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Ecology of the invasive neogobiids *Neogobius melanostomus* and *Ponticola kessleri* in the upper Danube River

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"One thing to remember is to talk to the animals.
If you do, they will talk back to you. But if you don't talk to the animals, they won't talk back to you, then you won't understand, and when you don't understand you will fear, and when you fear you will destroy the animals, and if you destroy the animals, you will destroy yourself."

Chief Dan George (1899 - 1981)

Native American Tsleil-Waututh Tribe

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III. Preface

This PhD-thesis “Ecology of the invasive neogobiids *Neogobius melanostomus* and *Ponticola kessleri* in the upper Danube River” was designed to contribute to a better understanding in fundamental mechanisms, ecological niche differentiation and effects of invasive fish species on the resident fauna in a large fluvial ecosystem. The opportunity to study both just arriving and recently established populations of two sympatric invasive gobiids at the same time in the upper Danube River offered a unique time-slot for analyzing the early stages of a biological invasion both at the population- and the specimen-level. This thesis, advancing basic ecology, molecular chemistry and invasion biology is structured into eleven chapters as follows:

As a general introduction, chapter one describes the importance of a better understanding of biological invasions in general and in invasive Ponto-Caspian gobies in special.

Chapter two presents the objectives of this study.

Chapter three introduces the Material and Methods applied.

The following chapters (four to seven) address four specific case studies in the context of the ecology and population biology of the invasive neogobiids *N. melanostomus* and *P. kessleri* in the upper Danube River, each representing an autonomous research paper. These papers are published in similar versions, depending on the specific journal requirements.

In chapter eight the specific research topics were discussed from a general perspective, providing a synthesis on the ecology of both invasive species.

Chapter nine to eleven comprise a list of publications that derived from this project, the references cited and last but not least the acknowledgements.

IV. Abstract

Worldwide, invasions of Ponto-Caspian gobiid fishes are suspected to cause regime shifts in freshwater ecosystems, however little is known about changes by time since invasion in the invaded ecosystem and the invading species itself.

This study analyzed the ecology of the sympatric invasive fish species round goby (*Neogobius melanostomus*) and bighead goby (*Ponticola kessleri*) outside their native ranges in the German section of an important European long-distance migration corridor for invasive aquatic species, the upper Danube River. Here, both neogobiids formed about 80% of the fish abundance and 60% of the fish biomass in rip-rap bank habitats after establishment. A comprehensive dataset, integrating population- and specimen-specific data was generated from established areas and a recently colonized location ("*invasion front*"), where also invasion dynamics from total absence to first arrival until establishment could be observed.

The identification of an effective and comparable sampling technique was an important first objective concerning field sampling of invasive gobies in a large river. This study also compared the trophic niche differentiation of both species using stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$), gut content analyses and morphometric analyses of the digestive tract with respect to seasonal determinants. The finding of an invasion front offered the opportunity to systematically investigate differences in feeding ecology, performance, morphology and parasitic load of invaders at different stages of an invasion process.

Point abundance sampling (PAS) of electrofishing was identified as the most effective and suitable sampling method to collect quantitative fish community data with the lowest selectivity (species, size, sex, feeding status) and the highest efficiency (catch, species). Comparisons of trophic niche differentiation identified both species as predacious omnivores with high dietary overlap. Amphipods were the most important and highly preferred food items, contributing to a generalist feeding strategy in both species. $\delta^{15}N$ signatures of *N. melanostomus* revealed an ontogenetic diet shift and significantly exceeded those in *P. kessleri*, indicating a niche separation of half a trophic level. *Ponticola kessleri* showed a higher degree of specialization and more stable feeding patterns across seasons, whereas *N. melanostomus* adapted its diet according to the natural prey availability, mirroring a great plasticity in this species. Both goby species consumed mainly other non-native species (~92% of gut contents) and seem to benefit from previous invasions of exotic prey species. Founder populations and invaders from the invasion front were found to be different from longer established round goby populations in demography, morphology, feeding behaviour, sex ratio and parasitic load. In contrast to the "*enemy release*"-hypothesis, higher abundance and density of acanthocephalan endoparasites were observed at the invasion front. Pioneering populations from the invasion front were dominated by females, comprising significantly larger individuals with highest condition and lowest gonado-somatic index. Especially pioneering female round gobies rather revealed an increased competitive ability than increased fecundity. The observed upstream-directed range expansion is seemingly not caused by out-migrating weak or juvenile individuals that were possibly forced to leave high density areas due to high competition. During this study, the invasive Ponto-Caspian goby *Babka gymnotrachelus* (Kessler, 1857) was recorded for the first time in Germany, indicating an effective sampling strategy.

Overall, pronounced changes in fish and invertebrate communities induced by the goby invasion suggest the occurrence of an “invasional meltdown” and a shift of the upper Danube River towards a novel ecosystem with communities and species that have greater resistance to goby predation. This seems to contribute to overcoming biological resistance and improve rapidity of dispersal. Such a complex change is also along the lines of what is happening to other aquatic systems in the world, i.e. the creation of novel ecosystems through the combination of environmental change and the impact of invasive species. As a result, novel ecosystems may provide different functional properties and ecosystem services, even though their persistence and values remain largely unknown. This also appears to be true for the Danube River, where a rapid ongoing shift from indigenous biodiversity towards a ubiquitous faunistic complex of potentially co-evolved exotic species which are adapted to human-altered aquatic systems was observed. Consequently, especially the success of Ponto-Caspian invaders reflects fundamental ecological changes in the large European freshwater ecosystems, which make a return to original communities almost impossible.

The upstream graded invasional meltdown and the possibility to observe characteristics of invaders at a distinct invasion front define the upper Danube River a unique study system worldwide. Here, a sampling design with adequate methods was established to examine the ecology of two sympatric benthic fish species and their prey in near shore habitats of a large river. Since suitable sampling procedures to systematically catch such fishes in deep water areas of fluvial ecosystems are still lacking, the established methodology can provide an important basis for the development of new methods.

V. Zusammenfassung

Weltweit gelten Invasionen von Schwarzmeergrundeln als Ursache für systemverändernde Vorgänge in Süßwasserökosystemen, jedoch ist nur wenig über zeitliche Veränderungen in betroffenen Ökosystemen sowie den invasiven Arten selbst bekannt.

Die vorliegende Studie untersucht die Ökologie der sympatrisch einwandernden Fischarten Schwarzmundgrundel *(Neogobius melanostomus)* und Kesslergrundel (*Ponticola kessleri*) außerhalb ihres natürlichen Verbreitungsgebietes in der deutschen Donau, einem wichtigen europäischen Langdistanz-Wanderkorridor für invasive aquatische Arten.

Beide Neogobiiden machen nach erfolgreicher Etablierung im Untersuchungsgebiet ungefähr 80% der gesamten Fischfauna, bzw. 60% der Fischbiomasse im blocksteingeprägten Uferbereich aus. Es wurde ein umfangreicher Datensatz erhoben, der populationsspezifische und individualisierte Daten aus längerfristig besiedelten und erst kürzlich kolonisierten Bereichen („Invasionsfront“) zusammenführt. Hier konnte auch die Invasionsdynamik vom Fehlen der Arten über die Ankunft bis hin zur Etablierung beobachtet werden.

Zunächst war die Ermittlung einer effektiven und vergleichbaren Methode zur Befischung von invasiven Grundeln in einem großen Fließgewässer nötig. Damit wurde die Einnischung ins Nahrungsnetz unter Verwendung stabiler Isotopenanalysen (δ^{13}C und δ^{15}N), Mageninhaltsuntersuchungen und morphometrischer Untersuchungen des Verdauungstraktes zwischen den beiden Arten unter Berücksichtigung jahreszeitlicher Unterschiede verglichen. Die Entdeckung einer Invasionsfront eröffnete die Möglichkeit, Unterschiede bezüglich Ernährungsökologie, Wachstumsleistung, Morphologie und Parasitenlast von Pionieren zu verschiedenen Zeitpunkten des Invasionsprozesses systematisch zu untersuchen.

Die „point abundance sampling“ (PAS)-Elektrobefischung wurde als effektivste und optimal geeignete Befischungsmethode identifiziert, um mit der niedrigsten Selektivität (Art, Größe, Geschlecht, Ernährungszustand) bei höchster Effizienz (Fang, Art) quantitative Daten zur Fischzönose zu erheben. Beide Arten waren demnach als räuberische Allesfresser mit einer sehr ähnlichen Nahrungszusammensetzung ins Nahrungsnetz eingenischt. Flohkrebse spielten als bevorzugte Beute und zugleich wichtigster Bestandteil einer generalistischen Ernährungsstrategie eine wichtige Rolle bei beiden Arten. Die δ^{15}N-Signatur von *N. melanostomus* ergab eine ontogenetisch bedingte Verschiebung in der Nahrungswahl und war signifikant höher als bei *P. kessleri*, was einer Nischenseparation von etwa einer halben trophische Ebene entspricht. *Ponticola kessleri* zeigte einen höheren Spezialisierungsgrad sowie ein saisonal stabileres Nahrungsmuster, während die Nahrungswahl bei *N. melanostomus* der natürlichen Verfügbarkeit folgte und damit eine hohe Plastizität dieser Art widerspiegelt. Beide Arten fraßen hauptsächlich andere nicht-heimische Spezies (~92% des

Verdauungstraktinhalts) und scheinen von vorausgegangenen Invasionen exotischer Beuteorganismen zu profitieren. Gründerpopulationen und Tiere von der Invasionsfront unterschieden sich von bereits länger etablierten Populationen hinsichtlich Altersstruktur, Morphologie, Ernährung, Geschlechterverhältnis und Parasitenlast. Im Gegensatz zur "*enemy release*"-Hypothese wurde bei Fischen von der Invasionsfront eine höhere Dichte und Häufigkeit endoparasitischer Kratzer (Acanthocephala) beobachtet. Pionierpopulationen von der Invasionsfront bestanden überwiegend aus signifikant größeren Individuen mit höchsten Konditionsfaktoren und niedrigstem Gonado-Somatischem Index. Insbesondere erst kürzlich eingewanderte Schwarzmundgrundel-Weibchen wiesen eher ein höheres Durchsetzungsvermögen als eine erhöhte Fruchtbarkeit auf. Wahrscheinlich wird die hier beobachtete flussaufwärts gerichtete Ausdehnung des ursprünglichen Verbreitungsgebiets nicht von abwandernden schwachen oder juvenilen Individuen (die möglicherweise Aufgrund von hohen Dichten und damit hoher Konkurrenz abwandern müssen) gesteuert.

Im Rahmen dieser Studie erfolgte der Erstnachweis der invasiven Ponton-kaspischen Nackthalsgrundel *Babka gymnotrachelus* (Kessler, 1857) in Deutschland. Dies verdeutlicht die Effizienz des angewandten Befischungsprogramms.

Insgesamt lassen die von der Grundelinvasion induzierten Veränderungen der Fisch- und Invertebratenfauna eine „invasive Kernschmelze" sowie eine Umwandlung der oberen Donau zu einem neuartigen Ökosystem (mit Artengemeinschaften, die gegenüber Grundelprädation widerstandsfähiger sind) vermuten. Dies scheint dazu beizutragen, den biologischen Widerstand des Ökosystems zu überwinden und damit die Ausbreitungsgeschwindigkeit zu beschleunigen. Derartig komplexe Veränderungen (Schaffung neuartiger Ökosysteme durch das Zusammenspiel von Umweltveränderungen und dem Einfluss invasiver Arten) geschehen derzeit in ähnlichen aquatischen Systemen weltweit. Letztlich gehen von diesen neuartigen Ökosystemen veränderte funktionelle Eigenschaften und Ökosystem-dienstleistungen aus, deren Persistenz und Wertschöpfung weitgehend unbekannt sind. Dies erscheint auch für die obere Donau zuzutreffen, wo eine rasch fortschreitende Veränderung der ursprünglich heimischen Artenvielfalt hin zu einem indifferenten Faunenkomplex potentiell ko-evolutionärer Exoten mit Anpassungen an anthropogen veränderte aquatische Systeme zu beobachten war.

Die flussaufwärts fortschreitende „invasive Kernschmelze" sowie die Möglichkeit, invasive Arten an einer Invasionsfront erforschen zu können, machen die obere Donau zu einem einzigartigen Forschungsobjekt weltweit. Die hier im Uferbereich etablierte Methodik zur Untersuchung der Ökologie benthischer Kleinfische und ihrer Beuteorganismen kann als Grundlage zur Entwicklung neuer Methoden genutzt werden, um künftig auch tiefere Bereiche von Fließgewässern systematisch beproben zu können.

1. The importance of a better understanding of biological invasions

1.1 Biological invasions and invasive species

Freshwaters in lakes, wetlands and rivers support ecosystems with diverse life forms, providing ecosystem services of essential importance to human societies on earth (Arthington *et al.*, 2010). Although, surface freshwater habitats do only contain about 0.01% of the world's water and cover only about 0.8% of the Earth's surface (Gleick, 1996), over 10000 fish species live in fresh water, i.e. approximately 40% of the global fish diversity and one quarter of the global vertebrate diversity (Lundberg *et al.*, 2000).

However, freshwater ecosystems belong to the most endangered ecosystems worldwide with declines in biodiversity being far greater than in the most affected terrestrial ecosystems (Sala *et al.*, 2000; Abell *et al.*, 2008). Thus, freshwater biodiversity became the over-riding conservation priority during the International Decade for Action ("Water for Life") from 2005 to 2015 (Dudgeon *et al.*, 2006).

Biological invasions are considered one of the major threats to global freshwater biodiversity: Besides destruction or degradation of habitats, overexploitation, water pollution and flow regime modification (Dudgeon *et al.*, 2006; Geist, 2011), alien invasive species have been identified as a central agent of human-caused global change (Van der Zanden, 2005).

Worldwide, and especially in in highly altered habitats, increasing numbers of ubiquitary invasive aliens and a decline in indigenous species is causing ecological communities to become increasingly similar (Van der Zanden, 2005). This globally proceeding bio-contamination is also known as '*homogenization of flora and fauna*' (Moyle & Mount, 2007), "*biomonotony*" or "*Mc Donaldization*" (Beisel & Devin, 2007). Driven by intended or unintended human mediated transportation of non-indigenous alien organisms, this process is not less than playing "*ecological roulette*" (Carlton & Geller, 1993), with "*alien invasive species"* being the principal performers in this game.

An *"alien invasive species"* is a non-native (non-indigenous, foreign, exotic) species, subspecies, or lower taxon, occurring outside of its natural range and dispersal potential (i.e. outside the range it occupies naturally or could not occupy without direct or indirect introduction or care by humans) which first manages to reproduce and becomes established by founding self-sustaining populations. Second, it works as an agent of change, threatening native biodiversity, but also entire ecosystems, habitats or species with economic or environmental value (McNeely *et al.*, 2001).

Biological invasions are highly complex processes consisting of different stages (Fig. 1.1), each with an independent probability of failure with cumulative failure rates being high (Kolar & Lodge, 2002).

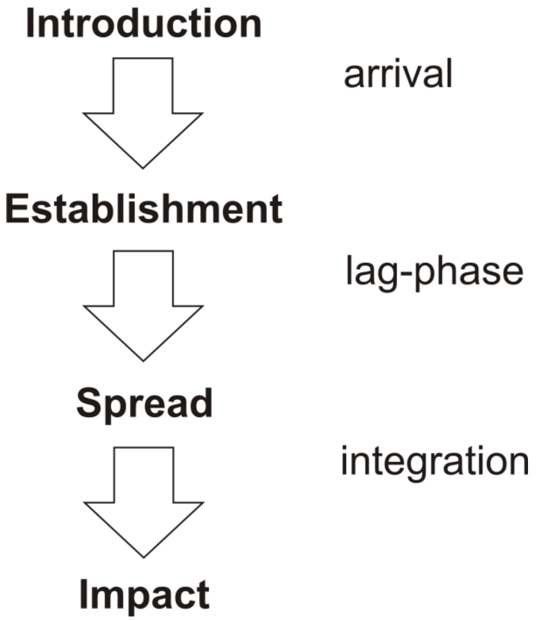

Fig. 1.1: The different stages of a biological invasion

Biological invasions are highly complex processes consisting of different stages, each with an independent probability of failure with cumulative failure rates being high (Kolar & Lodge, 2002). Introduction, establishment and spread have long been the characteristic phases of a biological invasion (e.g., "*community assembly*"-theory, Lodge, 1993a). The integrated conceptual model of Moyle & Light (1996) defined the phases arrival, establishment and integration. Kolar & Lodge (2002) added an impact-phase. The transition between establishment and spread (integration) can be diffuse, often containing an intermediate lag-phase, which is a latency period between arrival and exponential increase (Richardson, 2011) with unknown duration. Figure modified after Moyle & Light (1996) and Kolar & Lodge (2002).

The often cited "*tens rule*"-hypothesis (Williamson & Fitter, 1996) estimates that about one of ten imported species "escapes" to the wild, one of ten of those introduced species becomes established, and one of ten established species spreads and becomes a pest (Vander Zanden, 2005). On the other hand, for vertebrates approximately one of four introductions becomes invasive (Vander Zanden, 2005). Consequently, nearly every single species can potentially become invasive as soon as introduced to a bio-geographical region where it did not occur before, establish and spread. The transition between establishment and spread can be diffuse, often containing an intermediate lag-phase, which is a latency period between arrival and exponential increase (Richardson, 2011) with unknown duration. Arrival can result from intended introduction (pet, sport, aquaculture, bait species, trade), unintentional translocation (ballast water transport, ship hull transfer) and migration via artificial waterways. A key element in the invasion process to understanding why some introduced populations fail to establish whereas others succeed is "*propagule pressure*" (Blackburn & Duncan, 2001), this "*introduction effort*" is a composite measure of inoculation size (number of individuals released) and propagule number, i.e. the number of discrete release events to a new environment (Lockwood *et al.*, 2006). Propagule pressure has been shown to be a strong predictor of the establishment of non-indigenous species, but its effects on the ecological impacts of biological invasions have hardly been examined (Richardson, 2011).

Once non-indigenous species establish, autochthonous biodiversity becomes threatened (Mooney & Cleland, 2001; Kolar & Lodge, 2002; Strayer, 2009) and there is often no turning back to the status-quo because of the difficulty of eradication (Vander Zanden, 2005).

However, substantial populations of invasive non-indigenous species can occasionally collapse dramatically (Simberloff & Gibbons, 2004; Moore *et al.*, 2012), often following a “*boom-and-bust*”-cycle (Simberloff & Gibbons, 2005). However, even if a crash ultimately occurs, these species may already have caused persistent ecological damage (Simberloff & Gibbons, 2005). The “*biotic-resistance*”-theory by Elton (1958) predicts that systems that are more diverse should be more resistant to exotic species, apparently because species-rich communities were better adapted to a more completely and efficient utilization of available space and limiting resources. Declining biodiversity facilitates biological invasions, potentially accelerating the loss of biodiversity and the homogenization of biota (Stachowicz *et al.*, 1999), also known as “*invasional meltdown*” (Simberloff & Von Holle, 1999). On the other hand, Vander Zanden (2005) described a close coupling between invasive introductions and human migrations, which underlines the importance of translocation processes.

To date, more than 140 non-native aquatic species are known in German water ways, with approximately 20% of these being invasive (Gollasch & Nehring, 2006). Due to shipping, (artificial) waterway interconnection, ornamental trade and stocking actions introduction rates of non-native aquatic species have highly accelerated over the last decades (Geiter *et al.*, 2002, Gollasch & Nehring, 2006). The most successful invaders have been blamed for serious ecosystem impacts worldwide (Keller *et al.*, 2011).

In general, invasive non-indigenous species involve many challenges for a native biocoenosis as predation, competition for food and habitat resources as well as the introduction of new diseases and parasites. Furthermore, many invasive fish species include certain life-history traits such as a generalist feeding strategy or complex reproductive behavior, but also aspects of population structure, genetics and habitat use (e.g. Sakai *et al.*, 2001; Olden *et al.*, 2006; Gozlan *et al.*, 2010), as well as the ability of rapid range expansion (Gutowsky & Fox, 2011) facilitating their invasion success.

The Ponto-Caspian region (i.e. coastal areas of the Black- and Caspian Sea, the Sea of Azov and lower reaches and estuaries of large streams like Danube, Dnjestr, Bug, Djnepr and Don) seems to serve as an important donor ecosystem for invasions of exotic aquatic species worldwide (Ricciardi & MacIsaac, 2000; Reid & Orlova, 2002; Ojaveer *et al.*, 2002). Notorious examples for such potent Ponto-Caspian invaders, being among the world’s most aggressive and harmful aquatic invasive species, are the zebra mussel *Dreissena* *polymorpha* (Pallas, 1771)*,* the killer shrimp *Dikerogammarus villosus* and nowadays several gobiid fish species (Lederer *et al.*, 2006; Ward & Ricciardi, 2007; Pöckl, 2009; Strayer, 2009; Gozlan *et al.*, 2010; Borcherding *et al.*, 2012; Kalchhauser *et al.*, 2013).

In Europe, range expansions of invasive aquatic Ponto-Caspian species have been mainly facilitated by the interconnection of river basins (Bij de Vaate *et al.*, 2002), resulting in three migration corridors associated to the Rivers Danube, Volga and Dnjepr (Fig. 1.2).

Fig. 1.2: The migration corridors of Ponto-Caspian species in Europe

Range expansions of aquatic Ponto-Caspian species in Europe have mainly been facilitated by the interconnection of river basins by artificial canals. Three inland migration corridors can be distinguished:
A northern corridor: Volga → Lake Beloye → Lake Onega → Lake Ladoga → Neva → Baltic Sea, a central corridor connecting the rivers Dnjepr → Vistula → Oder → Elbe → Rhine, and a southern corridor connecting Danube → Main→ Rhine (Bij de Vaate *et al.*, 2002). Figure modified after Bij de Vaate *et al.* (2002).

The inter-connection of rivers by canals directly cross-linked previously separated drainage systems, with ships acting as vectors for invasion and (industrial) harbours being the main dispersal entry-points (Wiesner, 2005). For example, the surface area of the catchments that are directly connected by inland water ways to the River Rhine, which is considered the busiest waterway of the world, has increased by a factor of 22 over the last two centuries (Leuven *et al.*, 2009; Früh *et al.*, 2012a).

1.2 Invasive Ponto-Caspian gobies

Comprising about 2000 species, the gobiids (*Teleostei: Perciformes: Gobiidae*) represent one of the world's most diverse fish families. Many of these cryptical, bottom dwelling fishes are small growing (rarely over 25 cm in total length) and prefer near-shore marine or brackish habitats (Kornis *et al.*, 2012). Among these gobiids, several species with a high invasive potential (see Kottelat & Freyhof, 2007) have recently colonized both freshwater and marine ecosystems, some of them even on both sides of the Atlantic Ocean (Corkum *et al.*, 2004; reviewed in Roche *et al.*, 2013) (Table 1.1).

In the last two decades, an increasing number of rapid range expansions have been reported especially for Ponto-Caspian neogobiids (Fig. 1.4; Fig. 1.6) from the Laurentian Great Lakes watershed (Corkum *et al.*, 2004; Johnson *et al.*, 2005; Kornis & Vander Zanden, 2010; Pennuto *et al.*, 2010; Bronnenhuber *et al.*, 2011; Brownscombe *et al.*, 2012), from almost the entire Baltic Sea region (Sapota & Skóra, 2005; Ojaveer, 2006; Sokołowska & Fey, 2011, Björklund & Almqvist, 2010) and from many other large European waterbodies, including the Danube River (e.g. Stráňai & Andreji, 2004; Jurajda *et al.*, 2005; Harka & Bíró, 2007) and the River Rhine (Borcherding *et al.*, 2011; Kalchhauser *et al.*, 2013). Secondary invasions aside of the main navigation routes and migration corridors (e.g. Piria *et al.*, 2011) and the proceeding spread of invasive neogobiids worldwide highlight a new quality of potential threats especially to areas with high endemic aquatic biodiversity (Poos *et al.*, 2010).

Table 1.1: Range expanding perciforme gobies with a high invasive potential

Common Name	Taxon (Perciformes)	Family	Origin
Amur Sleeper	*Perccottus glenii* Dybowski, 1877	Odontobutidae	East Asian freshwaters
Bighead Goby	*Ponticola kessleri* (Günther, 1861)	Neogobiidae	Black Sea basin
Caspian Bighead Goby	*Ponticola gorlap* (Iljin 1949)	Neogobiidae	Caspian Sea basin
Caspian Monkey Goby	*Neogobius pallasi* (Pallas, 1814)	Neogobiidae	Caspian Sea basin
Pontian Monkey Goby	*Neogobius fluviatilis* (Pallas, 1814)	Neogobiidae	Black Sea basin
Racer Goby	*Babka gymnotrachelus* (Kessler, 1857)	Neogobiidae	Ponto-Caspian area
Round Goby	*Neogobius melanostomus* (Pallas, 1814)	Neogobiidae	Ponto-Caspian Area
Tubenose Goby	*Proterorhinus semilunaris* (Pallas, 1814)	Neogobiidae	Black Sea basin

In Germany, a continuous invasion of non-native gobiid species (Table 1.2) has been observed since the opening of the Rhine-Main-Danube Canal in the year 1992 (Wolter & Röhr, 2010). To date, gobiids of the genera *Proterorhinus*, *Neogobius*, *Ponticola* and *Babka* actively spread within the German waterway network mediated by channelized inter-connections between catchments, which are embanked with their highly preferred rip-rap structures (Sindilariu *et al.*, 2006; own observations) and navigation itself by biofouling and ballast water (Wolter & Röhr, 2010).

The tubenose goby *Proterorhinus semilunaris* (Pallas, 1814) was the first goby species arriving to Germany, first recorded in 1984 in the Danube River (Laßleben, 1985). Successful multiple invasions of the bighead goby, *Ponticola kessleri* (Günther, 1861) had been detected in 1999 (Seifert & Hartmann, 2000) followed by the round goby, *Neogobius melanostomus* (Pallas, 1814), which arrived there in 2004 (Paintner & Seifert, 2006). In the course of this study (see chapter 7), the racer goby *Babka gymnotrachelus* (Kessler, 1857) was discovered 2011 (Haertl *et al.*, 2012) in the Danube River for the first time in Germany. One more gobiid species had been reported for Germany, namely the monkey goby *Neogobius fluviatilis* (Pallas, 1814), found in the River Rhine in 2008 (Stemmer, 2008).

Table 1.2: Invasive perciforme gobies in German waterbodies and their first occurrence

Invasive Perciforme Gobies	**Presence in German Waterbodies**		
Taxon	First Record	Drainage	Reference
Proterorhinus semilunaris (Pallas, 1814)	1984	Danube	Laßleben (1985)
Ponticola kessleri (Günther, 1861)	1999	Danube	Seifert & Hartmann (2000)
Neogobius melanostomus (Pallas, 1814)	2004	Danube	Paintner & Seifert (2006)
Neogobius fluviatilis (Pallas, 1814)	2008	Rhine	Stemmer (2008)
Perccottus glenii Dybowski, 1877	2009	Danube	Reshetnikov & Schliewen (2013)
Babka gymnotrachelus (Kessler, 1857)	2011	Danube	Haertl *et al.* (2012)

These gobies are members of an enigmatic group native to the Ponto-Caspian region containing about 24 species, which have been variously termed "*neogobiins*". Despite their remarkable radiation, the systematic relationships of Ponto-Caspian neogobiin gobies have been disputed and remain unclear (Neilson & Stepien, 2009). Miller & Vasil'eva (2003) expressed a need for detailed cladistic revision and noted the poorly understood taxonomy.

Thus, fish taxonomy in this thesis is strongly following the "*Catalog of Fishes*" (Eschmeyer, 2010).

With more than 2800 kilometers, the Danube River is the second largest River in Europe, collecting water from 19 nations and linking more countries than any other river in the world (Tockner *et al.* 2009). Since the Greek period, the Danube River served as an important waterway, enabling migration of warriors, pilgrims and other pioneers as well as the transportation of goods until the development of the railway network. In the last few decades this river, interconnected to the River Rhine in 1992, also became one of the most important European long-distance dispersal routes for aquatic invasive species (Bij de Vaate et al 2002; Panov *et al.* 2009).

With a time-shift of about five years, an ongoing sympatric invasion of the neogobiids *N. melanostomus* and *P. kessleri* can be observed in the middle and upper sections of the Rivers Rhine and Danube only (Table 1.3).

