $\delta^{15}N$-$\delta^{13}C$ values (CD), mean nearest neighbour distance in $\delta^{15}N$-$\delta^{13}C$ space (NND), standard deviation (SDNND), and the corrected standard ellipse area (SEAc) (Jackson, Inger, Parnell, & Bearhop 2011). For *A. brama*, *C. carpio* and *S. squalus* only one specimen per species was collected and they were not included in the analysis.

Statistical analyses

To examine how the different food sources contributed to the isotopic signatures of collected individuals, Stable Isotope Mixing Models (SIMM) were used. Before applying SIMM, differences in isotopic values from different tissues were corrected following Phillips & Gregg (2003). Exploratory dual plot graphs of $\delta^{15}N$ versus $\delta^{13}C$ for consumer tissues and food sources were built to determine which potential prey could be included in the mixing model (source increments: 1%; mass balance tolerance: 0.1‰; Phillips & Gregg, 2003). Results are presented as the average percentages with their standard deviation, as well as the possible range percentage for each prey.

Pianka's index (P) (Pianka 1967) for niche overlap was estimated for both dietary and isotope data whereas patterns of niche overlap were statistically tested against a proper null model (Gotelli & Entsminger, 2011; Codron et al., 2015). RA2 (amphibians) and RA3 (fish and snakes) algorithms were used to test for non-random patterns of resource utilization (Albrecht & Gotelli, 2001; Luiselli, 2006; Vignoli & Luiselli, 2012; Vignoli, Bissattini, & Luiselli 2017). Differences in $\delta^{15}N$ and $\delta^{13}C$ signatures among species were analysed using a permutational multivariate analysis of variance (PERMANOVA) followed by pair-wise (post-hoc) tests between couples of species. A canonical analysis of principal coordinates CAP (Anderson, Gorley, & Clarke 2008) was conducted to analyse the detected differences whereas Spearman correlations between CAP axis and the original set of data were calculated to determine how the two variables affected the differences among species.

Statistical tests were performed using STATISTICA software (version 8.0 for Windows - Statsoft), and null models were built by using EcoSim software (Gotelli & Entsminger, 2011). Layman's metrics (Parnell, Inger, Bearhop, & Jackson 2010) were calculated with the R package SIAR (Stable Isotope Analysis in R). To visualize the overall network and interactions among sampled species, Cytoscape v.3.4.0 was used. PERMANOVA and CAP analysis were performed with PRIMER v. 6 (Clarke, 1993). All statistical tests were performed with two tails and alpha set to 0.05. Text gives means and standard deviation.

Results

Stomach content analysis

Overall, 38 *A. melas* (8.37±1.90 cm), 24 *C. auratus* (19.37±1.90 cm), 104 *L. gibbosus* (8.15±1.32 cm), 21 *P. parva* (6.08 ±2.38 cm), and 3 *R. rutilus* (15.73±0.17 cm) were collected. Plant material and aquatic insects were the most important food items, except for *A. melas* and *L. gibbosus* that fed also on crayfish and other fish (Appendix I). Low values of niche width were more frequent, occurring in *A. melas* (B = 0.394), *C. auratus* (B = 0.293) and *L. gibbosus* (B = 0.138), being intermediate for *P. parva* (B = 0.482) and high for *R. rutilus* (B = 0.941). Niche overlap among fish was extremely high (P_{obs}=0.53, P_{exp}=0.23, $P_{obs}>P_{exp}$ p=0.002). Plant materials and detritus mainly contributed to the diet of 33 analysed crayfish (6.64±1.78 cm), resulting in intermediate niche width values (B = 0.556) (Appendix I).

Twenty-eight stomachs from adult bullfrogs (19.15±1.14cm), 18 from juvenile bullfrogs (8.69 ±2.75 cm), and 21 from adult green frogs (6.15±1.49 cm) were collected. *P. clarkii* was the most important food item for adult bullfrogs whereas isopods mainly contributed to juvenile bullfrog and green frog diet (Appendix I). Adult bullfrogs showed lower niche width values (B = 0.137) than conspecific juveniles (B = 0.682) and green frogs (B = 0.340). Low diet overlap was found between adult and juvenile bullfrogs (P_{obs}=0.39, P_{exp}=0.51, $P_{ob}<P_{exp}$ p=0.83). Niche overlap values were high between juvenile bullfrogs and green frogs (P_{obs}=0.77, P_{exp}=0.56, $P_{obs}>P_{exp}$ p=0.04) and low between adult bullfrogs and green frogs (P_{obs}=0.08, P_{exp}=0.45, $P_{obs}<P_{exp}$ p=0<0.001).

Food resources for *N. natrix* (N=20, 71.78±9.97 cm) and for *N. tessellata* (N=11, 85.045±18.892) comprised *P. clarkii*, fish (*A. melas*, and *C. auratus*), and amphibians (*P.* kl. *esculentus*) (Appendix I). Both species *s*howed intermediate niche width values. No significant diet overlap was found between the two species (P_{obs}=0.40, P_{exp}=0.50, $P_{obs}>P_{exp}$ p=0.75). Trophic web reconstruction from stomach contents displayed a separation of the overall studied food web into two energy paths (aquatic and terrestrial) with *P. clarkii* and aquatic insects being in between (Figure 1).

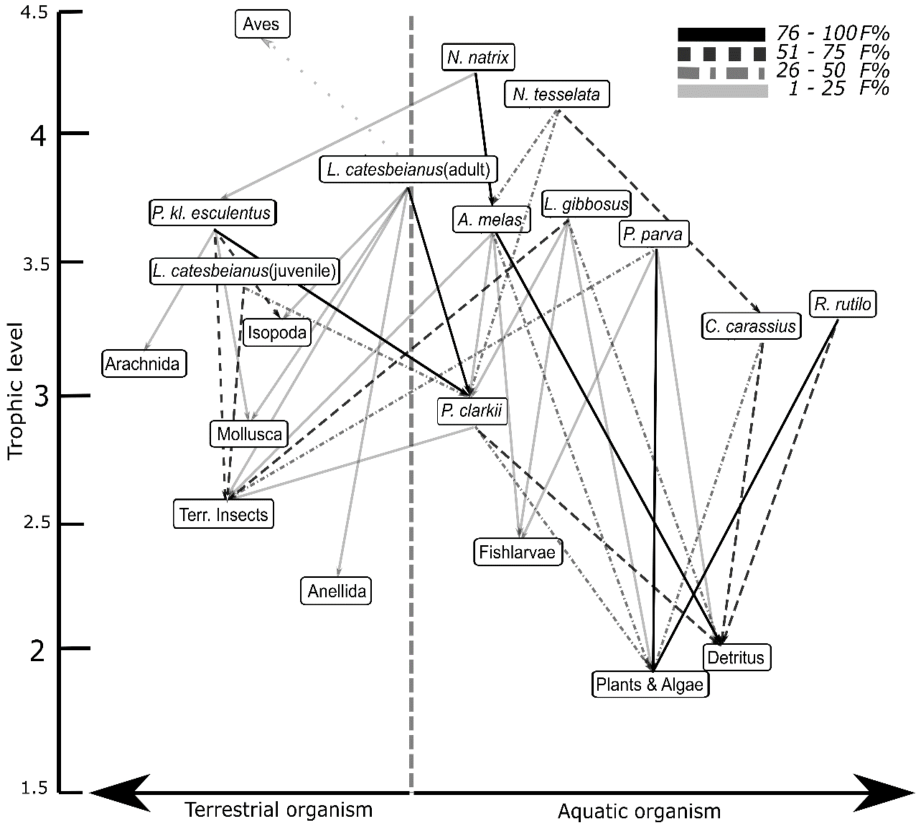

Figure 1: Trophic web obtained using stomach content of sampled species and ordered according to estimated trophic levels. Grey labels indicate taxa with trophic values; white labels show taxa. which trophic positions were not analysed and were ordered according to direct observations. The black dashed line indicates a relation with a not sampled taxon whose trophic position was estimated. Different grey tones are indicating varying percentages of frequencies found in the specific stomach.

Stable Isotopes Analysis

Results from Stable Isotope analysis and estimation of trophic positions are listed in Table 1. The application of SIMMs supported stomach content data (Table 2). Overall, five trophic levels with filamentous green algae and detritus as basal resources were identified. Primary consumers constituted the basal trophic group followed by carnivores 1 (crayfish, aquatic and terrestrial beetles), planktivorous and benthivorous fish species (*C. auratus* and *R. rutilus*) and bullfrog juveniles. Carnivores 2 included intermediate fish predators (*A. melas, L. gibbosus*, and *P. parva*), *P.* kl. *esculentus* and bullfrog adults. Carnivores 3 included top vertebrate predators *N. tessellata* and *N. natrix* (Table 2).

Table 1: Mean values (±SD) of nitrogen and carbon isotope ratios, estimated trophic position, and sample number of species collected in the study area.

SPECIES	^{15}N AIR (‰)	Trophic position	^{13}C PDB (‰)	N
Natrix natrix	10.902±1.098	4.341	-26.026±1.999	20
Natrix tessellata	10.385±1.075	4.189	-27.947±1.460	11
Lithobates catesbeianus adult	7.957±0.840	3.474	-24.394±0.662	20
Pelophylax kl. *esculentus*	7.636±0.854	3.380	-23.242±1.364	21
Lepomis gibbosus	7.526±0.433	3.348	-28.060±1.028	20
Ameiurus melas	7.318±0.300	3.286	-27.499±1.318	20
Pseudorasbora parva	6.661±0.607	3.093	-26.347±2.052	20
Lithobates catesbeianus juvenile	6.159±0.560	2.946	-22.838±0.754	10
Rutilus rutilus	5.577±0.147	2.774	-24.527± 0.823	3
Carassius carassius	5.438±0.717	2.733	-26.627±0.914	20
Terrestrial Insects	5.157±0.444	2.651	-20.813±5.037	22
Aquatic Insects	4.197± 0.323	2.368	-24.754±4.127	13
Procambarus clarkii	4.168±0.515	2.360	-24.427±2.181	21
Primary consumers	2.944± 1.105	2.000	-25.190±0.582	42
Detritus	2.700±0.315	1.928	-17.920±0.482	10
Algae	1.344±0.215	1.529	-7.145±0.380	10

Table 2: Mixing Model results for mean proportion of diet sources contributing to fish, crayfish, frog and snake diet in the study area

Ameiurus melas			***Lepomis gibbosus***		
PREY	%	RANGE	PREY	%	RANGE
Procambarus clarkii	28.7±16.1	0-59	Fish	17.8±2.3	14-22
Fish	17.8±2.5	13-22	Aquatic insects	27.9±15.2	0-58
Algae	27.7±0.5	27-28	*Procambarus clarkii*	26.9±15.9	0-59
Aquatic insects	25.9±15.6	0-58	Algae	27.4±0.5	27-28
Carassius carassius			***Pseudorasbora parva***		
PREY	%	RANGE	PREY	%	RANGE
Aquatic insects	38.6±9.1	22-54	Aquatic insects	45.5±2.4	42-49
Algae	36.4±5.8	25-46	Algae	35±0	35
Detritus	25±14.9	0-53	Fish	19.5±2.4	16-23
Rutilus rutilus			***Lithobates catesbeianus* adults**		
PREY	%	RANGE	PREY	%	RANGE
Aquatic insects	33.5±8.8	18-49	*P. clarkii*	60.7±5.6	47-73
Algae	33.4±5.7	23-44	Fish	18.6±11.2	0-42
Detritus	33.1±14.5	7-59	*P.* kl *esculentus*	13.9±8.4	0-31
			Terrestrial insects	6.8±2.9	0-13
***Lithobates catesbeianus* juveniles**			***Pelophylax* kl. *esculentus***		
PREY	%	RANGE	PREY	%	RANGE
Isopods	31±18.3	0-67	Isopods	30.7±18.1	0-67
Gastropods	27.1±16	0-59	Gastropods	26.8±15.8	0-58
Terrestrial insects	23.9±2.1	19-29	Terrestrial Insects	26.4±2	21-31
Diplopods	18±4.6	7-29	Diplopods	16.1±4.6	4-27
Natrix natrix			***Natrix tessellata***		
PREY	%	RANGE	PREY	%	RANGE
L. catesbeianus juvenile	25.8±15.2	0-54	*C. carassius*	27±11.6	0-57
A. melas	25.6±11.2	2-49	*L. gibbosus*	20.6±13.8	0-63
P. kl *esculentus*	24.4±14.3	0-51	*A. melas*	19.4±13.4	0-64
C. carassius	24.1±12.2	0-49	*P. clarkii*	16.6±8.7	0-4
Procambarus clarkii					
PREY	%	RANGE			
Detritus	28.9±17.1	0-63			
Fish	25.5±3.5	17-33			
Algae	23.5±6.7	10-35			
Aquatic insects	22.1±13.1	0-48			

PERMANOVA returned significant differences in species δ^{15}N and δ^{13}C levels, especially among top-predators (Appendix 2). CAP analysis (CAP1 and CAP2 axis squared canonical correlations equal to 0.80 and 0.34, respectively) revealed that δ^{15}N was inversely correlated with CAP1 (CAP1: δ15N rs= -0.99; δ13C rs = 0.20) whereas δ^{13}C was strongly correlated with CAP2 (CAP1: δ15N r_s= 0.05; δ^{13}C r_s = 0.98; Figure 2). The CAP plot showed that *Natrix* species were similar in δ^{15}N but differed in δ^{13}C. Bullfrog adults were similar in δ^{15}N to *P.* kl. *esculentus* but showed higher δ^{13}C levels; bullfrog juveniles were lower for δ^{15}N than both conspecific adults and green frogs but similar to *P. kl. esculentus* for δ^{13}C levels. Fish showed well defined values of isotopic signatures except for *A. melas, L. gibbosus* and *R. rutilus. P. clarkii* did not differ in isotope signatures from terrestrial and aquatic insects (Appendix 2 and Figure 2).

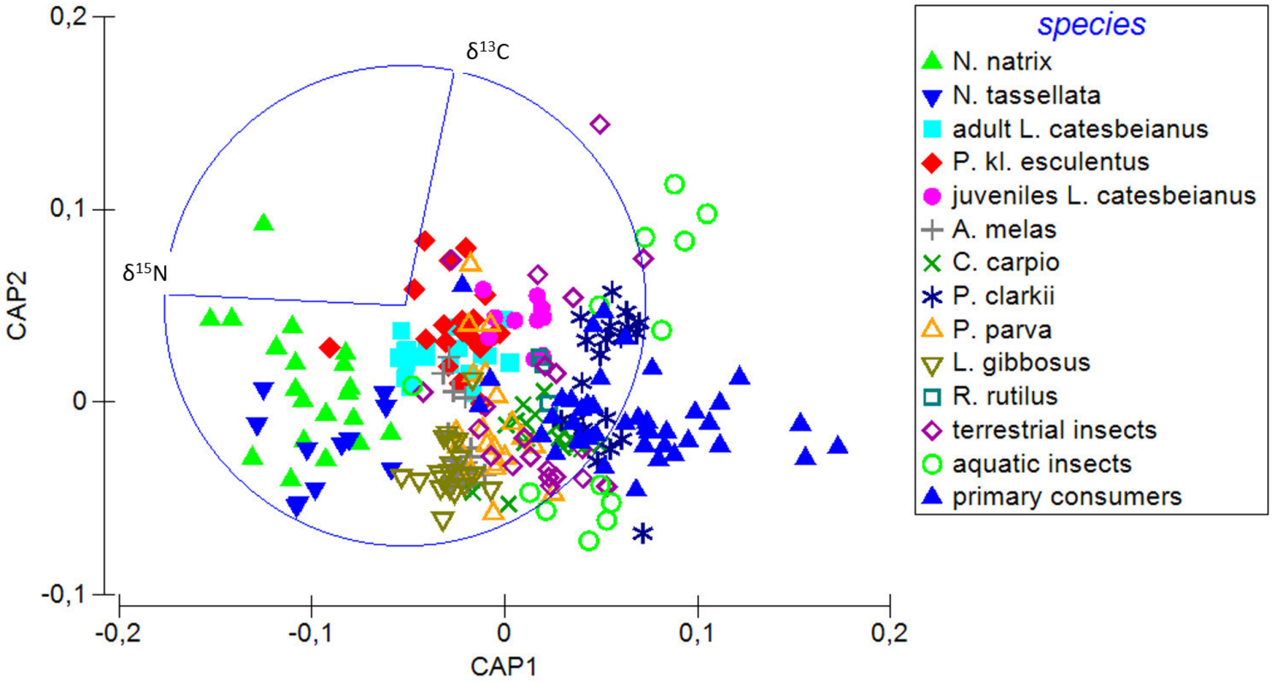

Figure 2: Two-dimensional scatter plot of the first and second principal coordinates axis (after resemblance matrix with Euclidean distance the raw matrix of data, N = 263, variables = δ^{15}N and δ^{13}C) for 'species' factor. Vectors of the linear correlations between individual variables are superimposed on the graph.

Layman's metrics showed high values indicating higher trophic diversity and low redundancy at our study site. *Natrix natrix* and *P.* kl *esculentus* showed the highest Layman's metrics values, suggesting that they (i) have a high dietary diversity (CD); (ii) feed on a great variety of basal resources (Cr) from different trophic levels (Nr); (iii) have a high niche width (TA and SEAc); (iii) high individual dispersion in isotopic space compared to *N. tessellata* and *L. catesbeianus* adults. Similar results were obtained comparing adult and juvenile bullfrogs, latter showing the lowest Layman's metrics. Among fish, the greatest TA, SEAc and CD were found for *P. parva*, while the lowest ones for *R. rutilus.* The niche width metrics for *P. clarkii* were even higher than those of *P. parva* (Table 3).

Table 3: Layman's metrics for the studied community; NR = $\delta^{15}N$ range, CR = $\delta^{13}C$ range, TA = convex hull area, CD = mean distance to centroid, MNND = mean nearest neighbour distance, SDNND = standard deviation of the nearest neighbour distance, SEAc = Standard Ellipse Area.

Species	NR	CR	TA	CD	MNND	SDNND	SEAc
Natrix natrix	4.28	8.51	18.66	1.89	0.75	0.67	6.57
Natrix tessellata	3.12	4.23	9.09	1.57	0.63	0.46	5.33
Lithobates catesbeianus adult	2.52	2.73	5.01	0.95	0.37	0.32	1.75
Pelophylax kl. esculentus	3.83	5.24	12.28	1.29	0.54	0.51	3.84
Lepomis gibbosus	2.04	4.89	5.00	0.88	0.38	0.43	1.46
Ameiurus melas	1.14	4.29	2.29	1.08	0.26	0.17	1.05
Pseudorasbora parva	2.30	8.32	8.99	1.72	0.58	0.50	3.61
Lithobates catesbeianus juvenile	1.46	2.15	2.43	0.81	0.41	0.25	1.47
Rutilus rutilus	0.29	1.52	0.04	0.64	0.60	0.62	0.15
Carassius carassius	2.81	3.98	5.54	0.94	0.33	0.24	2.06
Terrestrial insects	4.78	12.48	40.25	2.97	0.88	1.00	13.50
Aquatic insects	6.39	12.48	36.85	4.64	1.19	0.96	23.13
Procambarus clarkii	2.03	8.04	9.54	1.99	0.55	0.50	3.71
Primary consumers	8.78	6.11	32.57	1.94	0.59	0.51	7.97
Whole consumers community	7.34	5.22	23.52	2.43	1.04	0.61	11.35

Discussion

Our study demonstrated how invaders often become the main component of the food web, with the exception for top consumers represented by native species highly dependent on alien species (Strayer, 2010). Up to four IAS were present at each estimated trophic level, with the involvement of *P. clarkii* and *L. catesbeianus* being noteworthy. These IAS, interacting with each other in a predator/prey relationship, have a marginal role in affecting the entire community (Bissattini & Vignoli, 2017; Bissattini, Buono, & Vignoli, 2018a, b).

This is the first detailed study on the structure of an aquatic community dominated by invaders, except for Lake Naivasha (Kenya) (Gherardi et al., 2011). Similarly, Naivasha's food web is composed of up to three IAS at each trophic level except for top consumers represented by indigenous birds (Harper et al., 2002; Gherardi et al., 2011).

Stomach content and stable isotopes analysis

Both isotope and dietary data showed that trophic interactions are well established. Alien fish contributed the most to snake diet with N. natrix consuming also native and introduced amphibians. The species showed a limited dietary overlap confirming that aquatic snakes from temperate regions often partition prey resources and differ subtly in habitat use (Mushinsky, 1987; Luiselli, 2006).

Procambarus clarkii mostly contributed to adult bullfrog diet, as previously found in other invaded areas (Wu, Li, Wang, & Adams 2005; Liu et al., 2015; Bissattini & Vignoli, 2017; Bissattini et al., 2018a, b; Liu et al., 2018). The diet spectra of fish mostly consisted of detritus, aquatic insects and plant materials (Holopainen, Tonn, & Paszkowski, 1997; Wolfram-Wais, Wolfram, Auer, Mikschi, & Hain 1999) with the addition of crayfish and small size fish for *A. melas* and *L. gibbosus* (Leunda et al., 2008; Fobert et al., 2013). The observed high degree of dietary overlap was not surprising due to non-limiting food resources and the high trophic flexibility of the species. Even *P. clarkii* showed an opportunistic feeding behaviour by consuming detritus, plant materials and fish (probably their carrions), these latter being easy to catch and largely available due to intense water level fluctuations (Gherardi, 2006). Moreover, terrestrial food sources represented a significant portion of the diet for several species, as found in other aquatic assemblages from temperate regions (Huckembeck et al., 2014). Particularly, terrestrial invertebrates mostly contributed to juvenile bullfrog and green frog diet probably because of their similar sizes (Vignoli, Bissattini, & Luiselli 2017; Bissattini et al., 2018b) and/or the same micro-habitat preferences (D'Amore, Kirby, & McNicholas 2009; Bissattini et al., 2018b), being both collected on the pond banks and in shallow waters.

Our data also confirmed that *L. catesbeianus* undergoes ontogenetic habitat and dietary shifts (Bissattini et al., 2018b). As adults, bullfrogs heavily relied on aquatic prey, whereas as juveniles, they exploited several food resources, mainly of terrestrial origin, confirming the tendency of the species to have a less generalized feeding during growth (Diaz De Pascual & Guerrero, 2008; Bissattini et al., 2018b). Amphibians are well-known to be gape-limited predators (Macale, Vignoli, & Carpaneto, 2008; Vignoli et al., 2017) and ontogenetic shifts in prey size have been demonstrated in many species (Trakimas et al., 2011; Bishop, Drewes, & Vredenburg 2014).

Concerning SIA, five trophic levels were estimated with snakes as top-predators, which suggests a short food chain length (Post, 2002; Kupfer et al., 2006). However, an additional trophic level could likely occur which includes medium-sized mammals and birds capable of preying on both snakes and frogs (Kupfer et al., 2006). The food chain length seemed to be correlated to the relatively small extension and the characteristics of the study site, which is a 'fluctuating environment', often subjected to marked seasonal variations in hydroperiod due to inconstant rainfall (Post, Pace, & Hairston, 2000; Kupfer et al., 2006).

As expected, *N. tessellata* and *N. natrix* were reported as top-predators since ephemeral freshwater wetlands often show simplified food webs dominated by reptiles due to the recurrent drying-up events (Gibbons et al., 2006). Bullfrog adults and green frogs were defined as intermediate predators because of their considerable role in energy flow and biomass conversion (Pough, 1980; Stewart & Woolbright, 1996). Interesting is the double role of bullfrog life stages: the species increases the trophic position during the life cycle, linking terrestrial and aquatic food webs and affecting prey communities in both habitat types (Whiles et al., 2006; Trakimas et al., 2011; Huckembeck et al., 2014; Bissattini et al., 2018b). A significant niche overlap was found between *P.* kl. *esculentus* and bullfrog juveniles although they occupy close but different trophic positions (Bissattini et al., 2018b). However, diet-tissue fractioning can vary among species and upon transition to adulthood (Martínez del Rio, Wolf, Carleton, & Gannes 2009; Trakimas et al., 2011), being affected by body size, growth rate and protein turnover (Martínez del Rio et al., 2009; Murray & Wolf, 2013).

Ameiurus melas, *L. gibbosus*, and *P. parva* confirmed their invertivorous feeding habit as already observed in the Tiber river (Tancioni, Baldari, Ferrante, Scardi, & Mancini, 2001). Such species represented an essential intermediate link in the food chain by providing a continuum between higher and lower trophic levels being both predators and prey (Berra, 2001). *Carassius auratus* and *R. rutilus* confirmed their planktivorous and benthivorous habits selecting food in sediments and ejecting all but the retained food particles back into the water column (Richardson, Whoriskey, & Roy 1995). Aquatic and terrestrial insects played an important role in the food web as a main link between primary and secondary consumers (Pizzolotto, 1993). *Procambarus clarkii* played a multifunctional role, as they were probably most important as shredders, scavengers and prey for other predators from higher trophic levels (Momot, 1995). The present study suggests that top-consumers, by preying on IAS, do not exert top-down control on the food web through predation and competition, especially on and with native species (Duffy, 2002). Our finding agrees with Polis and Strong (1996) who suggested that bottom-up and top-down effects in communities are lost when allochthonous resources occur at both the basal and the top consumer levels. In this context, snakes partitioned the available food sources, with *N. tessellata* specialized on alien fish, and *N. natrix* preyed also on amphibians (Luiselli, Filippi, & Capula, 2005; Luiselli et al., 2007). The exploitation of alien fish and juvenile bullfrogs by *N. natrix* may also mitigate the predatory pressure of the species on native amphibians that usually represent its elective prey. Similarly, bullfrog adults, by consuming *P. clarkii,* may reduce their predatory impact on native amphibians (Wu et al., 2005; Liu et al., 2015, 2018; Bissattini et al., 2018a, b). In this regard, it would be interesting to evaluate if *P. clarkii* could also consume bullfrog eggs, larvae and tadpoles.

However, we cannot exclude the competition impact of juvenile bullfrogs on green frogs (Stewart & Sandison, 1972; Werner, Wellborn, & McPeek 1995; Bissattini et al., 2018b). Indeed, resource-limited conditions, likely occurring when our study ponds periodically dry up, may increase the trophic niche overlap between juvenile bullfrogs and green frogs (Kuzmin, 1995; Bissattini et al., 2018b), affecting their coexistence in the long term, resulting in green frog niche displacement, reduction in fitness, or even extinction at local scale (Kats & Ferrer, 2003; D’Amore et al., 2009).

It should be acknowledged that this study provided only a snapshot of the structure of the community from a trophic point of view, neither predicting how the current situation will evolve in the future as (i) the species composition may shift towards those not vulnerable to invaders or other invaders can enter the assemblage, (ii) effects of invaders may accumulate through time, or (iii) invaders may interact with others producing effects that change over time (Strayer, 2010).

Conclusions

Our study suggests that IAS occurring in keystone positions may not strongly influence the structure of the whole community when interacting with each other (Polis & Strong, 1996). However, we still ignore the long-term ecological and evolutionary feedbacks between IAS and the invaded communities (Strayer, 2010). Thus, large efforts should be devoted to deeply investigate multiply invaded communities to fully understand the impacts of invaders and to plan successful managements of IAS and the conservation of native ones (Nyström et al., 2001; Gherardi et al., 2011).

Author contributions

All the authors contributed critically to the different phases of the research and to the writing of this paper and gave final approval for publication. The authors report no potential conflicts of interest.

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Supplement I. The percent number and frequency of occurrence (in parentheses) of prey taxa found in fish, crayfish, frogs and snakes collected at the study site. a. = adults; j. = juveniles; l. = larvae; und. = undetermined.