Table 1.3: Initial colonization events of *N. melanostomus* and *P. kessleri* worldwide

		Neogobius melanostomus	***Ponticola kessleri***
Invaded Ecosystem	**Country**	**First Record (Reference)**	**First Record (Reference)**
Danube River	Serbia	1997 (Simonović *et al.*, 1998)	1986 (Janković *et al.*,1987)
Danube River	Austria	2000 (Wiesner *et al.*, 2000)	1994 (Wiesner, 2005)
Danube River	Slovakia	2003 (Stráňai & Andreji, 2004)	1997 (Stráňai, 1997)
Danube River	Hungary	2003 (Guti *et al.*, 2003)	1996 (Roche *et al.*, 2013)
Danube River	Germany	2004 (Paintner & Seifert, 2006)	1999 (Seifert & Hartmann, 2000)
River Rhine	Netherlands	2004 (van Beek, 2006)	2007 (van Kessel *et al.*, 2009)
River Rhine	Germany	2008 (Borcherding *et al.*, 2011)	2006 (Borcherding *et al.*, 2011)
River Rhine	Switzerland	2011 (Kalchhauser *et al.*, 2013)	2011 (Kalchhauser *et al.*, 2013)
Baltic Sea	Poland	1990 (Sapota, 2004)	no record
Baltic Sea	Finland	2005 (Ojaveer,2006)	no record
Baltic Sea	Latvia	2002 (Ojaveer, 2006)	no record
Baltic Sea	Sweden	2005 (Ojaveer, 2006)	no record
Baltic Sea	Denmark	2008 (Azour, 2011)	no record
Laurentian Great Lakes	Canada	1990 (Jude *et al.*, 1992)	no record
Aegean Sea	Turkey	2001 (Eryilmaz, 2002)	no record

The round goby *Neogobius melanostomus* (Pallas, 1814) (Fig. 1.3) is a small benthic fish - up to 20 cm in total length, native to the Black, Caspian, and Azov seas and the lower reaches of their large associated rivers (Charlebois *et al.*, 1997). It originally inhabited the lower sections of the Danube River as far upstream as the town of Vidin in Bulgaria (Simonović *et al.*, 1998). To date, *N. melanostomus* is one of the most wide-ranging invasive fish species worldwide (Fig. 1.4), with substantial abundance of introduced populations on both sides of the Atlantic Ocean: The Laurentian Great Lakes watershed, the Baltic Sea and several major European streams are affected by rapid range expansions and important ecosystem effects, initializing extensive research on this species (reviewed in Kornis *et al.*, 2012). In lotic habitats, *N. melanostomus* can comprise more than 50% of the total catch (Krakowiak & Pennuto, 2008), illustrating the potential of impact on the ecology of freshwater ecosystems.

Fig. 1.3: Round goby, *Neogobius melanostomus* (Pallas, 1814)

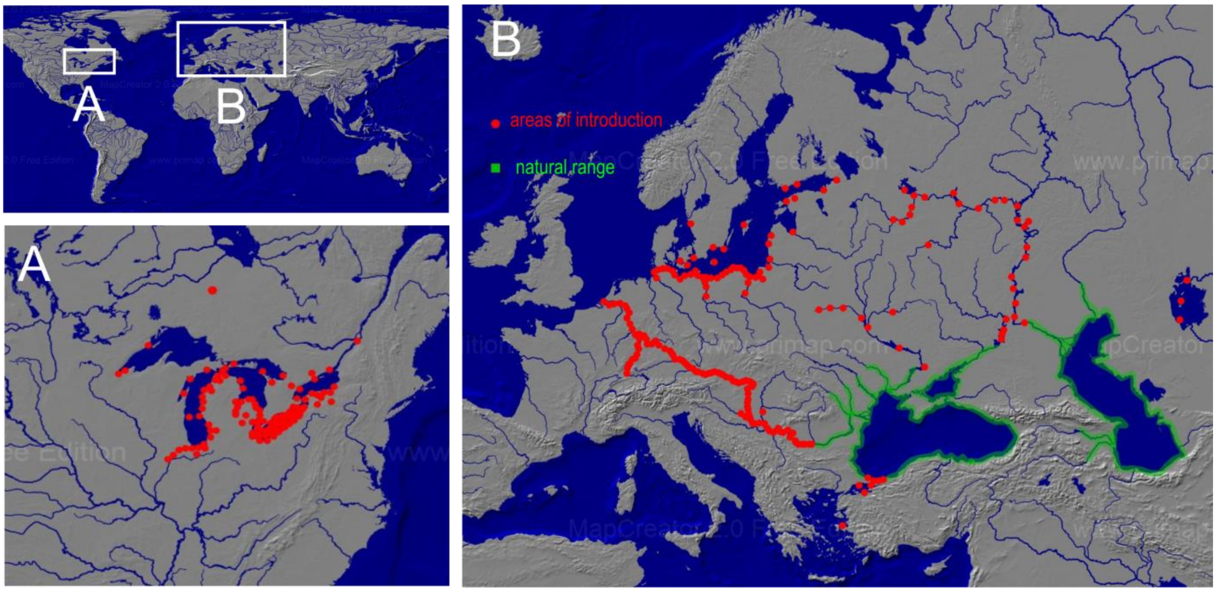

Fig. 1.4: *Neogobius melanostomus* - natural range and areas of introduction worldwide

The natural Ponto-Caspian range of *N. melanostomus* (indicated by the green areas) and introduced populations (indicated by red circles) worldwide (A: Laurentian Great Lakes area; B: European waterbodies). Distribution maps re-drawn after Miller & Vasil'eva (2003) and Kottelat & Freyhof (2007) with modifications after Jude *et al.* (1992), Simonović *et al.* (1998), Wiesner *et al.* (2000), Eryilmaz *et al.* (2002), Guti *et al.* (2003), Sapota (2004), Stráňai & Andreji (2004), Paintner & Seifert (2006), Copp *et al.* (2005), van Beek (2006), Ojaveer (2006), Azour (2011), Borcherding *et al.* (2011), Kornis *et al.* (2012), Kalchhauser *et al.* (2013), Roche *et al.*, 2013 and own observations.

The bighead goby *Ponticola kessleri* (Günther, 1861) (Fig. 1.5), is a Ponto-Caspian gobiid species (similar in size to *N. melanostomus*) that originally inhabited the brackish zone of the Black Sea (northern and western shores), and lower parts of rivers entering the Black Sea between the rivers Danube and Dnjepr (Svetovidov, 1964). The western most native distribution of *P. kessleri* in the Danube River was delineated by the mouths of the Rivers Velika Morava and Nera in Serbia (Ahnelt *et al.*, 1998; Jurajda *et al.*, 2005).This highly-invasive species was the first Ponto-Caspian gobiid invader of the middle Danube and previously one of the most abundant and widely distributed species of the invading gobiids also in the upper Danube (own observations), starting its range expansion in the early 1990s (Kováč *et al.*, 2009; reviewed in Roche *et al.*, 2013) (Fig. 1.6).

Fig. 1.5: Bighead goby, *Ponticola kessleri* (Günther, 1861)

Under competitive conditions, the bighead goby has been outnumbered in both abundance and distribution dynamics by the subsequently arriving *N. melanostomus* in the middle (Kováč *et al.*, 2009) and in the upper (own observations) Danube River. However, Kováč *et al.* (2009) also predicted specific differences in life history-traits favoring *N. melanostomus* potential success in novel environments over short time scales (several years) and *P. kessleri* over longer time scales (decades and longer).

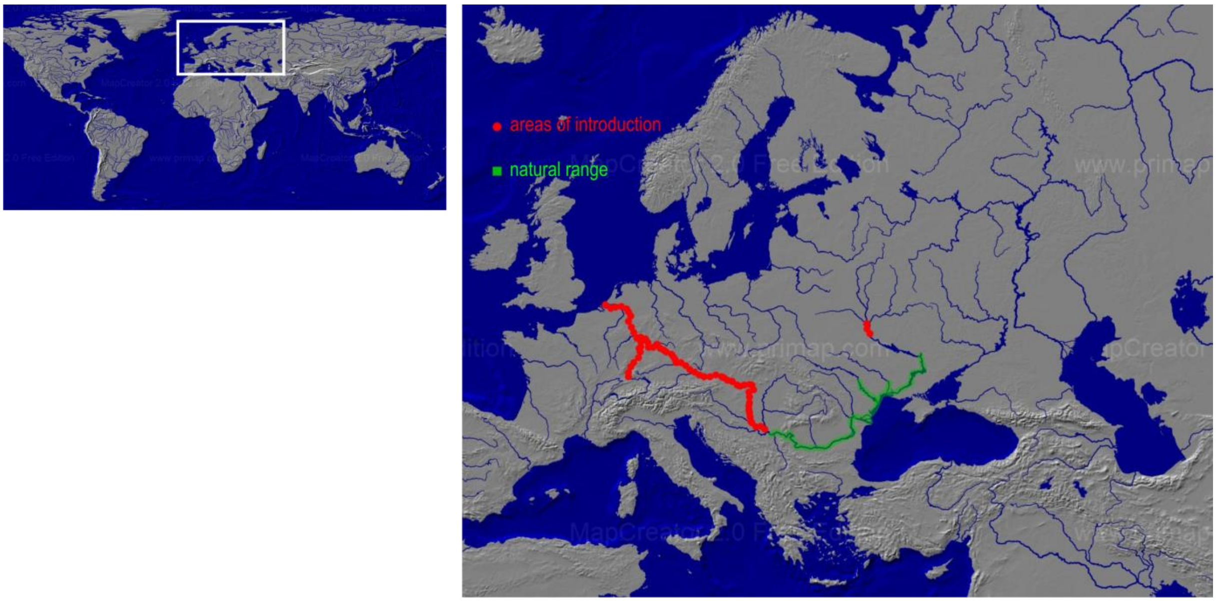

Fig. 1.6: *Ponticola kessleri* - natural range and areas of introduction worldwide

The natural Ponto-Caspian range of *P. kessleri* (indicated by the green areas) and introduced populations (indicated by red circles) worldwide (i.e., European waterbodies). Distribution map re-drawn after Miller & Vasil'eva (2003) and Kottelat & Freyhof (2007) with modifications after Stráňai (1997), Ahnelt *et al.* (1998), Seifert & Hartmann (2000), Jurajda *et al.* (2005), Copp *et al.* (2005), Wiesner (2005), van Kessel *et al.* (2009), Kováč *et al.* (2009), Borcherding *et al.* (2011), Kalchhauser *et al.* (2013) and own observations.

Since niche differentiation and effects of an invader can be modulated by evolutionary or ecological processes, time (since invasion) needs to be explicitly considered to understand the effects of many invaders (Strayer *et al.*, 2006).

According to our own observations, currently *N. melanostomus* and *P. kessleri* comprise more than 50% of the total fish-biomass and up to 80% of the fish-community abundance at rip-rap shoreline habitats of the upper Danube River and the River Rhine (Borcherding *et al.*, 2012), respectively.

These high densities and the lack of knowledge on their ecosystem impact mirror a high relevance for scientific research in both *N. melanostomus* and *P. kessleri* biology.

1.3 Status quo and current ecological research needs

One of the key challenges of invasion biology in aquatic systems is determining the environmental conditions under which non-indigenous species establish populations in new habitats and it is widely believed that environmental degradation of streams and rivers may facilitate susceptibility to invasion (Früh *et al.*, 2012b).

Degraded sites close to navigable waterbodies were exposed to an increased invasion risk by all major groups of alien species with temperature regime shifts, chloride and dissolved oxygen concentrations as major factors for invasibility (Früh *et al.*, 2012a). Besides such biogeochemical processes, diverse communities of aquatic biota regulate freshwater quality in ways that are not sufficiently acknowledged nor appreciated by the water resources management community. Also, the success of river protection and restoration depends upon understanding and accurately modelling relationships between hydrological patterns, fluvial disturbance and ecological responses (Arthington *et al.*, 2010). Even in case of the zebra mussel, which is known for its potential to fundamentally transform freshwater food webs and biogeochemistry, ecological research has been uneven with important research questions remaining unanswered, especially concerning the long-term, large-scale effects of the invasion (Strayer, 2009).

Moyle & Light (1996) demanded a growing need to generally understand the invasion process and its phases to predict the success and effects of invading species. Although invasive species were meanwhile identified as a major driver of global change, yet understanding their impacts still remains a critical challenge (Strayer *et al.* 2006; Simberloff 2011; Moore *et al.*, 2012).

A crucial requirement for understanding the effects of invasive alien species is the comparability of abundance data between studies, especially when different types of habitat were sampled. Thus, in any fish-biological investigation, decisions on sampling strategies and techniques are the first and most crucial steps (Bernhardt & Palmer, 2011; Jähnig *et al.*, 2011). A great variety of different sight-based and sight-independent, active and passive fishing methods have been applied to collect standardised fish population data (Casselman *et al.*, 1990) and many of those methods have been also applied in worldwide goby research. The most appropriate sampling method depends on the specific goals of the research project, as each gear type has its own advantages and biases (Kornis *et al.*, 2012). Note that catch rates, selectivity patterns, and species specificity may strongly differ depending on the sampling method chosen, which currently hampers quantitative comparisons due to a lack of method inter-calibration.

Secondary invasions of *N. melanostomus* into high diversity Great Lakes tributaries and species at risk hotspots may trigger potential new concerns for endangered freshwater species (Poos *et al.*, 2010). *Neogobius melanostomus* invasion of near shore habitats and coastal tributaries of the Laurentian Great Lakes, acting as a springboard for this invasive species to disperse into inland ecosystems has been predicted to continue (Kornis & Vander Zanden, 2010). These upstream directed range expansions and the invasions of tributaries by neogobiids still continue, also in the affected European Rivers (own observations; Kalchhauser *et al.*, 2013).

Climate-related species losses or replacements caused by biological invasions are not only the foremost drivers of fish population extirpations (Sharma *et al.*, 2011), they will moreover increasingly influence taxonomically based biological metrics and multimetric indices, which were frequently used by water-quality agencies to monitor the status of aquatic resources and to define ecological conditions of rivers (Hamilton *et al.*, 2010).

Currently, increasing numbers of non-indigenous species frequently dominate aquatic communities and thus cause problems in the present day water quality assessment both in Europe (European Community - Water-Framework Directive (EC-WFD), 2000) and North America (US Clean Water Act, 1972) as an invader-dominated community can only be considered as representative for a site if these species are included in the biological assessment (Orendt *et al.*, 2010). Thus, one future need is implementing established non-indigenous species into biological assessment procedures.

Invasive gobies have been suspected to cause serious and lasting changes of ecosystems by affecting native communities (Lodge, 1993b; Ricciardi, 2001; Minchin *et al.*, 2007; Van Riel *et al.*, 2007; Van Kleev *et al.*, 2008). Especially the rapid expansion of *N. melanostomus* has been linked to the decline of native fish diversity and abundance (Crossman *et al.*, 1992; Jude *et al.*, 1992; Freyhof, 2003; Jurajda *et al.*, 2005; Karlson *et al.*, 2007; Kornis *et al.*, 2012) and to negative population trends in prey species (Vanderploeg *et al.*, 2002; Barton *et al.*, 2005; Lederer *et al.*, 2006; Pennuto *et al.*, 2010). As invasive gobies do not only interact with native species, but also underlie intra- and interspecific competition due to their same sedentary life style (Števove & Kováč, 2013), data on these closely related, competing invasive aliens may provide further insight into invasion dynamics. Thus, a better understanding of the causes and underlying mechanisms of these biological invasions is hence of prime importance to mitigate these detrimental effects on biodiversity (Gozlan *et al.*, 2010; Rabitsch *et al.*, 2013).

To date however, many studies on the ecology of invasive gobies are limited by few examined specimens, single sampling time-points or incompatible sampling methods. They thus do not provide a reliable picture e.g. of the feeding ecology (Borza *et al.*, 2009). As most recent studies were focused on specific lotic or marine habitats, there is also limited knowledge on recently invaded headwater habitats, i.e. sampled before invasion-induced changes like food resource limitation or potential dietary adaptations occur.

The recent sympatric invasion of Ponto-Caspian gobiids in the upper Danube River could thus provide a close observation of the invasion dynamics of just arriving invaders by respecting time since invasion, both on specimen and population level.

2. Objectives

This study aimed to deduce general ecological objectives from the sympatric invasion of two fish species in a fluvial system, which may contribute to improve the conservation of autochthonous freshwater biodiversity. In particular this thesis was designed to analyze the ecology of *Neogobius melanostomus* and *Ponticola kessleri* (Pisces: Perciformes: Neogobiidae) outside their native range in the German section of an important European long-distance migration corridor for invasive aquatic species, the (upper) Danube River.

Choosing an integrative sampling approach, this survey aimed to

- identify an accurate and precise method suitable for the sampling of neogobiids in rip-rap and gravel habitats of large rivers.
- assess methodological effects of the most commonly applied sampling techniques (electrofishing, angling and fish traps) on population assessment of invasive neogobiids and on the associated fish assemblage.
- provide the trophic niches of both sympatric invading fishes using a combination of stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$), gut content analyses and morphometric analyses of the digestive tract considering seasonal patterns.
- determine food preferences, using a comparison of the natural occurrence of benthic invertebrates as prey with gut contents considering seasonal patterns.
- assess the role of invasive versus native prey species in the invasion success of both goby species considering seasonal patterns.
- identify phenotype-environment correlations in this sympatric fish invasion from initial absence until establishment.
- analyse potential differences between pioneering invaders and their established conspecifics at different stages of an invasion.
- assess potential impact of both invaders on the autochthonous freshwater biodiversity
- search for possibly undetected benthophiline gobiid species.

3. Material and Methods

3.1 Study area

In summer 2009, distribution of gobiids along a recently invaded 200 river kilometer invasion pathway in the upper Danube River between Austria and Germany was monitored during a presence-absence pilot study. This pre-study had been conducted to overview abundance and distribution as well as to identify the farthest upstream site where round goby and bighead goby were present ("*invasion front*") in order to design a representative sampling strategy for the main project. Analogously to Bronnenhuber *et al.* (2011), gobies were considered absent at a site where no specimens were caught at a minimum of 1200 electroshocking seconds.

The uppermost site where single specimens of *N. melanostomus* had been recorded was at river kilometer 2390.2 (N48°58'39.0''; E12°02'16.72'') on August 25th, 2009. The upstream edge of *P. kessleri* distribution was found at river kilometer 2395.3 (N48°57'04.16''; E11°59'19.06'').

The sampling design of all primary research studies conducted herein comprised three river sections with an "established area" where *N. melanostomus* had been recorded for the first time before 1st January 2007, an invasion front where a *N. melanostomus* invasion was expected to happen soon after the initiation of this study and an uppermost "negative control area" with *N. melanostomus* and *P. kessleri* absence during this study. Considering these findings, ten representatively distributed river stretches along the upper Danube River from Kelheim (river kilometer 2416, Germany) to Engelhartszell (river kilometer 2196, Austria) were selected (Fig. 3.1).

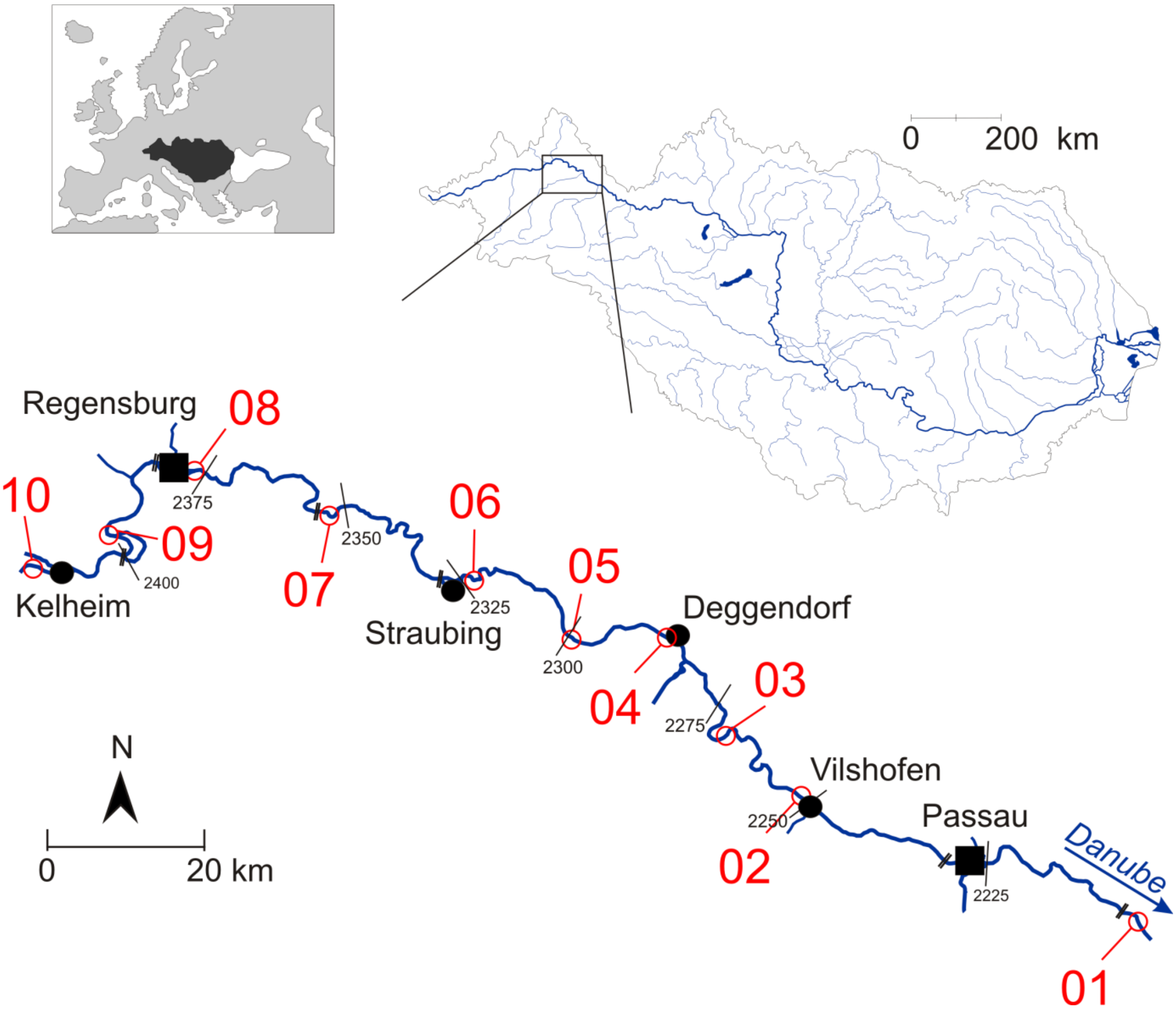

Fig. 3.1: Study area

Study area with ten sampling stretches (red circles) along the upper Danube River. European context and location within the drainage area of the Danube River are highlighted. Important cities are indicated by black circles and squares, river kilometers are represented by black numbers.

Each river stretch comprised four sampling sites covering the two meso-habitats semi-natural gravel-bars and artificial rip-rap shorelines, equally located on the right and on the left embankment of the Danube River (Table 3.1). Due to the lack of semi-natural habitats at the right shoreline, river stretch #8 “Bad Abbach” contained only three sampling sites. Within the study area, sampling covered a total river length of about 200 river kilometer of the upper River Danube.

Table 3.1: Location of ten representative river stretches along the upper Danube River

River Stretch					Lower Boundary		Upper Boundary	
№	**Notation**	**Mesohabitat**	**Bank**	**Length [m]**	**River km**	**GPS (wgs 84)**	**River km**	**GPS (wgs 84)**
1.1.	Engelhartszell	rip-rap	left	290	2196.190	E 13°46'29.60" N 48°28'31.85"	2197.280	E 13°46'19.25" N 48°28'40.30"
1.2.	Engelhartszell	rip-rap	right	290	2200.000	E 13°44'16.40" N 48°29'57.17"	2200.290	E 13°44'11.50" N 48°30'05.78"
1.3.	Engelhartszell	gravel	left	290	2201.300	E 13°43'57.09" N 48°30'37.98"	2201.590	E 13°43'48.16" N 48°30'45.67"
1.4.	Engelhartszell	gravel	right	290	2201.650	E 13°43'37.75" N 48°30'41.46"	2201.940	E 13°43'22.62" N 48°30'46.70"
2.1.	Vilshofen	rip-rap	left	310	2250.850	E 13°10'17.17" N 48°38'50.54"	2251.160	E 13°10'07.68" N 48°38'58.51"
2.2.	Vilshofen	rip-rap	right	290	2250.700	E 13°10'15.36" N 48°38'42.53"	2250.990	E 13°10'4.78" N 48°38'51.09"
2.3.	Vilshofen	gravel	left	330	2254.800	E 13°07'29.67" N 48°39'46.05"	2255.130	E 13°07'14.04" N 48°39'49.08"
2.4.	Vilshofen	gravel	right	330	2257.330	E 13°06'45.76" N 48°40'51.21"	2257.660	E 13°06'41.24" N 48°41'00.42"
3.1.	Aichet	rip-rap	left	300	2266.900	E 13°03'00.24" N 48°43'39.67"	2267.200	E 13°02'45.81" N 48°43'36.87"
3.2.	Aichet	rip-rap	right	290	2267.400	E 13°02'43.96" N 48°43'27.21"	2267.690	E 13°02'36.09" N 48°43'18.92"
3.3.	Aichet	gravel	left	290	2270.300	E 13°00'54.87" N 48°43'20.41"	2270.590	E 13°00'57.60" N 48°43'32.86"
3.4.	Aichet	gravel	right	340	2271.950	E 13°01'38.98" N 48°43'58.84"	2272.290	E 13°01'50.67" N 48°44'06.53"
4.1.	Deggendorf	rip-rap	left	290	2286.300	E 12°56'30.66" N 48°49'54.92"	2286.590	E 12°56'18.36" N 48°50'00.64"
4.2.	Deggendorf	rip-rap	right	290	2286.900	E 12°55'52.75" N 48°50'00.50"	2287.090	E 12°55'40.90" N 48°50'05.82"
4.3.	Deggendorf	gravel	left	310	2280.100	E 12°59'34.28" N 48°47'45.21"	2280.950	E 12°59'14.13" N 48°47'56.84"
4.4.	Deggendorf	gravel	right	290	2280.860	E 12°59'08.71" N 48°47'52.47"	2281.150	E 12°58'54.27" N 48°48'00.56"
5.1.	Mariaposching	rip-rap	left	300	2294.350	E 12°50'25.95" N 48°50'10.29"	2294.650	E 12°50'18.24" N 48°50'2.01"
5.2.	Mariaposching	rip-rap	right	290	2295.350	E 12°49'59.52" N 48°49'40.40"	2295.640	E 12°49'46.18" N 48°49'36.04"
5.3.	Mariaposching	gravel	left	290	2295.500	E 12°49'46.88" N 48°49'45.04"	2295.790	E 12°49'34.33" N 48°49'40.80"
5.4.	Mariaposching	gravel	right	290	2296.850	E 12°48'48.58" N 48°49'24.42"	2297.140	E 12°48'34.60" N 48°49'23.88"
6.1.	Straubing	rip-rap	left	300	2310.680	E 12°41'18.90" N 48°54'07.48"	2310.980	E 12°41'05.41" N 48°54'11.83"
6.2.	Straubing	rip-rap	right	350	2309.650	E 12°41'48.91" N 48°53'43.01"	2310.000	E 12°41'35.53" N 48°53'50.17"
6.3.	Straubing	gravel	left	300	2316.000	E 12°37'18.65" N 48°54'14.79"	2316.300	E 12°37'15.57" N 48°54'04.98"
6.4.	Straubing	gravel	right	290	2314.620	E 12°38'12.67" N 48°54'23.79"	2314.910	E 12°38'00.23" N 48°54'28.48"
7.1.	Geisling	rip-rap	left	300	2353.000	E 12°21'41.85" N 48°58'30.96"	2353.310	E 12°21'26.41" N 12°21'26.41"
7.2.	Geisling	rip-rap	right	290	2351.590	E 12°22'43.67" N 48°58'26.13"	2351.880	E 12°22'30.77" N 48°58'17.28"
7.3.	Geisling	gravel	left	560	2350.850	E 12°22'54.84" N 48°58'46.73"	2351.400	E 12°22'40.69" N 48°58'29.62"

7.4.	Geisling	gravel	right	790	2350.250	E 12°23'24.11" N 48°58'47.18"	2351.030	E 12°22'53.18" N 48°58'38.68"
8.1.	Regensburg	rip-rap	left	360	2375.700	E 12°08'57.41" N 49° 1'17.95"	2376.060	E 12°08'41.18" N 49°01'22.70"
8.2.	Regensburg	rip-rap	right	290	2375.000	E 12°09'22.73" N 49°01'01.27"	2375.290	E 12°09'10.06" N 49°01'07.08"
8.3.	Regensburg	gravel	left	300	2374.200	E 12°09'59.49" N 49°00'58.36"	2374.500	E 12°09'45.27" N 49°00'59.47"
8.4.	Regensburg	gravel	right	310	2373.950	E 12°10'11.04" N 49° 0'50.93"	2374.260	E 12°09'56.86" N 49° 0'54.44"
9.1.	Bad Abbach	rip-rap	left	340	2394.960	E 11°59'12.14" N 48°57'14.94"	2395.300	E 11°59'19.06" N 48°57'04.16"
9.2.	Bad Abbach	rip-rap	right	350	2394.975	E 11°59'16.07" N 48°57'15.31"	2395.325	E 11°59'24.73" N 48°57'05.31"
9.3.	Bad Abbach	gravel	left	360	2399.100	E 12°02'09.11" N 48°56'25.79"	2399.460	E 12°02'14.39" N 48°56'15.58"
9.4.	Bad Abbach	gravel	right		n.a.	n.a.	n.a.	n.a.
10.1.	Kelheim	rip-rap	left	300	2415.000	E 11°51'43.37" N 48°54'59.69"	2415.300	E 11°51'23.28" N 48°54'57.54"
10.2.	Kelheim	rip-rap	right	300	2412.730	E 11°53'28.79" N 48°54'26.74"	2413.030	E 11°53'14.52" N 48°54'28.36"
10.3.	Kelheim	gravel	left	300	2415.970	E 11°51'08.74" N 48°54'45.11"	2416.270	E 11°51'06.75" N 48°54'31.55"
10.4.	Kelheim	gravel	right	300	2415.000	E 11°51'44.00" N 48°54'56.50"	2415.300	E 11°51'24.35" N 48°54'55.34"

3.2 Field work

Fish assemblage and sampling of gobies on the population level

Sampling was conducted from October 2009 to October 2011, covering the early (March–June) and late (August–October) annual growth period of fish. In order to avoid the introduction of a systematic sampling bias (e.g. due to trends in water temperatures and discharge), even and uneven river stretches (first even and then uneven numbers of the consecutively numbered river stretches) were sampled.

Fishes were sampled during daylight from shorelines (in ~60 cm water depth) using a standardised electrofishing method, the multiple-point, fractional sampling strategy "*point abundance sampling of electrofishing*" (PAS-electrofishing), developed by Nelva *et al.* (1979). PAS-electrofishing considers the spatial structure of the population or community using numerous small samples instead of one or a few large samples and thus yields a high number of independent samples (Zalewski, 1985; Persat & Copp, 1990; Reynolds, 1992; Scholten, 2003; Lapointe *et al.*, 2006b). PAS-electrofishing was performed according to Persat & Copp (1990) with duration of 10 s electrical current flow (Scholten, 2003). The activated ring-shaped anode (diameter 31 cm), powered by a mobile 3.1 kW (continuous DC) electrofishing generator (ELT62-IID; Grassl GmbH, Berchtesgaden, Germany), was submerged into the water ~20 cm above the ground for 2 s and then slowly lifted up to the surface for the remaining 8 s (Fig. 3.2). Stunned fishes were caught using a round dip net (diameter: 48 cm, mesh size: 5 mm) by a second person.

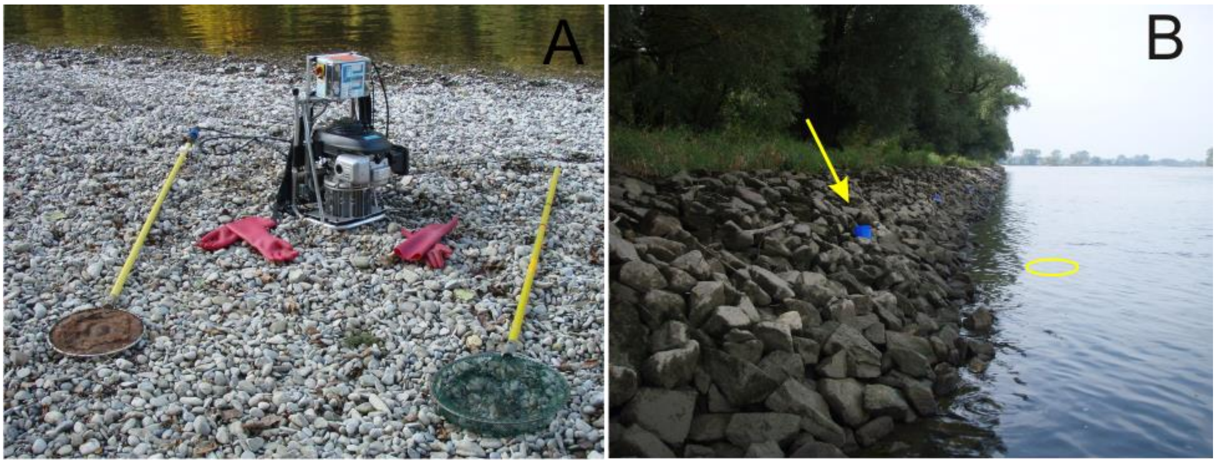

Fig. 3.2: Fishing gear (A) and exemplary river stretch (B)

(A) Fishing gear: Type: ELT62-IID mobile backpack unit (Grassl GmbH, Germany) with a power output of 3.1 kW continuous DC; anode left, dip net right.
(B) exemplary river stretch (#5.2 Mariaposching, right shoreline) with 10 m distanced PAS-points (marked by buckets ready to take up catch, yellow arrow) and first point-abundance sampling-point in front (yellow ellipse).

In order to avoid a systematic bias, all samples were taken by the same fishing-team (anode: A.F. Cerwenka, dip net: J. Brandner) and a distance of 10 meters between points to avoid disturbance. The 10 m distance between points is double the proposed distance for individual PAS-points according to peer-reviewed scientific literature (e.g. Watkins *et al.*, 1997). The effect radius of the applied fishing gear was about 0.5 to 1 m around the anode (own observations), mainly depending on the electrical conductivity.

Every river stretch comprised at least 30 PAS-points at each stream shoreline.

In total, 4297 PAS-points were sampled, comprising 2027 gravel, 2245 rip-rap and 25 other habitats (groin, sand).

All fishes were determined to species level, counted, measured (L_T to nearest mm) and weighted (M_T to nearest 0.2 g). Sex of gobiids was determined by an examination of the morphology of the urogenital papilla (Fig. 3.3) following Miller (1984) and Kornis *et al.* (2012).

All fishes were inspected for infection rates with ectoparasitic plathyhelminths of the genus *Rossicotrema spp.* (black spot disease) and assigned into four categories (0 = no black spots; 1 = few, i.e. < 5 spots; 2 = medium, i.e. 5-100 spots; 3 = high, i.e. > 100 spots).

Following federal fishing laws and sampling licensing, all native fishes were carefully returned to the river after sampling. All efforts were carried out in strict accordance with the legal obligations of the Federal Republic of Germany.

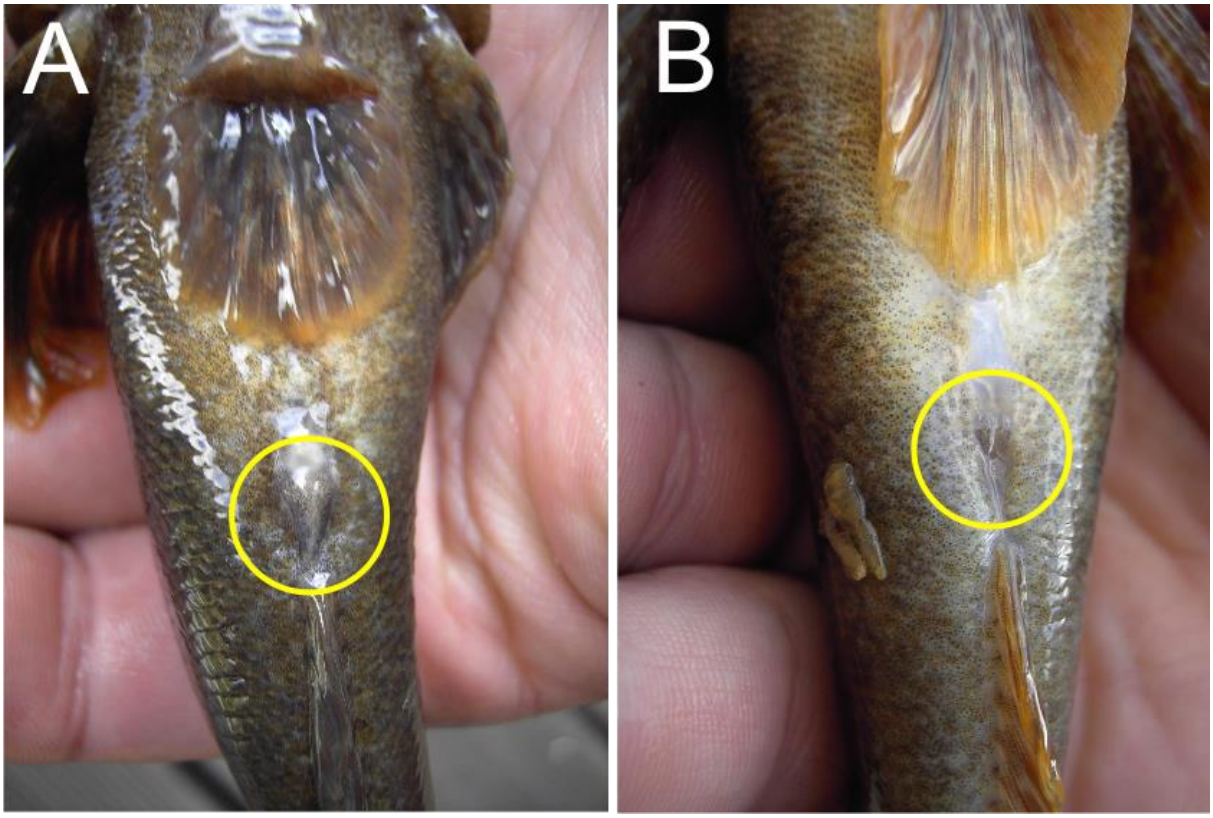

Fig. 3.3: Morphology of the urogenital papilla in male and female *P. kessleri*

The sexual dimorphism in the morphology of the urogenital papilla in neogobiids (here: *Ponticola kessleri*) allows the determination of males and females in the field. Both sexes have an erectile urogenital papilla between the anus and the base of the anal fin (marked by yellow circles). The female papilla is broad and blunt (B) whereas the male papilla (A) is longer, pointed and has a terminal slit (Kornis *et al.*, 2012).

Sampling of gobies on the specimen level

In addition to the demographic sampling for characterizations on the population level, a total number of 672 *N. melanostomus* specimens and 505 *P. kessleri* specimens were collected at danubian bank habitats in 2010 and 2011. Whenever possible, two male and two female specimens were collected from each single sampling site. Specimens were sacrificed using a lethal dose of anesthetic and immediately frozen on dry ice to avoid degradation of gut contents and muscle tissue. These specimens were dissected following a standardised protocol (see 3.3 Laboratory analyses) and were deposited (individualised by numbers) at the ichthyological collection of the Bavarian State Collection of Zoology in Munich (ZSM).

Several recent studies on neogobiids did not consider the effects of fish size on feeding behaviour (e.g. Adámek *et al.*, 2007; Polačik *et al.*, 2009; Borza *et al.*, 2009), whereas many other studies described ontogenetic diet shifts in *N. melanostomus* (French & Jude, 2001; Phillips *et al.*, 2003; Barton *et al.*, 2005; Johnson *et al.*, 2005a; Karlson *et al.*, 2007; Campbell *et al.*, 2009; Brandner *et al.*, 2013).

The known size-effects in at least one of the species were accounted for in two ways:

First, specimens were size-class selected (target size: 8 to 12 cm) with (mean ± S.D) L_T of 9.8 ± 1.7 cm (n = 505) in *P. kessleri* and 9.7 cm ± 1.3 cm (n = 672) in *N. melanostomus* (data pooled from 2010 and 2011).

Second, to test for this effect in *N. melanostomus* nutrition, additional samples (L_T of 2 - 17 cm) of 16 specimens (river stretch #08 “Regensburg”; 49°01'01.95"N, 12°09'21.09"E; October 15th, 2010) and 15 specimens (river stretch #10 ‘Kelheim’; 48°54'26.99"N, 11°53'24.56"E; September 9th, 2011) were collected (Fig. 5.1; Table 5.1).

Since many morphometric indices assume isometry of body proportions in fish of varying size (e.g. Anderson & Neumann, 1996; Froese 2006) and potential ontogenetic diet shifts may influence stable isotope signatures, differences in growth were tested for all selected specimen samples. To test for such differences in growth (and to estimate growth-associated bias) between the size-selected samples, length-weight regression analyses were computed using ANCOVA to compare slopes and to ensure comparability of data.

Sampling of benthic invertebrates

To obtain quantitative benthos samples, a suction sampling device was designed, modified from Brooks (1994) and Brown *et al.* (1989). This flow through system, driven by a water pump (18 l/min, 1.0 bar; Barwig, Germany) inside a duct, integrated a 1000 x 500 µm-mesh for filtering benthic organisms. A flexible tube (Ø = 16 mm) with a brush frontend was used to scrub and collect benthic invertebrates from surfaces and interstices. Efficiency was evaluated in laboratory tests, where mean catch rates of 40.2% (S.D. = 6.6%, n = 5, duration = 120 s; substratum Ø = 5 – 8 mm) and 26.4% (S.D. = 8.8%, n = 5, duration = 120 s; substratum Ø = 8 – 16 mm) of the amphipod *Gammarus pulex* (L., 1758) were observed. This suction sampling device allowed standardized sampling including the collection of gastropods and bivalves.

Suction samples were collected from the same sites where gobies were sampled (~60 cm water depth; duration = 120 s; n = 3 at each sampling site). Altogether, in 2010, 190 samples (early summer: n = 105, late summer: n = 85) and, in 2011, 222 samples (early summer: n = 117, late summer: n = 105) of benthic invertebrates were preserved in 70% ethanol immediately after capture.

Physico-chemical conditions and hydromorphology

Physico-chemical conditions were measured at all sampling sites (before and after sampling of fishes) using handheld multi 350i and Turb 355T (both WTW GmbH, Weilheim, Germany) to characterize the Danube River. Data were collected covering the early (March–June) and late (August–October) season during the annual fish sampling (Table 3.2).

Table 3.2: Physico-chemical conditions of the Danube River within the study area

		Early Season			Late Season		
Physico-Chemical Parameter	**Unit**	**Mean**	**S.D.**	**n**	**Mean**	**S.D.**	**n**
Water Temperature	°C	15.1	3.8	181	15.7	3.4	171
Oxygen Dissolved	mg/l	11.6	2.3	180	9.9	1.4	143
Oxygen Saturation	%	119	22	180	103	14	143
pH		8.3	0.2	180	8.1	0.2	144
Turbidity	NTU	8.4	7.8	180	4.7	2.6	152
Electrical Conductivity (at 25 °C)	µS/cm	506	70	181	531	56	171

At river stretch #08, “Regensburg”, the upper Danube River drains a catchment area of about 35450 km^2 with a mean annual discharge of about 450 m^3s^{-1} (hydrological data of the Bavarian Environment Agency, LfU).

3.3 Laboratory analyses

Dissection of specimens

Sex determined in the field was confirmed by standardized dissection for all specimens. The wet weights of liver, ovaries in females, testes and seminal vesicles in males were recorded to the nearest 0.001 g. Since both gobiids are known to serve as a paratenic host for acanthocephalans (Ondračková *et al.*, 2010), subadult acanthocephalans attached to liver, kidney, spleen, gonads and the surface of the intestinal tract were counted using a stereo-binocular. Ecological indicators of parasite infection were applied according to Ondračková *et al.* (2010), using mean abundance (i.e. mean number of parasites per host) and mean density (i.e. abundance per fish total mass).

Digestive tract analyses

The digestive tract was removed by cutting off the caudal end of the oesophagus (posterior pharyngeal teeth) and the anal aperture (Fig. 3.4). Oesophagus, oesogaster and intestine were separated from other organs and the length of the uncoiled dissected intestinal tract was measured to the nearest mm. Gut contents from the posterior intestine were not analyzed because of the progressed digestion process. Therefore, the posterior intestine was cut off at the intestinal-rectal sphincter level (Fig. 3.4) following morphological findings of Jaroszewska *et al.* (2008).

The gut from the esophagus to the middle intestine termination was weighed to the nearest 0.001 g before and after emptying to obtain the wet weight of gut contents. All food items from digestive tract samples were fixed in ethanol for later identification and counting.

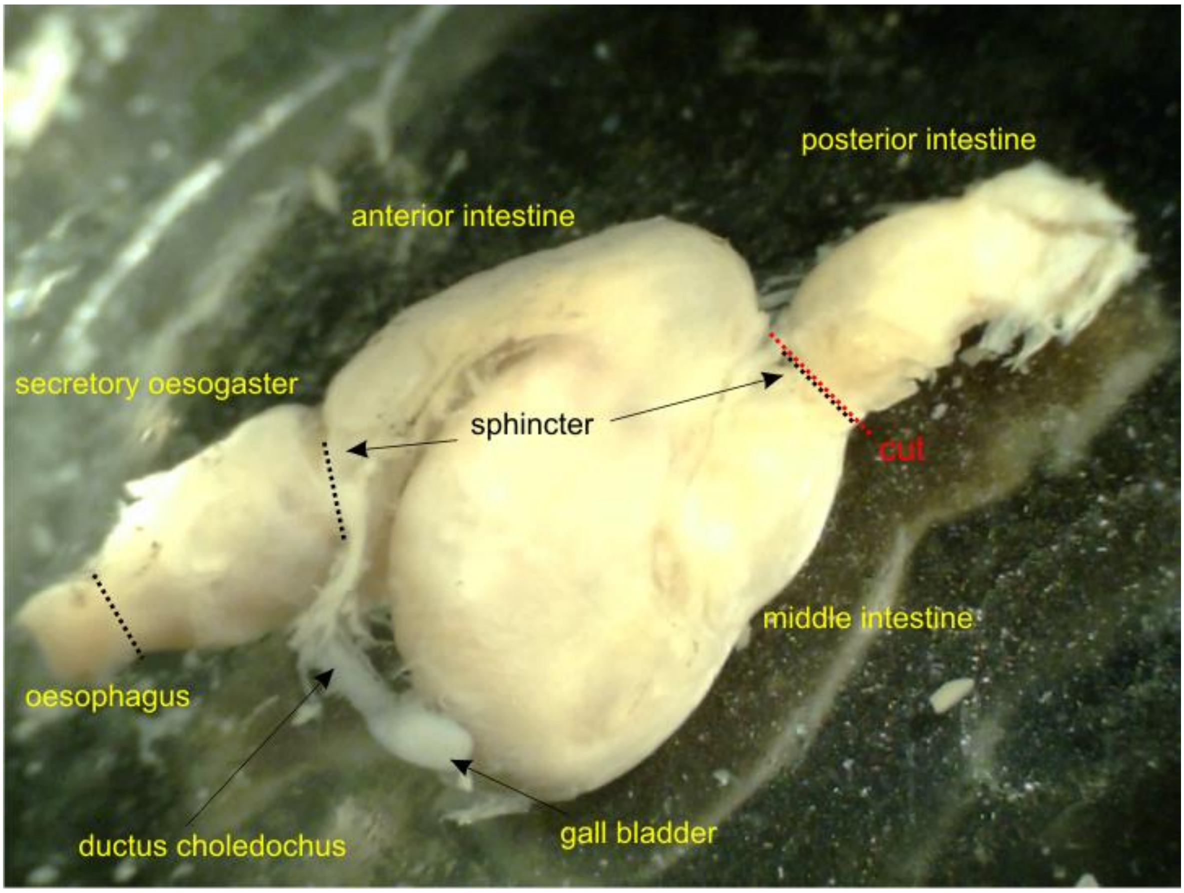

Fig. 3.4: Morphology of the digestive tract of *N. melanostomus*

As several relevant prey taxa in this study occurred in amounts too small for reliable weighting or volumetric measuring by water-displacement, the per cent contribution of all food items to the whole gut content was estimated using a stereo microscope following the procedure by McMahon *et al.* (2005) and Polačik *et al.* (2009). The contributions of individual food items were expressed as "visually estimated proportion of volume [%]". For methodological comparisons of fish stomach content analyses and visual estimation of volumes see also Hynes (1950) and Hyslop (1980). Additionally, we refer to Karlson *et al.* (2007), who showed that dry weight of food components can even be estimated with sufficient accuracy from only the numbers and maximum lengths of items by using conversion factors available from literature. Crushed bivalves and amphipods were reconstructed from the contents of the intestinal mucus hulls whenever possible to gather taxonomically relevant parts of their exoskeletons.

Determination of benthic invertebrates

Benthic invertebrates from environmental samples and digestive tracts were identified to the lowest possible taxon considering manageable taxonomical levels (e.g. Chironomidae, Oligochaeta). The literature used for the determination of benthic invertebrates comprised Bauernfeind & Humpesch (2001), Eggers & Martens (2001), Eggers & Martens (2004), Lechthaler (2005), Lechthaler & Car (2005), Lechthaler & Stockinger (2005), Lechthaler (2007), Lechthaler (2009), Glöer & Meier-Brook (2003), Habach (2006), Orendt & Spieß (2010), Reynoldson & Young (2000), Sundermann & Lohse (2006), Waringer & Graf (1997), Wood & Okamusa (2005) and Zwick (2004).

Due to immaturity and thus poorly developed identification characters, amphipods often could not be determined to species level and thus were counted as “Amphipoda”. The percent volumetric proportion of each taxon within a sample was visually estimated using a stereo microscope. The values were expressed as “visually estimated proportion of volume” [%].

In order to detect potential differences in the spatial distribution, the catch of organisms belonging to the same taxon or cumulative category was additionally expressed as catch per unit effort (CPUE [min^{-1}]) in environmental samples.

Stable isotope analysis

In aquatic ecosystems, stable isotope analysis (SIA) has been extensively used to study especially food web interactions and trophic relations (Brain, 2006). In fish ecology, particularly $\delta^{13}C$ and $\delta^{15}N$ SIA have been shown to be powerful markers for middle to long-term feeding patterns and trophic niche assessments (Vander Zanden *et al.*, 1997; Post, 2002; Perga & Gerdeaux, 2005; Brush *et al.*, 2012), but also for habitat use and migration (Mac Mahon, 2010). SIA relies on isotopic turnover, which is defined as the change in tissue isotope composition, attributable to growth and metabolic tissue replacement (McAvoy *et al.*, 2001; Auerswald *et al.*, 2010), and could thus be a potent species-specific trait useful for trophic niche assessment (Barbosa *et al.*, 2009).

To obtain markers for middle to long-term feeding pattern and trophic niche assessment, $\delta^{13}C$ and $\delta^{15}N$ stable isotope analyses of goby specimens and of the most abundant prey items were conducted. $\delta^{13}C$ and $\delta^{15}N$ are relative isotope ratios calculated as $(R_{sample}/R_{standard})-1$, where R is the ratio of the heavy and the light isotope and standard is Vienna-PeeDee Belemnite (V-PDB) in the case of carbon and atmospheric N_2 in the case of nitrogen. Benthic invertebrates were held in tap water for 24 h to empty their guts. Subsequently, pieces of fish flank muscle tissue (about 0.5 – 1.0 cm^3) were sampled, snap-frozen using liquid nitrogen and stored at -18 °C until analysis. The additional sets of samples with greater length variation were analyzed to test for (i) correlations between L_T and $\delta^{15}N$ signatures and (ii) a diet shift between muscle tissue and gut contents. The $\delta^{15}N$ values of the gut contents were calculated as averages weighted by their “index of food importance” from mean $\delta^{15}N$ signatures of benthic invertebrates collected from the upper Danube River (Table 5.3).

This approach was preferred over the direct determination of the isotopic composition of the gut content because a much larger number of replicated measurements for the different food items could be used, better reflecting the average of each food component than the snapshot found in the gut. Furthermore, assumptions on whether the gut content did still reflect food resources despite digestion or addition of mucus, which may have already changed the isotopic composition of the gut content, can be avoided by using this approach. After ultrasonic cleaning, all samples were defatted with a chloroform-methanol (2:1) solution, oven-dried (40 °C for 48 h) and ground to homogenous powder, using a mixer mill.

Samples of 0.3 – 0.4 mg were weighed into tin cups and combusted in an isotope ratio mass spectrometer (Delta plus, Finnigan MAT, MasCom GmbH, Bremen, Germany) interfaced (via ConFlo II, Finnigan MAT, MasCom GmbH, Bremen, Germany) with an elemental analyzer (EA 1108, Carlo Erba, Thermo Fisher SCIENTIFIC, Milan, Italy) and a pyrolysis unit (HT Sauerstoffanalysator, HEKAtech GmbH, Wegberg, Germany). Repeated analyses of a solid internal laboratory standard (bovine horn, run after each ten samples) showed maximum standard deviations of 0.15‰ for $\delta^{15}N$ and 0.15‰ for $\delta^{13}C$ values.

3.4 Morphometric indices and statistical analyses

Somatic mass (M_S) was calculated to compute the subsequently represented morphometric indices as

$$M_S = M_T - \left(M_{indexed\,organ} + M_g\right)$$

with M_g = gut content mass

To test for differences in important body mass indices between specimens of a population, the hepato-somatic index (HSI = 100 $M_{liver}\, M_S^{-1}$) and the gonado-somatic index (GSI = 100 $M_{gonads}\, M_S^{-1}$) were calculated for both sexes according to Marentette *et al.* (2009).

Length-weight relationships between populations and specimens were assessed using "*Fulton's condition factor*" (K), calculated (Anderson & Neumann, 1996; Froese, 2006) as

$$K = 100\frac{M_T - M_g}{{L_T}^3}$$

with L_T = total length [mm], M_T = total fish body mass [g], M_g = gut content mass [g]

According to Herder & Freyhof (2006), the relative importance of a food item i among all items j for a population was calculated as the "*Index of food importance*" (I_{FI}) using visually estimated volumes and counted numbers of food items:

$$I_{FI}(i) = 100\, O(i)\, V(i)\left(\sum_{n=1}^{j} O(i)\, V(i)\right)^{-1}$$

with O = % occurrence of prey i

and V = visually estimated proportion of volume [%] of prey i

I_{FI} varies from 0 to 100, with higher values corresponding to a larger contribution of one food item as compared to total gut content. Since macrobenthos samples were treated like gut content samples, importance of naturally available prey was also calculated following the above mentioned formula as "index of environmental importance" (I_{EI}) for each food item i.

Dietary overlap (O_D) between *N. melanostomus* and *P. kessleri* was calculated using the "Schoener-Index" (Schoener, 1970; see also Herder & Freyhof, 2006):

$$O_{\mathrm{D}} = 1 - \frac{\left(\frac{1}{2}\sum |\mathrm{p_a} - \mathrm{p_b}|\right)}{100}$$

with p_a = percentage of a food item in species a
and p_b = percentage of a food item in species b

O_D ranges from 0 to 1, with 0 meaning total dissimilarity and 1 representing identical gut contents.

The "*Zihler Index*" (Z_I) was calculated to assess digestive tract lengths as an indicator for diet adaptations (Zihler, 1982) according to Herder & Freyhof (2006):

$$Z_{\mathrm{I}} = \frac{L_{\mathrm{I}}}{10\sqrt[3]{M_{\mathrm{T}}}}$$

with L_I = length of the uncoiled intestinal tract [mm], M_T = total fish body mass [g]

Z_I was used since it avoids body shape-dependent bias when comparing uncoiled digestive tract lengths. Bibliographic data (Karachile & Stergiou, 2010) displayed a range in Z_I for omnivores with preference to animal material of 1.5 to 12.7 (n = 26, mean Z_I = 3.8, S.E. = 0.5), whereas Z_I of herbivores ranged from 4.5 to 53.6 (n = 5, mean Z_I = 20.3, S.E. = 9.2).

To assess food uptake and to test for potential food limitation effects on feeding behavior, the "*Index of stomach fullness*" (I_{SF}) was calculated following Hyslop (1980) as

$$I_{SF} = 100\,\frac{M_g}{M_T}$$

with M_T = total fish body mass [g], M_g = gut content mass [g]

As no trend in the I_{SF} (data from 2010), neither in *N. melanostomus* (regression analysis; $R^2 = 0.0283$, n = 142), nor in *P. kessleri* (regression analysis; $R^2 = 0.0199$, n =107) could be detected across daytime (Fig. 3.5), PAS-data from different time-points were pooled.