	Ameiurus melas	***Carassius carassius***	***Lepomis gibbosus***	***Pseudorasbora parva***	***Rutilus rutilus***	***Procambarus clarkii***	***Lithobates catesbeianus* a.**	***Lithobates catesbeianus* j.**	***Polyphylax esculentus***	***Natrix tessellata***	***Natrix natrix***
PREY	%N	%N	%N	%N	%N	%N					
Coleoptera	8.09 (22.50)	0	1.59 (3.85)	0	0	13.16 (15.15)	6.82 (8.70)	15.39 (25.00)	12.25 (30.77)	0	0
Diptera	30.64 (42.50)	0	58.20 (39.42)	36.11 (25.00)	0	7.90 (9.09)	0	0	2.04 (7.69)	0	0
Diptera l.	0	0	0	0	0	0	0	2.56 (6.25)	0	0	0
Ephemero	0	0	1.06 (1.92)	0	0	0	0	0	0	0	0
Hemiptera	0	0	0	0	0	0	0	0	4.08 (15.38)	0	0
Hymenopt	0	0	0.53 (0.96)	0	0	0	4.55 (4.35)	5.13 (12.50)	20.41 (23.08)	0	0
Lepidopter	0	0	0	0	0	0	2.27 (4.35)	2.56 (6.25)	4.08 (7.69)	0	0
Lepidopter	0	0	0	0	0	0	0	10.26 (25.00)	2.04 (7.69)	0	0
Mecoptera	0	0	0	0	0	0	0	0	2.04 (7.69)	0	0
Orthoptera	0	0	0	0	0	0	0	5.13 (12.50)	0	0	0
Eggs und.	1.73 (5.00)	0	2.12 (3.85)	2.78 (6.25)	0	0	0	0	0	0	0
Larvae	0.58 (2.50)	0	2.65 (4.81)	0	0	2.63 (3.03)	0	0	0	0	0
Araneae	0	0	0	0	0	0	0	7.69 (18.75)	6.12 (23.08)	0	0
Pulmonata	0	0	0	0	0	0	6.82 (13.04)	15.39 (31.25)	2.04 (7.69)	0	0
Isopoda	0	0	0	0	0	0	2.27 (4.35)	23.08 (31.25)	44.9 (61.54)	0	0
Decapoda	2.89 (12.50)	0	2.12 (3.85)	0	0	0	70.46 (95.65)	10.26 (25.00)	0	25 (9.09)	0
Haplotaxid	0	0	0	0	0	0	2.27 (4.35)	0	0	0	0
Julidae	0	0	0	0	0	0	0	2.56 (6.25)	0	0	0
Anura a.	0	0	0	0	0	0	0	0	0	0	20 (20.00)
Adults	0.58 (2.50)	0	2.12 (3.84)	2.78 (6.25)	0	0	0	0	0	0	0
Eggs	1.73 (5.00)	0	1.06 (0.96)	0	0	0	0	0	0	0	0
Cyprinidae	0	0	0	0	0	0	0	0	0	50 (18.18)	0
Ictaluridae	0	0	0	0	0	0	0	0	0	25 (9.09)	80 (5.00)
Aves	0	0	0	0	0	0	4.55 (8.70)	0	0	0	0
Algae	7.51 (30.00)	13.04 (12.50)	1.59 (2.88)	36.11 (81.25)	28.57	7.90 (12.12)	0	0	0	0	0
Plant	9.83 (32.50)	8.70 (8.33)	3.70 (6.73)	16.67 (37.50)	42.86	21.05 (24.24)	0	0	0	0	0
Plant seeds	18.50 (2.50)	0	0	0	0	13.16 (9.09)	0	0	0	0	0
Detritus	17.92 (77.50)	86.96 (83.33)	23.28 (42.31)	5.56 (12.50)	28.57	34.21 (39.39)	0	0	0	0	0

Supplement 2. Results of the pair-wise (post hoc) test between couples of levels of factor 'species', after PERMANOVA main test. Grey cells label significant differences between the two levels (row and column).

	Primary consumers	P.clarkii	Aquatic insects	Trrestrial insects	C. carpio	R.rutilus	juveniles L. catesbeianus	P. parva	A. melas	L. gibbosus	P. kl. esculentus	adult L. catesbeianus	N. tassellata
P. clarkii	t=2,035; p=0,021												
Aquatic insects	t=1,242; p=0,205	t=0,008; p=1											
Terrestrial insects	t=2,818; p=0,002	t=1,849; p=0,052	t=1,158; p=0,265										
C. carpio	t=4,005; p=0,001	t=4,521; p=0,001	t=2,183; p=0,029	t=1,479; p=0,127									
R.rutilus	t=1,667; p=0,081	t=1,063; p=0,302	t=0,471; p=0,72	t=0,410; p=0,803	t=2,995; p=0,005								
juveniles L. catesbeianus	t=5,002; p=0,001	t=3,430; p=0,001	t=1,577; p=0,123	t=2,263; p=0,037	t=9,092; p=0,001	t=2,946; p=0,005							
P. parva	t=5,260; p=0,001	t=4,594; p=0,001	t=2,485; p=0,007	t=1,580; p=0,111	t=2,306; p=0,012	t=1,670; p=0,09	t=4,971; p=0,001						
A. melas	t=7,654; p=0001	t=7,564; p=0,001	t=3,736; p=0001	t=3,220; p=0,002	t=5,199; p=0,001	t=4,242; p=0,001	t=10,047; p=0,001	t=2,342; p=0,021					
L. gibbosus	t=8,682; p=0,001	t=8,875; p=0,001	t=4,272; P=0,001	t=3,961; p=0,001	t= 7,030; p=0,001	t=5,967; p=0,001	t=13,122; p=0,001	t=3,554; p=0,001	t=1,479; p=0,134				
P. kl. esculentus	t=8,325; p=0,001	t=6,087; p=0,001	t=3,120; p=0,001	t=3,500; p=0,002	t=9,170; p=0,011	t=2,530; p=0,007	t=2,774; p=0,004	t=5,524; p=0,001	t=9,177; p=0,001	t=11,094; p=0,001			
adults L. catesebeianus	t=8,384; p=0,001	t=6,851; p=0,001	t=3,285; p=0,002	t=3,114; p=0,002	t=9,532; p=0,001	t=3,66; p=0,001	t=5,963; p=0,001	t=4,381; p=0,001	t=8,223; p=0,001	t=10,679; p=0,001	t=2,792; p=0,002		
N. tassellata	t=9,685; p=0,001	t=9,105; p=0,001	t=4,461; p=0,001	t=4,735; p=0,001	t=9,602; p=0,001	t=5,361; p=0,001	t=10,355; p=0,001	t=5,31; p=0,001	t=5,409; p=0,001	t=5,461; p=0,001	t=8,719; p=0,001	t=8,354; p=0,001	
N. natrix	t=11,824; p=0,001	t=9,800; p=0,001	t=5,394; p=0,001	t=5,737; p=0,001	t=9,604; p=0,001	t=4,090; p=0,001	t=7,556; p=0,001	t=6,081; p=0,001	t=6,536; p=0,001	t=6,942; p=0,001	t=6,992; p=0,001	t=5,978; p=0,001	t=2,487; p=0,011

Chapter 3.4

The Arno River in Florence, Italy: trophic analysis of a species assemblage dominated by alien species

Short title: Invasive species in the Arno river

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Abstract

The interactions occurring between species in multiply invaded freshwater ecosystems are difficult to investigate due to the covering nature of aquatic ecosystems. Especially for ecosystems, where the species community is exclusively composed of alien species, studies on interactions and eventual facilitation and hindering processes mostly focus on single species. Furthermore, information is even less available for ecosystems that have no native species. To investigate such an ecosystem, the species community in a high anthropogenically disturbed stretch of the Arno river was examined using the combined approach of stable isotope analyses incorporating dietary information. As a result, we show that the alien species established in the Arno river formed a unique pyramid shaped community with the European catfish *Silurus glanis* in the apex position, followed by primary predatory fish (*Ictalurus punctatus, Lepomis gibbosus*) as well as *Alburnus alburnus* and secondary consumer fish species (*P. parva, Padogobius* sp.). Moreover, these species feed on a variety of primary producers (*Myriphyllum* sp., *Potamogeton* sp., *Phragmites australis*) and primary consumers (*Dikerogammarus villosus*, *Procambarus clarkii*, *Cyprinus carpio*, *Palaemon* sp., etc.). Moreover, while some species compete with each other, others show mechanisms of avoidance., Especially the situation of *I. punctatus* is interesting, with juveniles occupying a higher trophic position than mature individuals. Furthermore, we highlight the value in using different baselines to estimate trophic positions and how the variable nature of interactions between alien species from the same or different geographic ranges underlines the need for assessments on a case by case basis.

Keywords: stable isotopes, community structure, alien fish assemblage, competition avoidance, mixing models, feeding ecology

In prep.

Introduction

The invasive potential distribution of an alien species depends on various abiotic and biotic factors such as its life history (Rejmánek 1996; Rejmánek & Richardson 1996; Kolar and Lodge 2001; Keller et al. 2007; Strayer 2010; Blackburn et al. 2011), predator-prey dynamics, adaptability and interactions with other species (Ricciardi 2003; Strayer et al. 2006; Ricciardi et al. 2013). The associated and intentional allopatry and following extensive dispersal of alien species changes the identities of entire species communities (Seebens et al. 2017). Due to effects across all levels of the recipient trophic web (Huxel et al. 2002; Strayer 2010). In the case that multiple invasive species are dominating one freshwater ecosystem, they tend to occupy different trophic levels (Gamradt and Kats 1996; Kiesecker and Blaustein 1998). But the presence of invasive species interacting across trophic levels increases the already-existing complex threat through direct and indirect effects (Peckarsky and McIntosh 1998), as the prevalence of omnivores (Pringle and Hamazaki 1998), and interactions among predators (Sih et al. 1998) are unforeseeable. These complex interactions among native and invasive species, foremost interactions among invasive species are not well understood (Parker et al. 1999; Huxel et al. 2002; Strayer 2010; Bissattini & Haubrock et al. 2018; Haubrock et al. 2018a) and as described in the “invasional meltdown hypothesis” by Simberloff and Von Holle (1999), can affect the chance of a species’ establishment success and increase the impact on the recipient environment (Simberloff and Von Holle 1999; Simberloff 2006) while in certain cases lowering the impact of predatory invasive species on native species (Soluk and Collins 1988a, b; Soluk 1993; Rosenheim 1998; Bissattini and Haubrock et al. 2018). Nonetheless, theoretical assessments of altered food chains are rare or only anecdotal (Polis and Strong 1996; Rosenheim 1998).

In the field of Invasion Biology, Stable Isotopes Analyses (SIA) proved to be a useful tool to investigate invasive species and their interaction with native biota (Vander Zanden et al. 1999a, b; Balzani et al. 2016). In particular, SIA proved to be a useful tool in investigating the impacts of non-native fish (Cucherousset et al. 2012).With the long-term and time-mediated information given by SIA of carbon and nitrogen, a snapshot of a community trophic structure can be taken (Boecklen et al. 2011; Layman et al. 2012; Middleburg 2014), trophic levels can be estimated (Post 2002), and feeding ecological niches finely quantified (Newsome et al. 2007). While carbon signatures identify the major energy sources, nitrogen signatures relate to the trophic position within a food web (Fry 2006; Layman et al. 2012). This relationship relies on predictable changes in the isotopic signal from prey to consumer, being enriched of 1‰ for C and 2.5-5‰ for N between consecutive trophic levels (Post 2002; Vanderklift and Ponsard 2003). Combined with the analysis of dietary contents, which provide a direct short temporal insight into the feeding habit of a species, relationships among species can be investigated (Bissattini and Haubrock et al. 2018).

The ichthyofauna of indigenous freshwater in Italy is composed of 48 taxa (Zerunian 2004). To these, several alien species were added. While some, such as the common carp *Cyprinus carpio*, are considered naturalized, many others are not, like the European catfish *Silurus glanis* that is object of local management efforts (Gualtieri &

Mecatti 2005). Overall, the number of alien freshwater species increased in the last 25 years to now 38 species. In the Arno river, species introductions occurred repeatedly, such as in the case of *Blicca bjoerkna*, *Rhodeus sericeus*, *Ictalurus punctatus* and *Silurus glanis* (Nocita 2002, 2007), species that are now naturalized (Nocita & Zerunian 2007). Moreover, anthropogenic effects have rendered native predators (e.g. *Esox luscious*) locally extinct (Nocita & Zerunian 2007). Multiply invaded freshwater ecosystems pose interesting research subjects, as they are characterized by unique species interactions and diverse introduction histories, and possibly their communities could be composed by ecologically vicariant species. Studies on these interactions and how they affect species' impacts are still scarce (but see Johnson et al. 2008; Gherardi et al. 2011; Bissattini & Haubrock et al. 2018), but are of utmost importance, as an increased understanding would be valuable for management and control efforts.

The aims of this work were: i) combining SIA, mixing models and dietary analyses to highlight feeding preferences and exerted pressure by predatory species; ii) compare species' trophic niches to infer the potential degree of feeding competition; iii) unravel important functional roles of organism (as pointed out for energy sources in Bissattini and Haubrock et al. 2018 for *P. clarkii*). This integrative approach presented not only insights into trophic interactions but will also help to improve our ability to understand interactions among invasive species and their impacts on surrounding freshwater communities.

Materials and Methods

Study area

After the Tiber, the 241 km long Arno river is the second most important river in Central Italy, with a drainage of more than 8200 km² and an effluent flow of 100 m²/s. It is the most important freshwater river in Central Italy after the Tiber river (see Nocita & Zerunian (2007) for detailed information on the species community). Furthermore, it is flowing through the major cities Florence and Pisa, and is anthropogenically influenced and sectioned by weirs, highly affecting the flow and limiting its connectivity. The sampling was conducted from April to June 2018, in a very influenced and for recreational angling used part of the inner-city (Florence) section of the river Arno (43.765606, 11.268234; DATUM WGS84).

Sampling

To collect a variety of species, a combined approach of several methods was applied. Fish were caught with standard fishing rods and the help of local fisherman using a variety of baits. Macroinvertebrates and frog tadpoles were collected using funnel traps and hand nets. Samples of the local aquatic vegetation were also taken as they represent the baseline of the ecosystem, making our results comparable with other studies. Sampled fish specimens were immediately euthanized with gil cuts and stored on ice before being transported for further processing. Overall, 232 specimens belonging to 9 fish species, 71 invertebrates from at least 6 species, 4 green frog tadpoles, and 13 samples from 3 different plant species (2 hydrophytes: *Potamogeton* sp. and *Myriophyllum* sp., and 1 helophyte: *Phragmites australis*) were collected. All sampled species and number of specimens, as well as means of morphological measures are listed in Table 1. Due to the rarity of both *Tinca tinca* and *Barbus barbus* in the study site, only one sample each was caught and included into the dataset to roughly indicate this species positioning in the community. However, these samples were not used for any statistical analysis.

Table1: List, mean length in cm and weight in g (+SE) as well as number (n) of sampled specimens for all collected species.

Species	Length [cm]	Weight [g]	Sex ratio [f:m]	n
Fish				
Silurus glanis	27.1 ± 1.06	122.84 ± 14.39	1:1	39
Ictalurus punctatus	33.82 ± 2.20	538.45 ± 106.27	1:1	40
Cyprinus carpio	31.2 ± 9.9	584.3 ± 506.7	3:1	4
Tinca tinca	9.8	11	-	1
Barbus barbus	17	36	-	1
Padogobius sp.	4.4 ± 0.1	2.3 ± 0.5	-	4
Lepomis gibbosus	9.1 ± 2.9	20.0 ± 16.6	1:1	20
Pseudorasbora parva	8.0 ± 1.3	4.8 ± 2.3	-	16
Alburnus alburnus	9.4 ± 1.0	7.3 ± 2.5	-	20
Crustaceans				
Procambarus clarkii	6.5 ±2.4	26.2 ± 1.7	1:1	10
Palaemon sp.				16
Dikerogammarus villosus				11
Macroinvertebrates				
Odonata				8
Ephemeroptera				9
Amphibians				
P. kl.*esculentus* (tadpoles)				4
Molluscs				
Gyraulus chinensis				9
Plants				
Potamogeton sp.				6
Phragmites australis				5
Myriophyllum sp.				2

Laboratory procedure

For all fish species, total length (TL) weight and, where possible, also sex was recorded. Length of fish and carapace total length (CTL) of crayfish were measured with a calliper (accuracy: 0.01 mm) and body mass (BM) was measured using an electronic balance (accuracy: 0.01 g) (see Table 1). Sampled species were collected under the consideration of an even spatial distribution across the sampled stretch of the river ecosystem (Wilson et al. 2010). After defrosting, stomachs were extracted, and prey items identified to the lowest possible taxon under a stereomicroscope. For isotopic analyses, muscle tissue was cleaned from fat, skin, scales, bones and carapace (for crayfish). For plants, a 5-10 cm leaf was cut off. Due to their small size, insects, crustaceans and tadpoles were treated as whole samples and for molluscs, only the soft body tissue was analysed without the shell. Samples were placed on separated glass trays and dried for 48 h in an oven at 60 °C. Once dry, all samples were grinded into fine powder with an agate mortar and pestle. For each sample of every samples species, two replicates of each 0.20-0.30 mg (respectively 1.00 – 1.10 mg for plants) were weighed on a Mettler Toledo AG245 microscale and enclosed into a tin capsule to be analyzed in continuous flow – isotope ratio mass spectrometry (CF-IRMS) with a Thermo FlashEA 1112 elemental analyser and a Thermo Finnigan Delta Plus isotope ratio mass spectrometer at Istituto di Geologia Ambientale e Geoingegneria (IGAG) of the National Research Council (CNR) in Montelibretti, Rome. Isotope compositions were expressed as ‰ with the δ notation (based on $\delta^{13}C$ or $\delta^{15}N= ((R_{sample}/R_{standard}) - 1) \times 1000$, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios). Results were referred to Vienna Pee Dee Belemnite for carbon and to atmospheric N_2 for nitrogen, with laboratory standards calibrated on IAEA international standards: N1, N2 and USGS25 for nitrogen and CH6, CH7 and USGS24 for carbon.

Statistical analyses

The arithmetic mean ± SE was calculated, and eventual correlations between $\delta^{15}N$ and $\delta^{13}C$ values and TL and BM measures were tested for each species using a linear model. The trophic positions of all species (TP_c) were calculated by applying the equation $TP_c= ((\delta^{15}N_c - \delta^{15}N_{base})/\Delta N) +\lambda$, where $\delta^{15}N_c$ is the mean $\delta^{15}N$ of the consumer, $\delta^{15}N_{base}$ is the mean $\delta^{15}N$ of the baseline, ΔN is the standard enrichment of 3.4 ‰ between trophic levels and λ is the basal trophic level (Post 2002; Britton et al. 2018). The TP of all species was calculated using each of the three as baseline collected plant species (λ=1) separately. Then, the final TP was calculated using the mean of the three TPs. Additionally, the TP was calculated using primary consumers (aquatic molluscs, λ=2) as baseline (Post 2002; Britton et al. 2018). To quantify intra-specific and community niche width, Layman metrics (Layman et al. 2007) were calculated with the R-package SIAR (Stable Isotope Analysis in R; Parnell et al. 2010). Additionally, the corrected standard ellipse areas (SEAc) and the corresponding 95% prediction ellipses (PE) for all species were calculated (Jackson et al. 2011).

The application of scatter plot for $\delta^{15}N$ versus $\delta^{13}C$ of consumer tissues and food sources enabled the determination of probable prey sources and combinations of prey contributing to the diet of predators (Phillips and Gregg 2003). Dietary analyses (DA) for catfish species (*I. punctatus* (mature and immature), *S. glanis*) were previously performed (Haubrock et al. 2018b), while DA for *L. gibbosus* was performed following the same protocol (Haubrock et al. 2018c) and are listed in Supplement 1. Stable isotope mixing models (SIMM; R package “SIMMr”) were applied for all fish and crayfish without consideration of priors but under consideration of potentially predated items, i.e. a predation on lower trophic levels. Thus, it was investigated how sampled species contribute to the isotopic signatures of each other, indicating the scenario with the highest probability (Parnell et al. 2013). Additionally, the same analyses were performed again for *L. gibbosus*, *I. punctatus* and *S. glanis* with results from DA as priors for the analyses. Results are presented as the average percent values with the possible range percentage for each prey item. With these, the trophic web of the studied ecosystem was reconstructed. Additionally, using the software PRIMER (Clarke & Warwick 2001) a Multi-Dimensional Scaling plot (MDS) was drawn and a Permutational Analysis of Variance (PERMANOVA;

Resemblance: Euclidean distance; Sums of squares type: Type III (partial); Fixed effects sum to zero for mixed terms; Permutation method: Unrestricted permutation of raw data; Levels: 18; Number of permutations: 999) was performed to test for significant differences in $\delta^{15}N$ and $\delta^{13}C$ among species and to eventually determine niche overlap between species.

Results

Correlations between stable isotope values and morphological measurements are shown in Supplement 2, however, statistically significant negative correlations were found between TL and $\delta^{13}C$ for *S. glanis* ($r_s = -0.512$; $p = 0.001$; $n = 39$), *I punctatus* ($r_s = -0.665$; $p < 0.001$; $n = 40$) as well as *P. clarkii* ($r_s = -0.693$; $p = 0.026$; $n = 10$), and a statistically significant positive correlation between these variables for *P. parva* ($r_s = 0.567$; $p = 0.022$; $n = 16$). Additionally, a statistically significant positive correlation between TL and $\delta^{15}N$ was found for *L. gibbosus* ($r_s = 0.917$; $p < 0.001$; $n = 20$).

Following the assumption of an isotopic enrichment of $\delta^{15}N$ by about 3.4 ‰ (Post 2002), the total community spanned over approximately 3 to 4 trophic levels, depending on the used method. Considering that molluscs are commonly used as baseline (1) (e.g. Xu et al. 2011), provided a higher estimation of TL in respect to that from baselines estimated using plants (2). The latter approach of using plants provided a three level community structure, with the 3° level represented by the two groups of *I. punctatus*, *S. glanis*, *L. gibbosus* and *A. alburnus*, the 2° level would be build-up by *C. carpio*, *B. barbus*, *Padogobius sp*., *P. parva*, *P. clarkii* and *Palaemon sp.* and the 1° level being comprised by *T. tinca*, *D. villosus*, molluscs, Odonata and Ephemeroptera. Finally, a negative TP respect to plants was recorded for tadpoles. However, the approach based on molluscs provided a more structured hierarchy. The 4° apex level was constituted by *S. glanis* and *I. punctatus* juveniles; the 3° level composed by *I. punctatus* adults, *C. carpio*, *B. barbus*, *Padogobius sp*., *L. gibbosus*, *P. parva*, *A. alburnus*, *P. clarkii* and *Palaemon*; the 2° level composed by *T. tinca*, *D. villosus*, molluscs, Odonata and Ephemeroptera larvae. This approach revealed the lowest value for frog tadpoles and highlights the lower values of *A. alburnus* in respect to *P. parva*.

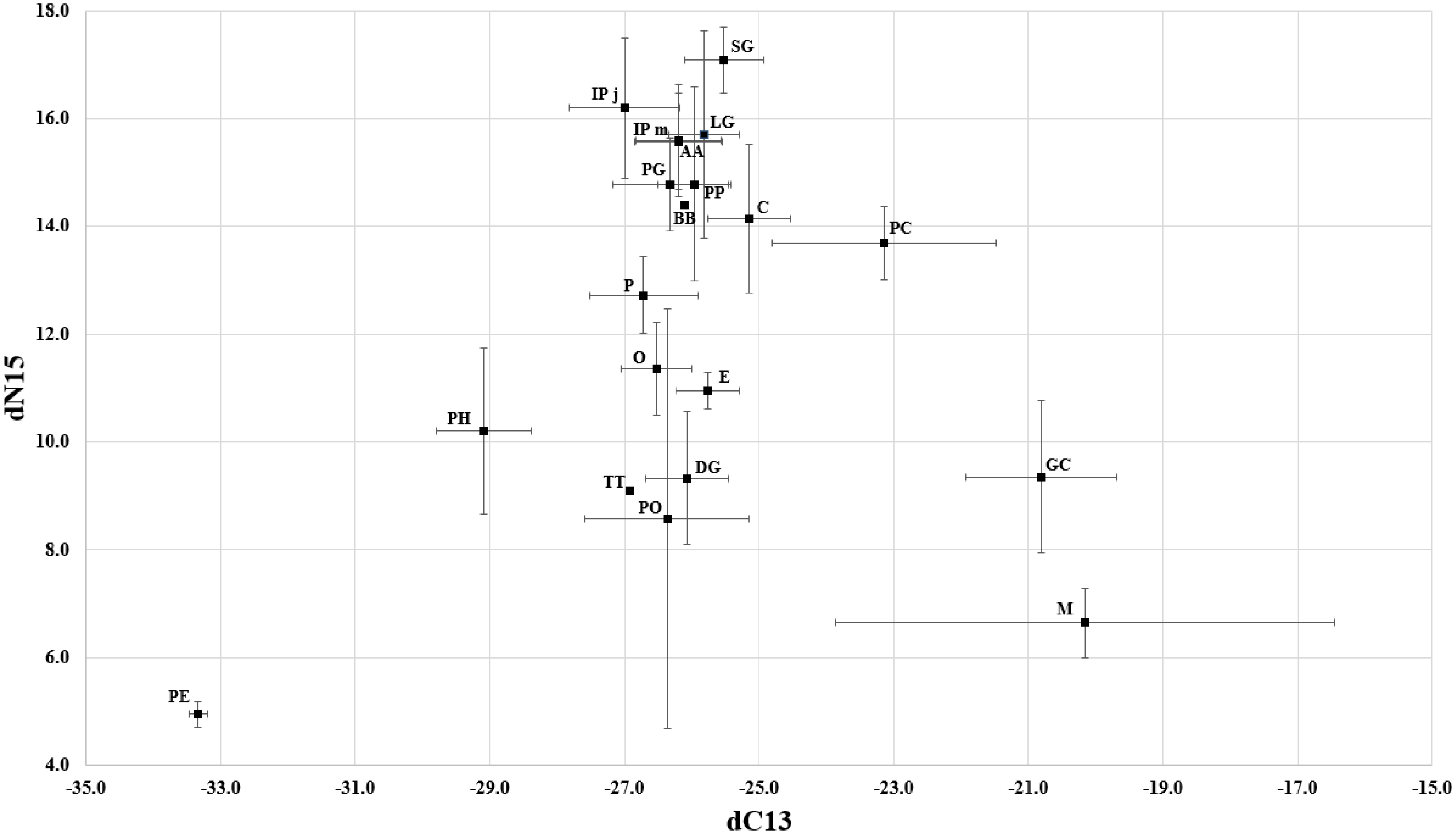

Figure 1: Isotopic space scatterplot of sampled species; Species IDs: SG = *S. glanis*; IPj = *I. punctatus* (juveniles); IPm = *I. punctatus* (matures); LG = *L. gibbosus*; AA = *A. alburnus*; PG = *Padogobius* sp.; PP = *P. parva*; BB = *B. barbus*; C = *C. carpio*; PC = *P. clarkii*; P = *Palaemon* sp.; O = Odonata; E = Ephemeroptera; DG = *D. villosus*; GC = *G. chinensis*; PH = *P. australis*; PE = *P.* kl. *esculentus* (tadpoles); TT = *T. tinca*; M = *Myriophyllum* sp.; PO = *Potamogeton* sp.

Layman's metrics are shown in Table 2. Among fish species, the greatest N-range (NR) was found for *P. parva* (7.51), followed by *L. gibbosus* (6.38) and adults of *I. punctatus* (4.60), while the narrowest values were presented by *Padogobius* sp. (1.79) and *S. glanis* (2.71). CR was largest for *A. alburnus* (3.24) and smallest for *C. carpio* (1.32). TA was highest in *P. parva* (9.64), followed by *I. punctatus* juveniles (8.76) and *L. gibbosus* (7.81), and was narrowest in *Padogobius* sp. (0.13) and *C. carpio* (1.53). SEAc, which is less influenced by extreme values, was highest in *L. gibbosus* (3.33), immediately followed by *P. parva* (3.27) then juveniles of *I. punctatus* (2.20); the lowest values of SEAc were fond for *Padogobius* sp. (0.17), *S. glanis* (0.87) and *C. carpio* (1.72). However, the small metrics for *Padogobius* sp. and *C. carpio* have to be considered with caution, due to the small number of collected individual (n=4 for both species). Among crustaceans, *D. villosus* had the highest NR (4.40), followed by *Palaemon* (2.11) and *P. clarkii* (2.07), while CR followed the exactly opposite hierarchy*: P. clarkii*=3.96, *Palaemon*=2.95 and *D. villousus*=1.90. TA was comparable between *P. clarkii* and *D. villosus* (4.60 and 4.49, respectively), while generally smaller in *Palaemon* (3.38). In contrast, SEAc was highest for *P. clarkii* (3.59), followed by *D. villosus* (2.36), then *Palaemon* (1.39). Finally, tadpoles showed the lowest Layman's metrics of the whole community.

The SEAc as well as the due to the consideration of 95% instead of 40% of data plots generally greater ellipse overlap values were estimated for all fish species with n > 5 as well as *P. clarkii* and are listed in Table 3. Considerable SEAc overlap was identified between juvenile *I. punctatus*, *A. alburnus* (0.50) and *L. gibbosus* (0.47) as well as between *A. alburnus* and *P. parva* (1.33). Moreover, it was generally low among all other fish species, while *P. clarkii* showed no relevant overlap with any fish species. Moreover, the estimated ellipse overlaps differed, as they indicated considerable overlap between life stages of *I. punctatus* (0.42), *P. parva* and *L. gibbosus* (0.59) and that minor overlap values were present between all species. Also, the ellipse overlaps between *A. alburnus* and *P. parva* was estimated as 0.46, lower than the SEAc overlap. Lastly, while ellipse overlap identified low overlap values between mature *I. punctatus* and *S. glanis* (0.09), *L. gibbosus* (0.17) and *S. glanis* (0.05), SEAc overlap did not identify them.

Tab 2. Mean values of nitrogen and carbon isotope ratios, estimated trophic positions (TP), Layman's metrics and sample number of species collected. The * indicates species not sufficiently represented for Layman's metrics estimations. NR = $\delta^{15}N$ range, CR = $\delta^{13}C$ range, TA = convex hull area, CD = mean distance to centroid, MNND = mean nearest neighbour distance, SDNND = standard deviation of the nearest neighbour distance, SEAc = Standard Ellipse Area.

SPECIES	**TP plants**	**TP molluscs**	**$\delta^{15}N$ (‰)**	**$\delta^{13}C$ (‰)**	**NR**	**CR**	**TA**	**CD**	**MNND**	**SDNND**	**SEAc**	**n**
Ictalurus punctatus (Immature)	3.3	4.0	16.2	-26.2	4.12	2.96	8.76	0.94	0.46	0.49	2.20	20
Ictalurus punctatus (Mature)	3.1	3.9	15.6	-27.0	4.60	1.96	5.39	1.06	0.43	0.25	1.84	20
Silurus glanis	3.5	4.3	17.1	-25.5	2.71	2.82	4.10	0.70	0.19	0.17	0.87	39
Cyprinus carpio	2.7	3.4	14.1	-25.2	3.11	1.32	1.53	0.93	0.89	0.65	1.72	4
Tinca tinca	1.2	1.9	9.1	-26.9	na							1
Barbusbarbus	2.7	3.5	14.4	-26.1	na							1
Padogobius sp.	2.8	3.6	14.8	-26.3	1.79	1.76	0.13	0.80	0.45	0.46	0.17	4
Lepomis gibbosus	3.1	3.9	15.7	-25.8	6.38	2.36	7.81	1.67	0.44	0.24	3.33	20
Pseudorasbora parva	2.8	3.6	14.8	-26.0	7.51	2.13	9.64	1.41	0.69	0.71	3.27	16
Alburnus alburnus	3.1	3.8	15.6	-26.2	3.01	3.24	5.27	0.95	0.37	0.29	1.80	20
Procambarus clarkii	2.5	3.3	13.7	-23.1	2.07	3.96	4.60	1.58	0.52	0.26	3.59	10
Palaemon sp.	2.2	3.0	12.7	-26.7	2.11	2.95	3.38	0.93	0.30	0.20	1.39	16
Dikerogammarus villosus	1.2	2.0	9.3	-26.1	4.40	1.90	4.49	1.20	0.51	0.42	2.36	11
P. kl. *esculentus* (tadpoles)	-0.0	0.7	4.9	-33.3	0.58	0.27	0.05	0.17	0.18	0.13	0.07	4
Giraulus chinensis	1.2	2.0	9.3	-20.9	4.15	3.48	7.67	1.51	0.97	0.34	5.13	9
Odonata	1.8	2.6	11.5	-26.2	2.01	0.89	0.92	0.55	0.36	0.50	0.71	15
Ephemeroptera	1.7	2.5	11.0	-25.8	1.11	1.61	0.87	0.49	0.37	0.14	0.51	9
Potamogeton sp.	na		8.6	-26.4	11.59	3.21	18.43	2.89	2.58	2.98	18.09	6
Phragmites australis	na		10.2	-29.1	3.65	1.62	1.79	1.37	0.92	0.62	2.35	5
Myriophyllum sp.	na		6.6	-20.2	na							2
Whole consumers community	na				12.14	12.53	62.86	3.29	1.53	2.12	25.83	232

Results of the applied SIMM without priors are showed in Figure 2. Both, *I. punctatus* juveniles and adults expressed a great reliance on *A. alburnus* with a higher proportion in matures in respect to juveniles, that consumed also other items. Also *S. glanis* SIMM showed a specialized piscivorous diet, focused mostly on the consumption of *L. gibbosus*. In contrast, *L. gibbosus* had a more generalist diet, based almost equally on tadpoles and various invertebrates: Ephemeroptera and Odonata larvae among insects, and *Palaemon* sp., *Dikerogammarus villosus* and the invasive *P. clarkii* among crustaceans. For *P. parva*, the estimated diet consisted mainly of *Palaemon* sp., followed by Ephemeroptera and then by *D. villosus*, Odonata and *P. clarkii*. Similarly, the diet of *A. alburnus*, except for the marked preference for *Palaemon*. Finally, *P. clarkii* showed a (diet) preference for molluscs, followed by plants (*Phragmites* and *Potamogeton*), insects (Ephemeroptera and Odonata) and tadpoles.

Tab 3. Estimated SEAc (40% of core data) and prediction ellipse overlap (95% of data) between all fish species with n > 5 and the present crayfish *P. clarkii*.

Pairwise niche overlap	95% prediction ellipses (PE) overlap	SEAc OVERLAP
Ictalurus punctatus total - *Silurus glanis*	0.1952842	0.00696969
Ictalurus punctatus juveniles - *Silurus glanis*	0.2783056	0.2237757
Ictalurus punctatus matures - *Silurus glanis*	0.08599398	0
Ictalurus punctatus juveniles – *Ictalurus punctatus* matures	0.4209284	0.2743763
Alburnus alburnus – *Pseudorasbora parva*	0.4619044	1.326478
Alburnus alburnus – *Lepomis gibbosus*	0.2180314	0.2067183
Pseudorasbora parva - *Lepomis gibbosus*	0.59065	0.4899332
Ictalurus punctatus juveniles - *Lepomis gibbosus*	0.1674263	0
Ictalurus punctatus matures – *Lepomis gibbosus*	0.2435497	0.4721647
Ictalurus punctatus juveniles – *Alburnus alburnus*	0.2538454	0.5012972
Ictalurus punctatus juveniles – *Pseudorasbora parva*	0.167938	0.1516686
Ictalurus punctatus matures – *Alburnus alburnus*	0.1868295	0.1908217
Ictalurus punctatus matures – *Pseudorasbora parva*	0.1252546	0.00160639261
Procambarus clarkii – *Alburnus alburnus*	0.05407385	0
Procambarus clarkii – *Pseudorasbora parva*	0.05718138	0
Procambarus clarkii – *Lepomis gibbosus*	0.0720494	0
Procambarus clarkii – *Ictalurus punctatus* juveniles	0.1064741	0
Procambarus clarkii – *Ictalurus punctatus* matures	0.1101311	0
Procambarus clarkii – *Silurus glanis*	0.00307070402	0.00040755778
Silurus glanis – *Alburnus alburnus*	0.2199789	0.00284873426
Silurus glanis – *Pseudorasbora parva*	0.1618313	0.0011838012
Silurus glanis – *Lepomis gibbosus*	0.2166511	0.2273793

The applied Mixing models under the consideration of the five most consumed prey items and their number of occurrence (Figure 4; 4 prey items in the case of *L. gibbosus*) showed differing trends than without the consideration of priors (Figure 3), highlighting a predation of *I. punctatus* (juveniles) on *Palaemon* sp., secondarily on small Cyprinidae (e.g. *A. alburnus*) and from *I. punctatus* (matures) on first Cyprinidiae and secondarily on *Palaemon* sp. and *D. villosus*. In the case of *S. glanis*, similar proportional contributions of observed prey items changed towards *I. punctatus* and *D. villosus* constituting mostly to its trophic position. Without numerical priors, *Palaemon* sp. and with priors, aquatic insects majorly contributed to the trophic level of *L. gibbosus*. Additionally, SIMMs including N% priors led to an increase in the estimated proportion of contributing prey items and less variability.

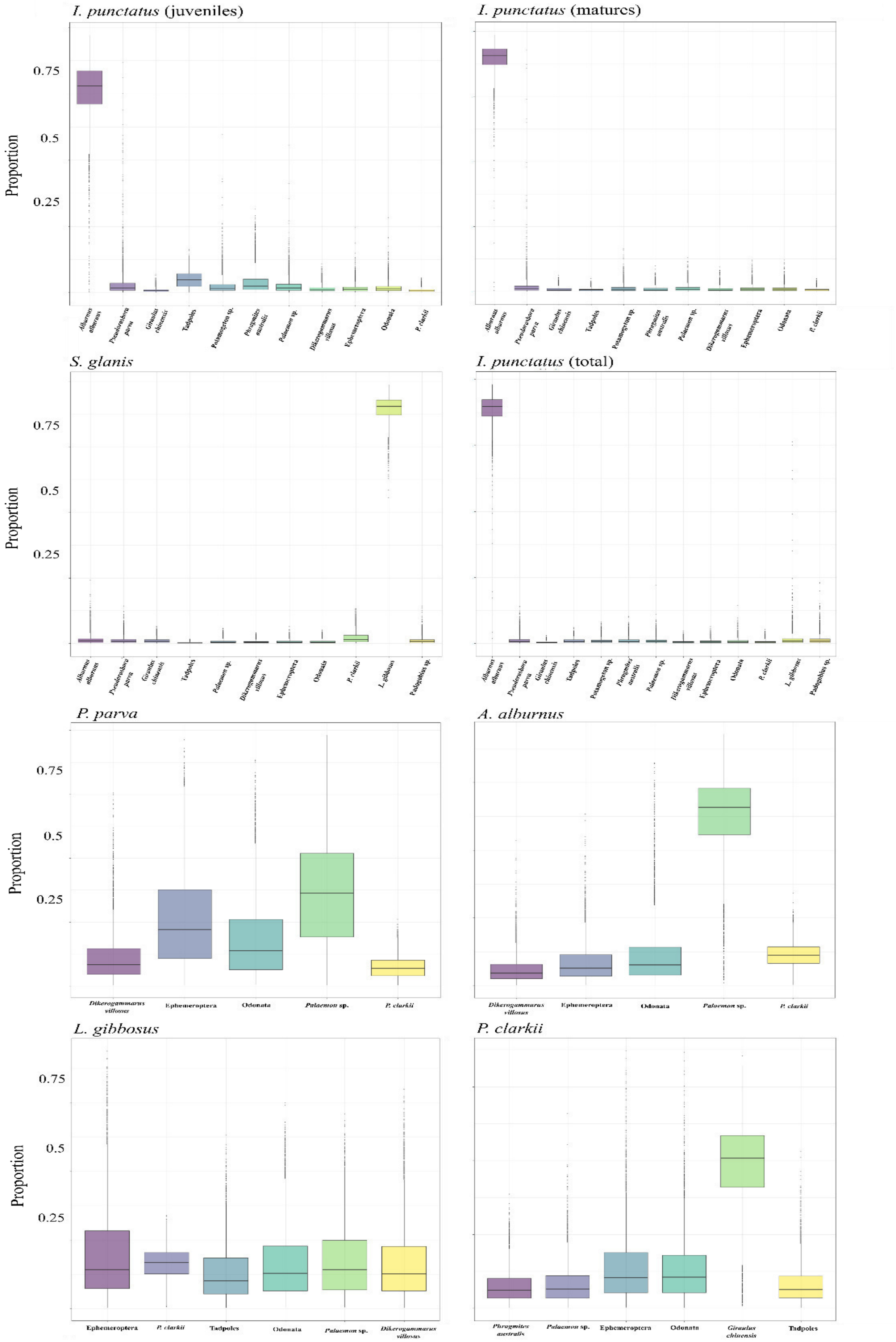

Figure 2: Estimated proportional contribution of diet items contributing to the trophic position of *I. punctatus* (juveniles), *I. punctatus* (matures), *I. punctatus* (total), *S. glanis*, *P. parva*, *A. alburnus*, *L. gibbosus* and *P. clarkii*. Considered items were chosen according to the possibility of consumption and predation on lower trophic prey.

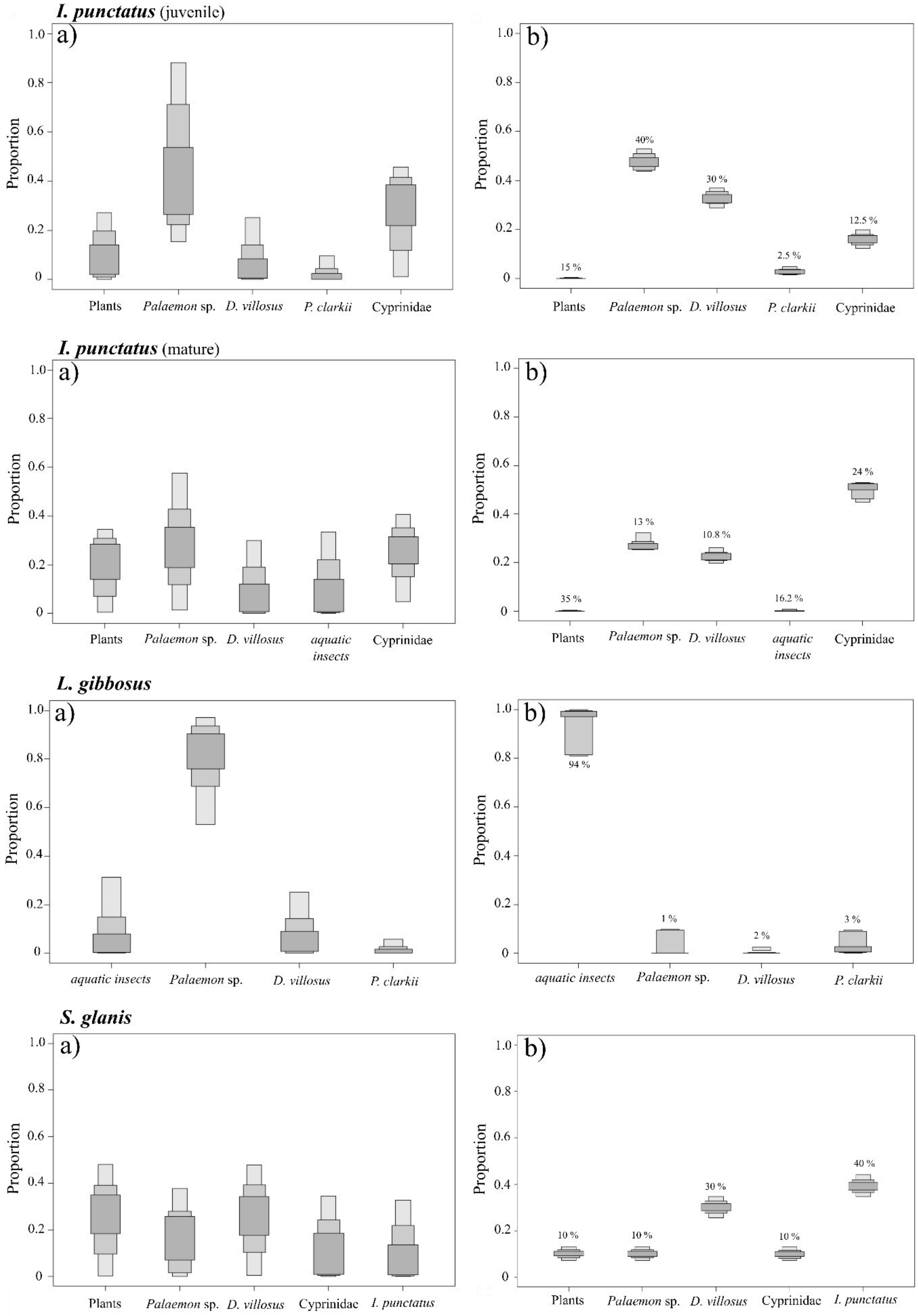

Figure 3: Proportional contribution of consumed prey items to the trophic position of *I. punctatus* (juvenile), *I. punctatus* (mature), *L. gibbosus* and *S. glanis*; a) based on the 5 mostly consumed prey items (N%); b) including the number of occurrences of the 5 most consumed prey items (indicted in %) included as priors.

Permanova

The applied PERMANOVA (Table 4) highlighted that most species are clearly distinct and separated from each other for carbon and nitrogen levels ($F_{3,52}$= 41.78, p = 0.001; see pairwise comparison in Supplement 3), while the applied post-hoc test (Supplement 3) indicated that some fish species were similar in their isotopic niches, confirming the results of trophic position estimations.

Table 4: PERMANOVA main test result for factor SPECIES

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
sp	17	3738.8	219.9	99.3	0.001	999	0.001
Res	215	476.4	2.2				
Total	232	4215.2					

Discussion

Multiple invaded communities are particularly relevant studies that are poorly documented (but see Preston et al. 2012; Jackson 2015), as most studies focus on the effects of a single introduced species. In the Mediterranean area, to our knowledge, only two papers explored the trophic interactions in highly invaded inland water community. Furthermore, they are both from lentic waters.

Bissattini & Haubrock et al. (2018) described the trophic interactions of a pond community in Monterotondo (Rome, Central Italy), composed by almost exclusively invasive species, except for top predators being two native snakes with combined stomachs and isotopes data. Haubrock et al. (2018a) also used SIA and stomach contents to investigate the relationships in the invasive species dominated Arreo Lake in Northern Spain. The present study was conducted in a completely different ecosystem, an important river with no seasonal drought and no native fish predators. Moreover, in the river Arno *P. clarkii* is present and widespread, but with low densities if compared with small ponds, shallow waters, ditch and small streams in Italy (Bissattini & Haubrock et al. 2018). Also, the fish assemblage composition varied considerably, with the absence of large predatory fish like *Ictalurus punctatus* and *Silurus glanis*.

Indeed, combining DA and SIA affirmed that the Arno river is an especially peculiar ecosystem, in which almost no native fish species are present. It in fact, can be seen as a unique melting pot of alien species from Asia and North America, mainly introduced voluntarily for sport fishing purposes. Consequently, manifold interactions that can only be observed in such an invaded range, were investigated with the use of stable isotopes and dietary analyses.

Sampling

While the sampling of all dominating fish species was successfully conducted and key species as well as functional groups were covered, some species were under-sampled due to their rarity (*i.e. Tinca tinca* and *Barbus barbus*) or status as endangered (*i.e.*

Padogobius sp.), while only a minimum of *Cyprinus carpio* individuals were sampled to meet the demands of local anglers. This was a similar case with mature *S. glanis* (>70cm). Another rare species that was not sampled was the omnivore *Squalius cephalus*, which presence is believed to be rare or locally extinct. Various present species (*e.g.* Chironomidae larvae) were not found. Nonetheless, a high abundance of invasive alien species in high trophic levels with divided niche and resource partitioning could minimizes the chance of recovery of the native macrozoobenthos. However, the particularly abundant pond slider *Trachemys scripta* as well as the occasionally found alligator snapping turtle *Macrochelys temminckii* were not sampled as the focus was on the fish community, and the sampling method was not suited to collect them.

Community structure

The reconstructed community presented a typical pyramidal shape, with the apex position being held by two invasive predatory fish, *Ictalurus punctatus* (immature) and *Silurus glanis*. Compared with the work of Bissattini and Haubrock et al. (2018), in which the community was composed –according to isotopic data – by approximately 2 trophic levels, in the Arno river it spans over at least 3 trophic levels, indicating a higher complexity. However, it is interesting to note that this complexity is reached with a high number of species, while in Arreo lake the same number of trophic levels was obtained with only 4 species. Moreover, these had clearly separated niches, with a primary role of N isotopes signatures (and therein the trophic level) in this partitioning (Haubrock et al. 2018a). These observations raise the question which factors affect the possibly present niches in river or lake ecosystems and thus, how the history of species introductions affect the trophic web. Moreover, the use of two different baselines, one from mediated-plants and one from primary consumers, indeed allowed the direct comparison with other studies using just one or the other type of baseline, as well as the comparison with other future studies (Xu et al. 2011). Moreover, the Arno river community showed some overlaps among fish trophic niches, similar to the by Bissattini and Haubrock et al. (2018) studied community, and not a clear segregation of all niches as observed in Arreo Lake (Haubrock et al. 2018a), being this latter the result of the low species number. Overall, the baseline constituted by molluscs seemed to more efficiently representing the functional groups of the studied community than in the case of a plant-based baseline due to the higher resolution presented by the results.

Compared TP estimations showed a strong top predator behavior for *S. glanis*, *I. punctatus* especially in the juvenile stage, and also *L. gibbosus*, coherently with their known biology and other studies (Werner et al. 1977; Vander Zanden et al. 1999a; Syväranta et al. 2010; Laughlin and Werner 2011). Compared with Haubrock et al. (2018a) where *L. gibbosus* had the role of primary predator, in the Arno River their trophic level was higher, consistent with the community studied by Bissattini and Haubrock et al. (2018), where they were concluded as secondary predators. The functional category of primary predatory fish was represented by *P. parva*, *A. alburnus*, and *Padogobius* sp., although for the latter this collocation has to be taken with caution due to the low number of sampled individuals (n= 4). Again, the trophic position of *P. parva* seemed to be approximately consistent with the Monterotondo pond community (Bissattini and Haubrock et al. 2018), although slightly higher and very broad. The common carp (n=4) and the only one specimen of *B. barbus*, can be considered true omnivores as they had a TP intermediate between that of primary predators and herbivores. However, the low number of individuals captured cannot allow other speculations, but rather give a rough indication of the positioning of these species trophic niche in the studied ecosystem. Among invertebrates, the invasive red swamp crayfish *P. clarkii* and *Palaemon* sp. were also included in this category. As discussed for *B. barbus*, the trophic position of *T. tinca* cannot be considered as precise. Moreover, in the same trophic level were also the invertebrate *G. chinensis* and larvae of predatory insects (Ephemeroptera and Odonata) as well as the invasive amphipod *Dikerogammarus villosus*, which is known to be a predator of other aquatic macroinvertebrates (Dick & Platvoet 2000). Finally, *P.* kl. *esculentus* tadpoles occupied the lowest trophic position likely reflecting that a) that no prey (e.g. algae) were consumed and b) collected specimens were very young.

Fascinatingly, juvenile *I. punctatus* occupied a higher trophic position than matures, which is unlikely for catfish species as they generally express higher N values with increasing total length (Syväranta et al. 2010). Considering the high level of eutrophication and the natural accumulation of nutrients (*i.e.* dead matter) in the river bottom, this can be potentially explained by the increased consumption of enriched detritus (Hendrix et al. 1986; Hansen & Kristensen 1998) by juveniles and offshore feeding of matures (as indicated by the difference in C values) or the potentially high competition for mature as well as increasingly piscivorous *I. punctatus* with other species like *S. glanis* (Fry et al. 1999).

Multiple species and foremost catfish species expressed a considerable correlation between TL and C, indicating a consumption on prey that feeds on different aquatic or even terrestrial vegetation. However, although Syvärantae et al. (2010) argued that in *S. glanis*, δ^{15}N increases with TL due to the ability to consume higher trophic prey, such an increase was only observed in *L. gibbosus*, indicating ontogenetic size-based shifts from benthic to pelagic prey.

Layman metrics and niche overlap

Among fish species, Layman's metrics (Layman et al. 2007) showed a great variability in N signatures, thus indicating the utilization of prey items from different trophic levels in *P. parva*, *L. gibbosus* and adults of *I. punctatus*, while the European catfish *S. glanis* was quite specialized in its trophic role. Carbon variability was largest in *A. alburnus*, suggesting the direct or indirect consumption of preys with different C signatures. Niche width measures showed the widest niche for *L. gibbosus, P. parva* and juveniles of *I. punctatus*, thus, these species express the greater intra-specific variability. In contrast, a more specialized niche was found for *S. glanis* and moreover, a niche partitioning among catfish species with the potential to compete due to the generalistic diet of *I. punctatus*. However, see Haubrock et al. (2018b) for a detailed discussion of the interactions and competition between *I. punctatus* and *S. glanis*. Moreover, the comparison between *A. alburnus* and *P. parva*, two ecologically similar species that showed considerable niche overlap, the latter showed a considerably wider niche width based on a considerable variability in δ^{15}N values, likely due to a higher feeding variability and adaptability to many ecosystems, a key factor in its invasion history. Rosecchi et al. (1993), Gozlan et al. (2010) and Yalçın-Özdilek et al. (2013) stated that *P. parva* is prone to predate larvae of fish as well as eggs, while exerting strong opportunism. However, while these were present during the time of the sampling, these were not observed in the diet, as previously observed by Annamaria Nocita (unpublished data). In contrast, *A. alburnus* showed significantly less inter-individual variability linked to a feeding mostly on microinvertebrates (Politou et al. 1993).

Similarly, it is interesting to compare the two size matched crustaceans *D. villosus* and *Palaemon* sp. The greater variability in δ^{15}N was found in the invasive *Dikerogammarus villosus*, suggesting the consumption of prey items from different trophic levels. However, *D. villosus* unexpected low trophic position raises questions on its specialized predatory behaviour, whilst *Palaemon* sp. despite being an omnivore occupies a higher position and narrower variability in δ^{15}N. On the other side, *Dikerogammarus* showed a narrower range of δ^{13}C compared to *Palaeomon* sp., suggesting a precise utilization of C sources. Overall, as for *P. parva*, *D. villosus* had a considerably wider niche than that of *Palaemon* sp., a factor potentially related to the success of this invasive crustacean.

The observed variability paired with the estimated trophic levels resulted in overall low niche overlaps between species. Indeed, the only considerable overlaps were identified between primary predatory fish species.

Mixing models and resource utilization

Standard MMs under the assumption of predation on lower trophic positions presented limited, but indicating information, as this theoretically estimated predation scenario is estimated based on the potential availability of prey and the possibility of its consumption (Phillips 2001; Phillips et al. 2005). Moreover, when considering only observed prey and its occurrences in the diet of the investigated *I. punctatus* presented generally more precise proportions of prey contributions. These were not only different from the MM in Figure 2, but also more specific and due to the combination of two different time mediated data, more accurate (Moore & Semmens 2008). However, including the high abundance of aquatic insects led to varying results for the MM of *L. gibbosus* and using the five most commonly present prey items in the diet of *S. glanis* led to a considerable presence of plants, which did not majorly contribute to its TP.

A case study on alien species communities?

The studied stretch of the inner-Florence Arno river is without doubt a peculiar ecosystem, as it is comprised of only alien fish species. Moreover, the communities' hierarchical order highlights the foregone interactions that have led to this structure.

Interestingly, SIA and DA revealed interactions differing according to weather alien species shared a common history of coevolution or not.

In the case of *I. punctatus* and *L gibbosus*, two species with a common life-history and overlapping native range as well as history of co-evolution, a considerable isotope overlap was identified and the PERMANOVA did not indicate a statistically significant difference in their trophic position. Moreover, the diet of both included a considerable percentage of aquatic insects, but the mixing models with and without prior indicated an entirely different contribution of prey items. Hence, it can be assumed that the competition is not direct, but rather based on the opportunistic nature of *I. punctatus* or other factors that lead to a lower trophic position in mature than juvenile *I. punctatus*. Furthermore, juvenile *I. punctatus* and *L. gibbosus* tend to occupy entirely different trophic positions as indicated by no considerable isotope overlap. Moreover, these species likely avoid competition with their different feeding activities (benthivorous generalist vs. pelagic opportunist).

In scenarios where potentially, competing alien species came from different geographical ranges, observations were slightly different. *P. parva* and *A. alburnus* showed considerable signs of competition for resources and major isotope overlap. However, densities of both species varied locally as well as according to sampling date while differences in $\delta^{13}C$ origin in both species are signs for competition avoidance mechanism, likely due to different ecological niches (Focken & Becker 1998).

In the case of the two apex predators, the competition has been analysed in depth and discussed previously (Haubrock et al.-2018b). The resulting data indicated, that while both, mature and juvenile *I. punctatus* show a medium to high diet overlap with *S. glanis*, a minor isotope overlap was only observed between immature *I. punctatus* and *S. glanis*. Furthermore, it has to be considered that *I. punctatus* is a primarily benthic forager, a factor that likely limits the competition with other species. These results led to the conclusion, that both life-stages of *I. punctatus* show a potential to compete with juvenile *S. glanis*. With the use of mixing models, different preferred prey items for both species were identified, while the use of priors, considering prey items identified in their diet, indicated considerable feeding on *I. punctatus*. However, it has to be stated that only 19 of 39 *S. glanis* had a full stomach.

Thus, it was shown that interactions differed according to involved species and geographic origin. Furthermore, the often described as keystone species *P. clarkii* (Bissattini and Haubrock et al. 2018; Liu et al. 2017) occurred in low densities, for what reason the more abundant *Palaemon* sp. and *D. villosus*, both highly abundant and present in the diet of multiple species as well as indicated by the mixing models results likely occupied a similar function. Therefore, the presence of *P. clarkii* shows no impact lowering effect on present species by predatory fish species.