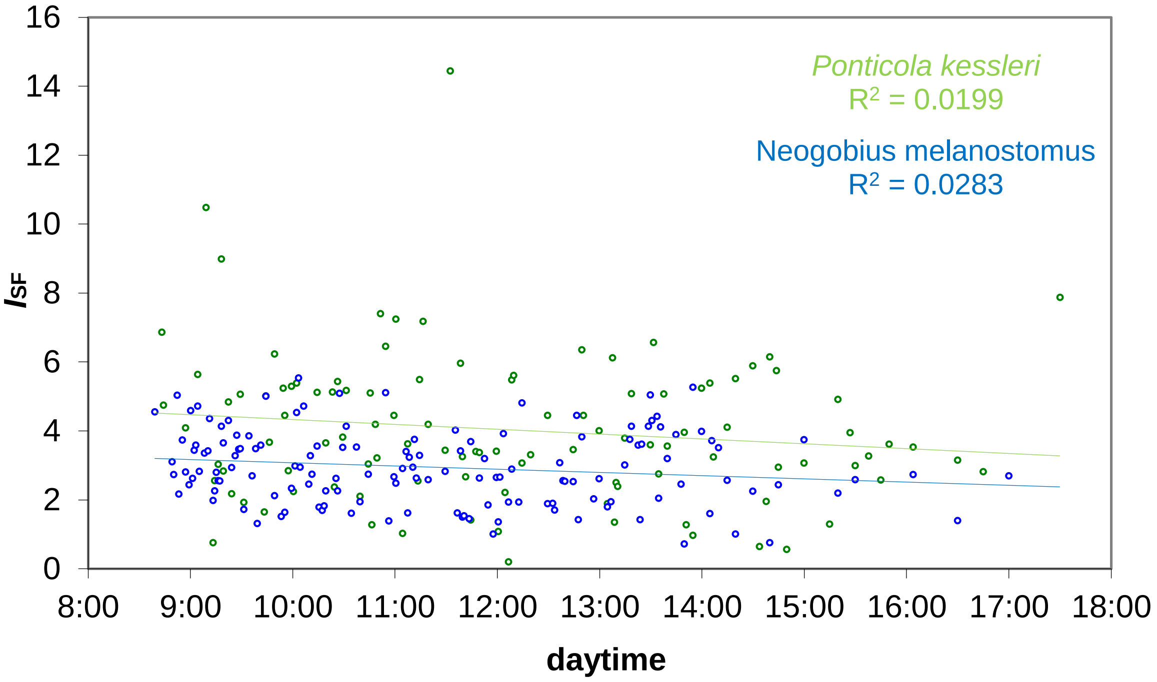

Fig. 3.5: Performance of the index of stomach fullness (I_{SF}) across daytime

Finally, a "*prey-specific index of food importance*" was calculated (I_P) to obtain a prey preference analysis independent from benthic invertebrate sampling and therefore containing fish as prey. Only guts of specimens of a population in which a specific prey i occurred, were considered:

$$I_{\mathrm{P}}(i) = \frac{1}{\sum I_{\mathrm{FI}}\,(i)\,n(i)}$$

with $I_P(i)$ = prey-specific index of food importance of prey i

$I_{FI}(i)$ = index of food importance of prey i

n(i) = number of guts containing prey i

The feeding strategies of gobies were then characterized in analogy to Costello´s method (Costello, 1990), modified by Amundsen *et al.* (1996) by plotting I_P of each prey versus its frequency of occurrence, given by its relative proportion n(i)%.

Benthic invertebrates and food taxa were classified according to their biogeograpical origin as “indigenous” and “invasive”, species too small for taxonomic identification and species with non-allocatable biogeographical origin were classified as “unassigned”. The proportions of these three classes were determined for the gut content samples of both goby species and for the benthic invertebrate samples.

For comparisons of mean values between species and seasons, one-way ANOVA (SIA) or t-tests (Z_I) were used if the criteria for parametric testing were fulfilled. Alternatively, non-parametric Mann-Whitney U-tests or Kruskal-Wallis-tests (Bonferroni corrected) were applied (I_{FI}, I_{EI}, I_{SF}, K, L_T, M_T). Significance was accepted at $\alpha \leq 0.05$). Statistical analyses and plots were computed using Statistica (version 6.1, StatSoft Inc., Tulsa, OK, USA), SPSS 11.0 (IBM SPSS Statistics, NY, USA), PAST (Hammer *et al.*, 2001) and Excel 2010 (Microsoft^{TM}). Maps were generated using the freeware “Map Creator 2” by primap software.

4. Effects of sampling techniques on population assessment

A similar version of this chapter was published as:

Brandner, J., J. Pander, M. Mueller, A. F. Cerwenka & J. Geist, 2013. Effects of sampling techniques on population assessment of invasive round goby. *Journal of Fish Biology* **82**: 2063-2079.

4.1 Abstract

In this study, a comparison of point abundance sampling (PAS) electrofishing, angling with two different hook sizes, and trap-based fishing was performed in a non-wadeable river to analyse their effects on catch per unit effort (CPUE) and population characteristics of invasive *N. melanostomus*. PAS-electrofishing was identified as the most effective (mean ± S.E. CPUE = 57 ± 4 *N. melanostomus* min^{-1}) and least selective method in terms of size, feeding status and species composition. Angling had the second highest CPUE, but was more size selective and resulted in a higher proportion of males compared to electrofishing (overall sex ratio angling (female to male) = 1:0.92, electrofishing 1:0.65). Due to low CPUE (0.012 ± 0.004) and low frequency-of-occurrence, minnow traps were least suitable for *N. melanostomus* population assessment. The results of this study suggest that a higher degree of standardisation and intercalibration is useful to achieve better comparability of population data of invasive *N. melanostomus* and other benthic fish species.

4.2 Introduction

Decisions on sampling strategies and techniques are the first and most crucial steps in any fish biological investigation (Bernhardt & Palmer, 2011; Jähnig *et al.*, 2011). A great variety of different sight-based and sight-independent, active and passive fishing methods have been applied to collect standardised fish population data in rivers (Casselman *et al.*, 1990). These include electrofishing (e.g. DeLury, 1947; Persat & Copp, 1990; Lapointe *et al.*, 2006a), hook-and-line methods (Gutowsky & Fox, 2011), net-based methods (seining, e.g. Kakareko *et al.*, 2009; gill netting, e.g. Diana *et al.*, 2006) and traps (Young *et al.*, 2010; Lynch & Mensinger, 2011) as well as visual estimation using SCUBA, remotely operated vehicles, snorkelling transects and underwater cameras (Gutowsky *et al.*, 2011). However, catch rates, selectivity patterns, and species specificity may strongly differ depending on the sampling method chosen. Thus, an accurate characterization of populations can be difficult

(Diana *et al.*, 2006; Lapointe et. al., 2006a). Only methodological comparisons allow the application of correction factors, consequently providing the possibility of intercalibration of density and population structure between methods.

Invasive gobies from the Ponto-Caspian region, especially the rapidly spreading round goby *Neogobius melanostomus* (Pallas 1814), are considered a major threat to the native fish fauna worldwide (Keller *et al.*, 2011; Kornis *et al.*, 2012). An increasing number of *N. melanostomus* invasions has been recorded in the Laurentian Great Lakes watershed (Corkum *et al.*, 2004; Johnson *et al.*, 2005b; Kornis & Vander Zanden, 2010; Pennuto *et al.*, 2010; Bronnenhuber *et al.*, 2011; Brownscombe & Fox, 2012), the Baltic Sea (Sapota & Skóra, 2005; Sokołowska & Fey, 2011), the Gulf of Finland (Ojaveer, 2006), Latvia, Estonia and southern Sweden (Corkum *et al.*, 2004; Björklund & Almqvist, 2010; Almqvist *et al.*, 2010), the Danube River (e.g. Stráňai & Andreji, 2004; Jurajda *et al.*, 2005; Harka & Biro, 2007; Brandner *et al.*, 2013a) and the River Rhine (Borcherding *et al.*, 2011a). Those recent invasions highlight a new quality of potential threats to areas with high endemic aquatic biodiversity (Poos *et al.*, 2010). Fish biologists in North America and Europe have gathered substantial information on densities and other population characteristics of *N. melanostomus* for two decades applying different sampling methods (reviewed by Kornis *et al.*, 2012). However, integration of these data from different regions and habitats is currently hampered by a lack of method intercalibration. Consequently, a precise and accurate estimation of densities and the determination of important demographic indicators (e.g. body size or sex ratio), which are crucial for population assessment, is still a major challenge in sampling of *N. melanostomus* and other fish species.

To our knowledge, this study for the first time provides an intercalibration of electrofishing, angling and minnow traps on boulder-dominated bank habitats (e.g. rip-rap) for larger (non-wadeable) water bodies. Specifically, we hypothesize that these fishing techniques differ in their effectiveness and selectivity for species, body size and sex. Therefore, catch rates for *N. melanostomus* and other species (bycatch), size and sex ratios, the degree of stomach fullness and diel variation of catch patterns were investigated and compared among sampling techniques.

4.3 Material and Methods

Sampling area

For the methodological comparison, a typical channelized, homogenous rip-rap section of the upper Danube River near the city of Regensburg (Bavaria, Germany), located between the river kilometers 2374.80 (E12°09'22.7", N49°01'01.1") and 2376.75 (E12°08'25.3", N49°01'17.3") was chosen (Fig. 4.1). Here, the upper Danube River drains a catchment area of 35450 km^2 with a mean annual discharge of about 450 $m^3 s^{-1}$ (hydrological data from 1924 to 2006, water gauge Regensburg, LfU Bayern). In this section of the Danube River, *N. melanostomus* was first recorded in Germany in 2004 (Paintner & Seifert, 2006). According to previous investigations from the years 2010 and 2011, rip-rap structures represent the most preferred habitat of invasive *N. melanostomus* in the upper Danube River, and previous screenings of *N. melanostomus* densities revealed (mean ± S.D.) 8 ± 4 *N. melanostomus* m^{-2} (Brandner & Cerwenka, unpubl. data). The sampling stretch, an artificial rip-rap bank without canopy cover and a total bank length of 1.3 km was partitioned into four segments of 320 m, comprising 33 sampling points each, with a distance of 10 meters between the points (Fig. 4.1). The different sampling methods applied were randomly distributed among the sampling points.

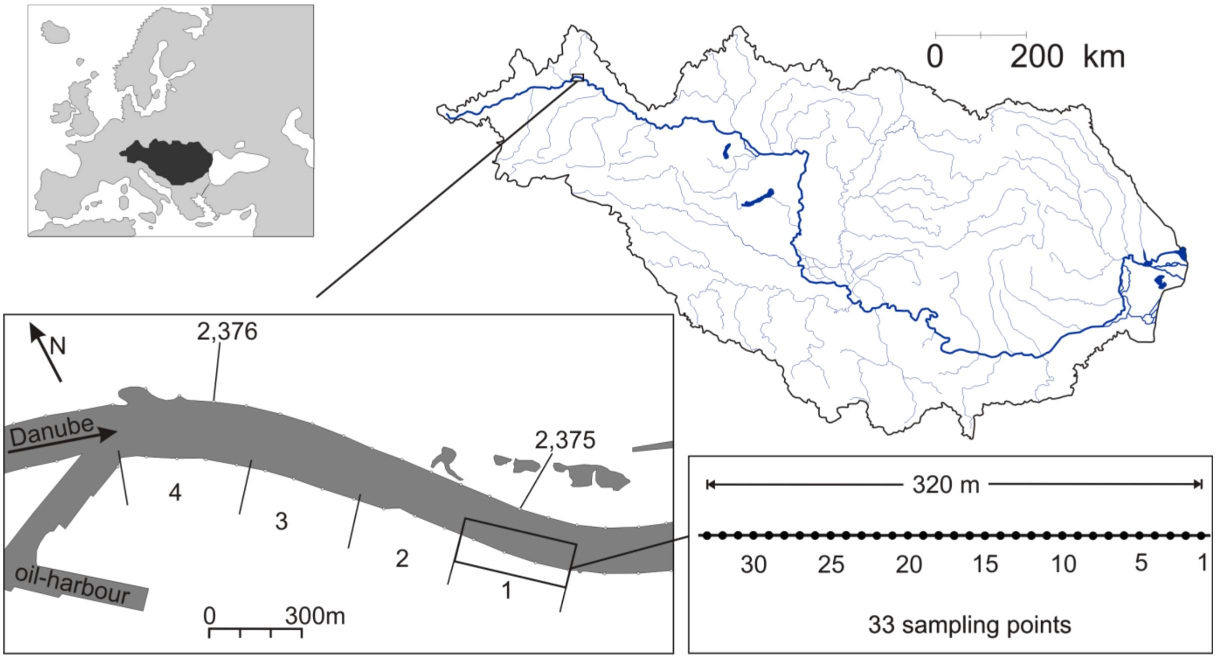

Fig. 4.1: Study area of *N. melanostomus* point-abundance sampling

Neogobius melanostomus study area, partitioned into four segments of 320m each, downstream of the city of Regensburg (Bavaria, Germany) in the upper Danube River. The Danube River basin and the location of the study area within the drainage area are highlighted. River kilometers 2376 and 2375 are marked. Each of the 33 sampling points (distance between points = 10 m), as shown in detail, was randomly assigned to one of the fishing methods. Note that an additional segment 5 (not shown) for the 4 and 8 h minnow trap exposure was located downstream of segment 1.

Physico-chemical conditions were measured using handheld multi 350i and Turb 355T (WTW GmbH) and stayed constant during the study period, with no significant differences between stretches [water temperature: 20.9-21.5 °C, electrical conductivity: 460-470 μS cm^{-1} (at 25 °C), dissolved oxygen: 7.3-8.1 mg/l, O_2-saturation 87-90%, turbidity: 3.7-4.7 NTU].

Sampling techniques

Electrofishing (active sampling technique, sight dependent), angling (active sampling technique, sight independent) and minnow traps (passive sampling technique, sight independent) are effective and commonly applied catch methods to assess *N. melanostomus* densities (Gutowsky & Fox, 2011; Kornis *et al.*, 2012) and were thus chosen for the method comparison. Four different sampling techniques (electrofishing, two variations of angling, and minnow traps) were applied in this study. Each sampling replicate for each technique comprised a 20-minute time interval.

As a standardised electrofishing technique, a multiple-point, fractional sampling strategy (point abundance sampling, PAS) developed by Nelva *et al.* (1979) was applied. PAS electrofishing considers the spatial structure of the population or the fish assemblage using numerous small samples instead of one or a few large samples and thus yields a high number of independent samples (Zalewski, 1985; Persat & Copp, 1990; Reynolds, 1992; Scholten, 2003; Lapointe *et al.*, 2006b). PAS electrofishing was performed according to Persat & Copp (1990) with duration of 10 s electrical current flow (Scholten, 2003). The activated ring-shaped anode without a net (diameter 31 cm), powered by a stationary 8 kW (continuous DC) electrofishing generator (FEG 8000; EFKO GmbH), was submerged into the water 20 cm above the ground for 2 s and then slowly lifted up to the surface for the remaining 8 s. Stunned fishes were caught using a round dip net (diameter of 48 cm, mesh size 5 mm) by a second person. A set of five PAS points was sampled within the 20 min fishing period.

The standardised angling gear consisted of a lightweight match-rod (Top Class 2, 4-m length, Neue Deutsche Angelgeräte Manufaktur int. GmbH (D·A·M) with a 10 g pencil shaped sinker attached to the end of the main line (monofilament, diameter 0.18 mm) equipped with one single barbless loop hook (TIEMCO fly hooks) mounted 10 cm above the sinker, fixed to the main line by a palomar knot. Analogously to Gutowsky *et al.* (2011), two variations of this angling method were applied using two different hook sizes, one with a size #16 hook ('TMC 900'; shank length 6 mm, throat 4 mm, gape width 3 mm) and the other with a bigger size #12 hook ('TMC 100'; shank length 6 mm, throat 5 mm, gape width 5 mm). Owing to the expected different size selectivity according to the hook size, the two variations of the angling method are subsequently referred to as two separate techniques. The rig with hook size #12 was baited with three live maggots, the hook size #16 was baited with one live maggot. The sinker was placed on the ground directly below the rod tip in 60 to 80 cm depth. According to previous studies investigating *N. melanostomus* abundance and population characteristics (Gutowsky & Fox, 2011; Brownscombe & Fox, 2012) each fishing period was 20 minutes.

Minnow traps (GEE´s improved wire minnow trap, 4 mm square metal mesh, 2.5 cm diameter entrance hole; tackle factory), baited with 30 g of cat-food (Sheba® Salmon taste, Mars GmbH) filled into a short nylon-stocking, were used according to Diana *et al.* (2006) and Young *et al.* (2010). Three minnow traps were placed at a depth of 60-80 cm with a distance of about 2 m between the single traps for each replicate and were emptied after each 20 minute fishing period. Three additional minnow trap triplets allocated to an additional segment five downstream the starting point of segment one were exposed four and eight hours to test if an elongated exposure time increases the total catch.

Captured invasive gobies were euthanized using an overdose of anaesthetic and were removed from the Danube River following federal fishing laws. All native fishes were returned to the river after sampling. All persons participating in the experiment had to familiarise with the fishing techniques during a separate training day one week prior to the study, in order to minimize potential learning effects.

Sampling schedule

To ensure comparability of the four sampling techniques, a strict sampling schedule was developed (Table 4.1). This design was chosen to allow the assessment of reproducibility and to test for the effect of daytime on catch rates and population characteristics. Fishes were sampled on 2nd and 3rd July 2012 from a total of 264 sampling points, randomly assigned to all methods. On each sampling day, two replicate sampling intervals were applied with sampling of segments one and two in the morning (t_1 - t_6) and segments three and four in the afternoon (t_7 - t_{12}). All sampling methods were carried out simultaneously by eleven persons, comprising four anglers, four electrofishers, and three trap fishers (Table 4.1). At each segment, 15 points were sampled by electrofishing, six points were sampled by angling with hook size #16 (baited with one maggot), six points were sampled by angling with hook size #12 (baited with three maggots) and six points were sampled by exposing three minnow traps per point. Two segments were sampled simultaneously, with the electrofishing generator being moved between segments after each set of five PAS points (Table 4.1). All sampling points were assigned randomly to the different techniques.

Table 4.1: Sampling schedule

All sampling points were assigned randomly. Segment 5 was located downstream of segment one and comprised five additional sampling points for the 4 and 8 h exposure of MT.
t_1 to t_{12}, replicated fishing periods with duration of 20 min each; A1, angler 1; A2, angler 2; A3, angler 3; A4, angler 4; E1, electrofisher 1 (anode); E2, electrofisher 2 (dip-net); E3, electrofisher 3 (anode); E4, electrofisher 4 (dip-net); T1, trap fisher 1; T2, trap fisher 2; T 3, trap fisher 3; #16, hook of the size #16 (baited with one life maggot) applied at one sampling point; #12, hook of the size #12 (baited with three life maggots) applied at one sampling point; 5 PAS, five point abundance samples at single sampling points; 3 MT, three minnow traps (baited with cat food) at one sampling point; 6 MT, six minnow traps at two sampling points (three traps each point).

		Segment 1 / 3					**Segment 2 / 4**					**Segment 5**	
	Timeline	A1	A2	E1	E2	T1	A3	A4	E3	E4	T2	T3	
Morning	t_1 (9:00-9:20)	#16	#16	5 PAS		3 MT	#16	#16	-		3 MT	6 MT (4h)	3 MT (8h)
	t_2 (9:30-9:50)	#12	#12	-		3 MT	#12	#12	5 PAS		3 MT		
	t_3 (10:00-10:20)	#16	#16	5 PAS		3 MT	#16	#16	-		3 MT		
	t_4 (10:30-10:50)	#12	#12	-		3 MT	#12	#12	5 PAS		3 MT		
	t_5 (11:00-11:20)	#16	#16	5 PAS		3 MT	#16	#16	-		3 MT		
	t_6 (11:30-11:50)	#12	#12	-		3 MT	#12	#12	5 PAS		3 MT		
Afternoon	t_7 (13:00-13:20	#16	#16	5 PAS		3 MT	#16	#16	-		3 MT	6 MT (4h)	
	t_8 (13:30-13:50)	#12	#12	-		3 MT	#12	#12	5 PAS		3 MT		
	t_9 (14:00-14:20)	#16	#16	5 PAS		3 MT	#16	#16	-		3 MT		
	t_{10} (14:30-14:50)	#12	#12	-		3 MT	#12	#12	5 PAS		3 MT		
	t_{11} (15:00-15:20)	#16	#16	5 PAS		3 MT	#16	#16	-		3 MT		
	t_{12} (15:30-15:50)	#12	#12	-		3 MT	#12	#12	5 PAS		3 MT		

Sampling of the four segments was repeated on the second sampling day with sampling points newly randomized, resulting in a total of 120 point abundance samples, 48 angling samples using hook #16, 48 angling samples using hook #12 and 144 minnow trap samples.

Data analysis

Data were immediately recorded after specimen catches by a separate documentation team. All fishes were determined to species level and counted. Their total length (L_T to nearest mm) and total body mass (M_T to the nearest 0.2 g) were measured. Sex of *N. melanostomus* was determined by an examination of the morphology of the urogenital papilla (Miller, 1984; Kornis *et al.*, 2012). The effectiveness of each method was expressed as the catch per unit effort [CPUE (min^{-1}), Nett *et al.* (2012)]. Fulton's condition factor K was calculated as $K = 100\ M_T\ L_T^{-3}$ (Anderson & Neumann, 1996). Size-class distributions (10 mm size classes) of all individuals and of females and males (pooled from all sampling points) were compared qualitatively among gears using histograms.

The index of stomach fullness (I_{SF}) was calculated as $I_{SF} = 100\ M_g\ M_T^{-1}$, where M_g is the gut content mass (Brandner *et al.*, 2013a) to assess possible effects of nutritional status on the effectiveness of different fishing methods. Therefore, a minimum of 15 male and 15 female *N. melanostomus* were collected at the first sampling day from the same time-point for electrofishing and angling. As catch numbers from the minnow traps were low, only 14 female and 15 male fish from both sampling days were available from this method. Fish were euthanized using a lethal dose of anaesthetic, snap-frozen on dry ice to avoid degradation of gut contents and stored at -18 °C until analysis.

Statistical analyses

Data were tested for normality using the Shapiro-Wilk test and homogeneity of variance was tested using Levene test. Differences in mean L_T, M_T, K (not normally distributed) of *N. melanostomus* between sampling methods were compared using Kruskal-Wallis test, followed by a post-hoc Mann-Whitney U-test (Bonferroni corrected). Within sampling techniques, differences between male and female fish in L_T, M_T and K were analyzed using the Mann-Whitney U-test. For sex-ratio analyses, differences in catch numbers between and within sampling methods were examined using chi-square tests (Wilson & Hardy, 2002). Differences in the I_{SF} (normally distributed data) between fishing methods were analyzed using one-way ANOVA followed by a Tukey post hoc test (Bonferroni corrected) for multiple comparisons. Differences in the I_{SF} between males and females within techniques were compared using t-tests. To test for possible differences in CPUE according to the time of sampling, catch data were plotted against sampling time and visually analyzed for each of the applied sampling techniques. Additionally, morning catch data from segments one and two (t_1-t_6) and afternoon catch data from segments three and four (t_7-t_{12}) of both days were compared using the Kruskal-Wallis test, followed by a post-hoc Mann-Whitney U-test (Bonferroni-corrected). Significance was accepted at $P \leq 0.05$ for all statistical tests. Statistical analyses and plots were computed using Statistica (version 6.1, StatSoft Inc.) and PAST (Hammer *et al.*, 2001).

4.4 Results

Fish community

A total number of 17 fish species comprising 1995 specimens was recorded all gears combined. *Neogobius melanostomus* represented 88% (n = 1757) of all specimens with a biomass of 57 (17.5 kg) of the total catch. The sex ratio of *N. melanostomus* catch ($n_{♀}$ = 1008, $n_{♂}$ = 739) was female dominated with 58% females and 42% males. Other invasive Ponto-Caspian gobies such as the bighead goby *Ponticola kessleri* (Günther, 1861) and the tubenose goby *Proterorhinus semilunaris* (Pallas, 1814) were caught in low abundance (less than 1% of total catch). Three specimens of the racer goby *Babka gymnotrachelus* (Kessler, 1857) were caught. This recently invading goby species had been first recorded in Germany in the upper Danube River about three river kilometers below the sampling stretch in 2011

(Haertl *et al.*, 2012). Bleak *Alburnus alburnus* (L. 1758), European perch *Perca fluviatilis* (L. 1758) and roach *Rutilus rutilus* (L. 1758) were the most abundant native fish species, comprising about 8.5% of all individuals. Furthermore, the bycatch comprised single individuals of chub *Squalius cephalus* (L. 1758), common nase *Chondrostoma nasus* (L. 1758), ide *Leuciscus idus* (L. 1758), asp *Leuciscus aspius* (L. 1758), European eel *Anguilla anguilla* (L. 1758), burbot *Lota lota* (L. 1758) and Wels catfish *Silurus glanis* (L. 1758).

Efficiency and specificity

Compared to the two angling techniques and the minnow traps, electrofishing was significantly (Kruskal-Wallis test, $P < 0.001$) the most effective approach to catch *N. melanostomus* with a mean CPUE of 57 *N. melanostomus* min^{-1} (Table 4.2, Fig. 4.2).

Table 4.2: Efficiency and selectivity of the fishing gears tested

Efficiency and selectivity [number of samples (n), mean ± S.E.) of point-abundance electrofishing (five point-abundance samplings (PAS) fished within each sampling period of 20 min with a duration of 10 s (pooled to one sample); 120 samples] angling (48 sampling periods of 20 min each) with two different rigs (hook sizes #12 and #16) and minnow traps (fished as triplets, 48 sampling periods with duration 20 min each) in a rip-rap shoreline of a large river (upper Danube River). Superscript letters denote significant differences between gears used, values highlighted in bold denote significant differences between females and males within gears. CPUE denotes catch per unit effort.

	electrofishing			angling (hook #12)			angling (hook #16)		minnow traps		
	n	Mean	S.E.	n	Mean	S.E.	Mean	S.E.	n	Mean	S.E.
N. melanostomus											
Total catch [n]	120	1135		48	371		204		144	34	
CPUE [min^{-1}]	120	56.750^{a}	3·930	48	0.387^{b}	0.062	0.213^{b}	0.029	144	0.012^{c}	0.004
CPUE$_{females}$ [min^{-1}]	120	**34.150**^{a}	3·620	48	0.195^{b}	0.238	0.114^{b}	0.014	144	0.006^{c}	0.002
CPUE$_{males}$ [min^{-1}]	120	**22.100**^{a}	2.750	48	0.189^{b}	0.031	0.099^{b}	0.020	144	0.006^{c}	0.003
Frequency of occurrence	120	97.5		48	81.2		85.4		144	11.1	
Biomass [%]	120	76.8^{a}	2.7	48	85.1^{b}	4.4	89.0^{c}	3.6	144	72.0^{a}	11.1
By-catch											
Species detected [n]	120	16		48	6		4		48	3	
Species detected [%]	120	94.1		48	35.3		23.5		48	17.6	
CPUE [min^{-1}]	120	9.250^{a}	1.260	48	0.025^{b}	0.006	0.021^{b}	0.007	48	0.002^{b}	0.001
By-catch : *N.melanostomus*		0.16			0.07		0.10			0.20	
Frequency of occurrence	120	52.5		48	33.3		25.0		48	3.5	
Biomass [%]	120	23.2^{a}	2.7	48	14.9^{b}	4.4	11.0^{c}	3.6	48	28.0^{b}	11.1

CPUE of PAS-electrofishing was 445-fold higher than for angling with hook size #16, 370-fold higher than for angling with hook size #12 and 3857-fold higher than per minnow trap exposed for 20 min (Kruskal-Wallis test, $P < 0.001$). Minnow traps exposed for 4 and 8 h yielded the lowest mean CPUEs of 0.004 *N. melanostomus* min^{-1} and 0.001 *N. melanostomus* min^{-1}, respectively.