Conclusion

The Arno river is a special study area, as the species community is almost entirely comprised of alien species while remaining a structure usually observed from intact species communities (McQueen et al. 1989; Polis 1994). Usually, alien species are considered a threat for native species. However, the main predatory species were observed to mostly feed on other alien species, resulting in complex and manifold species interactions. These differing interactions underline the need to be studied on a case to case basis. Especially as in the Arno river, diets of predatory fish vary according to season and availability of prey. However, it can be concluded, that the presence of competing and potentially known prey items can affect the interactions between alien species. In the case that two competing alien species originate from the same geographical range, competition seems to be present, but not direct. In the case that species from different geographical ranges contended with each other, the relationship was characterized by avoidance mechanisms (differing prey consumption, habitat use, etc.).

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Supplement 1. Detailed results of dietary analyses for predatory fish species*I.punctatus* and *S. glanis* from Haubrock et al. (2018b) plus equally analysed data for *L. gibbosus*. Observed prey items are listed as Frequency of Occurrence [F%], Number of Occurrences [N%] and the prominence value [PV].

Prey items	***Ictalurus punctatus* (immature)**		***Ictalurus punctatus* (mature)**		***Ictalurus punctatus* (total)**		***Silurus glanis* (immature)**		***Lepomis gibbosus***	
	[F%]	**[N%]**	**[F%]**	**[N%]**	**[F%]**	**[N%]**	**[F%]**	**[N%]**	**[F%]**	**[N%]**
Detritus	0.30	0.08	0.25	0.04	0.30	0.06	0.10	0.09	0.00	0.00
Plants	0.45	0.09	0.60	0.14	0.45	0.12	0.13	0.11	0.00	0.00
Detergent	0.20	0.08	0.05	0.01	0.20	0.04	0.00	0.00	0.00	0.00
*Palaemon*sp.	0.25	0.18	0.15	0.04	0.25	0.10	0.03	0.02	0.05	0.01
Dikerogammarusvillosus	0.18	0.14	0.20	0.03	0.18	0.08	0.05	0.07	0.13	0.03
Procambarus clarkii	0.10	0.01	0.15	0.03	0.10	0.02	0.00	0.00	0.13	0.03
unid. Crustaceans	0.08	0.14	0.00	0.00	0.08	0.06	0.03	0.02	0.00	0.00
Terr. Insects	0.43	0.16	0.45	0.29	0.43	0.23	0.00	0.00	0.00	0.00
Aq. Insects	0.13	0.00	0.25	0.06	0.13	0.03	0.03	0.02	0.75	0.90
Hirudinea	0.03	0.00	0.05	0.01	0.03	0.00	0.00	0.00	0.00	0.00
Fish larvae	0.08	0.02	0.05	0.01	0.08	0.01	0.00	0.00	0.00	0.00
unid. Fish	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.25	0.05
small cyprinids	0.25	0.05	0.30	0.08	0.25	0.06	0.18	0.16	0.00	0.00
large cyprinids	0.03	0.00	0.05	0.01	0.03	0.00	0.00	0.00	0.00	0.00
Ictalurus punctatus	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.09	0.00	0.00
Molluscs	0.05	0.00	0.10	0.04	0.05	0.02	0.00	0.00	0.00	0.00
Small mammals	0.03	0.00	0.05	0.01	0.03	0.00	0.03	0.02	0.00	0.00
small birds	0.08	0.01	0.10	0.02	0.08	0.01	0.03	0.02	0.00	0.00
Testudines	0.03	0.00	0.05	0.01	0.03	0.00	0.00	0.00	0.00	0.00
Fishing Baits	0.23	0.03	0.35	0.17	0.23	0.11	0.03	0.34	0.00	0.00

Supplement 2: Spearman correlation estimates for all fish species with n > 5 and crayfish

			TL	δ^{15}N	δ^{13}C
Silurus glanis	TL	Correlation coefficient		-.074	-.512**
		p		.653	.001
		N		39	39
	N	Correlation coefficient	-.074		.231
		p	.653		.158
		N	39		39
	C	Correlation coefficient	-.512**	.231	
		p	.001	.158	
		N	39	39	
Ictalurus punctatus	TL	Correlation coefficient		-.290	-.665**
		p		.069	.000
		N		40	40
	N	Correlation coefficient	-.290		-.044
		p	.069		.788
		N	40		40
	C	Correlation coefficient	-.665**	-.044	
		p	.000	.788	
		N	40	40	
Lepomis gibbosus	TL	Correlation coefficient		.917**	.074
		p		.000	.755
		N		20	20
	N	Correlation coefficient	.917**		.149
		p	.000		.531
		N	20		20
	C	Correlation coefficient	.074	.149	
		p	.755	.531	
		N	20	20	
Alburnus alburnus	TL	Correlation coefficient		-.160	-.103
		p		.501	.667
		N		20	20
	N	Correlation coefficient	-.160		-.460*
		p	.501		.041
		N	20		20
	C	Correlation coefficient	-.103	-.460*	
		p	.667	.041	
		N	20	20	
Pseudorasbora parva	TL	Correlation coefficient		.270	.567*
		p		.313	.022
		N		16	16
	N	Correlation coefficient	.270		-.026
		p	.313		.922
		N	16		16
	C	Correlation coefficient	.567*	-.026	
		p	.022	.922	
		N	16	16	
Procambarus clarkii	TL	Correlation coefficient		.280	-.693*
		p		.434	.026
		N		10	10
	N	Correlation coefficient	.280		-.503
		p	.434		.138
		N	10		10
	C	Correlation coefficient	-.693*	-.503	
		p	.026	.138	
		N	10	10	

*. The correlation is at the level of $P < 0.05$ significant

Supplement 3: Results of PERMANOVA pair-wise (post-hoc) between couples of levels of factor ‘species’, after PERMANOVA main test. AA = *Alburnus alburnus*; CC = *Cyprinus carpio*; DG = *Dikerogammarus villosus*; EPHEM = Ephemeroptera; FRAG = *Phragmites australis*; IP = *Ictalurus punctatus*; LG = *Lepomis gibbosus*; MIR = *Miriophyllum* sp.; MOL = *Giraulus chinensis*; ODON = Odonata; P = *Palaemon* sp.;PC = *Procambarus clarkii*; PG = *Padogobius* sp.; PP = *Pseudorasbora parva*; SG = *Silurus glanis*; POT = *Potamogeton* sp.; TAD = *Pelophylax* kl. *esculentus* (tadpoles).

Unique

Groups	t	P(perm)	perms	P(MC)
AA. CC	2.5309	0.023	658	0.007
AA. DG	13.936	0.001	966	0.001
AA. EPHEM	11.879	0.001	951	0.001
AA. FRAG	9.9816	0.001	884	0.001
AA. IN	9.1688	0.001	949	0.001
AA. IP	1.4736	0.109	886	0.112
AA. LG	0.77445	0.514	702	0.478
AA. MIR	10.617	0.005	215	0.001
AA. MOL	15.244	0.001	975	0.001
AA. ODON	9.4892	0.001	910	0.001
AA. P	7.877	0.001	875	0.001
AA. PC	23.35	0.001	960	0.001
AA. PG	1.3389	0.175	340	0.185
AA. POT	7.1405	0.001	951	0.001
AA. PP	1.6626	0.095	924	0.09
AA. SG	6.5311	0.001	937	0.001
AA. TAD	22.668	0.001	860	0.001
CC. DG	5.3422	0.003	324	0.001
CC. EPHEM	5.2918	0.008	197	0.001
CC. FRAG	4.5746	0.023	56	0.002
CC. IN	3.8863	0.011	91	0.002
CC. IP	2.9136	0.002	822	0.001
CC. LG	1.4196	0.171	704	0.164
CC. MIR	3.7225	0.096	10	0.024
CC. MOL	5.3311	0.006	211	0.001
CC. ODON	4.0799	0.008	148	0.001
CC. P	3.0454	0.005	545	0.004
CC. PC	9.0909	0.002	279	0.001
CC. PG	1.3213	0.181	25	0.225
CC. POT	2.2869	0.056	83	0.047
CC. PP	0.98462	0.32	537	0.348
CC. SG	5.402	0.001	673	0.001
CC. TAD	14.201	0.025	35	0.001
DG. EPHEM	3.3865	0.003	811	0.001
DG. FRAG	4.0126	0.002	792	0.001
DG. IN	3.1653	0.004	849	0.005
DG. IP	14.528	0.001	972	0.001
DG. LG	9.4397	0.001	965	0.001
DG. MIR	4.9007	0.011	77	0.001
DG. MOL	7.4822	0.001	967	0.001
DG. ODON	4.202	0.001	828	0.001
DG. P	7.348	0.001	949	0.001
DG. PC	9.2725	0.001	965	0.001
DG. PG	7.072	0.001	595	0.001
DG. POT	0.60709	0.701	888	0.625
DG. PP	8.2619	0.001	965	0.001
DG. SG	23.843	0.001	973	0.001
DG. TAD	12.131	0.002	662	0.001
EPHEM. FRAG	5.6542	0.002	721	0.001
EPHEM. IN	2.5303	0.007	702	0.009
EPHEM. IP	11.052	0.001	960	0.001
EPHEM. LG	6.9627	0.001	956	0.001
EPHEM. MIR	6.5682	0.013	54	0.001
EPHEM. MOL	8.2844	0.002	760	0.001
EPHEM. ODON	2.3511	0.006	671	0.015
EPHEM. P	5.1366	0.001	913	0.001
EPHEM. PC	12.4	0.001	959	0.001

EPHEM. PG	8.0993	0.004	418	0.001
EPHEM. POT	1.7972	0.017	769	0.08
EPHEM. PP	5.9448	0.001	933	0.001
EPHEM. SG	20.715	0.001	931	0.001
EPHEM. TAD	31.384	0.002	457	0.001
FRAG. IN	2.9447	0.002	459	0.002
FRAG. IP	9.6188	0.001	911	0.001
FRAG. LG	6.5862	0.001	909	0.001
FRAG. MIR	5.0624	0.052	21	0.001
FRAG. MOL	8.4814	0.001	759	0.001
FRAG. ODON	4.7809	0.002	630	0.001
FRAG. P	5.4832	0.001	899	0.001
FRAG. PC	9.1895	0.001	733	0.001
FRAG. PG	5.3224	0.007	91	0.001
FRAG. POT	1.6163	0.057	407	0.103
FRAG. PP	5.9076	0.001	931	0.001
FRAG. SG	17.297	0.001	897	0.001
FRAG.TAD	7.7497	0.016	121	0.001
IN. IP	8.9236	0.001	954	0.001
IN. LG	5.8383	0.001	950	0.001
IN. MIR	5.608	0.023	28	0.001
IN. MOL	8.0486	0.001	897	0.001
IN. ODON	1.6019	0.093	691	0.11
IN. P	3.1943	0.002	889	0.004
IN. PC	10.796	0.001	933	0.001
IN. PG	4.9949	0.006	179	0.001
IN. POT	1.6156	0.084	642	0.111
IN. PP	4.8844	0.001	935	0.001
IN. SG	16.788	0.001	906	0.001
IN. TAD	14.721	0.005	235	0.001
IP. LG	1.8447	0.048	910	0.059
IP. MIR	10.8	0.002	513	0.001
IP. MOL	16.721	0.001	981	0.001
IP. ODON	8.9866	0.001	859	0.001
IP. P	8.4653	0.001	943	0.001
IP. PC	25.251	0.001	973	0.001
IP. PG	1.6742	0.092	801	0.066
IP. POT	8.9909	0.001	962	0.001
IP. PP	2.8909	0.002	933	0.004
IP. SG	6.6457	0.001	969	0.001
IP. TAD	19.12	0.001	913	0.001
LG. MIR	6.7854	0.009	216	0.001
LG. MOL	10.405	0.001	984	0.001
LG. ODON	5.7077	0.001	911	0.001
LG. P	5.5833	0.001	928	0.001
LG. PC	16.49	0.001	972	0.001
LG. PG	1.0119	0.339	726	0.32
LG. POT	5.9607	0.001	956	0.001
LG. PP	1.4282	0.175	899	0.161
LG. SG	3.9563	0.001	959	0.001
LG. TAD	12.868	0.001	903	0.001
MIR. MOL	1.672	0.104	55	0.083
MIR. ODON	6.541	0.015	44	0.002
MIR. P	8.4044	0.007	145	0.001
MIR. PC	2.6309	0.032	66	0.013
MIR. PG	5.4435	0.071	11	0.006
MIR. POT	1.9789	0.078	28	0.061
MIR. PP	6.502	0.008	146	0.001
MIR. SG	15.936	0.001	441	0.001
MIR. TAD	8.0235	0.074	15	0.003
MOL. ODON	8.5407	0.001	943	0.001
MOL. P	11.854	0.001	965	0.001
MOL. PC	7.4583	0.001	966	0.001
MOL. PG	7.7655	0.001	443	0.001
MOL. POT	3.6846	0.001	870	0.001
MOL. PP	9.7235	0.001	981	0.001
MOL. SG	23.34	0.001	962	0.001

MOL. TAD	14.318	0.001	517	0.001
ODON. P	2.999	0.001	465	0.001
ODON. PC	12.524	0.001	955	0.001
ODON. PG	5.8829	0.001	245	0.001
ODON. POT	2.0416	0.015	758	0.065
ODON. PP	4.6767	0.001	866	0.001
ODON. SG	17.438	0.001	925	0.001
ODON. TAD	25.227	0.001	343	0.001
P. PC	17.36	0.001	984	0.001
P. PG	3.3591	0.002	625	0.003
P. POT	3.8635	0.001	924	0.001
P. PP	4.038	0.001	694	0.001
P. SG	16.824	0.001	978	0.001
P. TAD	18.236	0.001	762	0.001
PC. PG	11.775	0.001	527	0.001
PC. POT	4.0294	0.003	904	0.001
PC. PP	15.443	0.001	977	0.001
PC. SG	36.146	0.001	970	0.001
PC. TAD	11.189	0.005	605	0.001
PG. POT	2.8967	0.008	151	0.021
PG. PP	0.3642	0.832	713	0.821
PG. SG	5.3568	0.001	774	0.001
PG. TAD	19.663	0.022	25	0.001
POT. PP	4.9764	0.001	953	0.001
POT. SG	12.618	0.001	926	0.001
POT. TAD	3.7587	0.005	195	0.002
PP. SG	6.6282	0.001	927	0.001
PP. TAD	12.798	0.002	841	0.001
SG. TAD	33.611	0.001	814	0.001

Chapter 3.5

When alien catfish meet - Resource overlap between the North American *Ictalurus punctatus* and immature European *Silurus glanis* in the Arno River (Italy)

Short title: Overlapping catfish niches

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Abstract

Due to the increasing globalization and ongoing introduction of alien species specifically regarding European freshwater ecosystems, native and already present alien species will be confronted with competitors with unknown outcomes. One such case is the situation of the European catfish *Silurus glanis* introduced in the Arno River (Central Italy), a species sought after by anglers, which is facing competition from the later introduced alien North American channel catfish *Ictalurus punctatus*. Large catfish species are highly valued among anglers, but their interspecific interactions and potential ecosystem-level impacts are still poorly known. We used stomach contents and stable isotope analyses to study niche partitioning between these two alien catfish species, coexisting in the Arno River . The results suggest partial niche segregation, with immature *S. glanis* showing a narrower dietary and isotopic niche and a slightly higher trophic position than *I. punctatus*. Monitoring the catfish population sizes, trophic niches and effects on lower trophic levels are essential for future management and mitigation of their potential impacts on invaded freshwater ecosystems.

Keywords: Isotope analyses, dietary analysis, niche overlap, niche partitioning, interactions, alien species

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Introduction

In some cases, introduced species served human well-being (Ewel et al., 1999) without significantly affecting the recipient environment (Williamson & Fitter, 1996a, b; Jerscke & Strayer, 2005). Nonetheless, many alien species become invasive and dominate native communities (Kolar & Lodge, 2001; Crooks, 2002), leading towards a homogenisation of ecosystems (Mack et al., 2000; Rahel, 2000). While interactions among alien and native species have recently received increasing attention (Strayer 2010; Balzani et al., 2016; Busst & Britton, 2017), the current issue is to understand interactions among alien species (Ricciardi, 2001; Bissattini et al., 2018). Particularly interesting, even if difficult to address, is the scenario of a top predator confronted with the introduction of another potential alien top predator that originates from a different area, and how relationships with other present species are affected (Griffen et al., 2008; Wei et al., 2012).

As in many Europeans countries (Gherardi et al., 2009), alien fish rapidly increased over the past 25 years (e.g. Italy: 40 out of 105 species – 38% are alien species; Nocita & Zerunian, 2007; Gherardi et al., 2008). This process has reshaped most freshwater communities. Although some introductions might have occurred accidentally, a significant proportion of species has been voluntarily introduced by anglers and sport fishing associations to improve recreational fishing.

One such species is the European or Wels catfish *Silurus glanis* (Linnaeus 1758), a fish native to North-Eastern Europe and Asia. It is known to be the largest freshwater fish in Europe (max size 2.7 m and 130 kg ; Stone, 2007; Boulêtreau & Santoul, 2016). In the last decades, *S. glanis* experienced a drastic increase in population size and distribution due to angling activities (Copp et al., 2009; Cunico et al., 2014). It was introduced in Italy in the 90s and was subsequently spread throughout the Italian peninsula (Balma et al., 1989) with diverse impacts on native and also newly introduced species (Castaldelli et al., 2013). In the Arno River Basin, it was most likely introduced to Bilancino Lake and then spread downstream towards the Sieve River (a tributary of the Arno River; Gualtieri et al., 2010). In the Florentine stretch of the Arno River, *S. glanis* has been reported by anglers since 2004 (Gualtieri & Mecatti, 2005). In the course of a few years, this species has become the target of many anglers. The increased investigation into invasive *S. glanis* revealed considerable positive economic (Cucherousset et al., 2017; Rees et al., 2017) but negative environmental impacts (Copp et al., 2009; Castaldelli et al., 2013).

At the same time, the North American channel catfish *Ictalurus punctatus* (Rafinesque 1818) was also introduced to Italy. However, information on pathways and means of introduction remain scarce (Ligas, 2008, Haubrock et al., 2017, 2018a, b). In the Arno River, according to catches by local authorities and anglers, it is likely that *I. punctatus* was introduced near Pisa for the purpose of angling and was subsequently transported upriver (Giuseppe Castaldelli pers. comm.) as it is increasingly favoured by anglers due to its high reproduction rate and catchability (Thomas Busatto pers. comm.). In the inner-Florence stretch of the Arno River, it first appeared in 2004 (Annamaria Nocita pers. comm.).

Catfish such as *S. glanis* have specific functions in ecosystems, affecting various ecological groups with their presence (Vejrik et al., 2017). *S. glanis* is known to be a generalist that is capable of rapidly adapting to new prey sources (Vejrik et al., 2017). However, data about the diet of immature (<60 – 80 cm) *S. glanis* from alien populations (but see Gualtieri & Mecatti, 2005 and Syväranta et al., 2010 for a basic analysis of immature *S. glanis*' diet) and about interactions with other catfish species are scanty. Similarly, much more information is available about genetics and physiology of *I. punctatus* in regard to aquaculture usage, but most studies that investigated the behaviour of *I. punctatus* are considerably old, with a minority of studies focusing on its invasiveness and diet (Busbee, 1968; Rosen et al., 1995; Haubrock et al., 2018a, b).

Although these two catfish species are morphologically unequal, with *S. glanis* reaching larger sizes, they are highly tolerant to environmental factors and show high invasive capabilities (Hilge, 1985; Copp et al., 2009). For this reason, control activities on *S. glanis* have been carried out countries like Italy (see e.g. Pascale et al., 2013; LIFE-Project LIFE03NAT/IT/000113 and LIFE15 NAT/IT/000989). Furthermore, these two species of catfish have different feeding strategies. With a fast expansion of the mouth while keeping opercular valves closed in the beginning to maximise the sucked volume, *S. glanis* imbibes prey inside its oral opening (Bruton, 1996). In contrast, the exact morpho-dynamic prey mechanism of *I. punctatus* has not been studied, but a combination of sucking and pursuing-and-biting is possible (Pavlov & Kasumyan, 2002). Moreover, length-weight relationships and thus growth-rates might differ between both species, but as they are ecosystem and factor depending, information for both *I. punctatus* in Europe as well as *S. glanis* from the Arno River has not yet been assessed. Furthermore, such information is needed to better understand the impact these species have on recipient ecosystems (Haubrock et al. 2018b). However, the diet of *I. punctatus* has been recently analysed by collecting stomach content of specimens in the Arno River (Haubrock et al, 2017; Haubrock et al. 2018a, b), while recent and relevant data for *S. glanis'* feeding habit from Central Italy are preliminary or anecdotal (Gualtieri & Mecatti, 2005), making investigating facilitation processes or interfering competition processes difficult.

Interactions between alien top predators are very likely, because in fish communities, resource partitioning mainly occurs along the trophic dimensions (Ross, 1986). According to the competitive exclusion principle (Hardin, 1960), coexistence can only be possible if utilized resources and occupied dimensions (activity time, habitat use) are different (Schoener, 1986; Chase & Leibhold, 2003). Studies investigating dietary and, thus dietary niche partitioning, mostly focused on either mature life stages (Schulze et al., 2012; Zaia Alves et al., 2017) or ontogenetic variations (Davis et al.,2012; Tarkan et al., 2018). However, immature life-stages in catfish species can dominate an ecosystem and have a considerable impact on other species as well as the potential to become a target to manifold interaction with other species (Elrod et al., 1974; Persson, 1988; Alp et al., 2004; Alp et al., 2011).

Stomach content analyses can provide a direct insight into recently consumed items and, consequently, feeding behaviour of different life stages, while stable isotopes analysis (SIA) provides long-term dietary information. Both combined allow a reconstruction of the studied communities' trophic web and the investigation of niche partitioning (Boecklen et al., 2011; Layman et al., 2012). This is possible, because isotopic signatures relate with the trophic position (nitrogen, N) and with the main energy source (carbon, C) of a consumer (Layman et al., 2012). Additionally, SIA allows the estimation of trophic levels (Post, 2002) and quantification of feeding niches (Newsome et al., 2007). The overlap degree among isotopic niches of different species can therein be assessed to evaluate the potential food competition between species (Layman et al., 2012; Balzani et al., 2016) or the niche partitioning (Werner, 1979; Barbour et al., 2009).

Over the last years, many anglers and local authorities reported steadily decreasing catches of *S. glanis* (abundance and biomass) in the Arno River within Florence while simultaneously the amount of *I. punctatus* increased (Nocita, 2007; Nocita & Zerunian, 2007; Gherardi et al., 2008). Hence, the present work uses a combined approach of stomach content and stable isotope analyses from both co-occurring species (immature and mature *I. punctatus*, immature *S. glanis*) in order to investigate the degree of interspecific interactions (e.g. feeding competition or prey partitioning), thereby aiming to determine if the presence of *I. punctatus* potentially affects the population of *S. glanis*.

Material and Methods

Sampling

Sampling was conducted twice a week, once in the morning (3-8 am) and evening (4-11 pm) between May and June 2018, as these months are considered an ideal activity period for both species (samples were taken after the initial two-month period of resumed activity, following the inactive winter months). These times were chosen because *S. glanis* shows its activity mostly during the night with peaks of activity before sunrise and after sunset (Carol et al., 2007) while *I. punctatus* shows diurnal and nocturnal activity with highest feeding activity during dusk and dawn (Boujard & Leatherland, 1992). Moreover, February and March are typically periods of high-water currents and strong rains, negatively affecting the sampling. The far west end of the inner-Florence river stretch was chosen due to its accessibility for fishing and the low human activity on both riversides (Fig. 1). The sampling area was characterized by high turbidity and no aquatic vegetation with scarce vegetation on river banks during the first half of the sampling period due to seasonal patterns and anthropogenic disturbance. Fish were caught using standard fishing rods (2.20 – 3.90 m), 0.30 – 0.44 mm monofilament line and size 2 - 26 fishing hooks baited with a variety of food (maggots, worms, and freshly cut liver or bait-fish) placed i) on the bottom, ii) in the middle water and iii) below the surface. Caught specimens were euthanized (gill-cuts) and put on ice before being transported to the laboratory for further processing. In total, 40 individuals of *I. punctatus* were sampled and the stage of maturity was set according to Haubrock et al. (2018b) as 31.9 cm, resulting in 20 'immature *I. punctatus* and 20 'mature *I. punctatus*'. Additionally, 39 individuals of 'immature *S. glanis*', recognized by the lack of gonads and size ranges for immature individuals in this species identified by Copp et al. (2009), were sampled. Older and bigger *S. glanis* were not sampled due to i) their rarity in the sampled stretch and ii) local pressure by anglers.

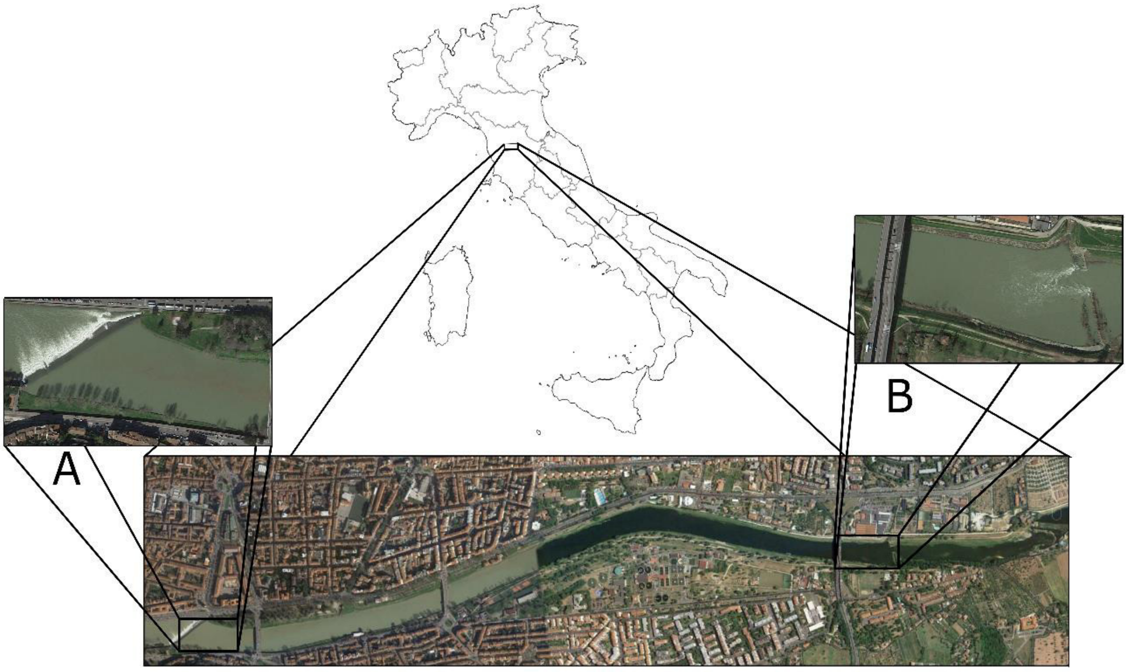

Figure 1: Sampling areas within the inner-city section of the Arno River in Tuscany (N43.7648903, E11.2697444).

Sample preparation

For all specimens, total length (TL; cm) was measured with an accuracy of 1 mm and body weight (F_W) with an accuracy of 1 g. Values reported in the text are average plus standard deviation. Specimens were stored in a freezer (-20 °C) until stomach contents were extracted for dietary analyses. For SIA, a sample of dorsal muscle tissue was taken from each specimen, preserved without any chemicals and stored in a freezer (-20°C). Samples were dried in an oven at 60°C for 48 h and ground into a fine and homogenized powder with an agate mortar and pestle. For each sample, approximately 0.25 mg of powder was weighed and packed into a tin capsule for isotopic analyses. Samples were analysed with an elemental analyser (FlashEA 1112) connected to an isotope ratio mass spectrometer (Thermo Finnigan Delta Plus Advantage) at the National Research Council in Montelibretti, Rome.

Growth and dietary analyses

As fish-size affects fish-weight, examination of the length-weight relationship was achieved with a logarithmic transformation and a linear regression model, enabling the use of the slope of both linear models as a proxy for the growth and growth type. Thus, the species-specific growth was compared by testing for homogeneity of slopes in an Analysis of Covariance (ANCOVA; Dhillon & Fox, 2004). To compare the weight gained with increased length, weight was set as the dependent variable and length as covariate.

The weight of ingested food (ST_W) was expressed as the percental weight of the respective fish (F_W) following the approach of Hureau (1969): $F_I = ST_W/F_W*1000$, where F_I is considered as the fullness index. Only cardiac, cecum and pyloric parts were considered without discarding contents of the intestine. The feeding intensity was calculated using the vacuity index (V_I), as the percentage of empty stomachs in respect to stomachs that contained prey items (Batistic et al., 2005). The diet breadth was estimated based on Levin's index formula (Whittaker et al., 1973): $B_i = \frac{1}{\sum p_i^2}$ where B_i is the standardized index of diet breadth for specimen i and p the squared sum of consumed prey items of specimen i. For further analyses, the stomach content data were expressed as Frequency of Occurrence (F%=number of stomachs containing each food item in relation to total number of full stomachs) and abundance (N%= the number of individuals of each food item with respect to the total number of individuals). With these, the prominence value (PV) for each dietary component was estimated following the approach of Hickley et al. (1994): $PV = N\% * \sqrt{F\%}$ to estimate the diet-overlap index α using the formula of Schoener (1986): $\propto = 1 - 0.5\left(\sum_{i=1}^{n}\left|PV_{xi} - PV_{yi}\right|\right)$, with n being the number of food items, PVx_i the prominence value of food item i in species x_i, PV_{yi} respectively the prominence values of food item i in species y. The estimated index varies between 0, i.e. no overlap, and 1, when diets are identical (Wallace, 1981). Pianka's measure of diet based niche overlap (Pianka, 1974) for species j and species k using the formula $O_{j\&k} = \frac{\sum_j^n p_j p_k}{\sqrt{\sum_i^n p_j^2 \sum_i^n p_k^2}}$ was calculated. Both estimates for diet overlap were used together, as Pianka's measure of niche overlap alone can be insufficient to identify a relation between niche overlap and competition, i.e. resource partitioning (Krebs, 1998). We utilized a Permutational Analysis of Variance (PERMANOVA; S17 Bray Curtis similarity, 3 fixed factor, three levels: 'immature *I. punctatus*', 'mature *I. punctatus*' and 'immature *S. glanis'; * Type III (partial) sum of squares, Unrestricted permutation of raw data, 1 dummy variable) and pairwise comparisons using the software PRIMER (Clarke & Warwick, 2001) to determine if diets varied between species and life-stages.

Stable Isotope Analyses

The trophic position of every individual (TP_c) was calculated by applying the equation $TP_c = ((\delta^{15}N_c - \delta^{15}N_{base})/\Delta N) + \lambda$, where $\delta^{15}N_c$ is the mean $\delta^{15}N$ of the consumer, $\delta^{15}N_{base}$ the mean $\delta^{15}N$ of primary producer (*Potamogeton nodosus*, n=5), ΔN the standard enrichment of 3.4 ‰ between trophic levels and λ the basal trophic level (=1 for plants) (Post, 2002; Britton et al., 2018). A primary producer was chosen rather than a primary consumer (e.g. molluscs) due to the lower variability of stable isotope values in plants (McClelland et al., 1997; Bissattini et al., 2018) and the possibility of high as well as variable $\delta^{15}N$ values in primary consumers due to the nitrogen-accumulating nature of the also present *Phragmites australis*.

A linear regression model was applied to investigate potential relationships between TL of *S. glanis* as well as *I. punctatus* and stable isotope values while the relationship between the two was analysed for potential correlations (Spearman, statistic: r_s). Additionally, to estimate and quantify intra-specific niche width, Layman's metrics (Layman et al., 2007) were calculated with the R-package SIAR (Stable Isotope Analysis in R; Parnell et al., 2010). While Layman's metrics are usually employed in community-wide analyses, they can be used to compare species present within one ecosystem without consideration of the entire species community (Layman, 2007; Jackson et al., 2011; Bissattini et al., 2018). Additionally, the corrected standard ellipse areas (SEAc; considering 40% of central data points) and the corresponding 95% prediction ellipses (SEAb) for both species and life stages for *I. punctatus* were calculated. With these, the degree of isotopic niche overlap (which lays between 0, i.e. no overlapping, and 1, completely overlapping) can be estimated and subsequently used as "a quantitative measure of dietary similarity among populations" (Jackson et al., 2012). Calculations were computed using the R package SIBER (Jackson et al., 2011). Furthermore, the percentual isotopic niche overlap between groups was calculated using the formula of Stasko et al. (2015).

The effect of TL on $\delta^{15}N$ was analysed for both, *I. punctatus* and *S. glanis* using the R-package "nicheROVER", which applies a Monte Carlo estimation on the potential overlap between *I. punctatus* (both life-stages) and *S. glanis* to estimate the probability that a random individual from either group would be found in the niche region of another group (Swanson et al., 2015).

Lastly, a PERMANOVA (1fixed factor, three levels: 'immature *I. punctatus*', 'mature *I. punctatus*' and 'immature *S. glanis'*; Type III (partial) sum of squares, Unrestricted permutation of raw data) was performed to test for significant differences in $\delta^{15}N$ and $\delta^{13}C$ levels among life stages as well as species and to eventually determine if groups and/or species are clearly distinguishable by either isotope. Additionally, a Canonical Analysis of Principal Coordinates (CAP) for factors whose levels were identified by the PERMANOVA test to be significantly different was applied to identify how $\delta^{15}N$ and $\delta^{13}C$ contribute to differentiate the levels. Pearson correlations with CAP axis for each variable are reported. For all tests, the level of significance under which the null hypothesis was rejected is $\alpha = 0.05$.

Results

Overall, 40 individuals of *I. punctatus* (mean TL = 33.82 ± 2.20 cm; mean F_W = 538.45 ± 672.1 g) consisting of 20 immatures (mean TL = 22.44 ± 1.35 cm; mean F_W = 117.75 ± 96.27 g) and 20 mature specimens (mean TL = 45.20 ± 2.09 cm; mean F_W = 959.15 ± 738.49g) were collected. *S. glanis* (n=39; mean TL ± SD = 27.1 ± 1.06 cm; mean F_W ± SD = 122.84 ± 89.85 g) matched the size range of sampled *I. punctatus,* enabling a direct comparison. The growth type of both species was although in both species positive, significantly different (ANCOVA, T = 74.376; df = 1; $p < 0.001$) (Fig. 2).

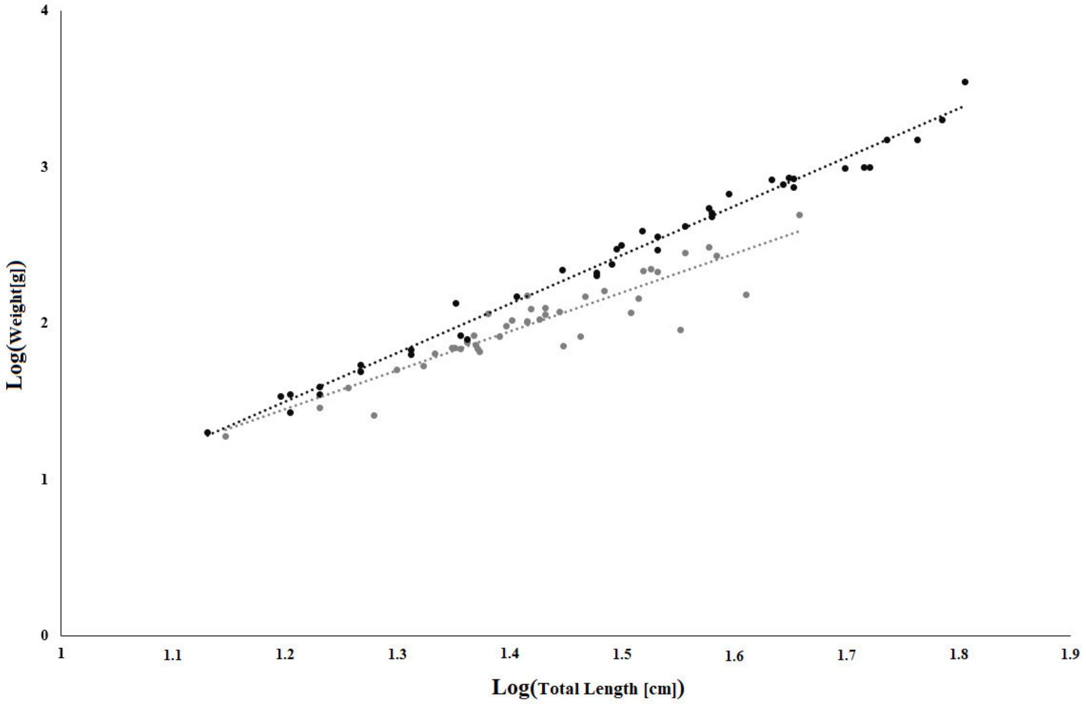

Figure 2: Log-transformed length to weight ratio of *Ictalurus punctatus* (black; y = 3.1177x – 2.2405; R² = 0.9874) and *Silurus glanis* (grey; y = 2.4836x – 1.5297; R² = 0.84)

Dietary analyses

From the 40 sampled *I. punctatus*, all contained prey items (V_I=100%), while from the collected *S. glanis*, 19 stomachs (V_I=48.72%) were found empty. F_I of *I. punctatus* was 842.97±330.1% (immature 817.42±352.8; mature 868.52±312.72) and 322.37±182.07 for *S. glanis*. The diet of both species as well as life-stages of *I. punctatus* were significantly different (PERMANOVA, F = 9.0434; df = 2; $p < 0.05$; post-hoc tests: t = 1.4282; $p < 0.05$). The most common food source for *I. punctatus* were the easily accessible prey items detritus and plants (F%=0.55; N%=0.28), followed by *Palaemonetes* sp. (F%=0.35; N%=0.20), terrestrial (F%=0.4; N%=0.18) and aquatic insects (F%=0.35; N%=0.09), and lastly small cyprinids (juvenile *Cyprinus carpio*, *Alburnus alburnus*, *Pseudorasbora parva*; F%=0.20; N%=0.05). The occurrence of detergent in *I. punctatus*, potentially remnants from water cleaning plants or fishermen's bait, was previously discussed (Haubrock et al. 2018). Utilizing the estimated prominence values (PV) as an indicator of the importance of a prey item, indicated that *Palaemonetes* sp., terrestrial insects and plants were most important for the diet of *I. punctatus*. For *S. glanis*, small cyprinids and plant material were dominant food items (Tab. 1). The diet-based niche breath for *I. punctatus* was 8.1 for immatures, 6.7 for mature specimens and 8.8 for the overall population, while *S. glanis* (B=5.6) showed a lower niche breath than *I. punctatus*. The niche overlap was moderately high between 'immature *I. punctatus*' and 'mature *I. punctatus*' as well as between 'mature *I. punctatus*' (respectively the total population of *I. punctatus*) and 'immature *S. glanis*'. In contrast, all groups of *I. punctatus* expressed with 0.6-0.8 a moderate to high diet overlap index with 'immature *S. glanis*' (Tab. 2).

Table 1: Frequency of occurrence (%F), abundance (%N), and prominence value (PV) of food items analysed for *Ictalurus punctatus* and *Silurus glanis*. "0.00"indicates that the specific prey items in the fish's diet were considerably low; "na" indicates that respective prey item was not present.

Prey items	***Ictalurus punctatus* (immature)**			***Ictalurus punctatus* (mature)**			***Ictalurus punctatus* (total)**			***Silurus glanis* (immature)**		
	[F%]	**[N%]**	**[PV]**	**[F%]**	**[N%]**	**[PV]**	**[F%]**	**[N%]**	**[PV]**	**[F%]**	**[N%]**	**[PV]**
Detritus	0.30	0.08	0.05	0.25	0.04	0.02	0.30	0.06	0.03	0.10	0.09	0.03
Plants	0.45	0.09	0.05	0.60	0.14	0.11	0.45	0.12	0.08	0.13	0.11	0.04
Detergent	0.20	0.08	0.05	0.05	0.01	0.00	0.20	0.04	0.02	0.00	0.00	0.00
Palaemonetes sp.	0.25	0.18	0.11	0.15	0.04	0.02	0.25	0.10	0.05	0.03	0.02	0.00
Dikerogammarus villosus	0.18	0.14	0.05	0.20	0.03	0.02	0.18	0.08	0.03	0.05	0.07	0.02
Procambarus clarkii	0.10	0.01	0.00	0.15	0.03	0.01	0.10	0.02	0.01	na	na	na
unid. Crustaceans	0.08	0.14	0.05	na	na	na	0.08	0.06	0.02	0.03	0.02	0.00
Terr. insects	0.43	0.16	0.10	0.45	0.29	0.19	0.43	0.23	0.15	na	na	na
Aq. insects	0.13	0.00	0.00	0.25	0.06	0.03	0.13	0.03	0.01	0.03	0.02	0.00
Hirudinea	0.03	0.00	0.00	0.05	0.01	0.00	0.03	0.00	0.00	0.00	0.00	0.00
Fish larvae	0.08	0.02	0.01	0.05	0.01	0.00	0.08	0.01	0.00	na	na	na
unid. fish	na	na	na	na	na	na	na	na	na	0.03	0.02	0.00
small cyprinids	0.25	0.05	0.02	0.30	0.08	0.04	0.25	0.06	0.03	0.18	0.16	0.07
large cyprinids	0.03	0.00	0.00	0.05	0.01	0.00	0.03	0.00	0.00	na	na	na
Ictalurus punctatus	na	na	na	na	na	na	na	na	na	0.08	0.09	0.03
Molluscs	0.05	0.00	0.00	0.10	0.04	0.01	0.05	0.02	0.01	na	na	na
Small mammals	0.03	0.00	0.00	0.05	0.01	0.00	0.03	0.00	0.00	0.03	0.02	0.00
Small birds	0.08	0.01	0.00	0.10	0.02	0.01	0.08	0.01	0.00	0.03	0.02	0.00
Testudines	0.03	0.00	0.00	0.05	0.01	0.00	0.03	0.00	0.00	na	na	na

Table 2: Estimated niche (Levins's Index) and diet overlap based on stomach content data.

Index	**X**	***Ictalurus punctatus*** (immature)	***Ictalurus punctatus*** (mature)	***Ictalurus punctatus*** (total)	***Silurus glanis*** (immature)
Pianka's measure of niche overlap	***Ictalurus punctatus*** (immature)	x	0.634	x	0.363
	Ictalurus punctatus (mature)	0.634	x	x	0.591
	Ictalurus punctatus (total)	x	x	x	0.549
	Silurus glanis (immature)	0.363	0.591	0.549	X
	X	***Ictalurus punctatus*** (immature)	***Ictalurus punctatus*** (mature)	***Ictalurus punctatus*** (total)	***Silurus glanis*** (immature)
Diet overlap index a	***Ictalurus punctatus*** (immature)	x	0.740	x	0.779
	Ictalurus punctatus (mature)	0.740	x	x	0.839
	Ictalurus punctatus (total)	x	x	x	0.612
	Silurus glanis (immature)	0.779	0.839	0.612	x

Stable Isotope analyses

The applied linear regression model for δ^{15}N and total length of both species indicated a decreasing trophic position with increasing size (Fig. 3), while statistically significant negative correlations were found between TL and δ^{13}C for *S. glanis* ($r_s = -0.512$; $n = 39$; $p = 0.001$) and *I. punctatus* ($r_s = -0.665$; $n = 40$; $p < 0.001$). With a trophic score of 3.3, *S. glanis* occupied a higher trophic position than immature (3.1) and mature *I. punctatus* (2.9). *S. glanis* however had a lower variability in Layman's metrics and lower N range compared to both, immature and mature *I. punctatus*. In contrast, both *I. punctatus* showed a narrower C range (Tab. 3). Calculating the overlap of SEAc and SEAb revealed different results (Tab. 3): while 'immature *I. punctatus'* and 'immature *S. glanis'* showed a considerable overlap (0.22/0.28), 'mature *I. punctatus"* overlap with 'immature *S. glanis*' was very low (0.00/0.09). Overall, immature and mature *I. punctatus* presented a medium overlap (0.27/0.42), considerably higher than the SEAc overlap of the overall population of *I. punctatus* with 'immature *S. glanis'* (0.01/0.19) (Tab. 4). Moreover, the percentual isotopic niche overlap was low between 'immature *I. punctatus'* and 'immature *S. glanis*' (14.3%) as well as between 'immature *I. punctatus*' and 'mature *I. punctatus*' (13.4%), while no overlap was estimated between 'mature *I. punctatus'* and 'immature *S. glanis*' or the total population of *I. punctatus* and 'immature *S. glanis*'.

Table 3: Estimated Layman's metrics and Stable Isotope results for δ^{15}N and δ^{13}C of all groups of *Ictalurus punctatus* and immature *Silurus glanis.* NR = δ15N range, CR = δ13C range, TA = convex hull area, CD = mean distance to centroid, MNND = mean nearest neighbour distance, SDNND = standard deviation of the nearest neighbour distance, SEAc = Standard Ellipse Area.

Group	***Layman metrics and Stable Isotope Analysis results***									
	Mean δ^{15}N	**Mean δ^{13}C**	**TP**	**NR**	**CR**	**TA**	**CD**	**MNND**	**SDNND**	**SEAc**
***Ictalurus punctatus* (immature)**	16.2	-27.0	3.1	4.1	3.0	8.76	0.93	0.45	0.49	2.20
***Ictalurus punctatus* (mature)**	15.6	-26.2	2.9	4.1	2.0	5..39	0.91	0.29	0.24	1.84
***Ictalurus punctatus* (total)**	15.9	-26.6	3.0	5.7	3.0	11.55	1.11	0.34	0.35	2.59
***Silurus glanis* (immature)**	17.1	-25.5	3.3	2.8	4.1	4.09	0.79	0.19	0.17	0.87

Pairwise overlaps between groups indicated that 'immature *I. punctatus*' showed the highest probability to occur in the niche of 'mature *I. punctatus*' (95% probability) and 'immature *S. glanis*' (97% probability), followed by the probability of 'mature *I. punctatus*' to fall into the niche of 'immature *I. punctatus*' (80%) (Tab. 4). However, *S. glanis* showed lower probabilities to fall in either niche of immature (68%) or mature (33%) *I. punctatus* (Tab. 4).

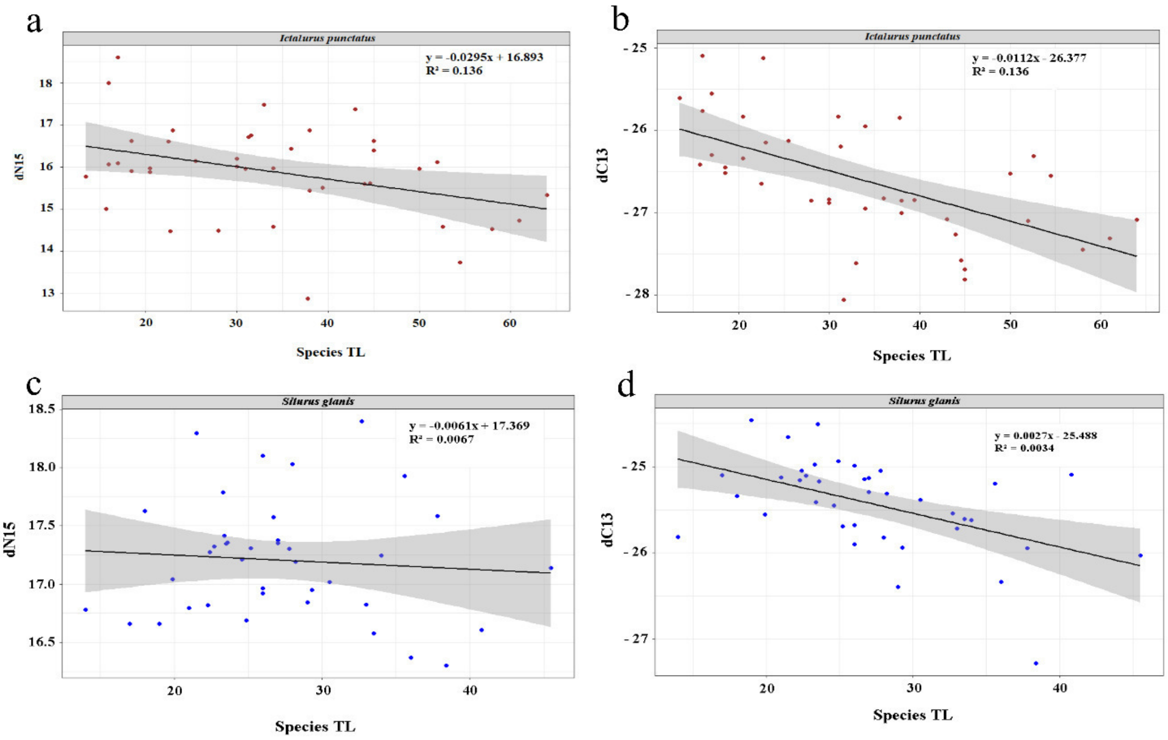

Figure 3: Linear regression between total length (TL) and δ^{15}N and δ^{13}C for *Ictalurus punctatus* (a,b) and *Silurus glanis* (c,d) from the inner-Florence section of the Arno river.

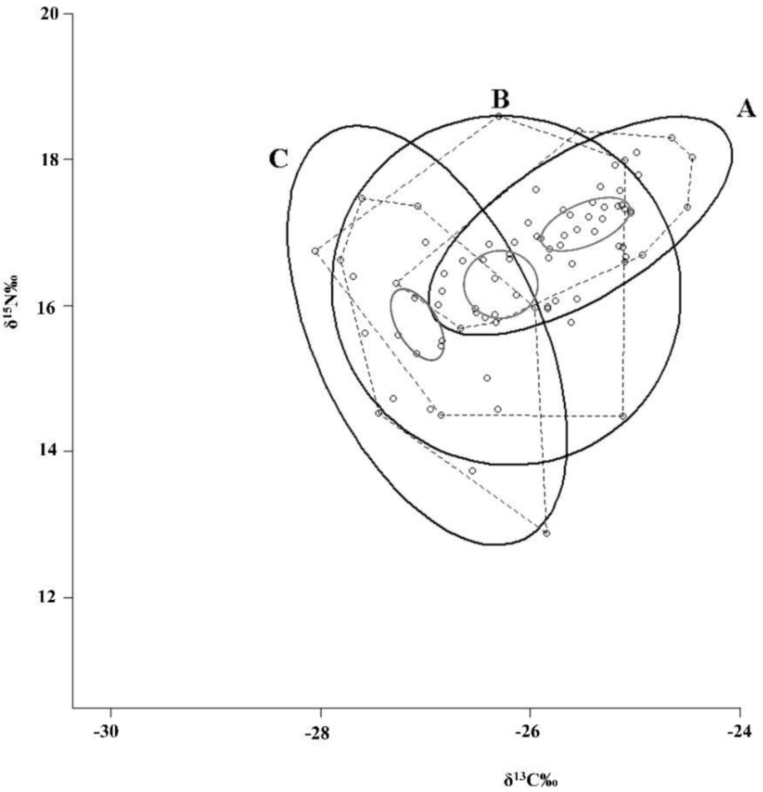

Figure 4: Standard Ellipse Area (SEAc; solid grey line); Total Convex Hull Area (TA; dashed black line) as well as 95% prediction ellipses (PE; solid black line) for *Silurus glanis* (A), immature (B) and mature (C) *Ictalurus punctatus*.

Table 4: Estimated niche overlap for all groups using the maximum likelihood fitted Standard Ellipse Area (SEAc) and the overlap between the corresponding 95% Prediction Ellipse (PE).

Index	**X**	***Ictalurus punctatus*** **(immature) (SEAc / PE overlap)**	***Ictalurus punctatus*** **(mature) (SEAc / PE overlap)**	***Ictalurus punctatus*** **(zotal) (SEAc / PE overlap)**	***Silurus glanis*** **(immature) (SEAc / PE overlap)**
SEAc overlap	***Ictalurus punctatus*** **(immature)**	x	0.27 / 0.42	x	0.22 / 0.28
	Ictalurus punctatus **(mature)**	0.27 / 0.42	x	x	0.00 / 0.09
	Ictalurus punctatus **(zotal)**	x	x	x	0.01 / 0.19
	Silurus glanis **(immature)**	0.22 / 0.28	0.00 / 0.09	0.01 0.19	x

The PERMANOVA main test confirmed significant differences among groups and the post-hoc comparisons showed that the three levels are clearly different (Tab. 5). Considering the results from CAP analysis (Fig. 5), groups differences are well explained by the first axis (CAP1, squared canonical correlation of $\delta_1{}^2$=0.5957; CAP 2 $\delta_2{}^2$=0.004). Both δ^{13}C (r_s = -0.926) and δ^{15}N (r_s = -0.771) are highly inversely correlated to CAP1 and effectively contribute to the differentiation of the groups, suggesting in particular that *S. glanis* is clearly different to other two groups for higher values for δ^{15}N and δ^{13}C. Overall, mature *I. punctatus* have lower values while immature *I. punctatus* lay in an intermediate position (see Fig. 4).

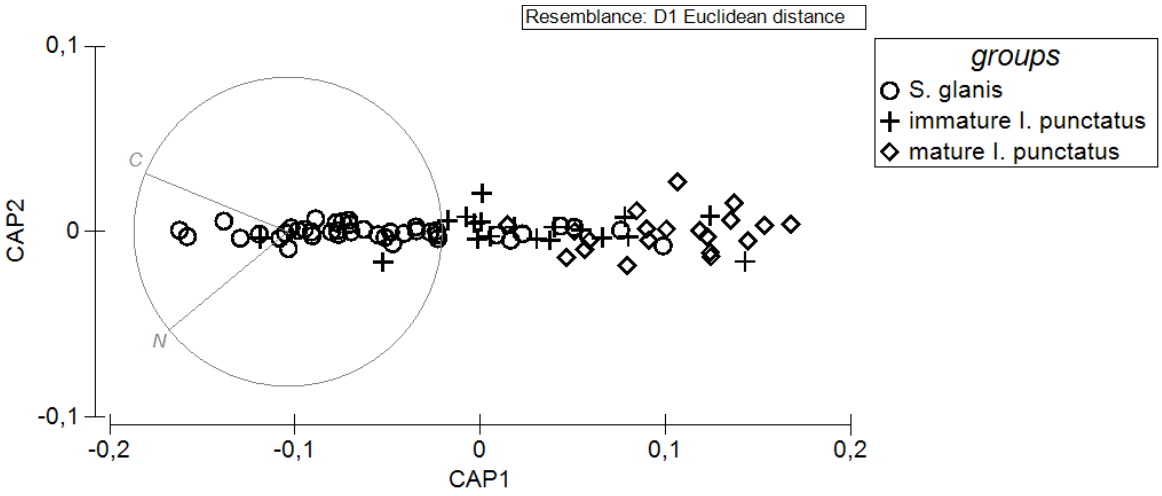

Figure 5: Two-dimensional scatter plot of the first and second principal coordinates axis (after resemblance matrix with Euclidean distance, n samples= 83, n variables = 2,) for immature and mature *Ictalurus punctatus* and *Silurus glanis*.

Table 5: PERMANOVA main and post-hoc tests results. Design:1 fixed factor, three levels; resemblance matrix measure of distance: Euclidean distance; type III (partial) sum of squares, unrestricted permutation of raw data.

	Source	Df	SS	MS	Pseudo-F	P (perm)	Unique perms
PERMANOVA main-test	Gr	2	63.519	31.76	28.53	0.001	999
	Res	80	89.057	1.1132			
	Total	82	152.58				

	Groups		t	P (perm)	Unique perms
Post-hoc tests	‘immature *I. punctatus*’	‘*S. glanis*’	4.3082	0.001	958
	‘mature *I. punctatus*’	‘*S. glanis*’	7.6838	0.001	969
	‘immature *I. punctatus*’	‘mature *I. punctatus*’	2.4983	0.003	747

Discussion

Interactions among alien fish species, especially with different life histories, are an understudied topic. More specifically, there is a lack of studies concerning trophic relationships among alien top predator’s native to different geographic areas while co-occurring in a new region, making this, at least to our knowledge, the first study assessing this issue.