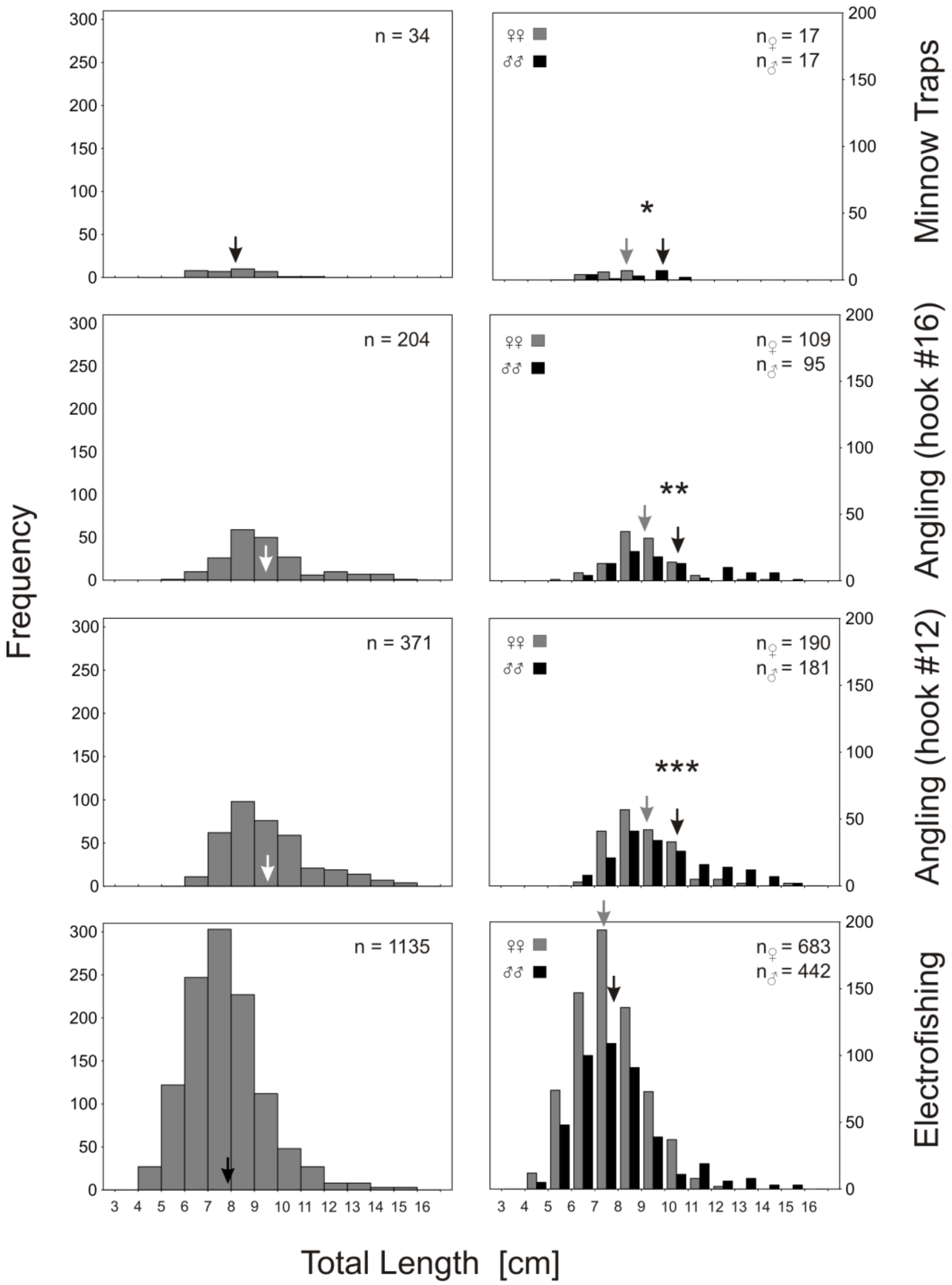

Fig. 4.2: Size-frequency distributions of *N. melanostomus* populations

Size-frequency distributions (1-cm size classes) of *N. melanostomus* populations sampled with electrofishing, angling (hook sizes #12 and #16) and minnow traps from a rip-rap bank of a non-wadeable large river. Histograms for all individuals (left row) and sex-specific histograms for mature *N. melanostomus* (right row) with females (grey bars) and males (black bars) were calculated from fish sampled on 2nd and 3rd of July 2012. Arrows in the left row indicate the mean L_T of the whole populations. Significant differences (Kruskal-Wallis test, $P < 0.001$; *post hoc:* Mann-Whitney U-test, Bonferroni-corrected, $P < 0.001$) between methods applied are indicated by the colour of the arrow. Arrows in the sex-specific histograms indicate the mean L_T for females (grey columns) and males (black columns), significant differences (Mann-Whitney U-test) are marked by asterisks.

Also, the number of species was higher in electrofishing than for all other methods (Table 4.2). The highest specificity for *N. melanostomus* (proportion of *N. melanostomus* to bycatch) was found for angling, with hook size # 12 capturing most *N. melanostomus* in relation to bycatch with a proportion of 1:0.07, followed by angling with hook size #16 (1:0.10) and electrofishing (1:0.16). Minnow traps caught *N. melanostomus* and *P. fluviatilis* in a proportion of 1:0.20, as well as a single specimen of *B. gymnotrachelus*.

A similar pattern of highest efficiency in PAS electrofishing was also evident from frequency-of-occurrence data. *Neogobius melanostomus* frequency-of-occurrence was 97.5% for electrofishing (120 PAS sites), 81.2% for angling with hook size #12 (48 sites), 85.4% for angling with hook size #16 (48 sites) and 11.1% for minnow-traps (48 sites, 144 traps). Bycatch frequency-of-occurrence was 52.5% for electrofishing, 33.3% for angling with hook size #12, 25.0% for angling with hook size #16 and 3.5% for minnow-traps.

Mean CPUE of morning (segments one and two) and afternoon (segments three and four) sampling did not differ significantly for both days and any sampling technique (Kruskal-Wallis tests, $P > 0.24$). Also, across daytime, no visually detectable temporal trend in the CPUE plots was found (data not shown).

Selectivity in L_T, M_T, K and I_{SF}

Both in males and females, significant (Kruskal-Wallis test, $P < 0.001$) differences between the applied sampling methods were detected in L_T (Table 4.3). In males, the mean L_T for angling was about 20% higher (both hook sizes: Mann-Whitney U test, $P < 0.001$) than for electrofishing and 14% higher for both hook sizes (however, not significantly) than for minnow traps. In males, no difference in L_T between electrofishing and minnow traps was observed. Also, for female round gobies, mean L_T did not differ between electrofishing and minnow traps but was about 15% higher for angling (both hook sizes: Mann-Whitney U, $P < 0.001$) than for minnow traps. Females caught by angling were about 20% larger (both hook sizes: Mann-Whitney U test, $P < 0.001$) compared with electrofishing. No significant differences in L_T were observed between both angling methods. In all techniques except for electrofishing, significant (Kruskal-Wallis test, $P < 0.001$) differences in L_T between male and female round gobies were found (Table 4.3). In angling, males were about 10% larger than females for hook size #16 (Mann-Whitney U, $P < 0.01$) and hook size #12 (Mann-Whitney U, $P < 0.001$). Similar results were observed for round gobies caught by minnow traps, where males were significantly (Mann-Whitney U test, $P < 0.05$) larger (12%) than females.

Table 4.3: Population parameters of *N. melanostomus* point-abundance sampling

Population variables of *N. melanostomus* point-abundance sampling (PAS; 20-min efforts), using electrofishing (five PAS with duration of 10 s) angling (two different gears) and minnow-traps (three minnow traps) along a rip-rap bank of a large river. The overall sex ratio (female : male) was calculated from the total catch numbers, the mean proportions of females and males were calculated from the 20-min samples. The Kruskal-Wallis P-values (differences between methods) are given [***P < 0.001; NS, non-significant (P > 0.05)]. Superscript letters denote significant differences (Mann-Whitney U-tests, Bonferroni-corrected) between the applied methods, values highlighted in bold denote significant differences (Mann-Whitney U-tests) between sexes within parameters of applied methods. Abbreviations denote total length (L_T), total mass (M_T), condition factor (K), index of stomach fullness (I_{SF}).

		PAS-electrofishing			angling (hook #12)			angling (hook #16)			minnow traps		
	P	n	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.
$L_{T\ females}$ [cm]	***	683	7.7^{a}	1.5	190	**9.2**^{b}	1.4	109	**9.1**^{b}	1.3	17	**7.7**^{a}	0.9
$L_{T\ males}$ [cm]	***	442	7.9^{a}	1·9	181	**10.0**^{b}	2.1	95	**10.1**^{b}	2.2	17	**8.7**^{c}	1.5
$M_{T\ females}$ [g]	***	683	7.4^{a}	4.8	190	**12.4**^{b}	7.3	109	**11.6**^{b}	5.6	17	**6.6**^{a}	2.5
$M_{T\ males}$ [g]	***	442	8.7^{a}	8.2	181	**16.5**^{b}	10.9	95	**17.6**^{b}	13.0	17	**9.9**^{c}	5.2
$K_{females}$	***	683	1.41^{a}	0.19	190	1.46^{b}	0.19	109	1.46^{b}	0.17	17	1.39^{a}	0.22
K_{males}	***	442	1.40^{a}	0.17	181	1.45^{b}	0.20	95	1.48^{b}	0.17	17	1.34^{c}	0.20
$I_{SF\ females}$	n.s.	16	2.74	0.91	15	2·62	0.93				14	2.64	0.94
$I_{SF\ males}$	n.s.	18	2.71	1.36	15	3.05	0.74				15	2.37	0.98
Overall sex ratio females : males		1125	**1:0.65**		371	**1:0.97**		204	**1:0.87**		34	1:1.00	
Females [%] (sample-based)		24	**61.5**	8.4	48	50.9	24.7	48	58.4	30.9	48	62.9	42.8
Males [%] (sample-based)		24	**37.4**	8.4	48	49.1	24.7	48	41.6	30.9	48	37.1	42.8

Mean M_T for male and female gobies was significantly (Mann-Whitney U test, $P < 0.001$) higher for both angling methods compared to electrofishing (1.8 to 2.0-fold). The difference in mean M_T between both angling methods and minnow traps (1.7 to 1.8-fold) was only significant (Mann-Whitney U test, $P < 0.001$) in females. No significant difference in M_T was observed between electrofishing and minnow traps (Table 4.3). Within each sampling method, significant (Kruskal-Wallis test, $P < 0.001$) differences in M_T between male and female gobies were observed for angling and minnow traps. For angling with hook size #12, mean M_T in males was significantly (Mann-Whitney U test, $P < 0.001$) higher (25%) than in females. Similarly, mean M_T in males was 33% higher (Mann-Whitney U test, $P < 0.01$) than in females for angling with hook size #16. The same result was found for minnow traps, with males being 33% heavier (Mann-Whitney U test, $P < 0.05$) than females. No significant difference in M_T between males and females was found for electrofishing.

In addition to higher L_T and M_T of angled gobies, angling also revealed a significantly (Kruskal-Wallis test, $P < 0.001$) higher K than electrofishing (Table 4.3). Mean K for male and female gobies was significantly (Mann-Whitney U test, $P < 0.01$) higher for angling with hook size #12 compared with electrofishing. Mean K was also higher for angling with hook size #16 compared with electrofishing in males (Mann-Whitney U test, $P < 0.001$) and females

(Mann-Whitney U test, $P < 0.01$). Between minnow traps and angling, the only significant difference in K was found for males and angling with hook size #16. Sex-specific differences in K were not significant within all methods applied.

No significant differences in the I_{SF} were observed for round gobies neither between the applied sampling methods (electrofishing: $I_{SF} = 2.7 \pm 1.1$ (S.D.); angling: $I_{SF} = 2.8 \pm 0.8$ (S.D.); minnow traps: $I_{SF} = 2.4 \pm 0.9$ (S.D.)) nor between males and females within the applied methods.

Sex ratio

Sex ratio (f : m) of round gobies differed between sampling methods with electrofishing (1 : 0.65) > angling (1 : 0.92) > minnow traps (1 : 1). Electrofishing yielded a higher proportion of females than angling with hook size #16 (χ^2 test, $P < 0.05$) and hook size #12 (χ^2 test, $P < 0.01$). No significant differences in sex ratio were detected between minnow traps and electrofishing as well as between minnow traps and both angling techniques (Table 4.3).

Within sampling techniques, sex ratios differed significantly from a 1 : 1 (f : m) ratio for electrofishing (χ^2 test, $P < 0.001$) and both angling techniques (hook #12: χ^2 test, $P < 0.001$, hook #16: χ^2 test, $P < 0.001$), with electrofishing yielding 1.64-fold more females than males. Angling with hook size #12 revealed 1.04-fold more females than males and angling with hook size #16 1.40-fold more females than males. No P-values are available for the comparison of minnow traps due to the high number of zero samples resulting from the low catch efficiency.

4.5 Discussion

N. melanostomus is suspected to cause serious and sustained changes of ecosystems by affecting native benthic invertebrate and fish communities (Jude *et al.*, 1992; Ricciardi, 2001; Vanderploeg *et al.*, 2002; Barton *et al.*, 2005; Jurajda *et al.*, 2005; Lederer *et al.*, 2006; Johnson *et al.*, 2008; Pennuto *et al.*, 2010; Kornis *et al.*, 2012). Consequently, effective techniques for sampling *N. melanostomus* have to be identified for the collection (Nett *et al.*, 2012) and interpretation of valid fish-population data. In large rivers, which seem to comprise one of the most important invasion pathways (Leuven *et al.*, 2009) and permanent habitats of invasive gobiids (Erös *et al.*, 2008), the relative efficiency of many sampling methods is largely unknown. In European rivers, artificial rip-rap structures comprise a large proportion of bank habitats (Schiemer *et al.*, 1991; Bram *et al.*, 2004) and seem to be a preferred habitat of *N. melanostomus* (Brownscombe & Fox, 2012). Rip-rap structures, especially in non-wadeable rivers, are difficult to sample (Casselman *et al.*, 1990; Lapointe *et al.*, 2006a). Herein, four commonly applied fishing techniques were investigated for their effectiveness on an artificial rip-rap bank habitat.

The present study identified PAS electrofishing as the most suitable method to comprehensively assess fish communities in rip-rap bank habitats of a large river, resulting in the largest body size spectrum and the highest number of species compared to all other techniques. Additionally, this method had the highest effectiveness to detect round gobies on the distinctive sampling sites, with a probability of 97.5%. This is in contrast to the results of Nett *et al.* (2012), who found a CPUE 400-fold lower (mean ± S.E. = 0.137 ± 0.043 round gob *N. melanostomus* min^{-1}) for electrofishing in wadeable streams. This discrepancy can result from different electroshocking gears (pulsed vs. continuous DC; different power output) and methods used (stretch vs. PAS). Additionally, different physico-chemical conditions such as electric conductance and water temperature can play an important role for the effective fishing range and thus the CPUE (Reynolds, 1992; Scholten, 2003). Since minnow traps provided nearly identical results in the study by Nett *et al.* (2012) and our study, and since Young *et al.* (2010) proposes that electrofishing and minnow traps are probably similar in CPUE, it seems likely that goby densities are underestimated if a pulsed electrofishing gear or other methods than PAS electrofishing are used. The high catch efficiency of PAS electrofishing may result from the vertical performance of the anode and simultaneous use of the dip net to catch all occurring fish. The vertical handling of the anode over the rip-rap leads to an increased emergence of gobies rather than swimming or drifting horizontally inside the rip-rap interstices (Polačik *et al.*, 2008a). Moreover, the disturbance of the site is reduced to a minimum because sampling points are approached by land. PAS electrofishing was also the most reliable method for the assessment of fish population characteristics on rip-rap habitats due to the catch of almost all size classes (Fig. 4.2) and the high variety of bycatch.

An assessment of the sex-selectivity of the different methods is difficult since the real sex ratio of *N. melanostomus* in the Danube River and other large rivers remains unknown. It is likely that the female-dominated population structure observed in the in the large number of specimens collected by PAS electrofishing most accurately mirrors the real sex ratio since this method was also least size- and species selective. This is supported by the findings of both angling techniques which also found a female-dominated population despite of the higher likelihood to catch males because of their greater aggressiveness, particularly during the nest-guarding period (Kornis *et al.*, 2012). The most balanced sex ratio found using minnow traps remains uncertain due to the small total catch with this technique.

Standardized angling yielded the second highest catch numbers of *N. melanostomus*. The efficiency found for angling from this study was similar to the findings (two-person 20-min angling) of Gutowsky *et al.* (2011), who reported a mean ± S.E. CPUE of 5.1 ± 1.2 *N. melanostomus* per 20 min to 12.0 ± 1.4 *N. melanostomus* per 20 min (S.E.) from the Trent River (Ontario, Canada). Unexpectedly, differences in catch efficiency between the two applied angling techniques were not observed. This makes angling results from different studies comparable within the herein applied range of gear specification. Since hook sizes are poorly standardised across manufacturers worldwide, this simplifies the international applicability of angling as a sampling method. Also, angling can be an effective sampling method in a wide range of environmental conditions, including off-shore habitats of deep streams where electrofishing is less effective (Gutowsky *et al.*, 2011; Brownscombe & Fox, 2012). However, for comprehensive population assessments this method is limited, since angling was selective for size and sex, as the catch was dominated by larger males and only *N. melanostomus* larger than 5.6 cm L_T were caught. These findings are in line with the results of Gutowsky *et al.* (2011) from the Trent River (Ontario, Canada) and may be explained by sex-specific behaviour, e.g. nest defence by male gobies. This can result from higher aggressiveness (Kornis *et al.*, 2012) and consequently higher number of bait attacks than for females. Surprisingly, similar to electrofishing the efficiency of angling was independent from the feeding status (starvation or saturation) of the caught gobies, indicating that this is independent from probably naturally occurring feeding times.

Baited minnow traps by far had the lowest efficiency among all methods tested. The high similarity in CPUE between this study and the findings of several other authors (Young *et al.*, 2010: mean ± S.E. maximum CPUE = 7 ± 0.5 *N. melanostomus* 24 h^{-1}, 24h exposure time; Diana *et al.*, 2006: three *N. melanostomus* h^{-1}, 1 h exposure time; Nett *et al.*, 2012: mean ± S.E. CPUE = 0.08 ± 0.02 *N. melanostomus* min^{-1}, 15 min exposure time) indicates that catch numbers are typically low for minnow traps and seem to be largely independent from sampling site and exposure time. This assumption is also confirmed by lab experiments (Johnson *et al.*, 2005b), where minnow traps performed poorly with little potential for sampling round gobies. Since these authors used different baits (Nett *et al.*, 2012: beef liver; Diana *et al.*, 2006: night crawlers; Young *et al.*, 2010: frozen corn) and the actual goby densities remain unknown, no recommendations on the most effective bait can be given so far. The very low catch numbers in this study may result from high visibility in the exposure depth of the traps, which might have prevented gobies from leaving the rip-rap interstices. Analogously to electrofishing and angling, the feeding status did not influence CPUE of minnow traps. Except for the very poor catch efficiency (CPUE) of minnow traps, the population characteristics (L_T, M_T, sex ratio) of this method were more similar to those observed from PAS electrofishing than angling. Due to low CPUE and low frequency-of-occurrence, minnow traps appear unsuitable to identify locations of invaded sites, which is the key first step in managing populations of an invasive species.

Conclusions

This study provides baseline data on intercalibration of different sampling techniques of *N. melanostomus* using relative comparisons. The selection of an adequate fishing method depends on the specific fisheries research questions since methodological comparisons in any large natural waterbodies necessarily depend on such relative comparisons. In case of comprehensively collecting quantitative fish community data, the method with the lowest selectivity (species, size, sex) and the highest efficiency (catch, species) is typically preferred. Low selectivity is particularly crucial for population characterizations in terms of length-frequency or age distribution, sex ratio and analyses of feeding patterns. Consequently, at least for the habitat structure investigated herein, PAS electrofishing is the most suitable sampling technique. Hook-and-line-based methods, despite of their greater size and sex selectivity, are particularly useful when the largest individuals are being targeted and when sampling needs to be carried out in case of lower visibility and greater water depth. However, there may be situations (e.g. tidal marshes, turbid waters, night sampling) when minnow traps can be effective sampling tools involving little cost. Based on the low catch rates of minnow traps, this method appears least suitable for many of the applied scientific research questions and requires further research in methodological standardisation (e.g. optimal bait, exposure time, depth, and inclusion of other habitats).

5. Comparative feeding ecology

A similar version of this chapter was published as:

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5.1 Abstract

Invasions of Ponto-Caspian gobiid fishes are suspected to cause regime shifts in freshwater ecosystems. This study compared the trophic niche differentiation of *Neogobius melanostomus* and *Ponticola kessleri* in the upper Danube River using stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$), gut content analyses and morphometric analyses of the digestive tract. Both species were identified as predacious omnivores with high dietary overlap and a generalist feeding strategy. Amphipods (especially invasive *Dikerogammarus* spp.) contributed 2/3 to the index of food importance. $\delta^{15}N$ signatures of *N. melanostomus* revealed an ontogenetic diet shift and significantly exceeded those in *P. kessleri* by ~1.5 ‰, indicating a niche separation of half a trophic level. *Ponticola kessleri* had shorter uncoiled intestinal tracts than *N. melanostomus*, indicating a narrower niche and adaptation to animal food. Trophic niches in both species expanded during the growth period with increasing intraguild predation and cannibalism in *P. kessleri* and increasing molluscivory in *N. melanostomus*. *Ponticola kessleri* showed a higher degree of specialization and more stable feeding patterns across seasons, whereas *N. melanostomus* adapted its diet according to the natural prey availability. The feeding patterns of both species observed in the upper Danube River strongly differ from those in their native ranges, underlining their great plasticity. Both goby species consumed mainly other non-native species (~92% of gut contents) and seem to benefit from previous invasions of prey species like *Dikerogammarus villosus*. The invasion success of gobies and their prey mirror fundamental ecological changes in large European freshwater ecosystems.

5.2 Introduction

Invasive species are considered one of the major threats to global freshwater biodiversity (Dudgeon *et al.*, 2006; Geist, 2011). Successful invasions of alien species often result in a homogenization of flora and fauna, leading to a "*global biomonotony*" (Mooney & Cleland, 2001; Beisel & Devin, 2007; Moyle & Mount, 2007).

Several invasive Ponto-Caspian gobiid fish species (Teleostei: Gobiidae) have colonized both freshwater and marine ecosystems worldwide with a high potential to cause ecological regime shifts. Goby invasions strongly affect the Laurentian Great Lakes area in North America (Jude *et al.*, 1992; Charlebois *et al.*, 1996; Ricciardi & MacIsaac, 2000; Gutowsky & Fox, 2011; Lynch & Mensinger, 2011) as well as European waterbodies (Corkum *et al.*, 2004; Sapota & Skóra, 2005; Kakareko *et al.*, 2009), including the River Rhine (Borcherding *et al.*, 2011a) and the Danube River (Ahnelt, 1998; Simonović *et al.*, 1998; Stráňai & Andreji, 2004; Jurajda *et al.*, 2005; Harka & Biro, 2007). The Danube River is the second largest river in Europe, with a total length over 2,800 kilometers. In 1992, the Rhine-Main-Danube junction (RMD-canal) connected the formerly separated major drainage systems of the Rhine-Main to the Danube and became one of the most important European shipping routes. Consequently, the Danube River became a part of the so-called Southern Invasive Corridor (Black Sea – Danube – RMD-canal – Main – Rhine – North Sea), one of the most important European long-distance dispersal routes for many aquatic invasive species (Bij de Vaate, 2002; Karatayev *et al.*, 2008; Leuven *et al.*, 2009; Panov *et al.*, 2009).

In the German section of the Danube River, the bighead goby. *Ponticola kessleri* (Günther, 1861), was first recorded in 1999 (Seifert & Hartmann, 2000), followed by an invasion of the round goby, *Neogobius melanostomus* (Pallas, 1814), which arrived in 2004 (Paintner & Seifert, 2006). According to our own observations, invasive gobies can be found in densities of up to 20 individuals per square meter and range expansion is still ongoing.

Both fish species have been suspected to cause serious and lasting changes of ecosystems by affecting native communities (Lodge, 1993b; Ricciardi, 2001; Minchin *et al.*, 2007; Van Riel *et al.*, 2007; Van Kleev *et al.*, 2008). Especially the rapid expansion of *N. melanostomus* has been linked to the decline of native fish diversity and abundance (Crossman *et al.*, 1992; Jude *et al.*, 1992; Freyhof, 2003; Jurajda *et al.*, 2005; Karlson *et al.*, 2007) and to negative population trends in prey species (Vanderploeg *et al.*, 2002; Barton *et al.*, 2005; Lederer *et al.*, 2006; Pennuto *et al.*, 2010). Recently, *N. melanostomus* also started to invade headwater habitats in the Great Lakes watershed of North America (Kornis & Vander Zanden, 2010; Bronnenhuber *et al.*, 2011), highlighting a new quality of potential threats to areas with high endemic aquatic biodiversity (Poos *et al.*, 2010). For understanding both the invasive potential and the ecosystem impact of gobies on recently invaded headwater habitats, a better understanding of their ecological and trophic niche differentiation is crucial. While feeding strategies, food resource utilization and food preferences of *N. melanostomus* and *P. kessleri* are documented for specific distribution areas such as the middle and lower Danube River (Simonović *et al.*, 2001; Borza *et al.*, 2009; Polačik *et al.*, 2009), the Laurentian Great Lakes and their tributaries (Johnson *et al.*, 2005a; Kornis *et al.*, 2012), or the Gulf of Gdansk (Skóra & Rzeznik, 2001; Karlson *et al.*, 2007), the trophic interactions between sympatric invasive gobies and benthic communities remain largely unknown. Stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) have been shown to be powerful markers for middle to long-term feeding pattern and trophic niche assessments (Vander Zanden *et al.*, 1997; Post, 2002; Perga & Gerdeaux, 2005) and can complement gut content analyses which provide information on short-term feeding patterns. To date, no study has combined stable isotope analyses with gut content analyses of invasive gobies in freshwater habitats. Seasonal differences between the feeding habits of both gobies (Borza *et al.*, 2009) suggest an ontogenetic diet shift in *N. melanostomus* nutrition (Campbell *et al.*, 2009) and underline the importance of better understanding the trophic niche separation of gobies and their impacts on aquatic food webs and endemic aquatic biodiversity.

To date, studies on the feeding ecology of gobies, particularly of *P. kessleri*, are limited by only few examined specimens and single sampling timepoints. They thus do not provide a reliable picture (Borza *et al.*, 2009). As most recent studies were focused on specific lotic or marine habitats, there is also limited knowledge on recently invaded (headwater) habitats, i.e. sampled before invasion-induced changes like food resource limitation or potential dietary adaptations occur.

The objectives of this study were to (i) compare the trophic niche differentiation between *N. melanostomus* and *P. kessleri* using a combination of stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$), gut content analyses and morphometric analyses of the digestive tract, (ii) determine food preferences using a comparison of the natural occurrence of benthic invertebrates as prey with gut contents and to (iii) assess the role of invasive vs. native prey species in the invasion success of both goby species, considering seasonal patterns. We hypothesize that the invasion success of both species can be largely explained by unspecific feeding patterns.

5.3 Material and Methods

Fishes and benthic invertebrates were sampled in early summer (March 29th – June 18th) and late summer (August 16th – October 18th) 2010 at ten representatively distributed river stretches along the recently invaded headwater reaches of the Danube River, Germany (Fig. 5.1, Table 3.1).

Table 5.1: Sampling stretches

Ten representatively distributed sampling stretches along the upper Danube River with first recordings of *P. kessleri* (Pk) and *N. melanostomus* (Nm), river kilometers (rkm) and GPS-coordinates (wgs-84) of upper and lower boundaries, sorted in upstream to downstream order at the upper Danube River, [1] own observations, [2] Seifert & Hartmann (2000), [3] Paintner & Seifert (2006), [4] Zauner (pers. com.), u = uncertain first recordings.

Sampling Stretch		First Record	Lower Boundary		Upper Boundary	
№	River Stretch	Pk / Nm	rkm	GPS	rkm	GPS
10	Kelheim	2010[1] / 2010[1]	2409	E 11°56'27" N 48°54'29"	2418	E 11°50'12" N 48°54'01"
09	Bad Abbach	2008[1] / 2009[1]	2393	E 12°00'13" N 48°57'57"	2400	E 12°02'05" N 48°56'03"
08	Regensburg	u / u	2373	E 12°10'41" N 49°00'34"	2377	E 12°08'29" N 49°01'22"
07	Geisling	u / u	2350	E 12°23'37" N 48°58'51"	2354	E 12°21'02" N 48°58'36"
06	Straubing	1999[2] / 2004[3]	2309	E 12°42'26" N 48°53'34"	2317	E 12°36'56" N 48°53'49"
05	Mariaposching	u / u	2292	E 12°52'12" N 48°50'28"	2298	E 12°47'46" N 48°49'33"
04	Deggendorf	u / u	2280	E 12°59'50" N 48°47'31"	2289	E 12°54'26" N 48°50'40"
03	Aichet	u / u	2267	E 13°03'08" N 48°43'37"	2273	E 13°02'15" N 48°44'32"
02	Vilshofen	u / 2004[3]	2250	E 13°10'44" N 48°38'24"	2259	E 13°05'41" N 48°41'02"
01	Engelhartszell	2002[4] / 2003[4]	2196	E 13°46'29" N 48°28'32"	2202	E 13°43'21" N 48°30'48"

Sampling covered a total river length of about 200 km within the early and late phases of one growth period. In order to avoid the introduction of a systematic sampling bias (e.g. due to trends in water temperatures), even and uneven river stretches (first even and then uneven numbers) of the numbered river stretches (Fig. 5.1) were sampled consecutively. A total number of 235 specimens of *P. kessleri* and 283 *N. melanostomus* were collected from shorelines (in ~60 cm water depth) by point abundance electrofishing (ELT62-IID; Grassl GmbH, Berchtesgaden, Germany).