In this study, *S. glanis* was observed to mainly feed on small cyprinids and *D. villosus*, fitting to this species early developing piscivorous diet (Copp et al., 2009; Syväranta et al., 2010). Moreover, *S. glanis* was identified to be less generalistic than *I. punctatus*, which showed a more diverse diet including insects, plant material, crayfish and fish that was highly variable among life stages. Additionally, all specimens of *I. punctatus* contained prey in their stomach, while almost half of *S. glanis*’ stomachs were empty. The observed high number of empty stomachs in immature *S. glanis* was unexpected as especially smaller fish feed every day. However, this result could be linked to the utilized sampling methods, inducing a lot of stress on hooked specimens and potentially resulting in vomiting of previously ingested prey, rather than on the time of sampling. Moreover, the length to weight ratio of *I. punctatus* was higher than that of *S. glanis* and the fullness index estimated for *I. punctatus* was in all three cases (immature, mature and all specimens) more than twice than that estimated for *S. glanis*. Concerning *I. punctatus*, omnivorous feeding with insectivorous tendency agrees with its diet and feeding behaviour from the native area (Busbee, 1968; Franssen & Gido, 2006). The high occurrence of plants (terrestrial as well as aquatic leaves) within the diet of sampled *S. glanis* is highly unusual (Carol et al., 2009). However, alien species (and especially *S. glanis*) are known to adapt to new and easily accessible prey (Copp et al., 2009; Strayer, 2010). Abundant algae or plants could potentially be a response to either scarce animal prey or an adaptation to abundant vegetation as a replacement to avoid intra- as well as interspecific competition (Copp et al., 2009; Davidson et al., 2011; Haubrock et al., 2018). Nonetheless, the used fullness index is potentially affected by physiological or morphological differences, e.g. differing weight to length ratios, but since both species belong to the order Siluriformes, these can be considered of secondary importance. However, these results indicate an advantageous adaptation to the invaded ecosystem, more efficient predation by *I. punctatus* or a comparably higher growth rate commonly found in majorly produced aquaculture fish species.

Diet and niche overlap

Schoener's diet overlap between all groups was with >0.6 considerably high, but while Pianka's diet-based niche overlap was similar between 'mature *I. punctatus*' and 'immature *S. glanis*', 'immature *I. punctatus*' and 'immature *S. glanis*' showed a lower diet-based niche overlap value (0.36) in respect to Schoener's diet overlap value. This difference can be due to the "prominence value" in the used formula, weighing the importance of prey items and, thus, being affected by the sample size. In contrast, SIA and the derived estimated ellipse overlay of SEAc indicated a higher similarity between niches occupied by 'immature *I. punctatus*' and '*S. glanis*' than between 'mature *I. punctatus*' and '*S. glanis*'.

Opposed to the narrow time constrained information presented by dietary analysis, SIA offers a wider mediated information on diet as well as, with SEAc and SEAb, occupied feeding niches. Thus, a moderate to high niche overlap can indicate that the compared species compete for similar resources, while not stating if they can coexist over a longer time period nor whether they feed on similar prey resources due to limited interference competition. Hence, neither dietary similarity between groups estimated with the overlap of SEAc, SEAb, nor percentual isotopic niche overlap reflected the results gained from direct dietary information. Analyses of diets are very focused and dependable on observable ingested items, while isotopes analyses consider previously consumed and assimilated prey. Additionally, all three groups belong to approximately the same estimated trophic position as expected from *I. punctatus* and *S. glanis* (Gido & Franssen, 2007; Syväranta et al., 2010), but 'immature *S. glanis'* had a slightly wider δ^{13}C range than the two life-stages of *I. punctatus*. This observation reflects the consumption of a greater variety of carbon sources. A study on *S. glanis* in France found that *S. glanis* was considerably enriched and variable in δ^{13}C in respect to other fishes, explainable with the frequent predation on terrestrial birds and / or mammals (Syväranta et al., 2010). However, this study lacked large-bodied individuals. Nonetheless, it seems reasonable to expect similar results, as anglers' observations confirm the active predation on e.g. pigeons by large *S. glanis* in the Arno River (Cucherousset et al., 2012; Thomas Busatto, pers. comm.). Similarly, δ^{15}N values tend to increase with the length of catfish (Syväranta et al., 2010), reflecting an increased piscivorous or animal diet. While this might be true for *S. glanis* (Syväranta et al., 2010), our results show generally lower mean δ^{15}N values and lower trophic position of 'mature *I. punctatus*' in respect to 'immature *I. punctatus*'. This can potentially be explained by the high level of eutrophication (Romero et al., 2013) and the natural accumulation of nutrients (i.e. dead matter) in the river bottom (Hendrix et al., 1986; Hansen & Kristensen, 1998). Immature *I. punctatus* are benthivorous and often detrivorous while mature individuals exert increased offshore feeding (as indicated by the difference in δ^{13}C values; Gido et al., 2006). Additionally, the lower mean δ^{15}N values could be linked to high competition for increasingly piscivorous mature *I. punctatus* with other species like mature *S. glanis* (Fry et al., 1999). Moreover, Fry et al. (1999) observed that especially δ^{15}N values of *I. punctatus* tend to remain constant with increasing size, while sampling at several occasions resulted in two mature groups with clearly distinct niches; they linked this observation to varying feeding activities of different schools of *I. punctatus*. Furthermore, a higher δ^{15}N range was found in *I. punctatus,* underlying its generalist and opportunistic behaviour. Thus, it is possible that the relationship of shared niche space continues to weaken as larger age-classes of S. glanis are included, because prey fish size increases with age and size of *S. glanis*. This seems to suggest that the difference in niche space may be exaggerated further by including larger size classes and that *S. glanis* prey preference and feeding strategy may change as they grow from immature to mature. Indeed, *S. glanis*' Layman's metrics showed a less variable niche occupation linked to a more specialized diet and niche placement compared to *I. punctatus*. The lower δ^{15}N values in 'mature *I. punctatus*' could be due to the sample, other disturbances (lack of prey, competition, etc.) or the opportunistic feeding of individual 'immature *I. punctatus*', therefore needing further investigations.

The results presented by SIA indicate that 'mature *I. punctatus'* and 'immature *S. glanis'* likely occupy different trophic niches with generally low similarity in their diet. However, both species show some degree of diet overlap for those common prey items and Pianka's diet-based measure for niche overlap, an observation likely linked to gape size limited predation in catfishes (Johnson et al., 2008). Moreover, this overlap could be related to the opportunistic nature of very generalist *I. punctatus* (Busbee, 1968; Rosen et al., 1995) while 'immature *S. glanis'* showed a lower diet and diet-based niche overlap but a more similar isotopic niche occupation. Considering the estimation of higher probability for *I. punctatus* (immature and mature) to occur within the niche of each other and the niche of *S. glanis* while *S. glanis* presenting lower possibilities, *I. punctatus* shows a higher plasticity in its niche occupation.

The high number of empty stomachs in *S. glanis* and high frequencies of certain prey items (e.g. detritus, plants, algae, small cyprinids) in the diet of *I. punctatu*s result in an overall similar prey assortment for the diet analyses, while the associated energy intake and thus isotope assimilation might differ. However, the estimated overlap and thus arising competitions (aside from competition for shelter) are only potential explanations for the observed decrease in *S. glanis*. Furthermore, it is possible that the feeding mechanism of "sucking-in" of small sized *S. glanis* is less efficient compared to the more active predation mechanism used by *I. punctatus*. Also, Menzel (1945) observed that especially immature *I. punctatus* predate eggs and larvae or fish species, as they commonly share the same habitat, enabling this species to threaten entire populations in relative short time after its introduction (Nesler, 1995; Narsh & Brooks, 1989; Lentsch et al., 1996). In the case of the Arno River, *I. punctatus* is seemingly more efficient in its prey consumption and shows signs of an advantageous biology, making it better adapted to the non-native ecosystem that slower-growing *S. glanis* of similar lengths. Furthermore, this hypothesis is supported by the wider trophic niche occupation and a less specialised diet paired with the observed higher fullness index as well as a higher length weight ratio in *I. punctatus*. Additionally, while both species are usually active during the night, *I. punctatus* (especially immatures) tend to extent their activity time into the day, thus giving it an advantage over *S. glanis* (Noeske-Hallin et al., 1985; Boujard & Leatherland, 1992; Boujard, 1995).

The observed differences in stomach content analyses and SIA, based on the different information they provide, underline how the opportunistic feeding behaviour of *I. punctatus* leads to diet and niche overlap while also highlighting the potential for those two species that share a common habitat and overlap in their activity time to compete for resources. Moreover, this observed niche overlap likely affects the more specialized *S. glanis* and could increase in times when resources are increasingly limited. Therefore, the competition between both alien catfish species could be one of the main reasons why a decrease in the abundance of mature *S. glanis* was observed after the introduction of *I. punctatus* but more long-term studies covering the entire year while also including mature *S. glanis* will be needed.

Conclusion

The presence of the alien North American catfish *I. punctatus* was previously believed to have a negative impact on the population of *S. glanis*, indicated by the decreasing abundance of *S. glanis* and an increase in the catchments of *I. punctatus* (Arno River: Annamaria Nocita and Thomas Busatto pers. obs.; Po River: Max Fiedler, Andrea Battaglini and Phillip Haubrock pers. obs.). The presented results indicate that this observation might be due to the potential for immature *I. punctatus*, which are the dominating life-stage (Ligas, 2007; Haubrock et al., 2017, 2018b), and immature *S. glanis*, a species that is more specialised in its niche occupation, to compete. Although both species are alien, *S. glanis* is a large body-sized fish (Copp et al., 2009) and cherished among “catch-and-release anglers” (Arlinghaus et al., 2007) as a trophy fish. However, with the increasing distribution of the fast growing and comparably more generalist as well as opportunistic *I. punctatus* (Tucker & Hargreaves, 2004; Olden & Poff, 2005), the population of *S. glanis* could be negatively affected through competition and overlapping niche partitioning. Further studies should focus on the predation degree between big-sized *Silurus glanis* (>~70 cm) and the two life-stages of North American catfish under the consideration of age and length classes, considering and investigating also the presence other species.

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Authors‘ Contribution Statement

Conceived and designed the investigation: PJH, ET. Performed field work: MA, PB. Analysed the data: MA, PJH, AFI. Wrote the paper: PJH, AFI, PB.

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CONCLUSION

Due to the restrictive nature and scarce availability of financial resources, one of the main challenges for ecologists working with invasive species is the prioritization of IAS according to the magnitude of their impact and ability to spread. To do so, assessments have to be based on a general understanding of the biology of an invader, followed by a thorough quantification of impacts. But impacts are diverse, manifold and differ according to species and ecosystem.

In this work, impacts and methods to assess impacts were reviewed, and different approaches to quantify and assess impacts were applied on selected species.

As shown in Chapter 1, considerable progress has been made, as not only single species' impacts, but rather interactions and thus, facilitations and even obstructing processes are investigated. Moreover, the way "impacts" are perceived has developed from strictly ecological or economically positive or negative effects to consideration of socio-economic impacts affecting human well-being. Thus, assessment tools like EICAT and SEICAT are the logical consequence that cope with the increased understanding.

Chapter 2

Hypothesis 1: "*Data and studies on per-capita effects of intensively studied species (e.g. Procambarus clarkii) are still needed. Simultaneously, many so far unstudied alien species (e.g. Ictalurus punctatus) also pose a significant threat to their recipient ecosystems.*"

In many cases, alien species have been the target of intense investigations, mostly due to their attractiveness or their biology, making them ideal model species. However, even in regard to species like *P. clarkii*, where one might expect that most aspects of this species invasion biology have been assessed, several questions remained unanswered. Reasons for this might be a high specificity of impacts on local scales or difficulties in their assessment due to variables that need to be controlled. However, as unlikely as it seems, even the burrowing activity of this species, which is well-known for its ability to periodically sustain life in hostile environments due to exactly this ability is not well understood. Moreover, even less was known about the way this "burrowing behaviour" effects the structure and stability of river banks and how different sexes interact when burrowing, especially as this behaviour might vary according to ecosystem and region. With the usage of model systems, this behaviour was investigated, revealing the extent of crayfish burrowing on river levees. Additionally, it could be observed that crayfish form "couples" fairly fast and continue to interact with each other, even going as far as working together on the construction of burrows and sharing duties of burrow protections; behavioural aspects that were previously unknown for this species.

On the other side, many species have not been assessed, despite the likeliness of causing significant harm to the ecosystem they invade. Just recently, increasing studies investigated the European catfish *Silurus glanis* or black bullhead *Ameiurus melas* in

Europe. However, these two being just few of the many species that are introduced to European freshwater ecosystems and succeed at establishing and often spreading. Considering the abundant North American channel catfish *Ictalurus punctatus* in Italy, it becomes clear that many species with potentially major impacts have been neglected, often willingly, being allowed to spread due to the hiding nature of aquatic environments.

Hypothesis 2: "*Data and studies on per-capita effects of intensively studied species are still needed. one species is the red swamp crayfish Procambarus clarkii. Despite being one of the most studied crayfish species, burrowing behavior is still poorly documented. However, this behavior can induce relevant impacts on the invaded habitat. Simultaneously, many so far unstudied alien species (e.g. Ictalurus punctatus) also pose a significant threat to their recipient ecosystems.*"

Whenever a new alien species makes its appearance or becomes noticeable, a repeatable scheme or protocol needs to be applied and the risks this species presents must be assessed. For this purpose, a literature review was conducted, compiling data from the native habitat, invaded habitats (if available) and potentially available information on the species in the geographical area of interest, in this case European freshwater ecosystems. With these, the major risk *I. punctatus* poses for European freshwaters was identified and the lack of studies in Europe underlined.

Furthermore, with the collection of as much data as possible as well as the application of fairly old but powerful dietary studies, the predation and competition impact *I. punctatus* exerts was assessed. Additionally, all information (diet, growth, population structure, competition) were complemented with more advanced statistical analyses and sampling of potentially competing species (e.g. *S. glanis*) and other present species for an analysis of Stable Isotopes to investigate the effect of *I. punctatus*' presence on the ecosystem. Not only did the studies reveal a notable impact based on predation on other species, but furthermore a competition with the also alien catfish species due to the unexpected generalist and opportunistic feeding behaviour exhibited by *I. punctatus*, thus likely also an effect on socio-economic aspect such as local angling practice.

Thus, based on *I. punctatus* ability to spread and capability to rapidly reproductive, paired with its overall broad environmental tolerance, it is likely that its impact on European freshwater species will increase over the years, leading to not only negative ecological impacts, but also economic losses and should therefore be considered as invasive.

Chapter 3

Hypothesis 3: "*The presence of multiple invasive species with or without common life-history and history of co-evolution can affect (i.e. lower or increase) the magnitude of exerted impacts, while these interactions might be specific to species and ecosystems*."

With the use of different techniques, interactions among alien species can be investigated and consecutively displayed, often revealing their unique nature and ecosystem- as well as species-specificity. However, it is this context-specificity that make species interactions important to investigate, as they can be the key to understanding facilitation or impeding processes and thus, the establishment of alien species.

Due to their context-dependency, interactions with already present species can alter the impact of an invader on the recipient flora and fauna. In the case of Arreo Lake, species originating from North America were introduced one after another to produce a sustainable fishery for the black bass *M. salmoides* and associated angling activities. These introductions have led to the reduction and even extinction of native fish species and macrozoobenthos. Lastly, due to the strong links between alien species, eradications have become increasingly difficult as the high presence of *P. clarkii*, a species difficult to control and manage facilitates the recovery of *L. gibbosus* and *M. salmoides* populations.

Hence, one might expect that the introduction of a second "high-impact" invader like the North American Bullfrog *L. catesbeianus* would add the impact on e.g. native amphibians of the already present *P. clarkii*. However, it has been previously observed that predator-prey interactions between these two species originating from their common life-history are re-established in the introduced range and, in fact, lower the impact of both species on native amphibian. Somewhat similar, it was observed that not only do alien *L. catesbeianus* establish rapidly when prey items are abundant, but also adapt to the presence and availability of *P. clarkii*, thus limiting their predation on other amphibians. Moreover, differences in the diet between immature and mature individuals resulted in lowered competition and, thus, increased potential for this species establishment. However, bullfrogs were not able to establish as top-predators when grass snakes were present. Another result gained from studying trophic interactions was the exploitation of *P. clarkii* by most alien and native species, centring around this abundant omnivore. Hence, observed interactions between alien species can be seen as facilitating for *L. catesbeianus*, while beneficial for native species that would be impacted more when presented by only one, *L. catesbeianus* or *P. clarkii*. Such interactions are not only interesting from a biological viewpoint.

Attempts to control and potentially eradicate alien species after they successfully established and after they successfully developed tight interactions with other species is increasingly difficult. In ecosystems where *P. clarkii*, a species difficult to control, becomes the main prey item for various species, it becomes increasingly hard to reduce predatory alien species due to their feeding relationship with *P. clarkii*. This underlines the importance of understanding interactions and of the impact an IAS can have on other species.

A different scenario is presented when alien species from different geographic origins interact, as in the observed situation between the North American channel catfish *I. punctatus* and the European catfish *S. glanis* in the Arno river. Although catfishes tend to fill specific roles within the ecosystem they are part of, these two species are occupying similar ecological niches, despite being morphologically very different (i.e. in terms of size, growth, predation mechanism). However, they were introduced to the river Arno in approximately the same year, but at different locations. In the inner-Florence section of the Arno river, *S. glanis* arrived first and established an abundant population, but declined in its abundance shortly after *I. punctatus* was introduced by local anglers around the year 2004.

With the use of dietary and stable isotope analyses, immature *S. glanis* and immature *I. punctatus* were identified to occupy similar trophic niches, but adult *I. punctatus* tend to overlap in their diet spectrum with *S. glanis* due to its generalistic and opportunistic nature, leading to an observable decline in the abundance of *S. glanis* in the study area. However, such interactions are object to the availability of prey, and, while SIA indicated a differing main prey item for both species, direct diet analyses showed that the annually changing water cycle affects the activity and abundance of prey items (and thus the diet of *I. punctatus*), resulting in increased competition. These kind of hindering interactions between species however do not result in lower impacts on native species, but rather lead to a decrease in the abundance of one species, while the other is dominating in terms of numbers and biomass, seemingly unaffected by this relationship. Furthermore, the interaction between these two catfish species nor the predation of other alien species has to be considered as the mainly negative impact; it is more the abundance, reproductive capability and ability to spread paired with its defense mechanism (the venomous pectoral and dorsal spines) that not only result in no predation by other species on *I. punctatus*, but also make the recovery of diminished or even locally extinct native species unlikely without extensive control efforts on alien species.

Therefore, interactions between species are seemingly unpredictable, as they are influenced by various factors and should be assessed on case-by case basis. With increased knowledge and understanding of interactions between alien species, mechanisms that determine the outcome, e.g. negative or positive affects for one or multiple alien species, lowering or increasing effect on present native species, could become assessable. With such information, more reasonable predictions could positively affect our understanding of mechanisms supporting the invasive ability of alien species. Therefore, it should be noted that the behaviour of invasions is indeed very complex and must be studied on a case by case basis, because their effect on e.g. successful management is immediate.

Concluding, it can be stated that with the available data, the above-mentioned hypotheses can be clearly confirmed.

FURTHER QUESTIONS AND REVEALED GAPS

Future efforts will need to focus on the assessment of impacts and the production of tools to scale aquatic species' invasiveness to determine the effectiveness of management and control attempts.

Nonetheless, some further questions arose while conducting the studies for the present thesis and could not be answered in any so far conducted study. Furthermore, certain gaps in our understanding of studied species were revealed. In the case of *P. clarkii*, the conducted study showed how male and female crayfish both participate in burrowing activity and form partnerships for the reproductive season. Additionally, it became obvious that the burrowing behaviour under different environmental conditions and *P. clarkii*'s response to them is not entirely understood. For *L. catesbeianus*, it was observed that despite the highly negative impact it has on food webs, the presence of other species can facilitate its establishment (as in the case of *L. gibbosus* feeding on typical predators of bullfrog tadpoles; i.e. dragonfly larvae) or lower its impact of co-occurring amphibians like the green frog *P.* kl. *esculentus* (due to the predation on *P. clarkii*). As studies were focusing on seasonal circumstances, it was not applicable to investigate the relationship between tadpoles and native species as well as other alien species.

For *I. punctatus*, the shortage of literature regarding its presence in Europe can be considered a huge gap, especially because it was introduced to Europe about two decades ago and is now widespread due to its use in aquaculture and angling. While our understanding of this species behaviour, adaptation and impacts increased significantly through this work, it is still unclear as how the interaction and competition with other species such as *L. gibbosus* or *P. clarkii* affects its establishment or how it adapts to different climates in Europe. Moreover, it is still unclear how, how often, when and where *I. punctatus* was introduced, making assessments of its future spread rather difficult. Last, but not least, it is of utmost importance to identify species at risk, since studies in the Western US showed the vulnerability of many endemic and economically important species such as *Oncorhynchus gorbuscha* to the predation of introduced channel catfish, but such assessments for Europe have yet to be made.

In conclusion, it has been shown that various aspects related to alien species' impacts need to be assessed in the future. Moreover, this work underlines the potential of IAS to impact recipient ecosystems without being noticed by the scientific community and thus, managed and controlled. Lastly, as ecosystems and the residing species' interactions are complex, it was revealed that differences according to the origin or interacting species are characterizing for the relationship.

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Lastly, I need to thank my daughters Ilaria and Sophia who have and will always be my motivation, because their fascination of nature became the reason why conservation is of such importance to me.

Thank you all for your unwavering support.

> “If the bricks aren’t well made, the wall falls down. This is an awefully big wall I’m building here, so I need a lot of bricks. Fortunately, I know a lot of brickmakers, and all sorts of other useful folks as wells.”
>
> ~ George R. R. Martin

However, it has to be pointed out, underlined and highlighted that this thesis and all the included projects were made possible by the Aquainvad-ED project (2020 Marie Sklodowska-Curie ITN-2014-ETN-642197).

Annex

A1

Declaration of Independent Work

I hereby declare, that I have authored this thesis independently, that I have not used other than the declared sources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources. According to my knowledge, the content or parts of this thesis have not been presented to any other examination authority. I am aware that the respective work can be considered as a "fail" in the event of a false declaration. In case of justified suspicion, the thesis in digital form can be examined with the help of suitable programs. For the comparison of my work with existing sources or future works, I agree to store a copy in the institutional repository.

Phillip J. Haubrock   Florence, 28.11.2018

Signature   City / Date   /

A2

STUDENT ETHICS CHECK LIST

1. Does your study involve humans as the focus of research, or make use of data collected from human subjects?

   ☐ Yes ☒ No

2. Does your study involve any species protected in Italy, EU, or international law, or work in protected areas including Special Areas of Conservation (SACs), Sites of importance for Nature Conservation (SINCs), Sites of Community Importance (SCIs) in Italy?

   ☐ Yes ☒ No

3. Does your study involve work on animal falling under the EU directive 63/2010 (protection of animals used for scientific purposes)?

   ☐ Yes ☒ No

Student Phillip J. Haubrock Supervisor Elena Tricarico

Phillip J. Haubrock Elena Tricarico

Date 08.07.2016

A3

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The effects of two different preservation methods on morphological characteristics of the alien channel catfish *Ictalurus punctatus* (Ictaluridae, Siluriformes) in European freshwater.

Short title: Preservation methods in channel catfish

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Abstract

Different methods used to store specimens, even for short periods, can notably affect morphometric characteristics such as colour, length, and shape. As a consequence, in the case of samples that have to be analysed morphometrically, the right preservation method is crucial. In this study, two comparably easy applicable methods, the effects of freezing and moderate cool storage on morphological traits, were studied on specimens of the scaleless North American channel catfish, *Ictalurus punctatus*, an alien species to European freshwaters of increasing concern. Results revealed that freezing, like preservation with alcohol or formalin, affected the generally considered characteristics (colour, standard length, fork length, total length, shape), while cool storage was shown to preserve most traits without notable alteration, an important difference when studying potentially adapting alien species. Implications are discussed.

Key Words: fish morphology, freezing, cooling, colour, alteration

Published in: Croatian Journal of Fisheries

Introduction

Samples of different taxonomic groups are generally preserved under varying conditions (Asahida et al. 1996, Gould 1996, Ghaly et al. 2010). For fish specimens, ichthyologists usually use formalin, alcohol, or freezing (Ghaly et al. 2010). Storing samples in formalin and alcohol as a long-term measure is reasonable, but short-term storage of large quantities (e.g. transport) can be delicate and is not always the most efficient method. However, the availability, transportation, and storage of ice is not always a feasible option.

Different short and long-term storage techniques have been shown to affect taxonomically important morphometric characteristics of fish, such as length (e.g. Al-Hassan and Shawafi 2003). Additionally, when considering the water content ratios of white and red muscles, the effect of storage and preservation method may cause variations between scale-less and scaled fish (Leslie and Moore 1986). Few studies deal with the effect of varying preservation methods on pigmentation and morphometric characteristics in different species of fish (see e.g. Jawad et al. 2001, Neave et al. 2006). This variability in morphology among different preserved species and samples can be due to several factors such as time elapsing until preservation, the applied preservation method, concentration of preservative, preservation duration and temperature, morphological variability among specimens, and species-specific factors (age, size, state, the osmoregulatory activity of the fish at the point of death; Yeh and Hodson 1975). As the North American channel catfish, *Ictalurus punctatus,* is receiving an increasing scientific attention in Europe as an alien species (Banha et al. 2017; Haubrock et al. 2017) and due to the potential influx of further North American catfish species that are difficult to distinguish by the untrained eye, a preservation method that does not impact morphological characteristics is needed.

The present work aims at investigating the effect of two easy applicable methods, freezing and cooling, on morphological characteristics. These options were chosen as two commonly applied and feasible methods for transporting and storing freshly caught specimens.

Material and Methods

Specimens of *Ictalurus punctatus* were collected from the river Arno (Central Italy). Immediately after the capture, morphological characters of all samples (total length, standard length and fork length, body shape and colour) were measured or photographed. Subsequently, specimens were euthanized with a standard gill cut. Twenty samples per preservation method were stored in two batches of ten specimens each. Specimens in the freezing treatment, were directly laid on and covered with ice cubes and were then transported to a standard freezer within one hour and frozen at -20 °C. For the cooling treatment, cooling down without freezing was achieved by transferring specimens into a reefer-container and loosely placing them between cloth-wrapped cold packs. Cold packs were replaced with new replenished packs after eight hours to keep the temperature between 2 and 4 °C. After 96 hours, cooled and frozen specimens were measured again, and measurements were compared. In the case of frozen samples, defrosting was achieved at ambient temperature over a period of 2 – 4 hours. Specimens were straightened up and subsequently measured. Measurements from before and after preservation for both methods were analysed using paired t-test (statistic: t); changes in length before and after preservation were compared between the two methods using Mann-Whitney test (statistic: U). The level of significance under which the null hypothesis was rejected was α = 0.05. Table 1 provides mean values or median values plus standard error or 1° - 3° interquartile. SPSS®, Statistical Package for Social Science 13.0 for Windows was used for the statistical analyses.

Results

Freezing and cooling led to different effects on morphological characters. In the case of freezing, a degradation in colour from goldish-brown to grey-blackish was visible in every case, while the body shape was unaffected overall, except for the belly being less elevated, soft, and pliable after defrosting. The standard length of frozen fish increased between 0.1 and 1.9 cm (~3.3 %), the distance between standard length and fork length by 0.1 – 0.4 cm and total length respectively by 0.1- 0.5 cm. Results of statistical analysis are displayed in Table 1. Additionally, the changes in size of specimens is linked to increases in all length categories (Figure 1). Comparably in cooled specimens, the increase in all length measures was between 0 and 0.2 cm, bellies kept their firm shape, and although colour change, it maintained a lighter tone (including the typical punctuation of *I. punctatus*) (Figure 2).

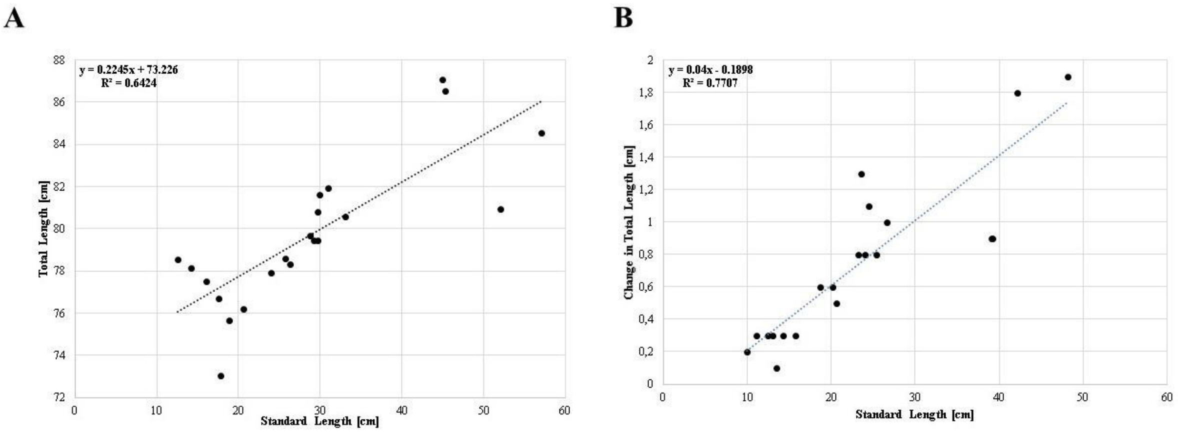

Figure 1: A) Standard length (X-Axis) in relation to total length (Y-Axis) before freezing. B) Standard length (X-Axis) in relation to increase of total length (Y-Axis) after 96 hours of freezing

Table 1: Comparisons of changes in Standard- Fork- and Total-Length between frozen and cooled specimens using Mann-Whitney-U test [U], and lengths before and after freezing and cooling using paired t-tests [t]. For paired t-tests, means ± standard errors and for Mann-Whitney-U Tests medians and 1°-3° interquartiles are reported. Significant values are highlighted in bold.