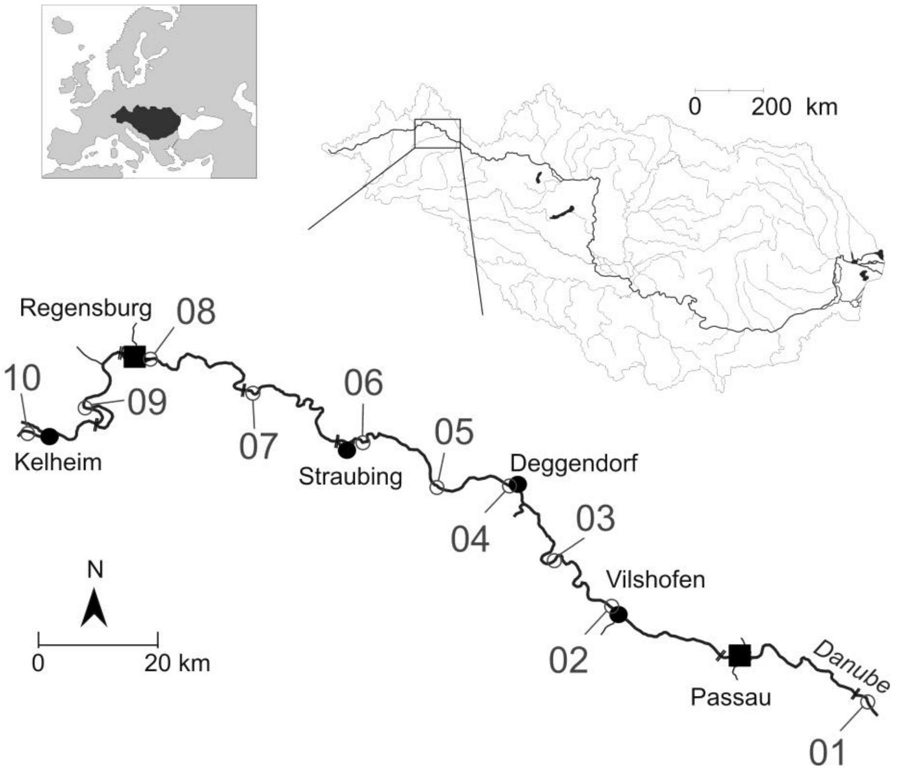

Fig. 5.1: Study area of the comparative feeding ecology study

Study area with ten sampling stretches covering the goby invasion front along the headwater reaches of the Danube River. European context and location within the drainage area of the Danube River are highlighted.

Several recent studies on neogobiids did not consider the effects of fish size on feeding habits (e.g. Adámek *et al.*, 2007; Polačik *et al.*, 2009; Borza *et al.*, 2009), whereas many other studies described ontogenetic diet shifts in *N. melanostomus* (French & Jude, 2001; Phillips *et al.*, 2003; Barton *et al.*, 2005; Johnson *et al.*, 2005a; Karlson *et al.*, 2007; Campbell *et al.*, 2009). The known size effects in at least one of the species analyzed were accounted for in two ways: (i) specimens were size-class selected (8 – 12 cm) with mean total lengths (L_T) of 10.0 cm (S.D. = 1.9 cm) for *P. kessleri* and 9.6 cm (S.D. = 1.3 cm) for *N. melanostomus* (see Table 5.2). (ii) To test for this effect in *N. melanostomus* nutrition, an additional sample of 16 specimens (L_T of 2 - 14 cm) was collected at river stretch no. 08_Regensburg (N49°01'01.95"; E12°09'21.09") on October 15th, 2010 (Fig. 5.1, Table 5.1). L_T was measured to the nearest mm, total wet body mass (M_T) was weighted to the nearest 0.2 g and sex was determined by the morphology of the urogenital papilla (Miller, 1984; Marentette *et al.*, 2009). The gobies were sacrificed using a lethal dose of anesthetic and immediately frozen on dry ice to avoid degradation of gut contents and muscle tissue.

Table 5.2: Numbers and performance indicators of *P. kessleri* and *N. melanostomus*

Numbers and performance indicators of *P. kessleri* and *N. melanostomus* from the upper Danube River in early and late summer 2010: Sex, Mean and corresponding standard deviation (S.D.) of total length (L_T), total wet body mass (M_T), index of stomach fullness (I_{SF}) and Fulton´s condition factor (K).

				L_T [cm]		M_T [g]			I_{SF}			K [g/cm^3]	
Species	Season	Sex	n	Mean	S.D.	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.
	Early	f	64	10.3	2.0	14.8	9.4	50	4.7	3.6	49	1.15	0.18
P. kessleri	Summer	m	39	9.7	1.5	12.1	6.1	31	4.0	1.9	31	1.15	0.14
	Late	f	75	10.2	2.1	14.6	9.9	75	3.6	2.0	75	1.17	0.32
	Summer	m	57	9.7	1.7	12.4	8.9	57	4.0	2.4	57	1.11	0.15
			235	**10.0**	**1.9**	**13.7**	**9.1**	**213**	**3.9**	**2.6**	**212**	**1.15**	**0.23**
	Early	f	64	9.3	1.3	12.9	6.2	57	3.6	1.6	57	1.42	0.15
N. melanostomus	Summer	m	80	9.3	1.7	12.6	6.1	67	3.8	1.7	67	1.38	0.11
	Late	f	71	9.7	0.8	13.3	4.0	71	2.8	1.3	71	1.41	0.14
	Summer	m	68	10.0	1.3	15.0	6.0	68	2.8	1.3	68	1.37	0.12
			283	**9.7**	**1.3**	**13.4**	**5.7**	**263**	**3.2**	**1.5**	**263**	**1.39**	**0.13**

To obtain quantitative benthos samples, a suction sampling device was designed, modified from Brooks (1994) and Brown *et al.* (1989). This flow through system, driven by a water pump (18 l/min. 1.0 bar; Barwig, Germany) inside a duct, integrated a 1000 x 500 µm-mesh for filtering benthic organisms. A flexible tube (Ø = 16 mm) with a brush frontend was used to scrub and collect benthic invertebrates from surfaces and interstices. Efficiency was evaluated in laboratory tests, where mean catch rates of 40.2% (S.D. = 6.6%. n = 5. duration = 120 s; substratum Ø = 5 – 8 mm) and 26.4% (S.D. = 8.8%. n = 5. duration = 120 s. substratum Ø = 8 – 16 mm) of the amphipod *Gammarus pulex* (L., 1758) were observed. This suction sampling device allowed standardized sampling including the collection of gastropods and bivalves. Suction samples were collected from the same sites where gobies were sampled (~60 cm water depth, duration = 120 s). Altogether 190 samples (early summer: n = 105, late summer: n = 85) of benthic invertebrates were preserved in 70% ethanol immediately after capture. Benthic invertebrates were identified to the lowest possible taxon considering manageable taxonomical levels (e.g. Chironomidae, Oligochaeta). Due to immaturity and thus poorly developed identification characters, amphipods often could not be determined to species level and thus were counted as “Amphipoda”. Organisms belonging to the same taxon or cumulative category were counted and expressed as catch per unit effort (CPUE [min^{-1}]). The percent volumetric proportion of each taxon within the sample was visually estimated using a stereo microscope. The values were expressed as “visually estimated proportion of volume” [%].

Stable isotope analysis

To obtain markers for middle to long-term feeding pattern and trophic niche assessment, $\delta^{13}C$ and $\delta^{15}N$ stable isotope analyses of goby specimens and of the most abundant prey items were conducted. $\delta^{13}C$ and $\delta^{15}N$ are relative isotope ratios calculated as $(R_{sample}/R_{standard})$–1, where R is the ratio of the heavy and the light isotope and standard is Vienna-PeeDee Belemnite (V-PDB) in the case of carbon and atmospheric N_2 in the case of nitrogen. Pieces of fish flank muscle tissue (about 0.5 – 1.0 cm³) were sampled and defatted with a chloroform - methanol (2:1) solution. Benthic invertebrates were held in tap water for 24 h to empty their guts. Subsequently, samples were snap-frozen using liquid nitrogen and stored at -18 °C until analysis. The additional set of samples with greater length variation was analyzed to test for (i) correlations between L_T and $\delta^{15}N$ signatures and (ii) a diet shift between muscle tissue and gut contents. The $\delta^{15}N$ values of the gut contents were calculated as averages weighted by their “index of food importance” from mean $\delta^{15}N$ signatures of benthic invertebrates collected from the upper Danube River (Table 5.3).

This approach was preferred over the direct determination of the isotopic composition of the gut content because a much larger number of replicated measurements for the different food items could be used, better reflecting the average of each food component than the snapshot found in the gut. Furthermore, assumptions on whether the gut content did still reflect food resources despite digestion or addition of mucus, which may have already changed the isotopic composition of the gut content, can be avoided by using this approach. After ultrasonic cleaning, all samples were oven-dried (40 °C for 48 h) and ground to homogenous powder, using a mixer mill. Samples of 0.3 – 0.4 mg were weighed into tin cups and combusted in an isotope ratio mass spectrometer (Delta plus, Finnigan MAT, MasCom GmbH, Bremen, Germany) interfaced (via ConFlo II, Finnigan MAT, MasCom GmbH, Bremen, Germany) with an elemental analyzer (EA 1108, Carlo Erba, Thermo Fisher SCIENTIFIC, Milan, Italy) and a pyrolysis unit (HT Sauerstoffanalysator, HEKAtech GmbH, Wegberg, Germany). Repeated analyses of a solid internal laboratory standard (bovine horn, run after each ten samples) showed maximum standard deviations of 0.15‰ for $\delta^{15}N$ and 0.15‰ for $\delta^{13}C$ values.

Fish gut analyses

The digestive tract was removed by cutting off the caudal end of the oesophagus (posterior pharyngeal teeth) and the anal aperture. Oesophagus, oesogaster and intestine were separated from other organs and the length of the uncoiled dissected intestinal tract was measured to the nearest mm. Gut contents from the posterior intestine were not analyzed because of progressed digestion process. Therefore, the posterior intestine was cut off at the intestinal-rectal sphincter level following morphological findings of Jaroszewska *et al.* (2008). The gut from the esophagus to the middle intestine termination was weighed to the nearest 0.001 g before and after emptying to obtain the wet mass of gut contents. All food items from digestive tract samples were fixed in ethanol, identified and counted. As several relevant prey taxa in this study occurred in amounts too small for reliable weighing or volumetric measuring by water-displacement, the per cent contribution of all food items to the whole gut content was estimated using a stereo microscope following the procedure by McMahon *et al.* (2005) and Polačik *et al.* (2009). The contributions of individual food items were expressed as "visually estimated proportion of volume [%]". For methodological comparisons of fish stomach content analyses and visual estimation of volumes see also Hynes (1950) and Hyslop (1980). Additionally, we refer to Karlson *et al.* (2007), who showed that dry mass of food components can even be estimated with sufficient accuracy from only the numbers and maximum lengths of items by using conversion factors available from literature. Crushed bivalves and amphipods were reconstructed from the contents of the intestinal mucus hulls whenever possible to gather taxonomically relevant parts of the exoskeletons.

Statistical analyses

According to Herder & Freyhof (2006), the relative importance of a food item i among all items j for a population was calculated as the "*Index of food importance*" (I_{FI}) using visually estimated volumes and counted numbers of food items:

$$I_{FI}(i) = 100\, O(i)\, V(i) \left(\sum_{n=1}^{j} O(i)\, V(i) \right)^{-1}$$

with O = % occurrence of prey i

and V = visually estimated proportion of volume [%] of prey i

I_{FI} varies from 0 to 100, with higher values corresponding to a larger contribution of one food item as compared to total gut content. Since macrobenthos samples were treated like gut content samples, importance of naturally available prey was also calculated following the above mentioned formula as "index of environmental importance" (I_{EI}) for each food item i.

Dietary Overlap (O_D) between *N. melanostomus* and *P. kessleri* was calculated using the "*Schoener-Index*" (Schoener, 1970; see also Herder & Freyhof, 2006):

$$O_{\mathrm{D}} = 1 - \frac{\left(\frac{1}{2} \sum |\mathrm{p_a} - \mathrm{p_b}|\right)}{100}$$

with p_a = percentage of a food item in species a
and p_b = percentage of a food item in species b

O_D ranges from 0 to 1, with 0 meaning total dissimilarity and 1 representing identical gut contents.

The "*Zihler Index*" (Z_I, Zihler 1982) was calculated to assess digestive tract lengths as an indicator for diet adaptations according to Herder & Freyhof (2006):

$$Z_{\mathrm{I}} = \frac{L_{\mathrm{I}}}{10\sqrt[3]{M_{\mathrm{T}}}}$$

with L = length of the uncoiled intestinal tract [mm], M_T = total fish body mass [g]

Z_I was used since it avoids body shape-dependent bias when comparing uncoiled digestive tract lengths. Bibliographic data (Karachile & Stergiou, 2010) displayed a range in Z_I for omnivores with preference to animal material of 1.5 to 12.7 (n = 26, mean Z_I = 3.8, S.E. = 0.5), whereas Z_I of herbivores ranged from 4.5 to 53.6 (n = 5, mean Z_I = 20.3, S.E. = 9.2).

Finally, a "*prey-specific index of food importance*" was calculated (I_P) to obtain a prey preference analysis independent from benthic invertebrate sampling and therefore containing fish as prey. Only guts of specimens of a population in which a specific prey i occurred, were considered:

$$I_{\mathrm{P}}(i) = \frac{1}{\sum I_{\mathrm{FI}}(i)\, n(i)}$$

with $I_P(i)$ = prey-specific index of food importance of prey i
$I_{FI}(i)$ = index of food importance of prey i
n(i) = number of guts containing prey i

The feeding strategies of gobies were then characterized in analogy to Costello´s method (Costello, 1990), modified by Amundsen *et al.* (1996) by plotting I_P of each prey versus its frequency of occurrence, given by its relative proportion n(i)%.

"*Fulton´s condition factor*" (K [g/cm^3]) was calculated according to Anderson & Neumann (1996), subtracting the gut content mass:

$$K = 100 \frac{(M_T - M_g)}{{L_T}^3}$$

with L_T = total length [mm], M_T = total fish body mass [g], M_g = gut content mass [g]

The slope of the regression between length and weight for the selected specimens was 3.0 ($R^2 = 0.941$) for *N. melanostomus* and 3.3 ($R^2 = 0.949$) for *P. kessleri*, indicating completely isometric growth for *N. melanostomus* (Anderson & Neumann, 1996).

To assess food uptake and to test for the effects of the daytime of sampling on feeding behavior, the "*Index of stomach fullness*" (I_{SF}) was calculated following Moku *et al.* (2000) and Tudela & Palomera (1995):

$$I_{SF} = 100 \frac{M_g}{M_T}$$

with M_T = total fish body mass [mm], M_g = gut content mass [g]

Benthic invertebrates and food taxa were classified according to their biogeograpical origin as "indigenous" and "invasive", species too small for taxonomic identification and species with non-allocatable biogeographical origin were classified as "unassigned". The proportions of these three classes were determined for the gut content samples of both goby species and for the benthic invertebrate samples.

For comparisons of mean values between species and seasons, One-Way ANOVA (SIA) or t-tests (Z_I) were used if the criteria for parametric testing were fulfilled. Alternatively, non-parametric Mann-Whitney U-tests or Kruskal-Wallis-tests (Bonferroni corrected) were applied (I_{FI}, I_{EI}, I_{SF}, K, L_T, M_T). Significance was accepted at $\alpha = 0.05$). Statistical analyses and plots were computed using SPSS 11.0 (IBM SPSS Statistics, N.Y., U.S.A.), PAST (Hammer *et al.*, 2001) and Excel 2010 (Microsoft^{TM}).

5.4 Results

Both goby species were present throughout the sampling area except for the most upstream sampling stretch where the first records were made in late summer 2010. The time elapsed since their first recordings (Table 5.1) at downstream river stretches of the sampled river section were up to 6 years for *N. melanostomus* and up to 11 years for *P. kessleri*. Of all fishes captured, both goby species comprised 58% of all specimens in early summer (56% *N. melanostomus*, 2% *P. kessleri*) and 56% in late summer (52% *N. melanostomus,* 4% *P. kessleri*). Other species mainly comprised autochthonous cyprinids and percids, *Anguilla anguilla* (L., 1758) and to some extent *Lota lota* (L., 1758), *Silurus glanis* L., 1758 and *Esox lucius* L., 1758.

Stable isotope analysis

Highest δ^{15}N values of all investigated species were found in *N. melanostomus* with maximum values of 16.2‰ in early summer and 16.4‰ in late summer (Table 5.3).

Table 5.3: Mean δ^{15}N and δ^{13}C values of *N. melanostomus*, *P. kessleri* and their prey

Mean δ^{15}N and δ^{13}C values with corresponding standard deviations and ranges for *N. melanostomus*, *P. kessleri* and important food items (with $^{\#}$ = pooled samples, $n^{\#}$ = size of pooled samples, na = not analyzed) of early and late summer 2010.

Species	**Season**	**System**	**n**	**$n^{\#}$**	**δ^{15}N [‰]**			**δ^{13}C [‰]**		
					Mean	S.D.	Range	Mean	S.D.	Range
N. melanostomus	early summer	Pisces	61		15.07	0.48	13.65 - 16.24	-28.98	1.03	-30.74 to -26.67
	late summer	Pisces	63		15.04	0.72	11.97 - 16.43	-28.98	0.94	-30.24 to -26.92
P. kessleri	early summer	Pisces	57		13.78	0.68	11.60 - 15.19	-28.80	0.72	-30.62 to -27.30
	late summer	Pisces	58		13.32	0.90	11.52 - 15.77	-28.58	0.87	-32.26 to -26.67
Jaera sarsi	late summer	Pericarida	$2^{\#}$	17, 26	12.95	0.85	12.10 - 13.81	-27.92	0.49	-28.41 to -27.44
Leuciscus idus	late summer	Pisces	1		12.33	na	na	-27.45	na	na
Dikerogammarus villosus	early summer	Amphipoda	6		11.55	1.04	10.03 - 12.86	-28.67	0.57	-29.25 to -27.67
Potamopyrgus antipodarum	late summer	Gastropoda	$2^{\#}$	19, 22	11.44	0.01	11.43 - 11.46	-17.59	0.11	-17.70 to -17.47
Theodoxus fluviatilis	late summer	Gastropoda	$2^{\#}$	3, 4	11.16	0.66	10.50 - 11.83	-31.16	0.74	-31.90 to -30.42
Dreissena polymorpha	late summer	Bivalvia	$2^{\#}$	3, 5	9.85	0.11	9.74 - 9.96	-31.22	0.54	-31.77 to -30.68
Chelicorophium curvispinum	late summer	Amphipoda	$1^{\#}$	11	9.75	na	na	-29.80	na	na
Corbicula fluminea	late summer	Bivalvia	$2^{\#}$	8, 20	9.74	0.23	9.51 - 9.58	-32.15	0.70	-32.84 to -31.45

The mean $\delta^{15}N$ value in *N. melanostomus* of 15.1‰ significantly (ANOVA, $F_{1,116} = 142.630$, $P < 0.001$) exceeded that in *P. kessleri* in early summer by 1.3‰. A similar pattern was observed at the end of the growth period in late summer, when the mean $\delta^{15}N$ value in *N. melanostomus* (mean = 15.0‰, S.D. = 0.7) significantly (ANOVA, $F_{1,119} = 136.069$, $P < 0.001$) exceeded that in *P. kessleri* (mean = 13.3‰, S.D. = 0.9) by 1.7 ‰. Considering a constant enrichment of ^{15}N by max. 3.4‰ ± 1.0‰ (Post, 2002) and by min. 2.3‰ ± 0.2‰ (McCutchan *et al.*, 2003) per trophic level (i.e. between prey and predator) in aquatic organisms, the significant differences in $\delta^{15}N$ values in both gobies (early summer $\Delta\ \delta^{15}N$ = 1.3‰; late summer $\Delta\ \delta^{15}N$ = 1.7‰) indicated a significantly lower trophic position of about half a trophic level in *P. kessleri* compared to *N. melanostomus*.

$\delta^{15}N$ values in the analyzed benthic invertebrates ranged from 9.7‰ to 13.8‰. Filter feeders like the amphipod *Chelicorophium curvispinum* Sars, 1895 as well as the bivalves *Dreissena polymorpha* Pallas, 1771 and *Corbicula fluminea* (Müller, 1771) had lowest $\delta^{15}N$ values (means ranging from 9.7‰ to 9.9‰). Omnivorous *Dikerogammarus villosus* (Sovinskij, 1894) and the grazing gastropods *Theodoxus fluviatilis* (L., 1758) and *Potamopyrgus antipodarum* J. E. Gray, 1843 had medium level $\delta^{15}N$ values (11.4‰ to 11.6‰). Highest invertebrate $\delta^{15}N$ values (12.9‰ to 14.3‰) were observed in the grazing isopod *J. sarsi* (12.1‰ to 13.8‰).

No differences in $\delta^{13}C$ values were observed in both gobies (means ranging from -8.6‰ to -29.0‰) despite the large range found in benthic invertebrates (Table 5.3). The $\delta^{13}C$ values of most food items ranged from -32‰ to -27‰, except for *P. antipodarum,* which was less depleted (mean ± S.D. = -17.6‰ ± 0.1‰).

The δ15N values of muscle tissue and gut contents of the additional *N. melanostomus* sample set followed similar functions and were strongly dependent on L_T (Fig. 5.2). Both data were highly significantly ($r^2 = 0.82$) described by a parabolic regression with size and a diet-tissue shift of 3.1‰ (S.E. 0.3‰). The residuals of the regression indicated that diet and muscle were predicted equally well without any trend in the residuals (Fig. 5.2, upper panel). The δ15N value of the gut content of *N. melanostomus* changed with L_T during the observed growth-phase. Up to a L_T of 10 cm, δ15N values increased by about 2.5‰ and then decreased again (Fig. 5.2, lower panel). Notably, the mean $\delta^{15}N$ value of the gut content was calculated from the mean $\delta^{15}N$ values of the detected species and thus reflects the change in the composition of the prey species but not an isotopic change within the individual prey species.

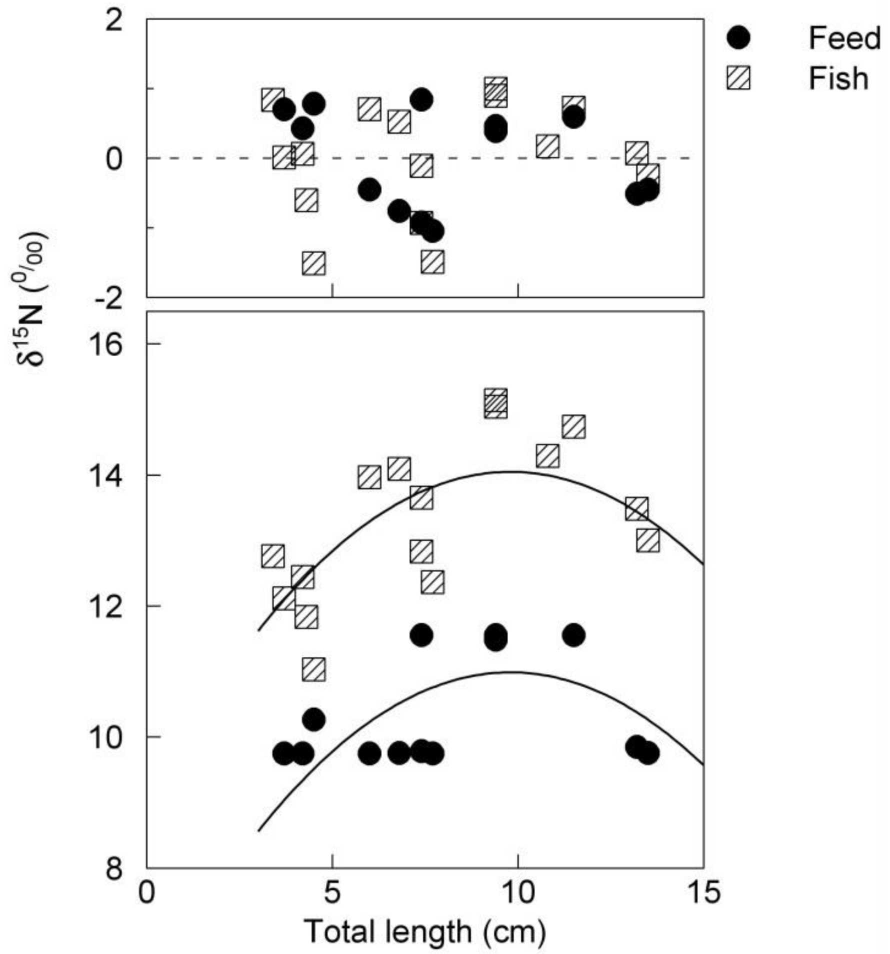

Fig. 5.2: Diet-tissue shift and ontogenetic diet shift in *N. melanostomus*

Diet-tissue shift and ontogenetic diet shift in N. melanostomus (additional sample, n = 16) from sampling stretch no. 08_Regensburg (N49°01'01.95"; E 12°09'21.09"), October 15th, 2010. Lower panel: Change in the relative nitrogen isotope ratio of gut content (calculated, “Feed”, filled circles) and muscle tissue of *N. melanostomus* (measured, “Fish”, squares) in relation to total length; lines are a parabolic regression ($r^2 = 0.82$; $P < 0.001$) based on total length and the type of tissue. The δ15N values of the gut contents (Feed) were calculated as averages weighted by their I_{FI} from mean δ15N signatures of benthic invertebrates collected from the upper Danube River (Table 5.3) Upper panel: residuals of the parabolic regression.

Diet and dietary overlap

Interspecific dietary overlap between both species was high and similar in early ($O_D = 0.66$) and late summer ($O_D = 0.69$). Crustacea were the dominant taxon consumed by *N. melanostomus* (about ⅔ of total) and *P. kessleri* (about ¾ of total) in both parts of the growth period (Fig. 5.3).

Dikerogammarus spp. and invasive Amphipoda represented the most important food items, contributing 73% (early summer) and 79% (late summer) of the total index of food importance in *P. kessleri*, 46% (early summer) and 60% (late summer) in *N. melanostomus*. Importance of Crustacea (v/o amphipods) increased from early to late summer. To a lesser extent, insect larvae (mainly chironomids, but also Ephemeroptera, Plecoptera, Trichoptera (EPT) and other aquatic insects) were consumed by both fish species. Especially in *N.*

melanostomus, consumption of chironomids was high in early summer (33%) and decreased to late summer (5%).