Cooling vs. Freezing

Parameter	**U**	**n**	**p**	**Freezing**	**Cooling**
Change Standard Length (cm)	208	20 20 40	**< 0.01**	0.74 (0.3 – 0.98)	0.1 (0.0 – 0.2)
Change Fork Length (cm)	207.5	20 20 40	**< 0.01**	0.64 (0.3 – 0.8)	0.1 (0.0 – 0.18)
Change Total Length (cm)	190	20 20 40	**< 0.01**	0.62 (0.3 – 0.7)	0.1 (0.0 – 0.1)

Before and After preservation

Preservation method	**Parameter**	**t**	**df**	**p**	**Before**	**After**
Freezing	Standard Length (cm)	-6.579	20	**< 0.01**	23.26 ± 2.47	24.00 ± 2.56
	Fork Length (cm)	-6.312	20	**< 0.01**	25.59 ± 2.70	26.22 ± 2.79
	Total Length (cm)	-7.132	20	**< 0.01**	28.77 ± 2.80	29.39 ± 2.88
Cooling	Standard Length (cm)	-5.667	20	**< 0.01**	26.25 ± 2.59	26.33 ± 2.60
	Fork Length (cm)	-5.596	20	**< 0.01**	28.42 ± 2.66	28.52 ± 2.68
	Total Length (cm)	[-5.6270	20	**< 0.01**	32. 40 ± 2.92	32.50 ± 2.93

Discussion

Differences in size, shape or size of morphological characters can be of outmost importance in studies assessing the morphology of a target species (Sayers 1987, Al-Hassan and Shawafi 2003). This is crucial when studying similar species with overlapping distributions, such as *I. punctatus*, *Ictalurus furcatus* and *Ameiurus catus*. These species can be morphologically variable, as for instance in the case of genetic introgression in *I. punctatus*, causing anal fin ray reduction up to 23 cm, making it morphologically more similar to *A. catus*. Another example might occur in the future, when alien species which are genetically compatible and morphologically very similar species with almost identical juvenile life stages, such as *I. punctatus* and *I. furcatus*, hybridize, making morphological distinction crucial. Furthermore, to the best of our knowledge, there are no standard approaches to calculate the size of specimens while alive based on only the size of preserved specimens. There is no evidence of standard correlation factors between living length and preserved length (Yeh and Hodson 1975).

Figure 2: Comparison between a specimen of *Ictalurus punctatus* after A) 96 hours of cooling and B) 96 hours of freezing.

In the present study, both preservation by freezing and by cooling had a significant effect on fish size. However, freezing caused a significantly higher increase compared to cooled specimens. Furthermore, we observed a change in skin colour and pigmentation in frozen specimens, leading to a much darker tone and invisibility of the typical black dots of the species. This pigmentation change has also been similarly observed in rainbow trout (Chen et al. 1984, No and Storebakken 1991). Conversely, cooling down preserved most traits in a more reliable way.

Hossaini et al. (2016) found that as an alternative to the described cooling and freezing, preservation with alcohol affected colour and body proportion of Zagros Tooth-Carp, *Aphanius vladykovi.* Additionally, Jawad et al. (2001) states there is shrinkage of multiple characteristics caused by different concentrations of formalin and alcohol, and also freezing, in sparid-fishes. In contrast, Al-Hassan and Abdullah (1982) observed the opposite, a slight increase in preserved specimen sizes. Although the impact of preservation using alcohol and formalin have not been the target of this study, differing results and the most often observed shrinkage in length can be explained by varying chemical concentrations in the body (e.g. a gradient in the tail), genetically based differences in white to red muscles ratios, and variable fluid content (Leslie and Moore 1986, Hossaini et al. 2016). Hence, different preservatives may influence characteristics differently and thus, while alcohol may damage proteins and therefore the tissue, formalin will negatively affect the colour while sustaining colour pattern. Therefore, a standardized and repeatable preservation is of interest for the correct identification and analysis of morphology.

Subsequently, it can be stated that the applicability of preservation with alcohol, formalin and freezing can be considered as more difficult to apply considering the hardship of transporting sufficiently sized container and proper amounts of preservative when dealing with multiple bigger sized specimens. We therefore suggest that morphometric measures on channel catfish should be taken directly on fresh or cooled specimens, while the use of frozen and defrosted fishes should be avoided for potential misidentification of species.

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Age determination in the Channel Catfish *Ictalurus punctatus* (Rafinesque, 1818) using pectoral spines: a technical report

Short title: Determining catfish age

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Abstract

The impacts of non-native species might be noticeable years after their initial introduction. Determining the age of a recently established non-native fish population can be important to identify the year of its introduction. Although well-established, the accuracy and reproducibility of age determination techniques are not often discussed. In this manuscript, we propose a standardised method for the determination of age in scale-less fishes. We use the Channel Catfish *Ictalurus punctatus* as a model species and we highlight the importance of the pectoral spine section location to produce accurate age determinations. Our results show that decalcification is not necessarily needed and that cuts to the basal section of the spine shaft produce the clearest and most reliable results.

keywords: Pectoral spine section location; annuli; population assessment; age determination; scale-less fish

Published in: FiSHMED; Fishes in Mediterranean Environments

Introduction

Invasions of freshwater ecosystems by non-native species may go unnoticed due to the hidden nature of aquatic habitats (García-Berthou et al. 2005; Brundu et al. 2013). Invasions can often be stopped shortly after the introduction of a non-native species (Simberloff et al. 2013), however, due to delayed detection within aquatic ecosystems, eradication frequently becomes unfeasible.

One such invasion is by the North American Channel Catfish *Ictalurus punctatus* (Rafinesque, 1818), a species that has inconspicuously established dense populations in various European waters (Ligas 2008). It was widely introduced into various European countries, for example: Serbia (Olenin et al. 2008); Slovakia (Kosco et al. 2004); Greece (Zenetos et al. 2009); and Germany (Kottelat & Freyhof 2007). Moreover, non-native populations have also been known to occur in Mediterranean regions such as the Californian watersheds (Moyle 2002), Portugal (Banha et al. 2017) and Italy (Ligas 2008; Haubrock et al. 2018a, b). This species is thought to have been introduced in Europe for the purposes of angling (Appelget & Smith 1951; Rezk et al. 2003; Kottelat & Freyhof 2007) and aquaculture (Welcomme 1988; Elvira & Almodóvar 2001; Savini et al. 2010), however, due to its tolerance to various environmental conditions and high fertility rates, often becomes invasive (Moyle 2002). Although the optimal water temperature for this species is between 28 and 30 °C (Cheetham et al. 1976), it shows a wide tolerance for very low (2 - 4 °C; Moss & Scott 1961) and very high (36.6 – 37.8 °C; Allen & Strawn 1968) temperatures. This tolerance has allowed this species to endure the highly variable temperatures of Mediterranean riverine systems, thereby allowing it to become established.

Since the 1980s, *I. punctatus* has been reported in Spain, however, it has recently spread into other European freshwater systems including those in Portugal and Italy (Elvira & Almodóvar 2001; Lopez et al. 2012; Banha et al. 2017). Following its introduction to Northern Italy in 1986 for aquaculture purposes (Nocita & Lenuzza 2016), *I. punctatus* now occurs in dense populations within the Po, Piave, Brante and Arno rivers. *Ictalurus punctatus* was first observed within the Arno River (Pisa, Italy) in 1998 (Nocita, personal communication). By 2004, its distribution had spread into the inner-Florence section of the Arno River (Nocita, personal communication), and is now one of several alien species that dominate this river's fish communities (Table 1). Furthermore, *I.* punctatus is the dominant species within the inner-city section of the Arno River and is likely causing adverse negative impacts on the native ecosystem through predation (Boersma et al. 2006; Endo et al. 2015), competition (Hackney 1975; Durham et al. 2005; Shephard & Jackson 2006) and disease transmission (Tyus & Nikirk 1990; Townsend & Winterbourne 1992; Nocita 2007; Nocita & Zerunian 2007; Pool 2007).

Identifying the age of non-native fishes can be a valuable tool when confronted with a new occurrence, as it can provide an indication of when a species was introduced (e.g. Gkenas et al. 2015). Moreover, age determinations can be used to determine if a species is reproducing and how its population (in respect to age-classes or maturity) is structured. Thus, it can be shown if a studied population behaves differently, i.e. in different growth patterns, population structure or age at maturity compared to populations in the native environment (De Roth 1965; Ribeiro & Collares-Pereira 2010). Lastly, knowing the age structure of a population can improve the evaluation of a species' invasiveness through specific characteristics (e.g. age at maturity, length-weight relationships) compared with native populations parameters (Bøhn et al. 2004). Hence, information on the age structure of a non-native population is often of considerable importance for management and stake holders but must be comparable among the study and research field.

Table 1: Fish-species present in the sampled inner-Florence section of the Mediterranean Arno river (Nocita 2001; Nocita 2007; Nocita & Zerunian 2007; pers. observation Phillip Haubrock)

Family	Species	Common name	Abundance	Status
Anguillidae	*Anguilla anguilla* (Linnaeus, 1758)	European eel	Low	Native
Cyprinidae	*Abramis brama* (Linnaeus, 1758)	Common bream	Low	Non-native
	Alburnus alburnus (Linnaeus, 1758)	Common bleak	High	Non-native
	Barbus barbus (Linnaeus, 1758)	Common barbel	Low	Non-native
	Carassius auratus (Linnaeus, 1758)	Goldfish	Low	Non-native
	Cyprinus carpio Linnaeus, 1758	Common carp	High	Naturalized
	Pseudorasbora parva (Temminck & Schlegel, 1846)	Topmouth gudgeon	High	Non-native
	Squalius cephalus (Linnaeus, 1758)	Common chub	Low	Non-native
	Tinca tinca (Linnaeus, 1758)	Tench	Low	Non-native
Ictaluridae	*Ictalurus punctatus* (Rafinesque, 1818)	Channel catfish	High	Non-native
Siluridae	*Silurus glanis* (Linnaeus, 1758)	European catfish	High	Non-native
Poeciliidae	*Gambusia holbrooki* Girard, 1859	Eastern mosquitofish	Low	Non-native
Gobiidae	*Padogobius bonelli* (Bonaparte, 1846)	"Ghiozzi Padano"	Locally high	Non-native
	Padogobius nigricans (Canestrini, 1867)	"Ghiozzo dell' Arno"	Low	Native
Centrarchidae	*Lepomis gibbosus* (Linnaeus, 1758)	Pumpkinseed	Locally high	Non-native
Percidae	*Sander lucioperca* (Linnaeus, 1758)	Pikeperch	Low	Native

Little is known about the use of different age determination methods in non-native scale-less fishes, particularly those that have recently been introduced into southern European watersheds, including members of the Ictaluridae Gill, 1861: *Ameiurus melas* (Rafinesque, 1820), *Ameiurus nebulosus* (Lesueur, 1819), *Ictalurus furcatus* (Valenciennes, 1840)*, I. punctatus* (Rafinesque, 1818); the Siluridae Cuvier, 1816: *Silurus glanis* Linnaeus, 1758; and the Cobitidae Swainson, 1838: *Misgurnus anguillicaudatus* (Cantor, 1842). Compared to scaled fish (see e.g. Lux 1971), determining the age of scale-less fish, such as catfish, is usually accomplished by an analysis of vertebrae (Marzolf 1955), otoliths (Sakaris & Irwin 2008) or the less conventional pectoral spines (see e.g. Penha et al. 2004; Carol et al. 2009). Since Siluriformes have relatively small otoliths (Sakaris & Irwin 2008), vertebrae and pectoral spines have been the method of choice in determining their age (Michaletz et al. 2009; Colombo et al. 2010). However, pectoral spines are generally considered as being easier to analyse due to their external positioning (Alexander 1966; Olivev et al. 2011). Surprisingly, various studies use the pectoral spine age determination method but fail at explicitly explaining how samples were prepared (e.g. if samples were decalcified or not; Ashley & Garling 1980; Buckmeier et al. 2002; Penha et al. 2004). The use of pectoral spines has its advantages (e.g. spines are easily accessible, the use of annuli is well practiced) and disadvantages (e.g. discrepancies from estimations using otoliths, variability in young and old specimens), but can be considered the easiest method to apply (Ashley & Garling 1980; Sakaris & Irwin 2008; Olivev et al. 2011). However, there is a lack of detailed information regarding the location of the section used for analysis in order to increase accuracy in age determination (Buckmeier et al. 2002).

With the invasion by *I. punctatus* is gaining more attention in Europe (Ligas 2007, 2008; Banha et al. 2017; Haubrock et al. 2017, 2018a, b), a clear description of how pectoral spines can be analysed for an exact age determination is needed for future studies. For this purpose, we compared age determination based on different spine characteristics, such as section location and thickness. We also present the first age information about this non-native fish in a Mediterranean freshwater ecosystem in Southern Europe. With this information, we aim to propose a replicable basis for future age and growth studies on scale-less fish.

Materials and Methods

Study Area

The Arno River is 241 km long and its watershed covers approximately 8200 km². It is the most important freshwater river in Central Italy after the Tiber River, with a mean annual discharge of about 110 m³/s (see Nocita & Zerunian 2007). It presents a discharge regime that is typical of Mediterranean systems due to the severe flow reduction during summer. The Arno River is sectioned by various weirs in Florence and presents a high density of aquatic vegetation during spring, although the riparian vegetation is almost always absent from riverbanks. Furthermore, the Bilancino reservoir in the north of Tuscany opens into the Sieve River, a tributary of the Arno river. With the use of floodgates, the water regime is controlled during winter periods to prevent floods and in summer, to regulate water levels in dry periods. In summer, the water of the Arno River in the inner-city section of Florence has a mean temperature of 29.6 °C (min: 27.2, max: 32.4; data from 2016-2018 http://www.arpat.toscana.it), while information on water temperatures from the winter period are not available. However, sheets of ice and temperatures below the thermocline are not uncommon in January (personal communication Gianna Innocenti).

Sampling

Samples of *I. punctatus* from the inner-city section of the Arno River (Figure 1; 43°45'49.9"N, 11°18'04.2"E) were collected in April 2018 using standard fishing rods (2.40 m), 0.42 mm braided line and size 2 fishing hooks baited with various items (cut-fish, worms, liver, etc.). Bait was cast into the faster flowing current in the middle-water to drift downstream with the current. A total of 28 fish were caught, placed on ice, and transported to the laboratory of the Department of Biology at the Natural History Museum “La Specola” in Florence.

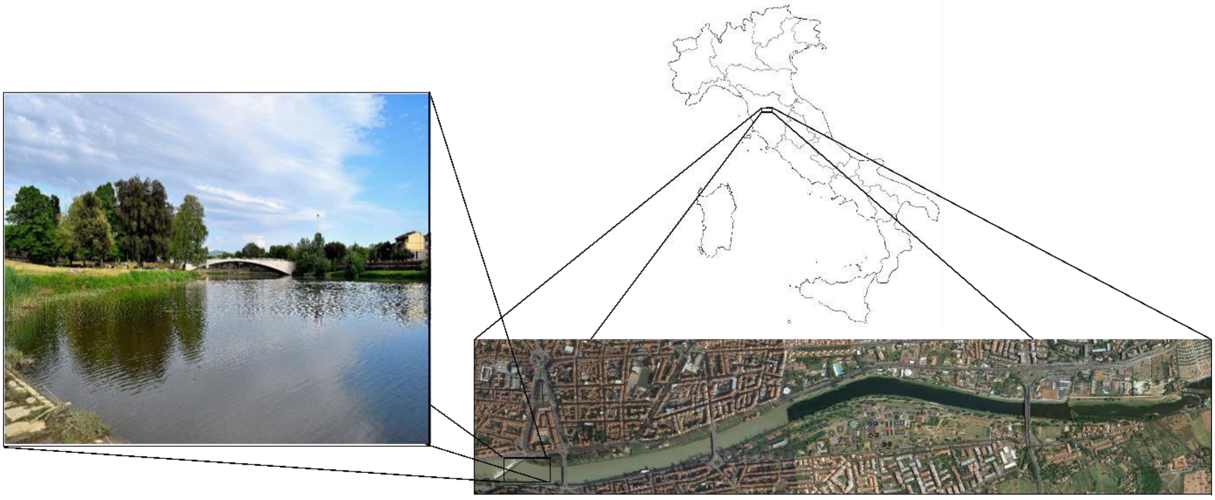


Figure 1: Sampled inner-Florence stretch of the Arno river.

Sample preparation

Total length (TL, nearest cm) as well as Total weight (TW, nearest g) were measured and are here reported as mean ± SD. Sex was determined using pelvic fin morphology (see Norton et al. 1976) before the pectoral spines were disarticulated from the pectoral girdle by clockwise twisting (Mayhew 1969). Spines were manually cleaned to remove all soft tissues and air-dried overnight without storage in ethanol before being placed into resin filled silicone cups. Once the resin had cured, spines were sectioned several times (Figure 2). From each section, a thin slice (ideally close to 2mm) was cut off with a standard jewellery saw and adhered to a microscope slide. These sections were sanded down to ~0.5mm, photographed and subsequently further ground down to ~0.2mm using fine sandpaper. All spine slices were analysed under a standard stereoscopic microscope with ×6.3 – ×20 magnification. The annuli were identified along the anterior radius (SA), previously indicated by Marzolf (1995) as the most reliable section. Each annulus was assumed to be the end of one growth period. All size measurements were taken with an electronic calliper.

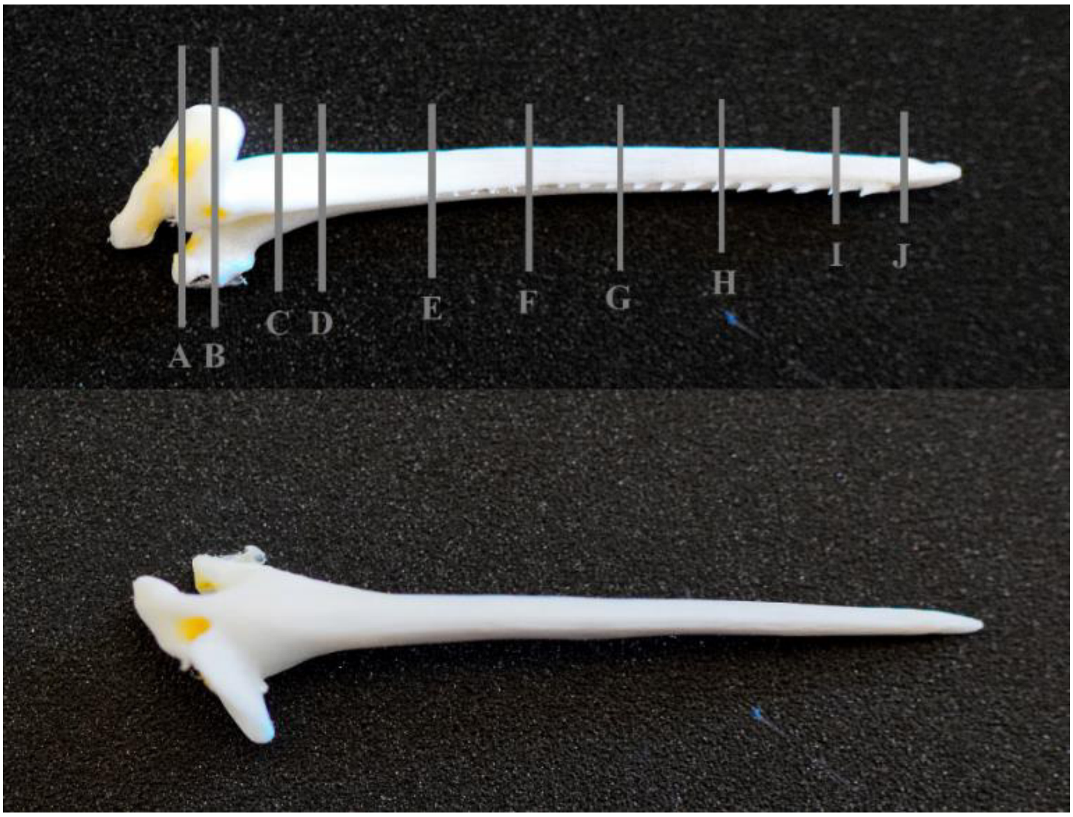


Figure 2: Spine of *Ictalurus punctatus* and indications of cuts (A-J) made to analyse the annuli, i.e. age

The observed results were then used to determine if differences in the positioning of the cuts can lead to varying age determinations and how the thickness of the spine (~0.2-0.5mm) slides can affect the readability. Lastly, with this information on the readability of different sections, the estimated ages of most reliable identified section were plotted against two sections that provided highly differing results. The reliability of sections was evaluated based on i) the easiness of preparation and ii) the readability determined as the ability to differentiate / count annuli and an observed consistent distance between annuli.

Results

The sampled specimens (n = 28; 17 females, 11 males) had total lengths ranging from 13.5 to 64 cm (33.5 ± 14.4 cm) and total weights between 20 and 3500 g (511.9 ± 106.3 g). From one individual (TL = 64 cm; TW = 3503 g; sex = female), ten successful cuts were produced for each spine and analysed at ~5 and ~0.2 mm (Table 2), revealing equal age determinations between cuts of both pectoral spines and a better readability at ~0.5 mm.

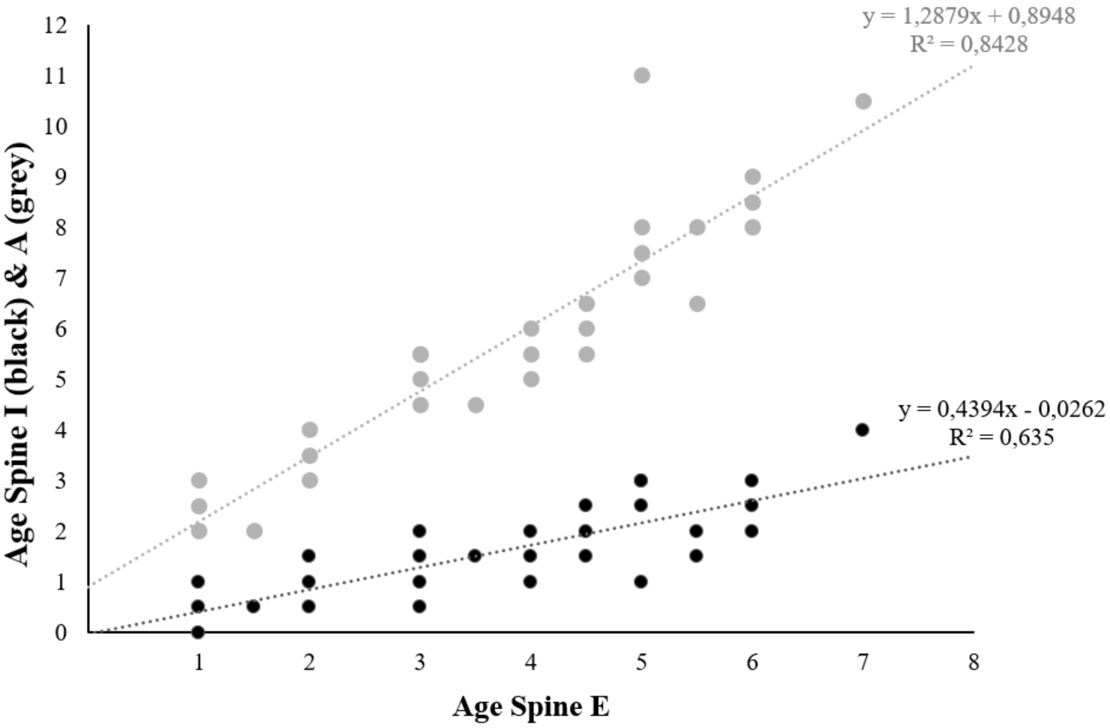
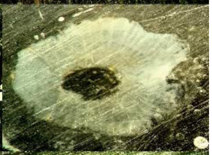


Figure 3: Age determined for the as most reliable determined section E plotted against the two variable sections I (black) and A (grey).

In 28 specimens, we compared the estimated ages among the cut sections / thickness between 1+ (cut J) and 5+ (cut A, B) years. Considering the spine sections from all specimens, cut section E at the beginning of the closed lumen with a thickness of ~0.5 mm produced the most reliable results. This was based on the clear display of readable annuli at the anterior radius, as well as closed and clearly distinguishable annuli. In contrast, annuli in cut sections A-D were not closed, negatively affecting the cutting process and readability. Moreover, annuli at different radiuses were variable in their clarity and definition. Plotting the estimated ages (~0.5 mm) at cut position E from all specimens against the respective ages at cut sections I (distal) and A (basal) revealed considerably varying results for the determination of age (figure 3, table 3). The varying trends highlighted higher age determinations when using cut section A, compared to that of the relied upon distal cut section I.

Table 2: Results from visual determination of the age from specimen #1. 10 cut sections were produced (A-J) and analysed at a thickness of ~0.5mm and ~0.2mm.

Section	**Thickness**		**Estimated age**	
	~0.5mm	**~0.2mm**	**~0.5mm**	**~0.2mm**
A			4+	4+
B			4+	4+
C			4+	3+
D			3+	3+
E			3+	3+
F			4+	2+
G			2+	2+
H			2+	2+
I			1+	2+
J			1+	1+

Discussion

Our results highlight the importance of a standardized method in determining the age of scale-less fishes, by selecting the same spine section and a repeatable protocol in spine section thickness. Moreover, this work presents, for the first-time, data on age and length (and thus, growth) of *I. punctatus* in Europe, which must be addressed in more detail in future research.

Considering the high abundance of *I. punctatus* in the study area, suitable growing conditions can be assumed. The investigated individual's spines that were cut at ten different sections was identified to be 3+ years of age. This individual was considerably larger than the other sampled individuals of the same age (Fig. 4) as well as individuals from native populations (Finnel & Jenkins 1954; Bouska et al. 2011). The growth rates of *I. punctatus* tend to be affected by water temperature, length of vegetative season, availability of prey and competition, accounting for the high variability in growth rates of this species in its native range. Therefore, warmer water released from pipelines at multiple locations in the Arno River offers abundant prey throughout the year (algae, small fish, etc.), allowing for faster growth. This links to observations by Reebs (2002), who states that these warmer water areas are monopolized by large *I. punctatus* individuals, potentially leading to exaggerated growth rates in individual specimens.