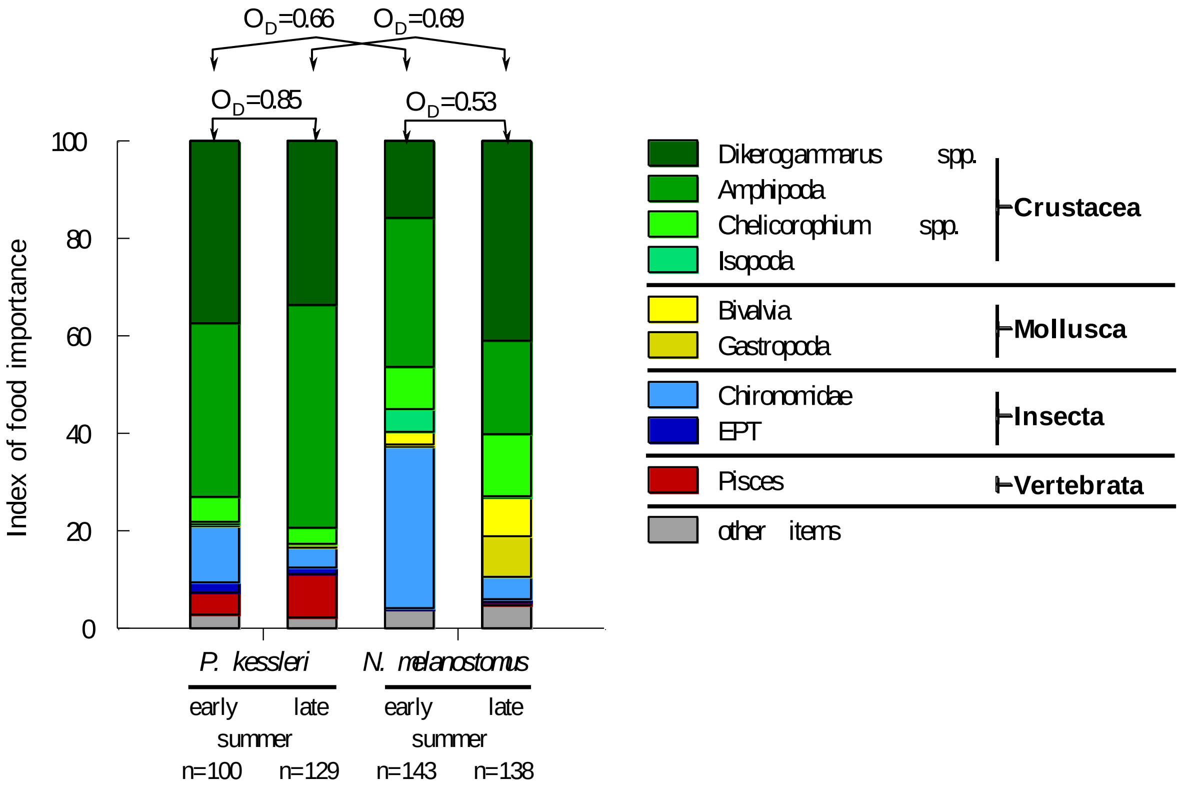

Fig. 5.3: Mean seasonal dietary compositions of *N. melanostomus* and *P. kessleri*

Mean seasonal dietary compositions of *N. melanostomus* and *P. kessleri*, as indicated by the index of food importance. Food items were combined to higher taxonomical groups. Ephemeroptera, Plecoptera and Trichoptera were combined to the group “EPT”; “other items” consisted of debris, detritus, terrestrial insects, leaves and sand. Intra- and interspecific dietary overlaps (O_D) within and between seasons were calculated by the Schoener-Index (Schoener, 1970).

Only six out of 235 (2.1%) *P. kessleri* and two out of 283 (0.9%) *N. melanostomus* specimens had empty guts. Daytime did not affect the index of stomach fullness in *N. melanostomus* (R^2 = 0.014) and *P. kessleri* (R^2 = 0.137). Highest values of the index of stomach fullness were found in *P. kessleri* with maximum values of 21.4 in early summer and 12.5 in late summer. The index of stomach fullness of *P. kessleri* (3.9 ± 2.6) significantly (Mann-Whitney U, $P < 0.05$) exceeded that of *N. melanostomus* (3.2 ± 1.5) in early summer but not in late summer. The intraspecific index of stomach fullness was seasonal in both species and additionally depended on sex in *P. kessleri* (Table 5.2). The index of stomach fullness of *N. melanostomus* in early summer was significantly (Kruskal-Wallis, $P < 0.01$) higher in females (3.6 ± 1.6) and (Kruskal-Wallis, $P < 0.001$) males (3.8 ± 1.7) than in late summer (females: 2.8 ± 1.2, males: 2.8 ± 1.3). *P. kessleri* females revealed significantly (Kruskal-Wallis, $P < 0.05$) higher index of stomach fullness in early summer (4.7 ± 3.6) than in late summer (3.4 ± 2.0), whereas no significant seasonal difference in the index of stomach fullness was found in male *P. kessleri* (early summer: 4.0 ± 1.9, late summer: 4.0 ± 2.4).

There was a distinct intraspecific seasonal shift in dietary composition of *N. melanostomus* (O_D = 0.53), since the index of food importance of molluscs in the diet significantly increased (Mann-Whitney U, $P < 0.001$) by a factor of five from early (3.1%) to late summer (16.2%), with a contrary picture in chironomids, where the index of food importance significantly decreased (Mann-Whitney U, $P < 0.001$) by a factor of seven. In *N. melanostomus*, significant changes between seasons were detected in 10 out of 14 food items (71%) resulting in a low seasonal dietary overlap (O_D = 0.53). In contrast, *P. kessleri* showed only significant changes in 4 out of 14 food items (29%) between seasons leading to a very high seasonal dietary overlap (O_D = 0.85). While intraspecific niche separation in *P. kessleri* remained stable from early to late summer, the trophic niche of *N. melanostomus* increased, as indicated by the decrease in niche overlap (O_D), demonstrating a greater plasticity in this species. The index of food importance of fishes as food items in *P. kessleri* was two-fold higher in late summer (about 10%) than in early summer (about 5%), but this difference was not significant (Mann-Whitney U, $P < 0.7$). Considering only fishes as prey, *P. kessleri* consumed *N. melanostomus* (25%), cyprinids (15%), *P. kessleri* (10%), European Perch (5%) and other fishes (45%). Consumption of Bryozoa was strictly limited to *N. melanostomus* and mysids were only consumed by *P. kessleri*, but contributed less than 2%. *Chelicorophium* spp., isopods, especially *Jaera sarsi (Valkanov, 1938*), zooplankton, oligochaetes and other items (terrestrial insects, debris, detritus, leaves, sand) were consumed by both species in overall low proportions.

Food availability and selection of food items

In early summer, abundance of benthic invertebrates was double the value from late summer (early summer: mean CPUE = 61 min^{-1}, S.D. = 68 min^{-1}; late summer: mean CPUE = 35 min^{-1}, S.D. = 30 min^{-1}; Kruskal-Wallis, $P < 0.01$). *Dikerogammarus* spp. and Amphipoda were dominant in benthic invertebrate samples in both parts of the growth period (Fig. 5.3). Their availability decreased most from early to late summer among all invertebrates (Δ CPUE = 18 min^{-1}; Mann-Whitney U, $P = 0.1$). Also the availability of Chironomidae significantly decreased from early to late summer (Δ CPUE = 13 min^{-1}; Mann-Whitney U, $P < 0.001$). The only significant increases from early to late summer in availability were detected in molluscs (Δ CPUE = 3.0 min^{-1}; Mann-Whitney U, $P < 0.001$) and *Chelicorophium* spp. (Δ CPUE = 7.5 min^{-1}; Mann-Whitney U, $P < 0.001$).

In late summer, feeding on Gastropoda ($\Delta\ I_{FI}$(Gas) = 7.9%; Mann-Whitney U, $P < 0.001$) and Bivalvia ($\Delta\ I_{FI}$(Biv) = 5.3%; Mann-Whitney U, $P < 0.05$) significantly increased in *N. melanostomus* diet, while the increase was not significant in *P. kessleri* diet ($\Delta\ I_{FI}$(Gas) = 0.2%; Mann-Whitney U, $P = 0.8$ and $\Delta\ I_{FI}$(Biv) = 0.2%; Mann-Whitney U, $P = 0.8$). *Chelicorophium* spp. contributed 8.7% in early summer and 12.8% in late summer of *N. melanostomus* diet, but this increase was not significant (Mann-Whitney U, $P = 0.5$). The index of food importance of *Chelicorophium* spp. significantly (Mann-Whitney U, $P < 0.01$) decreased (early summer: 5.1%, late summer: 3.3%) in the diet of *P. kessleri*.

A comparison of the index of environmental importance with the index of food importance revealed seasonal changes of selectivity (preferences: ratio > 1, avoidance: ratio < 1) in both fish species (Fig. 5.4).

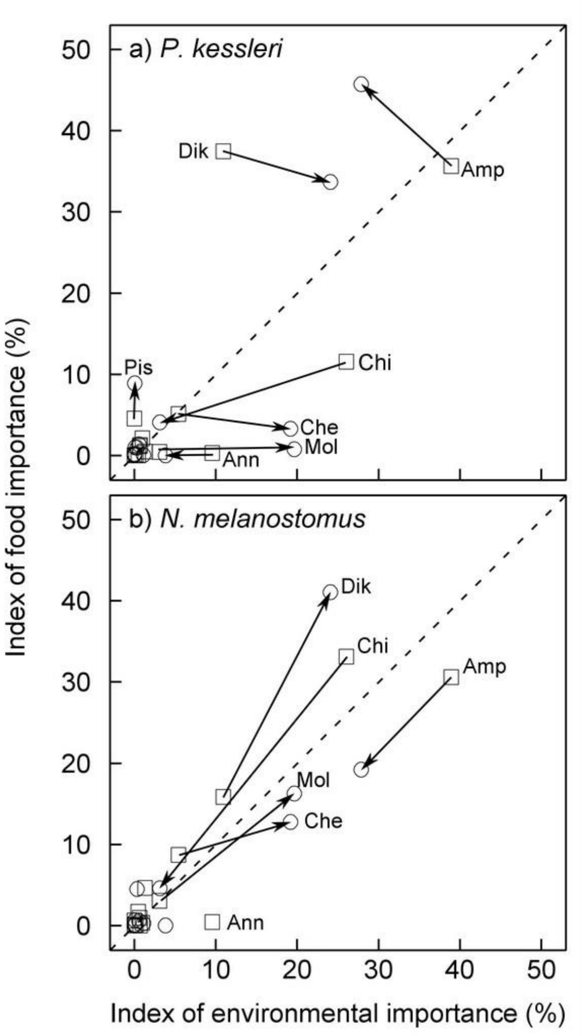

Fig. 5.4: Electivity plot of seasonal preferences in *P. kessleri* and *N. melanostomus*

Electivity plot displaying seasonal preferences of *P. kessleri* (a) and *N. melanostomus* (b) in early summer (squares) and late summer (circles). Points beyond the angle bisector indicate preference (positive selection of food items), whereas points below indicate avoidance (negative selection of food items). Arrows denote the change of important items from early to late summer. Abbreviations: Dik, *Dikerogammarus* spp.; Amp, Amphipoda; Chi, Chironomidae; Mol, Mollusca; Pis, Pisces; Che, *Chelicorophium* spp.; Ann, Annelida.

Even when environmental availability of food changed, *P. kessleri* maintained its dietary composition (as indicated by almost horizontal arrows in Fig. 5.4 a) *P. kessleri* positively selected *Dikerogammarus* spp. both in early and late summer. The index of food importance of Amphipoda increased in *P. kessleri* (Mann-Whitney U, $P = 0.1$) and Amphipoda became the most selected food item in late summer. Consumption of fish doubled from early to late summer. Chironomids were consumed in either season, but were avoided in early summer (Mann-Whitney U, $P < 0.001$). Annelids and molluscs were almost completely refused. Other items were consumed corresponding to their environmental availability.

In contrast, feeding behaviour of *N. melanostomus* was more opportunistic with a seasonal diet shift proportional to the changes in environmental availability as indicated by the arrows in Fig. 5.4 b, which run almost parallel to the diagonal line. Nevertheless, *N. melanostomus* exhibited some selectivity. At the beginning of the growth period, chironomids were an important resource and the most preferred prey, but their index of food importance decreased significantly (Mann-Whitney U Test, $P < 0.001$) in late summer. Low densities in late summer were compensated by significantly increased feeding on *Dikerogammarus* spp. ($\Delta\ I_{FI}$(Dik) = 25.2%; Mann-Whitney U, $P < 0.001$), while Amphipoda were sub-proportionally consumed. *Dikerogammarus* spp. was an important resource and the most selected food item of *N. melanostomus* in late summer, while molluscs and *Chelicorophium* spp. were avoided (Fig. 5.4 b). Annelids were rarely consumed. In spite of increasing importance due to higher availability in late summer, other food items were consumed in proportions mirroring their relative abundance in the environment.

Feeding strategies

The Costello´s plot technique revealed similar generalized feeding strategies and a relatively wide niche with a broad diet spectrum for both investigated goby species (Fig. 5.5). *Dikerogammarus* spp. and other amphipods as prey were of high importance for both fish populations. Most of the individuals of the *N. melanostomus* population utilized many resource types simultaneously with a prey-specific index of food importance almost entirely below 40% (Fig. 5.5 b), whereas different *P. kessleri* individuals specialized on few resource types with prey-specific index of food importance > 50% for *Dikerogammarus* spp., Pisces and Amphipoda (Fig. 5.5 a). Fishes were consumed by 13 out of 100 (13%) *P. kessleri* in early summer and 19 out of 129 (15%) specimens in late summer. Also, the prey-specific index of food importance for fish increased about one third from early to late summer and reached 66% (Fig. 5.5 a).

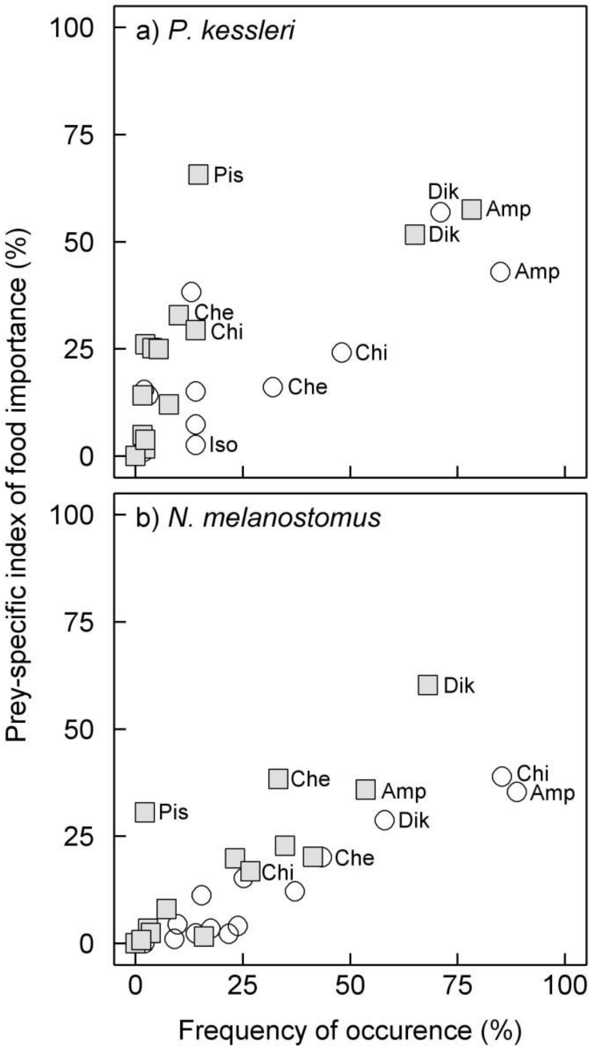

Fig. 5.5: Feeding strategies of *P. kessleri* and *N. melanostomus*

Feeding strategies of *P. kessleri* (a) and *N. melanostomus* (b) in early (filled squares) and late summer (open circles) plotted according to Amundsen *et al.* (1996). The vertical axis represents the feeding strategy in terms of specialization or generalization, where specialization increases with increasing height. The proportion of goby individuals within the population preying upon a food item is explained by the horizontal axis. Thus overall importance of a prey increases from the lower left to the upper right of the diagram. Abbreviations denote major food items: Dik, *Dikerogammarus* spp.; Amp, Amphipoda; Chi, Chironomidae; Pis, Pisces; Che, *Chelicorophium* spp.; Iso, Isopoda.

Invasive species as prey items

Invasive species dominated (I_{FI} > 45%) the diets of *N. melanostomus* and *P. kessleri*. while indigenous ones only played a significantly (Mann-Whitney U, $P < 0.001$) minor role (I_{FI} < 5%). The investigated danubian invertebrate community consisted of a high fraction of invasive aquatic invertebrates (I_{EI} = 39%) and a marginal fraction of indigenous ones (I_{EI} = 3 %). Considering taxa with unassigned origin ("unassigned", Fig. 5.6) as missing values, ratios in indices of importance of invasive to indigenous species were 11:1 in *P. kessleri* (I_{FI} =

92%), 14:1 in *N. melanostomus* (I_{FI} = 93%), and 12:1 in benthic invertebrates (I_{FI} = 92%). The dominant group within macroinvertebrate samples, the Gammaroidea, comprised 99.8% invasive taxa with Ponto-Caspian origin (*Dikerogammarus* spp. 90.2%, *Echinogammarus* spp. 7.5%, *Pontogammarus* spp. 1.9%, *Obesogammarus* spp. 0.2%) and only 0.2% autochthonous *Gammarus roeseli* (Gervais, 1835). Food resources utilized by gobies were not selected according to their biogeographical origin as no differences between the index of food importance and the index of environmental importance were observed in either category (Fig. 5.6).

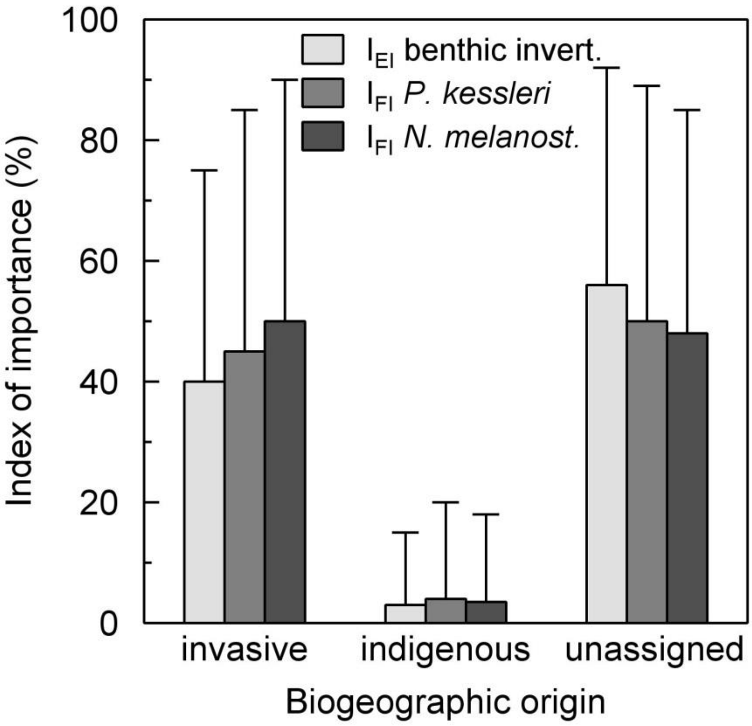

Fig. 5.6: Importance of invasive species in the diets of *N. melanostomus* and *P. kessleri*

Mean importance and standard deviation (S.D.) of invasive species in the diets of *N. melanostomus* and *P. kessleri* compared to the mean environmental availability of benthic invertebrates as food resources in the headwater reaches of the Danube River, displayed by the index of food importance (I_{FI}, gobies, grey) and the index of environmental importance (I_{EI}, benthic invertebrates, black). Species too small for taxonomic identification and species with non-allocatable biogeographical origin were classified as “unassigned”.

Morphometric analyses

In line with the results of the gut content analyses, the Zihler-Index characterized both goby species as omnivores with preference for animal food items (predacious omnivores). The uncoiled intestinal tract in *P. kessleri* was significantly shorter (t-test, $P < 0.001$) than in *N. melanostomus* (n = 207, $Z_I = 3.12 \pm 0.41$ and n = 254, $Z_I = 3.94 \pm 0.70$ for *P. kessleri* and *N.*

melanostomus, respectively), indicating a slightly narrower niche by a higher adaptation of the digestive tract to animal food.

Fulton´s condition factor was significantly (Kruskal-Wallis, $P < 0.001$) lower in *P. kessleri* (1.15 ± 0.23) than in *N. melanostomus* (1.39 ± 0.14) in both seasons. Within species, no seasonal differences in L_T, M_T and K were detected (Table 5.2).

5.5 Discussion

Trophic relations and niche differentiation

This study characterized the trophic niches of two closely related goby species in the recently invaded headwater reaches of the Danube River. As evident from stable isotope, gut content and morphometric gut data, both species are predacious omnivores with similar trophic niches. As hypothesized, their great dietary breadths and opportunistic feeding strategies characterize both gobies as generalists, which may be important in explaining their invasion success. However, there are some important restrictions. Feeding on benthic invertebrates was not completely random, as amphipods, especially *Dikerogammarus* spp. were the most important and commonly preferred prey of both fishes and consumption of these taxa exceeded their environmental availability. The observed importance of amphipods may also be influenced by the size class (8-12 cm L_T) of fishes used to assess diets with the consequence of excluding the ontogenetic diet shift. This improved the detection of a seasonal diet shift in *N. melanostomus*, highlighting their great plasticity, and a wide niche overlap between both gobiids. However, the transition from an amphipod-dominated diet to a mollusk-based feeding (found in this study for the additional sample of *N. melanostomus*) occurred at the chosen length and thus at least partly also captures the ontogenetically determined niche width. Also, the study design used herein was selected for the main purpose of comparing the same sizes of different species.

Ponticola kessleri showed a moderately higher degree of specialization and a more stable feeding pattern across seasons compared to *N. melanostomus*. In late summer, both gobies increased selective feeding. Although availability of *Chelicorophium* spp. and molluscs increased from early to late summer, *P. kessleri* food choice remained constant, since it strongly relied on *Dikerogammarus* spp. and amphipods during the whole growth period, while *N. melanostomus* increased feeding on items with increased availability. This indicates a narrower trophic niche in *P. kessleri* compared to *N. melanostomus*, whose feeding varied between seasons following resource availability leading to a niche expansion in this species from early to late summer. The increasing isotopic spacing ($\delta^{15}N$) between both species from early to late summer (by a factor of 1.3) corroborates the trophic niche expansion in *N. melanostomus*. Since gut lengths depend on diet specification (Balfour, 1988), the longer digestive tract of *N. melanostomus* also indicates a more omnivorous generalist feeding than the more predacious niche associated with the shorter digestive tract of *P. kessleri*. This is corroborated by the observed wider spectrum of food items (including hard-shelled molluscs. even bryozoans and plant material) in *N. melanostomus*, while *P. kessleri* feeding is more restricted and contains a more carnivorous, easier digestible diet consisting mainly of amphipods and fish. The higher index of stomach fullness in *P. kessleri* also supports a narrower niche since it indicates a generally higher food uptake in this species. In other words, feeding behaviour of *P. kessleri* is more stenophag compared to the more euryphag *N. melanostomus*.

Neogobius melanostomus exhibits a pronounced and continuous ontogenetic diet shift, which determines a broad dietary niche at the population level. At a total length of about 10 cm, it switches from preying upon insects and crustaceans (increasing limb, Fig. 5.2) to a mainly mollusc dominated diet (decreasing limb, Fig. 5.2), which can also be interpreted as an increasing specialization at the individual level. The findings from the corresponding gut content analyses corroborate these results and mirror earlier findings from Campbell *et al.* (2009), who detected a diet shift from amphipods to molluscs at about 11 cm L_T in Lake Erie by mass balance simulation, assuming a fractionation of 3.4‰ (Post. 2002). Such dietary changes, based upon gut content analyses, have been also reported from other waterbodies in the Great Lakes area (French & Jude, 2001; Barton *et al.*, 2005) and the Baltic Sea (Karlson *et al.*, 2007) where this shift seems to occur between 6 and 11 cm L_T.

The trophic relations assessed by stable isotope analysis in this study are in line with the findings of Van Riel *et al.* (2006) from the lower River Rhine, where three trophic levels were distinguished with particular organic matter (POM) and phytoplankton as a base, primary and secondary consumers at medium level and top predators as highest level. Gobies were not considered in that study, as they had not invaded the River Rhine at that time.

The $\delta^{15}N$ values of the present study indicated a significantly lower trophic position (of about half a trophic level) of *P. kessleri* compared to *N. melanostomus* despite the fact that the gut content analyses would have predicted the opposite results since more fish (mainly *N. melanostomus*) was consumed by *P. kessleri* and more bivalves were consumed by *N. melanostomus*. This mismatch between the more predatory *P. kessleri* and one of their prey, *N. melanostomus*, could derive from differences in growth rates, excretion, digestive tract anatomy and physiology. In addition, digestibility of food, parasites and diseases might also play a role. The other $\delta^{15}N$ values of this study were within the range of values expected. with the bivalves as filter feeders at the lower end and both gobies as top predators at the upper end of the analyzed trophic chain being about 3 to 4‰ above the bivalves. The $\delta^{15}N$ values of *N. melanostomus* were similar to those measured in the Gulf of Gdansk (about 14.2‰), where small *N. melanostomus* (L_T of 60 – 120 mm) tended to be more enriched in ^{15}N than medium-sized and large ones (Karlson *et al.*, 2007). Analogously, $\delta^{15}N$ values clearly indicated an isotopic change with increasing L_T in *N. melanostomus* in this study, too. Both gut contents and muscle tissue exhibited the same behaviour, which indicates that the picture derived from the instantaneous gut content analyses matches the long-term feeding picture derived from the muscle isotopic analyses. This interpretation is further supported by two findings: First, the diet-tissue shift is close to the expected diet-tissue shift for one trophic level with a consistent enrichment of ^{15}N within a range of 2.3‰ (McCutchan *et al.*, 2003) to 3.4‰ (Post, 2002) per trophic level, between prey and predator which would not be true if the present food had differed from the previous food (Auerswald *et al.*, 2010). Second, the residuals exhibited no trend. Such a trend with opposite sign for both tissues would evolve after a (isotopic) diet change until a new equilibrium between food and tissue is achieved. Such opposite trends were neither visible for the increasing nor the falling limb of the parabolic relation. Half-life times for marine goby muscles are reported to range around 25 days (Guelinckx *et al.*, 2007, 2008) where new tissue contributes most to this fast apparent turnover (Maruyama *et al.*, 2001). The increase by 2.5‰ when gobies grew from 4 to 10 cm may have occurred over a period of about half a year assuming a growth rate of one cm per month (Guelinckx *et al.*, 2008), which agrees with the observations of this study. This provides sufficient time for equilibration.

Their flexible, generalist feeding strategy and the observed capability of an ontogenetic diet shift probably both contribute to a greater plasticity in realized trophic niche of *N. melanostomus*, which reduces intraspecific competition and which may add to the greater success (i.e. a factor 25 greater abundance) of this species compared to *P. kessleri*. These specializing mechanisms, which increase resource utilization and thus reduced competition, have not been found in *P. kessleri*, which might cause a lower competitive ability against *N. melanostomus*.

Comparisons with other goby populations

In their native ranges, pronounced feeding on fish and amphipods has been observed in *P. kessleri*, Vasil´Eva & Vasil´Ev (2003) reported that in the Dniester estuary region, *P. kessleri* predominantly fed on small fishes, mainly small gobies (78-92%) and crustaceans (about 7% mainly mysids and chelicorophiids), while molluscs, polychaetes and chironomids were less important. Additionally, a seasonal variation in food composition with small fishes comprising one third in spring and up to 100% in summer and autumn was observed there for *P. kessleri*. The diet of native *P. kessleri* populations from the lower Danube was largely based on fish and amphipods (Polačik *et al.*, 2009). A diet with a clear dominance of amphipods and low proportions of fish was found in non-native populations from the middle Danube River (Adámek *et al.*, 2007; Polačik *et al.*, 2009; Borza *et al.*, 2009). Consequently, *P. kessleri* dietary niche is characterized by a restriction to fish and amphipods as prey, with a lower specialized feeding on fish in non-native populations, which is in line with the findings of this study from the headwater reaches of the Danube River.

The feeding behaviour of native and most invasive *N. melanostomus* populations differ from those of the non-native populations of the upper Danube River (analyzed here), supporting their great dietary plasticity during invasion of new areas. A diet with an essential importance of molluscs (mainly bivalves) has been reported for *N. melanostomus* inside their native distribution range: Molluscs were most important food items (about 86% of gut contents) in the Sea of Azov area (Kovtun *et al.*, 1974) and in Ponto-Caspian habitats such as the Bug estuary (~90%), the Grigoryevskiy estuary (~88%), the eastern Dnieper estuary (~83%) and the Azov Utlukskiy estuary (~68%), while crustaceans (gammarids, chelicorophiids), chironomids, annelids and fishes were of low importance (Pinchuk *et al.*, 2003). Similarly, invasive *N. melanostomus* populations in the North American Laurentian Great Lakes (French & Jude, 2001; Janssen & Jude, 2001; Barton *et al.*, 2005; Johnson *et al.*, 2005; Lederer *et al.*, 2006; Kornis *et al.*, 2012) and coastal waters of the Baltic Sea (Skóra & Rzeznik, 2001; Karlson *et al.*, 2007) mostly fed on bivalves (*Dreissena* spp.) with occurrence of an ontogenetic diet shift. Shemonaev & Kirilenko (2008) observed intense molluscivory (~90% of diet by weight) for invasive *N. melanostomus* in a lentic reservoir of the River Volga.