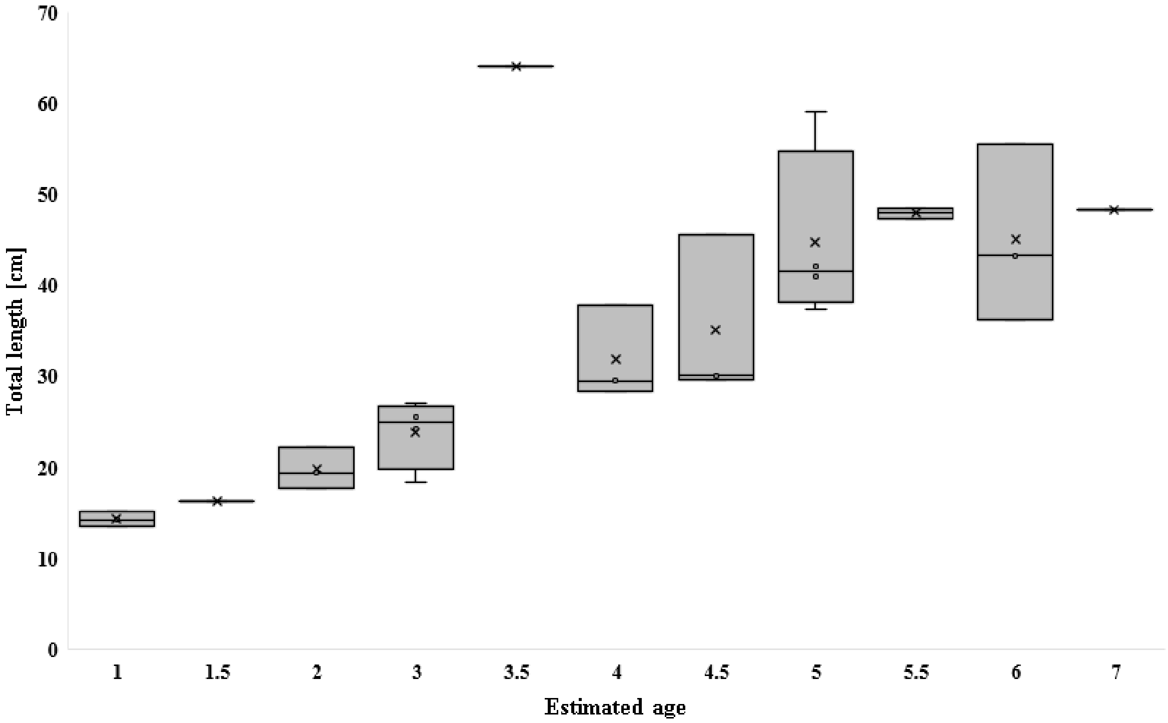


Figure 4: Channel catfish ages determined at section E in relation to total length of the respective age groups. Cross and dots mean ? also boxes mean quartiles or confidence intervals? And whiskers what they mean (min and max)

Our results highlight the high variability between the number of observed annuli at the basal sections A-C in respect to the more distal sections G-J. However, the readability of annuli is considerably affected by the open shaft at basal cut sections, limiting the surface at which annuli can be counted. Additionally, the thickness of produced cuts affected the readability, because cuts that were too thin resulted in indistinguishable annuli. This high variability between estimated ages among sections means that the introduction of *I. punctatus* goes back between 11 (section A), 7 (section E) or 3 years (section I). As no individuals older than 14 years were identified in our study, our results imply that this species either reproduced or it was continually reintroduced after 2004, the year it was first observed in the inner-city of Florence. Furthermore, the observed 8-year variability in specific sections of spines underpins the importance of exact age determination by following a reproducible protocol. Moreover, the observed differences have implications for other studies investigating the age of Ictaluridae by means of pectoral spines. While Buckmeier et al. (2002) followed a similar procedure for the preparation and reading of the pectoral spines for the age determination of *I. punctatus*, the authors used the distal end of the spine and cut through the dorsal and anterior processes. As a result, it was noted that spines and otoliths led to varying but not significantly different results. The differences between the otoliths and pectoral spines highlighted by these authors likely originated because section A was the spine used for age determination. Our results verify this due to the variability of annuli among different spine sections, specifically section A.

In conclusion, we highlight the variability of results obtained from different sections of *I. punctatus* spines and difficulties interpreting the annuli, especially those in cuts from distal spine sections. We propose a standardized procedure to accurately determine the age of scale-less fish by cutting the pectoral spine along the upper most distal shaft with a closed lumen (position E), without the need for decalcification. Lastly, due to the small sampling size, future studies should enlarge the dataset and broaden the number fish species specifically, catfishes. Future research in this field could aid in the evaluation of the invasiveness of this species, particularly in Mediterranean rivers, allowing for the improvement of management and risk assessments in areas with highly endangered endemic fishes (Smith & Darwall 2006).

Table 3: Age determination of 28 specimens of *I. punctatus* at a spine thickness of ~0.5 mm for three different pectoral spine cut sections (E, I & A)

Specimen	Age			Total Length (cm)
	Section I	Section E	Section A	
1	1+	3+	4+	64.0
2	1	3	5	27.1
3	2	3	4+	18.4
4	0+	2	3+	17.8
5	1	2	3	22.3
6	1+	2	4	19.4
7	1	1	2	13.5
8	0+	1	2+	15.2
9	0	1	3	14.2
10	0+	1+	2	16.3
11	1	4	6	29.5
12	1+	4+	5+	45.5
13	2	4	5	28.3
14	2+	4+	6+	29.7
15	3	5	11	42.1
16	2+	5	7	40.9
17	2	5+	6+	47.4
18	1+	5+	8	48.5
19	1	5	7+	37.3
20	0+	3	5+	24.3
21	2+	6	8	43.3
22	2	6	8+	36.2
23	3	6	9	55.6
24	1+	4	5+	37.8
25	1+	3	5+	25.6
26	2	4+	6	30.1
27	3	5	8	59.0
28	4	7	10+	48.3

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Authors' contributions

E.T., A.I. and P.J.H. designed the study and E.T. and A.I. supervised the concept and laboratory work. A.N. supervised the sampling. P.J.H., P.B. and I.F. conducted the laboratory work with valuable input from I.J. and A.N. Spines were analysed and interpreted by P.J.H., P.B., I.F., I.J. N.J.B. and A.N. P.J.H. and N.J.B. were responsible for the writing and editing of the manuscript.

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Special Issue: Management of Invasive Species in Inland Waters

Short Communication

Developing innovative methods to face aquatic invasions in Europe: the Aquainvad-ED project

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Abstract

Aquatic Invasive Alien Species (AIAS) are increasing due to the synergistic effects of climate change and habitat destruction. AIAS can heavily impact biodiversity and human health, causing a loss of ecosystem services; therefore, their control and management have now become a priority, particularly in the light of the new EU regulation 1143/2014 on invasive alien species. The main research goal of the Innovative Training Network Marie Skłodowska-Curie Aquainvad-ED is to exploit the application of innovative tools and the power of citizen science for early detection, control and management of AIAS. Eight early stageresearchers are involved in Aquainvad-ED, engaged in four main research themes: (1) development and application of novel methods for early detection of AIAS; (2) identification of vectors of introduction and pathways of dispersal; (3) impacts of freshwater and marine invaders; and (4) risk assessment and control of AIAS. In order to develop multidisciplinary approaches to address these issues, the fellows are working within an international consortium (UK, Spain, Italy) composed of scientists and conservation practitioners from three universities (Swansea University, Universidad de Oviedo, Università degli Studi di Firenze), one technological institute (AZTI), two governmental agencies (Natural Resources Wales and Cardiff Harbour Authority), one NGO (Wye & Usk Foundation) and five SMEs working in fundamental and applied aspects of AIAS (Neoalgae, Natural Applications, NEMO, Ecohydros, and Itinera C.E.R.T.A).

Key words: alien species, invasive, early detection, control, pathway

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Introduction

Globally, as a result of the advancing breakdown of biogeographic barriers, the introduction of alien invasive species is greatly contributing to biodiversity decline, ecosystem homogenization, and loss of ecosystem services, heavily impacting human health and economic activities (Kettunen et al. 2009; Simberloff et al. 2013; Jeschke et al. 2014; Mazza et al. 2014; Roy et al. 2016). The problems posed by aquatic invasive alien species (AIAS) are particularly dramatic due to the synergistic effects of climate change and habitat destruction. Aquatic ecosystems, especially freshwater ones, are vulnerable to biological invasions due to the strong affinity of humans to water (e.g. alteration, exploitation, utilization) and the intrinsic dispersal ability of aquatic species compared to terrestrial ones (Gherardi et al. 2009; Strayer 2010; Havel et al. 2015; Tricarico et al. 2016).

Over the last centuries, aquatic ecosystems in Europe have been colonized by highly invasive alien species. A total of 1,369 alien species have been reported in the European seas (Katsanevakis et al. 2013), mostly introduced through the Suez Canal (with an increase since 1990s) that was recently enlarged, facilitating the arrival of new species (Zenetos et al. 2012; Galil et al. 2015). There are 756 alien species in European fresh waters (Nunes et al. 2015), introduced mainly through aquaculture, the pet/aquarium trade (an emerging important pathway since 2000s: Maceda-Veiga et al. 2013; Mazza et al. 2015) and through sport fishing (Nunes et al. 2015). In both aquatic realms, many notable invaders are present, causing substantial damage to invaded ecosystems (Katsanevakis et al. 2013; Nunes et al. 2015).

The management of AIAS has become a priority, particularly in the light of the new EU regulation 1143/2014 (EU 2014) on the prevention and management of the introduction and spread of invasive alien species (art. 25). More than half (57%) of invasive alien species included in the EU concern list are freshwater species (Implementing Regulation EU 2016/1141 of 13 July 2016). Successful management of AIAS requires several steps: early detection, identification of introduction routes and dispersal pathways, and development of efficient control measures (CBD 2002). Public awareness and stakeholder involvement are also critical for preventing new introductions and for mitigating the impact of existing ones (CBD 2002).

Several projects (e.g. DAISIE, IMPASSE, COST Action TD1209 Alien Challenge) and initiatives (e.g. Essl et al. 2015; Latombe et al. 2016; Lucy et al. 2016) have addressed and are addressing different issues concerning invasive alien species in Europe and worldwide in order to harmonize terminology and optimize actions (databases, pathways, monitoring process, fostering collaboration). In this context, the project Marie Sklodowska Curie 2014 ITN (Innovative Training Network) H2020 Aquainvad-ED (AQUAtic INVaders: Early Detection, Control and Management; 2015–2019; http://www.aquainvad-ed.com/) was developed to tackle AIAS in Europe and to harmonize with the Marine Strategy Framework Directive (2008) and the Water Framework Directive (2000). ITN projects bring together universities, research centres and companies from different European countries to train a new generation of researchers. The funding boosts scientific excellence and business innovation and enhances researchers' career prospects through developing their skills in entrepreneurship, creativity and innovation. The main research goal of Aquainvad-ED is to exploit novel tools combined with the power of crowd data sourcing (citizen science) to develop innovative methods for early detection, control and management of AIAS.

The project

In order to develop multidisciplinary approaches to address AIAS issues, Aquainvad-ED involves an international consortium of three European countries (UK, Spain, Italy), composed by scientists and professionals from three universities (Swansea University, project leader, Universidad de Oviedo, Università degli Studi di Firenze); one technological institute (AZTI); two governmental agencies (Natural Resources Wales and Cardiff Harbour Authority); one NGO (Wye & Usk Foundation) and five SMEs (Small and medium-sized enterprises) working in fundamental and applied aspects of AIAS (Neoalgae, Natural Applications, NEMO srl, Ecohydros and Itinera C.E.R.T.A scarl). Together, the Aquainvad-ED network offers a multidisciplinary approach (genetics, behaviour, ecology, citizen science, risk assessment) and the expertise of academic and non-academic partners to the assessment and management of biological invasions in aquatic habitats, through the enhancement of unique skills (e.g. technical, research and analytical competences), knowledge-sharing and capacity building.

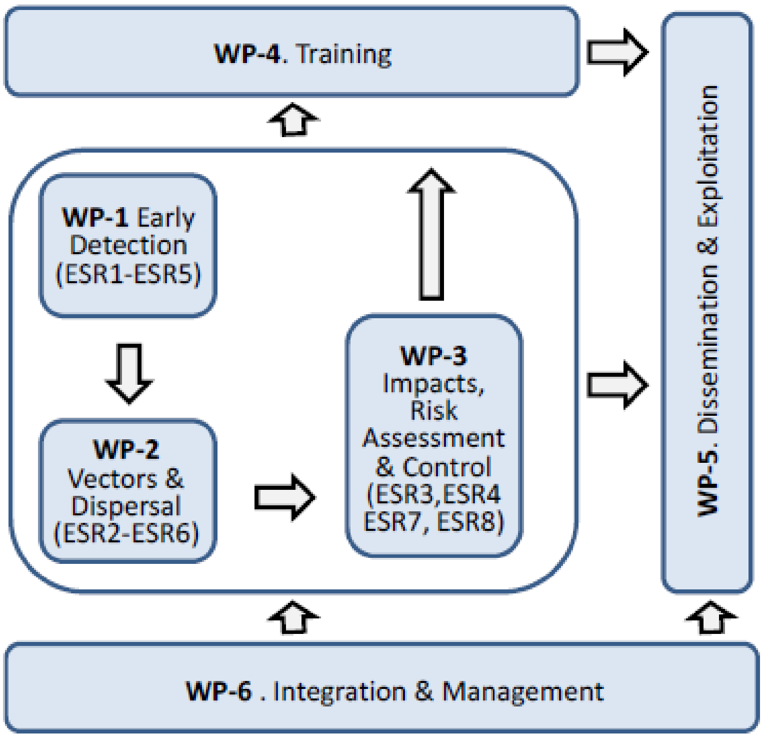

Figure 1: The six Aquainvad-ED Work Packages (WPs) along with the corresponding Early Stage Researchers (ESRs).

The specific goals of the project are: i) developing, optimising and trialling innovative methods for early detection of freshwater and marine invaders; ii) identifying ecological and demographic factors determining AIAS establishment and spread; iii) recommending novel procedures for AIAS control which are applicable to natural and managed aquatic systems (e.g. rivers, estuaries, artificial reservoirs); iv) integrating information on location, dispersion and control measures into management plans in order to prevent further AIAS introductions and dispersal in Europe, and v) raising public awareness about introduction routes and dispersal pathways, as well as about ecological and socio-economic impacts caused by AIAS.

In order to accomplish these goals, Aquainvad-ED is structured into six complementary Work Packages (WPs; Figure 1): WP1, dealing with the development and application of novel methods for early detection for AIAS; WP2, focussing on identification of introduction vectors and dispersal pathways; WP3, concerning the assessment of impacts of selected freshwater and marine invaders, as well as risk assessment and control of AIAS; WP4 on organizing training activities devoted for the recruited fellows; WP5 on dissemination and exploitation; and WP6 dedicated to the integration and management of the whole project.

The Fellows

Eight Early Stage Researchers (ESRs) are involved in Aquainvad-ED, and are dedicated to four projects linked to WP1, WP2 and WP3 (Figure 1): (1) development and application of novel methods for early detection AIAS; (2) identification of introduction vectors and dispersal pathways; (3) impacts of aquatic invaders; and (4) risk assessment and control of AIAS. Each ESR has academic and non-academic supervisors, and two planned secondments within the consortium partners in order to acquire multidisciplinary and multi-sectorial skills. WPs 4, 5 and 6 are dealing with training activities, dissemination and project management, involving the supervisors coordinated by Swansea University (WPS 4, 6) and by Wye & Usk Foundation (WP5).

Development and application of novel methods for early detection AIAS

Teja Muha (Swansea University, UK) and Anaïs Rey (AZTI, Spain) are developing molecular methods based on metabarcoding, able to detect the overall community, and qPCR, suitable for detecting specific species in freshwater [for detection of the killer shrimp *Dikerogammarus villosus* (Sowinsky, 1894), the zebra mussel Dreissena polymorpha (Pallas, 1771), alien macrophytes and fish] and marine environments (for detection of invaders as required by the “Ballast Water Convention”). To achieve this, laboratory and field calibration of molecular methods are applied to a range of freshwater and marine systems in the UK, Spain and Italy. As part of a citizen science programme, a smartphone app (AquaInvaders) is being used to promote citizen science programs for the early detection of AIAS.

Identification of vectors of introduction and pathways of dispersal

Marta Rodríguez-Rey (Swansea University, UK) and Sabine Rech (Universidad de Oviedo, Spain) are working on the identification of physical and ecological constraints for the survival of AIAS. Rodríguez-Rey is mainly addressing the different role of natural vs. anthropic variables in the dispersal of non-native invasive species, as well as the social perception towards alien species. Rech is focussing on floating objects and marine litter as potential vectors of AIAS (Rech et al. 2016a). The fellows will compile an inventory of AIAS arriving to selected freshwater and marine systems in the three project countries. They will estimate optimal conditions, potential floating vectors, and high-risk activities and source and sink areas for invasion and dispersal of AIAS, before mapping the main routes of introduction and dispersal, based on floating and stranded samples of rafting biota (Rech et al. 2016b), traffic research, meta-barcoding profiles (deriving from Teja and Anaïs), fouling experiments, using eDNA and experiments under controlled conditions. In this way, it will be possible to develop recommendations and guidelines for identifying vectors of introduction and pathways of spread of key aquatic invaders.

Impacts of aquatic invaders

To quantify current ecological and socio-economic impacts and the relationships among invaders, Matteo Rolla (Swansea University, UK) and Phillip J. Haubrock (NEMO srl, Italy) are studying selected freshwater invaders [such as *D. villosus*, *D. polymorpha*, the red swamp crayfish *Procambarus clarkii* (Girard, 1852), the channel catfish *Ictalurus punctatus* (Rafinesque, 1818), the bullfrog *Lithobates catesbeianus* (Shaw, 1802); Haubrock et al. 2016a] through laboratory and field experiments (Haubrock et al. 2016b; Rolla et al. 2016). Moreover, they are assessing the ecosystem services affected by these AIAS in order to quantify the economic costs. These fellows will develop guidelines for estimating current and future AIAS impacts in aquatic environments under a range of future climate and environmental scenarios.

Risk assessment and control of AIAS

To accomplish the last step of AIAS management, Iva Johović (Università degli Studi di Firenze, Italy) and Roberta Skukan (Neoalgae, Spain) are modelling and assessing the risk of invasion for a range of fresh-water (e.g. *P. clarkii*, *L. catesbeianus*) and marine invaders [the invasive seaweeds *Codium spp.*, *Sargassum muticum* (Yendo) Fensholt, 1955, and *Undaria pinnatifida*, Harvey (Suringar), 1873], respectively (Haubrock et al. 2016a). They are also testing different control techniques and mitigation measures to prevent the spread of selected AIAS (Johović et al. 2016). In order to assess the risk of marine invasion, molecular species identifications and biogeography data will also be integrated in Roberta's research as a valuable tool for effective management strategies (Skukan et al. 2016a). Citizen science programs as a useful tool for early detections and prevention of algae invasions will be also implemented (i.e. Skukan et al. 2016b). Their final aim will be to develop guidelines for mitigating biological and socio-economic impacts caused by freshwater and marine invaders, as well as predictive models for the identification of vulnerable areas under current and future climate change.

Network and training activities

Aquainvad-ED partners meet annually to review progress and provide an update on project status. They also engage in specific training activities to enable ESRs to develop new skills. For example, in December 2015, they attended the Inaugural Training Event on Entrepreneurship Skills at Swansea (UK), and the Rivers Trust Spring Conference at Hay-on-Wye (UK) in May 2016, where they also participated in a training event on Citizen Science and Communication. In April 2017, they attended a training workshop in Spain on early detection methods for aquatic invaders, and in spring 2018 they will attend a training workshop on strategies and methods for AIAS management in Italy.

Conclusion

As introductions of alien species in Europe increases, new legislation requires more efficient management tools for AIAS. The Aquainvad-ED project will contribute to this task, not only through the production of science-based guidelines and deliverables, but also through the training of a new generation of multidisciplinary researchers who will be able to face biological invasions from different perspectives. The project will benefit from the outputs of the previous cited projects and initiatives and will surely establish a collaboration with the recently developed INVASIVESNET network (ET is part of COST Action Alien Challenge and INVASIVESNET).

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MODULO DI PRESENTAZIONE DELLA TESI DI DOTTORATO DI RICERCA (da allegare alla domanda di Esame Finale e da consegnare all'ufficio Dottorato e Alta Formazione entro i termini previsti)

Corso di Dottorato di Ricerca in BIOLOGIA EVOLUZIONISTICA ED ECOLOGIA (D.M. 45/2013) - ciclo 842-31

N. di Matricola 127442

Dissertazione di Esame Finale di Dottorato di Ricerca nella Sessione Ordinaria Ciclo 31 dell'a.a. 2017

presentata da HAUBROCK PHILLIP JOSCHKA

NATO A Kassel - GERMANIA - IL 22/02/1989

Titolo della Tesi: ASSESSING THE IMPACTS OF AQUATIC INVASIVE SPECIES IN FRESHWATER ECOSYSTEMS
Lingua: INGLESE
Settore scientifico disciplinare: BIO/05
Tutore: SCAPINI FELICITA
Tutore: INGHILESI ALBERTO FRANCESCO
Tutore: TRICARICO ELENA
Parole Chiave: invasive species, freshwater ecosystem, fish, impact assessment, risk assessment
Carattere della Tesi: Elaborato Scritto

Firma del Tutore Felicita Scapini

Firma dell' eventuale Cotutore: Elena Tricarico Alberto Francesco Inghilesi

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FERRARA, li 16/02/2019 Firma del Dottorando Phillip J. Haubrock

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Richiedo il rilascio del diploma di Dottorato di Ricerca.

FERRARA, li 16/02/2019 Firma del Dottorando Phillip J. Haubrock

Ricevuta di compilazione del questionario fine corso

dal 1994 Consorzio Interuniversitario
ALMALAUREA
Soluzioni con lode per l'Università e il mondo del lavoro

HAUBROCK PHILLIP JOSCHKA
Data di nascita 22/2/1989
Luogo di nascita GERMANIA
Codice fiscale HBRPLL89B22Z112S

UNIVERSITÀ DEGLI STUDI DI FERRARA
BIOLOGIA EVOLUZIONISTICA ED ECOLOGIA (D.M. 45/2013)
Tipo corso: Dottorato
Username AlmaLaurea: PHILLIP.HAUBROCK

Consenso al trattamento dei dati personali
per le finalità e nelle modalità indicate di seguito: **SI**

Dichiaro di acconsentire al trattamento dei dati per le finalità e nelle modalità indicate di seguito:

Informativa sul trattamento dei dati personali – creazione del Curriculum Vitae
Il Consorzio Interuniversitario AlmaLaurea, ai sensi dell'art. 13 del Regolamento (UE) 2016/679, La informa che:

Soggetti del trattamento: titolare del trattamento dei dati è il Consorzio Interuniversitario AlmaLaurea, viale Masini 36, 40126, Bologna – Italia, Tel. +39 051 6088919, Fax +39 051 6088988; il Responsabile per la Protezione dei Dati (RPD) del Consorzio, individuato alla pagina http://www.almalaurea.it/info/condizioni/privacy, può essere contattato all'indirizzo rpd@almalaurea.it ;

Finalità, base giuridica e conservazione dei dati forniti: i dati forniti sono trattati per favorire l'inserimento nel mondo del lavoro e la formazione professionale. Questa finalità è perseguita attraverso la possibilità di creare e mantenere costantemente aggiornato, all'interno della banca di dati AlmaLaurea, il Suo Curriculum Vitae et studiorum. I dati da Lei di volta in volta inseriti per aggiornare il Suo Curriculum Vitae sono trattati per la medesima finalità; per tale finalità, i Suoi dati personali sono trattati, ai sensi dell'art. 6, 1° comma, lett. a) del Regolamento (UE) 2016/679, solo in caso Lei fornisca il Suo consenso, che ha diritto di revocare in qualsiasi momento, senza incidere sui trattamenti già effettuati; i dati da Lei conferiti, inoltre, possono essere trattati per elaborare rapporti statistici ed effettuare eventuali ulteriori rilevazioni (mediante la fornitura e la facoltà di compilare specifici questionari), così da monitorare a distanza di tempo la condizione di studio e di lavoro degli studenti, dei diplomati e dei laureati; per tali finalità, i Suoi dati personali sono tratti, ai sensi dell'art. 6, 1° comma, lett. a) del Regolamento (UE) 2016/679, solo qualora Lei ci fornisca il Suo consenso, che ha il diritto di revocare in qualsiasi momento, senza incidere sui trattamenti già effettuati; per le finalità sopra indicate, i Suoi dati sono conservati fino a quando Lei intenda mantenere il Suo Curriculum Vitae nella banca dati AlmaLaurea.

Destinatari o categorie di destinatari dei dati personali: per le finalità di favorire l'inserimento nel mondo del lavoro e la formazione professionale, possono avere accesso ai dati contenuti nel Suo Curriculum Vitae i seguenti soggetti o categorie di soggetti: il Suo Ateneo o la Sua Istituzione AFAM, in qualità di titolari autonomi del trattamento, se aderenti al Consorzio o se hanno stipulato con esso specifici accordi, consultabili al seguente link [http://www.almalaurea.it/info/chisiamo/chisiamo_soci]; i soggetti (pubblici e privati, italiani ed esteri) e le Associazioni di Categoria che dichiarano espressamente di essere interessati ai dati per l'avviamento nel mondo del lavoro e di formazione professionale; la Società AlmaLaurea S.r.l., in qualità di responsabile del trattamento, nonché altri soggetti incaricati della fornitura di supporto tecnologico . La comunicazione dei dati può avvenire, eventualmente, previo un rimborso spese. L'elenco dei responsabili del trattamento di cui è titolare il Consorzio Interuniversitario AlmaLaurea è pubblicato sul sito https://www.almalaurea.it/info/condizioni/privacy ;

Trasferimento dei dati all'estero: per le finalità di favorire l'inserimento nel mondo del lavoro e la formazione professionale, i dati contenuti nel Suo Curriculum Vitae possono essere comunicati, su specifica richiesta, a soggetti (imprese o organizzazioni) situati in Paesi fuori dall'Unione europea. In tali casi, qualora l'impresa sia situata in un Paese per il quale non è stata adottata una decisione di adeguatezza della Commissione europea, ai sensi dell'art. 45 del Regolamento (UE) 2016/679, garantiremo l'accesso al Suo Curriculum Vitae solo nel caso in cui Lei ci fornisca il Suo espresso consenso;

Esercizio dei diritti dell'interessato: può esercitare i diritti a Lei riconosciuti dalla vigente normativa in materia di protezione dei dati personali, tra cui il diritto di chiedere al titolare del trattamento l'accesso ai dati personali e la rettifica o la cancellazione degli stessi, la limitazione o opposizione al trattamento che La riguarda, oltre al diritto alla portabilità dei dati, nei limiti di quanto previsto agli artt. 15-21 del Regolamento 2016/679; ai sensi dell'art. 77 del Regolamento, qualora ritenga che il trattamento che La riguarda violi la normativa in materia di protezione dei dati personali, Lei ha diritto di proporre reclamo al Garante italiano per la protezione dei dati personali ovvero all'autorità di controllo dello Stato membro Ue in cui risiede abitualmente, lavora ovvero del luogo ove si è verificata la presunta violazione; per l'esercizio dei Suoi diritti può rivolgersi al titolare del trattamento, inviando una comunicazione all'Ufficio Assistenza agli Utenti, al seguente recapito: supporto.laureati@almalaurea.it.

20/11/2018
(data)

Phillip J. Haubrock
(firma per esteso)

UNIVERSITÀ
DEGLI STUDI
DI FERRARA
- EX LABORE FRUCTUS -

pagoPA

QUIETANZA di PAGAMENTO

In data 16/02/2019 00:16:26 Universita Ferrara ha ricevuto la notifica dell'avvenuto pagamento per conto di:

HAUBROCK PHILLIP JOSCHKA

Codice Fiscale: HBRPLL89B22Z112S

dell'importo di € 56,00 con causale Tasse per servizio erogato

relativo all'avviso di pagamento 001000000019106684

con IUV 000000019106684

Il pagamento è stato effettuato tramite PagoPA dal PSP:

B - BCITITMM

Intesa Sanpaolo

- () -

Unità Operativa: -

Il pagamento, identificato dall'indicatore univoco IUR 190461052032 , è stato effettuato da HAUBROCK PHILLIP JOSCHKA in data 16/02/2019 .

UNIVERSITÀ DEGLI STUDI DI FERRARA

HAUBROCK PHILLIP JOSCHKA

Nato/a a Kassel ( ) - GERMANIA il 22/02/1989, nell'a.a. 2015/2016

è stato/a immatricolato/a a Corso di Dottorato in
BIOLOGIA EVOLUZIONISTICA ED ECOLOGIA (D.M. 45/2013)

Il Responsabile

Il Rettore
PASQUALE NAPPI

Firma omessa ai sensi art. D.Lgs. 12/02/93 n. 39

Stampato il 02/11/2015

Esami sostenuti	Data	Voto	Firma

11. Wohnort/Residence/Domicile

~~FLORENZ~~

11. Wohnort/Residence/Domicile

Gelnhausen

Gelnhausen, den 04. Dez. 2018

11. Wohnort/Residence/Domicile

12. Größe/Height/Taille 194 cm

13. Augenfarbe/Colour of eyes/Couleur des yeux

BLAUGRÜN

14. Ordens- oder Künstlername/Religious name or pseudonym/Nom de religion ou pseudonyme

C4VGX3T9V

Typ/Type/Type
P

Kode/Code/Code
D

Pass-Nr./Passport No./Passeport Nº
C4VGX3T9V

1. Name/Surname/Nom
HAUBROCK

2. Vornamen/Given names/Prénoms
PHILLIP JOSCHKA

3. Staatsangehörigkeit/Nationality/Nationalité
DEUTSCH

4. Geburtstag/Date of birth/Date de naissance
22.02.1989

5. Geschlecht/Sex/Sexe
M

6. Geburtsort/Place of birth/Lieu de naissance
MÜNSTER

7. Ausstellungsdatum/Date of issue/Date de délivrance
22.08.2016

8. Gültig bis/Date of expiry/Date d'expiration
21.08.2026

9. Behörde/Authority/Autorité
GENERALKONSULAT
MAILAND

10. Unterschrift der Inhaberin/des Inhabers-Signature of bearer-Signature de la titulaire/du titulaire

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P<D<<HAUBROCK<<PHILLIP<JOSCHKA<<<<<<<<<<<<<<<<
C4VGX3T9V2D<<8902225M2608215<<<<<<<<<<<<<<<<<0
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