In the lower Danube River, *N. melanostomus* preyed to a similar extent (prey specific importance) on molluscs and amphipods (Polačik *et al.*, 2009). Simonović *et al.* (2001) observed pronounced molluscivory in *N. melanostomus* in the middle Danube. However, a great contribution of amphipods and chironomids to a broad diet were found in other non-native populations from the middle Danube River (Polačik *et al.*, 2009; Borza *et al.*, 2009). Similarly, in Great Lakes tributaries, which did not contain *Dreissena spp.*, Phillips *et al.* (2003) observed a diet without amphipods, instead being dominated by chironomids (up to ⅔ of diet by volume, depending on fish size), while Pennuto *et al.* (2010) observed the opposite in this area (amphipods up to ⅔ of diet by wet weight and nearly no chironomids). The results of these studies and those reported herein strongly suggest that feeding of *N. melanostomus* largely depends on availability and abundance of prey organisms in the ecosystem. It appears that in lotic habitats, diets of *N. melanostomus* are typically dominated by non-mollusc benthic invertebrates (~71% by mass, Laurentian Great Lakes tributaries) which mirrors the findings from our study, while in lentic or marine ones, molluscs are usually the primary diet component comprising 57- 65% of the food biomass uptake (Kornis *et al.*, 2012).

In addition to the differences according to various habitat types, time since invasion (in other words, ecosystem impact) also seems to be important. Most of the habitats in which *N. melanostomus* has been reported to primarily feed on molluscs and not on amphipods have been occupied for more than a decade, whereas their invasion in the upper Danube River (investigated herein) with an amphipod-based diet is a rather recent phenomenon. Similarly, in recently invaded areas of the Baltic Sea studied two years after first recordings, decapod shrimp (up to 72% of food biomass uptake) and snails were the most important food items (Azour, 2011) even in large *N. melanostomus*, whereas there was a shift towards bivalves in earlier colonized habitats of the Baltic sea, studied 5-14 years after invasion (Skóra & Rzeznik, 2001; Karlson *et al.*, 2007). Consequently, the currently underestimated factor of time since invasion deserves better consideration in analyses of feeding habits of invasive Ponto-Caspian gobies and other invasive species since niche differentiation and effects of an invader can be modulated by evolutionary or ecological processes (Strayer *et al.*, 2006).

In recently invaded habitats studied herein, both goby populations probably have not yet reached maximum densities limited by the carrying capacity. It is therefore likely that the feeding ecology patterns found are close to their fundamental niches.

The role of invasive prey species in goby feeding and ecosystem impacts

As evident from this study, *D. villosus*, and few other invasive amphipods have already replaced native amphipods in the headwater reaches of the Danube River. Amphipods, especially invasive *D. villosus*, were identified as main energy suppliers for Ponto-Caspian gobies in the upper Danube River and thus seem to facilitate the ongoing invasion by "fuelling". Invasive species contribute ten times more to the feeding of the gobies than indigenous species. Both goby species consumed mainly other non-native species (~92% of gut contents) and thus seem to benefit from previous invasions of prey species such as *Dikerogammarus villosus, Chelicorophium curvispinum, Dreissena polymorpha and Corbicula fluminea*.

Invasions of the Ponto-Caspian gobiid fishes *Neogobius melanostomus* and *Ponticola kessleri* are suspected to cause serious regime shifts in freshwater ecosystems by e.g. affecting fish diversity and benthic invertebrate community (see citations in the introduction). As these species continue to spread throughout European rivers and in the case of round gobies, in North America, a new quality of potential threats can affect areas with high endemic aquatic biodiversity (Keller *et al.*, 2011). Hereby, invasion success doses not necessarily depend on the presence of *Dreissena* spp., which might complicate potential countermeasures. However, substantial populations of invasive species can occasionally collapse dramatically (Simberloff & Gibbons, 2004). Ontogenetically determined niche extension in *N. melanostomus* as well as intraguild predation and cannibalism in *P. kessleri* can be characterized as a feedback regulation process (“closed loop omnivory”, Polis *et al.*, 1989) which is likely to hamper spontaneous short-term population collapses of *P. kessleri* and *N. melanostomus*, potentially caused by overexploitation of food resources. Hence, these mechanisms are more likely to stabilise both fish populations and thus will consolidate their status quo by preventing from competitive exclusion or boom-and-bust.

Based on life history traits, *P. kessleri* is supposed to win the competition (Kováč *et al.*, 2009). However, based on trophic interactions, *N. melanostomus* is likely to have advantages under changing food resource availabilities. A monitoring of the future success of both species in the danubian headwaters may provide valuable insights into the relative importance of both factors.

According to Borza *et al.* (2009), the food web of the Danube River is currently approximating the Ponto-Caspian one, a process supporting the “*invasive meltdown*” theory (Simberloff & Von Holle, 1999). This is also supported by our data and analogous predictions were made for the River Rhine (Van Riel *et al.*, 2006). The benthic community of the analyzed headwater reaches of the Danube River has been altered by invasions of benthic invertebrate species within the last two decades (see Tittizer *et al.*, 2000), apparently indicating such an invasive meltdown. As a consequence, the indigenous, lotic invertebrate biocoenosis of danubian headwaters has been altered to a non-native, lenitic one, now harbouring a major brackish and marine fauna. It is unlikely that this effect was primarily caused by the gobies, which have just arrived at the headwater reaches of the Danube. It is more likely that this alteration now provides food web conditions suitable for (further) Ponto-Caspian goby invasions, particularly since they almost entirely prey upon invasive species. However, given the differences in feeding strategies of the two goby species, their wide dietary niches and their plasticity regarding diet selection, it is likely that food is not the most important factor for the invasion success of the gobies. The success of the gobies and their prey rather reflects a change in environmental conditions favouring both prey and predator. The invasion of the gobies and their prey species may thus only mirror the fundamental ecological food web changes in large European freshwater ecosystems.

Implications for future research and management

The observed feeding patterns of invasive gobies and their interactions with the danubian food web support previous findings that both species are highly competitive due to their generalist and opportunistic feeding strategies. At the same time, invasive gobies have a strong potential to alter current food web structures. Strategies to stop a further spread of both species are unlikely to be effective at this stage. Future research may be expanded to also address the feeding patterns of different size classes, as well as different habitat types within the Danube River. Only recently, the arrival of a another goby species, *Babka gymnotrachelus* (Kessler, 1857), was detected for the first time in Germany (Haertl *et al.*, 2012) whose trophic interactions with the extant species and the aquatic food web will help to compare the feeding plasticity and other factors which govern invasion success in these species.

6. Bigger is better: Characteristics of round gobies at the invasion front

A similar version of this chapter was published as:

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6.1 Abstract

Few studies have systematically investigated differences in performance, morphology and parasitic load of invaders at different stages of an invasion. This study analyzed phenotype-environment correlations in a fish invasion from initial absence until establishment in the headwater reach of the second largest European river, the Danube. Here, the round goby (*Neogobius melanostomus*) formed 73% of the fish abundance and 58% of the fish biomass in rip-rap bank habitats after establishment. The time from invasion until establishment was only about two years, indicating rapid expansion. Founder populations from the invasion front were different from longer established round goby populations in demography, morphology, feeding behaviour, sex ratio and parasitic load, indicating that plasticity in these traits determines invasion success. Competitive ability was mostly dependent on growth/size-related traits rather than on fecundity. As revealed by stable isotope analyses, specimens at the invasion front had a higher trophic position in the food web, and seem to benefit from lower food competition. Somatic performance seems to be more important than investment in reproduction during the early stages of the invasion process and upstream-directed range expansion is not caused by out-migrating weak or juvenile individuals that were forced to leave high density areas due to high competition. This mechanism might be true for downstream introductions via drift. Greater abundance and densities of acanthocephalan endoparasites were observed at the invasion front, which contradicts the expectation that invasion success is determined by lower parasitic pressure in newly invaded areas. Overall, the pronounced changes in fish and invertebrate communities with a dominance of alien species suggest invasional meltdown and a shift of the upper Danube River towards a novel ecosystem with species that have greater resistance to goby predation. This seems to contribute to overcoming biological resistance and improve rapidity of dispersal.

6.2 Introduction

Invasive species are important drivers of global biodiversity loss (Sala *et al.*, 2000; Mooney & Cleland, 2001) and one of the major threats to global freshwater biodiversity (Dudgeon *et al.*, 2006; Geist 2011; Keller *et al.*, 2011). Successful invaders are not a random selection of species (Karatayev *et al.*, 2009). Instead, they often have certain life history traits in common, including a generalist feeding strategy, complex reproductive behaviour involving e.g. nest guarding, the ability of rapid range expansion but also aspects of population structure, genetics and habitat use (e.g., Olden *et al.*, 2006; Gutowsky & Fox, 2011; Kolar & Lodge, 2002). Most of these successful invaders, including the zebra mussel Dreissena polymorpha Pallas, 1771 and the so-called ´killer shrimp´ Dikerogammarus villosus (Sovinskij, 1894) have been blamed for serious ecosystem impacts worldwide (Strayer, 2009; Pöckl, 2009). Current studies identified plasticity in life history traits to be an important advantage to the success of invasive species, allowing them to easily adapt to different environments throughout the different stages of the invasion process (Bøhn *et al.*, 2004; Brandner *et al.*, 2013a; Záhorská & Kováč, 2013; Kováč *et al.*, 2009; Brownscombe & Fox, 2012). Since several evolutionary and ecological processes can change life history strategies of invaders advancing from one stage to the next (Feiner *et al.*, 2012; Groen *et al.*, 2012; Gutowsky & Fox, 2012), time since invasion needs to be considered to identify and quantify the role of these factors (Strayer *et al.*, 2006). However, to our knowledge, no study has yet systematically investigated biological invasion processes from total absence until the dominance of an invasive species, focusing on life history plasticity over time.

Recently, a benthic Ponto-Caspian gobiid fish species (Teleostei: Gobiidae), the round goby Neogobius melanostomus (Pallas, 1814), has colonized both freshwater and marine ecosystems on both sides of the Atlantic Ocean (Corkum *et al.*, 2004). Its rapid spread and the high potential to cause ecological regime-shifts (e.g., Janssen & Jude, 2001; Bergstrom & Mensinger, 2009; Karlson *et al.*, 2007; Kipp & Ricciardi, 2012) have mobilized substantial scientific interest in this species as a model to study invasion biology processes worldwide (reviewed in Kornis *et al.*, 2012). In the last two decades, an increasing number of rapid range expansions of N. melanostomus have been reported from the Laurentian Great Lakes watershed (Corkum *et al.*, 2004; Johnson *et al.*, 2005b; Kornis & Vander Zanden, 2010; Pennuto *et al.*, 2010; Bronnenhuber *et al.*, 2011; Brownscombe *et al.*, 2012), from almost the entire Baltic Sea region (Sapota & Skóra, 2005; Ojaveer, 2006; Sokołowska & Fey, 2011) and from many other large European waterbodies, including the Danube River (Brandner *et al.*, 2013a; Stráňai & Andreji, 2004; Jurajda *et al.*, 2005; Harka & Bíró, 2007) and the River Rhine (Borcherding *et al.*, 2011a). Secondary invasions aside of the main navigation routes and migration corridors (e.g., Piria *et al.*, 2011) and the proceeding spread of round goby worldwide highlight a new quality of potential threats especially to areas with high endemic aquatic biodiversity (Poos *et al.*, 2010). In lotic habitats, round goby was found to comprise more than 50 % of the total fish catch (Krakowiak & Pennuto, 2008), illustrating the potential impact on aquatic food webs. Therefore, a better knowledge of round goby ecology at all stages of the invasion is crucial to estimate associated ecosystem impacts (Taraborelli *et al.*, 2010).

Comparisons between native and non-native round goby populations revealed differences in distribution and abundance (Polačik *et al.*, 2008b), as well as in the capability to generate phenotypic differences in life history traits. In particular, shifts in population characteristics, somatic condition, growth rate, diet and maturity as well as in external morphology were observed between the native range and newly invaded habitats (Polačik *et al.*, 2009; Polačik *et al.*, 2012; Bergstrom *et al.*, 2008). However, none of these studies considered spatio-temporal effects on plasticity. Recent studies from the Trent-Severn-Waterway (Ontario, Canada) characterized initially invaded areas, subsequently referred to as “invasion front”, by having a lower proportion of sites containing round gobies, lower densities, larger individuals and male-biased sex-ratios (Gutowsky & Fox, 2011; Gutowsky *et al.*, 2011). Consequently, demographics, life history-traits and growth at newly invaded areas seem to differ from relatively long-established areas. To date, little is known about invasions that are independent from ballast water transport and ship hull transfer. Additionally, population dynamics and life-history characteristics of round goby pioneers from other habitats such as large rivers are underrepresented in scientific studies on this topic.

In the German section of the Danube River, which is one of the most important European long-distance dispersal routes for aquatic invasive species (Bij de Vaate *et al.*, 2002; Panov *et al.*, 2009), N. melanostomus was first recorded in 2004 (Paintner & Seifert 2006). Here round goby can be found both at established areas in densities of up to 20 individuals per square meter and not far upstream, at a distinct invasion front (Brandner *et al.*, 2013a), where introduction is not directly related to navigational vessel traffic. Thus, the present invasion of N. melanostomus within the upper Danube River offered the opportunity to quantitatively study early (introduction) and late (establishment, spread and impact) phases of a round goby invasion. In particular, the greater availability of food resources and habitat structures in newly invaded areas may cause differences in demographic parameters of round goby populations such as length and weight distributions, and the proportion of sexes, but also in feeding behaviour, reproduction, parasitic load and fitness compared to areas with established populations. The great degree of phenotypic plasticity in *N. melanostomus* among distinct geographical regions is evident from several life trait variables such as length-weight relationships with b-values varying between 2.4 in the Sea of Azov (Kovtun *et al.*, 1974) and 3.3 in the Sea of Marmara (Tarkan *et al.*, 2006). On the other hand, there are currently very few field studies available that were able to compare the plasticity of invasive species within the same ecosystem or habitat over time by comparing populations or sub-populations at recently invaded sites with established ones within the same system. This is, to our knowledge, the first study, examining a recent round goby invasion from total absence to the first occurrence until establishment.

The general objectives of this study were to (i) compare early and late phases of a round goby invasion at population- and specimen-level in a recently invaded, lotic ecosystem, (ii) test for phenotypic differences (length, weight and condition factor, hepato-somatic and gonado-somatic indices) between fish representing those early and late population stages, and (iii) analyze founder traits and demographic effects with respect to the time since invasion, considering abundance, sex ratio, parasitic load, and feeding patterns. Analogous to invasive plants (Blossey & Nötzold 1995) this study hypothesized that also animal invaders from recently invaded areas differ from their conspecifics in established populations by possessing an increased competitive ability, including greater body sizes and condition factors, reduced parasitic load and different feeding strategies. This study compared phenotypic characteristics of round gobies in pioneering and established populations within one of the most important European invasion pathways, the Danube River.

6.3 Material and Methods

Ethics Statement

All specimens in the current study were sampled using electrofishing, which was conducted under license number 31-7563/2 to the Aquatic Systems Biology Unit of Technische Universität München (TUM). All specimens used for analyses were killed using an overdose of anaesthetic and immediately frozen on dry ice to avoid degradation of gut contents and muscle tissue. Following federal fishing laws and sampling licensing, all invasive gobies were removed from the Danube River, whereas all native fishes were carefully returned to the river after sampling. All efforts were carried out in strict accordance with the legal obligations of the Federal Republic of Germany.

Field sampling

To explore potential differences between newly invaded and established “populations” (i.e. sub-populations in sensu stricto), the round goby distribution along a 200 river-km invasion pathway in the upper Danube River was monitored during a pilot study. In summer 2009, this investigation was conducted to identify the upstream border to which round gobies had reached (invasion front). Analogously to the sampling of Bronnenhuber *et al.* (2011) at three Great Lakes tributaries, round gobies were considered absent at a site where no individuals were caught at a minimum of 1200 electroshocking seconds. The uppermost site where single individuals of *N. melanostomus* had been recorded (August 25th, 2009) was river-km 2,390.2 (N48°58'39.03"; E12°02'16.72"). The intended sampling design comprised three river sections with an "established area", where round goby had been recorded for the first time before January 1st, 2007, an invasion front, where a round goby invasion was expected to happen soon after the initiation of this study, and an uppermost "negative control area" with round goby absence during this study. Considering these findings, ten representatively distributed river stretches along the upper Danube River were selected (Fig. 6.1, Table 6.1).

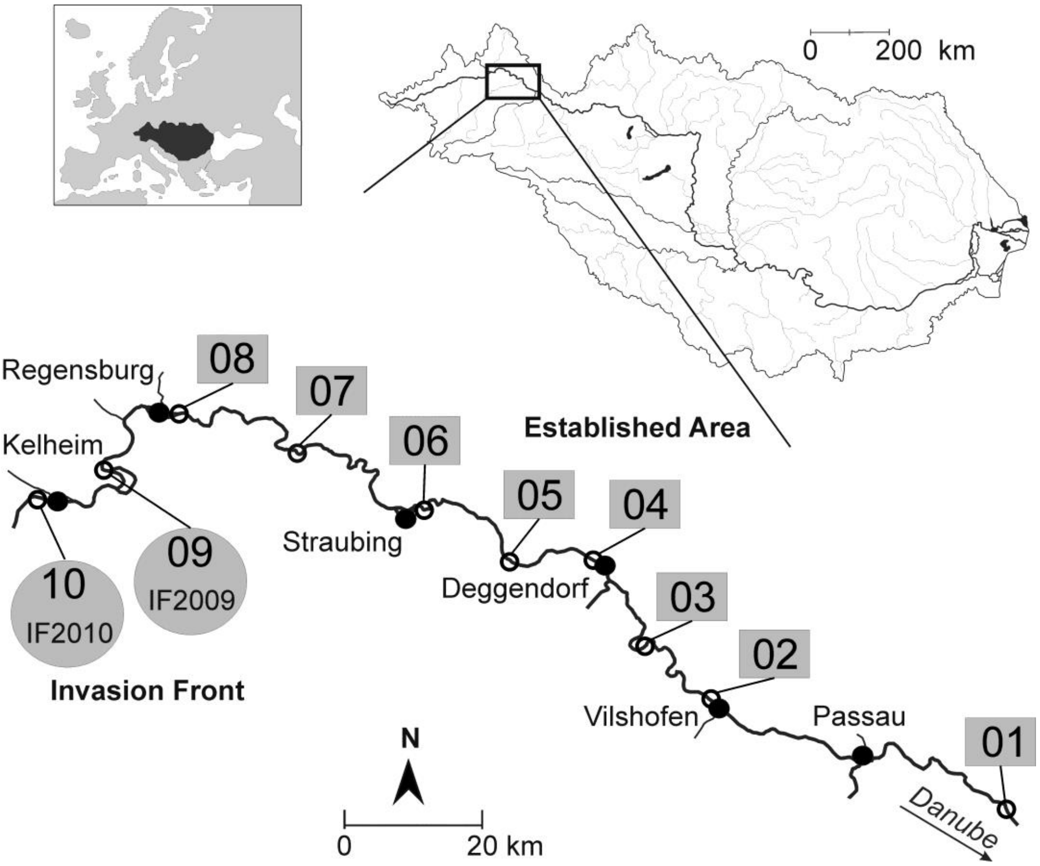

Fig. 6.1: Study area at the upper Danube River between Austria and Germany.

Study area with ten representatively chosen rip-rap sampling stretches covering a recent round goby invasion along the headwater reaches of the upper Danube River between Austria and Germany. The consecutive numbers denote two newly colonizing sub-populations at a recent invasion front (grey circles) "IF2010" (sampling stretch #10, first record: September 2010) and "IF2009" (sampling stretch #09, first record: August 2009) as well as eight established sub-populations from the "established area" (sampling stretches #08 to #01; grey rectangles). The Danube basin and the location of the study area within the drainage area are highlighted. Filled black circles denote important cities along the Danube River.

The established area comprised eight river stretches (populations #01 to #08) from Engelhartszell (Austria) to the city of Regensburg (Germany). As round goby started to invade the river stretch #09 “Bad Abbach” in autumn 2009, this area was defined “IF2009” (invasion front 2009). Due to round goby invasion in the intended negative control area #10 “Kelheim” in autumn 2010, this river stretch was defined “IF2010” (invasion front 2010).

The sampling was conducted from October 2009 to October 2011, covering the early (March–June) and late (August–October) annual growth period of fish as suggested from previous studies (e.g., Brandner *et al.*, 2013a). In order to avoid the introduction of a systematic sampling bias (e.g. due to trends in water temperatures), even and uneven river stretches (first even and then uneven numbered river stretches) were sampled consecutively. According to Sindilariu *et al.* (2006) and our own observations, rip-rap structures are the preferred habitat of invasive round goby in the Danube River, representing about ⅔ of the available bank habitat in the study area. Thus, to exclude a possible bias due to different mesohabitat structures, only rip-rap habitats were sampled.

Fishes were collected during daylight from shorelines (in ~60 cm water depth) by electrofishing (ELT62-IID; Grassl GmbH, Berchtesgaden, Germany) using the point abundance sampling (PAS) technique (Nelva *et al.*, 1979; Persat & Copp, 1990) with a duration of 10 s and a distance of 10 meters between individual points. Every river stretch comprised at least 30 PAS-points at both shorelines. In total, 2135 PAS-points were collected at 72 rip-rap samplings (Table 6.1).

Table 6.1: Sampling design and location of river stretches

Consecutive number, name, total number of rip-rap samplings (n), total number of point abundance samples (PAS), population status (defined after time since invasion), first recording of *N. melanostomus*, river kilometers (rkm) and GPS-coordinates (WGS84) of upper and lower boundaries (sorted in upstream to downstream order) of ten representatively distributed rip-rap river stretches from both river shorelines along the upper Danube River. [1] own observations, [2] Paintner & Seifert (2006), [3] Zauner (pers. com.) , u = exact time point uncertain, but first record clearly before 2007.

Sampling Design						Lower Boundary		Upper Boundary	
№	River Stretch	n	PAS	First Record	Population Status	rkm	GPS	rkm	GPS
10	Kelheim	10	300	2010[1]	newly colonizing	2409	E 11°56'27" N 48°54'29"	2418	E 11°50'12" N 48°54'01"
09	Bad Abbach	10	301	2009[1]	newly colonizing	2393	E 12°00'13" N 48°57'57"	2400	E 12°02'05" N 48°56'03"
08	Regensburg	9	270	u	established	2373	E 12°10'41" N 49°00'34"	2377	E 12°08'29" N 49°01'22"
07	Geisling	6	180	u	established	2350	E 12°23'37" N 48°58'51"	2354	E 12°21'02" N 48°58'36"
06	Straubing	4	110	2004[2]	established	2309	E 12°42'26" N 48°53'34"	2317	E 12°36'56" N 48°53'49"
05	Mariaposching	5	131	u	established	2292	E 12°52'12" N 48°50'28"	2298	E 12°47'46" N 48°49'33"
04	Deggendorf	3	90	u	established	2280	E 12°59'50" N 48°47'31"	2289	E 12°54'26" N 48°50'40"
03	Aichet	8	240	u	established	2267	E 13°03'08" N 48°43'37"	2273	E 13°02'15" N 48°44'32"
02	Vilshofen	9	273	2004[2]	established	2250	E 13°10'44" N 48°38'24"	2259	E 13°05'41" N 48°41'02"
01	Engelhartszell	8	240	2003[3]	established	2196	E 13°46'29" N 48°28'32"	2202	E 13°43'21" N 48°30'48"

All fish species were inspected for infection rates with ectoparasitic plathyhelminths of the genus *Rossicotrema spp.* (black spot disease) and assigned into four categories (0 = no black spots; 1 = few, i.e. < 5; 2 = medium, i.e. 5-100; 3 = high, i.e. > 100).

In addition to the demographic sampling for characterizations on the population level, an additional 365 round goby specimens were collected (targeting two females and two males from every single river stretch) for characterization of specimen level data. This sample subset was size-class selected (target 8 – 12 cm), as many morphometric indices assume isometry of body proportions in fish of varying size (e.g., Froese, 2006) and stable isotope signatures in *N. melanostomus* can be influenced by ontogenetic diet shifts (Brandner *et al.*, 2013a). The mean total length (L_T) of all chosen specimens was 9.82 cm (SD = 1.15 cm) with a slope (b) of the length-weight-regression of about 3.0 (b = 3.045; $R^2 = 0.927$; $p<0.001$), indicating isometric growth (Froese, 2006) for the chosen specimens. To test for site-, sex- and season-specific differences in length-weight relationships and to assess the possibility of pooling samples within the established area, slope comparison of length-weight regressions were computed and tested using ANCOVA. No statistically significant differences between slopes were identified, with p-values >0.05 in all cases. Due to the spatial shift of the invasion front between 2010 and 2011, specimens from those two years were analyzed separately.

As several recent studies described ontogenetic diet shifts in N. melanostomus (Brandner *et al.*, 2013a; Karlson *et al.*, 2007; Johnson *et al.*, 2005; Campbell *et al.*, 2009, one additional sample of 16 specimens (L_T of 4 - 14 cm) was collected at an established population (#08, “Regensburg”; N49°01'01.95"; E12°09'21.09") on October 15th, 2010 and another additional sample of 15 specimens (L_T of 8 - 17 cm) was collected at IF2010 (#10, “Kelheim”; N48°54'26.99"; E11°53'24.56") on September 9th, 2011 (Fig. 6.1, Table 6.1) to test for this size effect at different stages of the invasion process. All specimens were deposited at the ichthyological collection of the Bavarian State Collection of Zoology (ZSM).

The wet weights of liver, gut contents, ovaries in females, testes and seminal vesicles in males were recorded to the nearest 0.001 g. As round goby is known to serve as a paratenic host for acanthocephalans (Ondračková *et al.*, 2010; Emde *et al.*, 2012) subadult acanthocephalans attached to liver, kidney, spleen, gonads and the surface of the intestinal tract were counted using a stereo-microscope. In order to test the “*enemy release”*-hypothesis, suggesting that invasive species carry less parasites in newly invaded areas than in established or original areas of distribution (see e.g. Williamson, 1996; Keane & Crawley, 2002), ecological indicators of parasite infection were applied according to Ondračková *et al.* (2005), using mean abundance (i.e. mean number of parasites per host) and mean density (i.e. abundance per fish total mass).

Stable isotope analysis

To obtain markers for middle to long-term feeding patterns, $\delta^{13}C$ and $\delta^{15}N$ stable isotope analyses of round goby flank muscle tissue (about 0.5 – 1.0 cm^3, defatted with chloroform - methanol (2:1) solution) were conducted as described in Brandner *et al.* (2013a). The additional sets of samples with greater length variation were analyzed to test for (i) correlations between L_T and $\delta^{15}N$ signatures, and (ii) a diet shift between muscle tissue and gut contents. The $\delta^{15}N$ values of the gut contents were calculated as averages weighted by their index of food importance (see I_{FI} below) from mean $\delta^{15}N$ signatures of benthic invertebrates collected from the upper Danube River (Brandner *et al.*, 2013a). Repeated analyses of a solid internal laboratory standard (bovine horn, run after each ten samples) showed maximum standard deviations of 0.15 ‰ for $\delta^{15}N$ and 0.15 ‰ for $\delta^{13}C$ values.

Fish gut analyses

Digestive tract dissection and processing was conducted following Brandner *et al.* (2013) with the anterior digestive tract being weighted to the nearest 0.001 g before and after emptying to obtain the wet weight of gut contents. All food items from the digestive tract samples were fixed in ethanol, identified to the lowest possible taxon considering manageable taxonomic levels, counted and visually estimated to the nearest % proportion of volume, using a stereo microscope.

Benthic invertebrates

Quantitative samples of benthic invertebrates were collected using a suction sampler [as described in Brandner *et al.* (2013)] from the same sites where gobies were sampled (~60 cm water depth, duration = 120 s, three replicates). Altogether 250 samples of benthic invertebrates were preserved in 70 % ethanol immediately after capture. A total of about 46,500 benthic invertebrates were identified to the lowest possible taxon considering manageable taxonomical levels. Organisms belonging to the same taxon or cumulative category were counted and expressed as catch per unit effort (CPUE [min^{-1}]). The percent volumetric proportion of each taxon within a sample was visually estimated analogously to the gut analysis in fish.

Indexing and statistical analyses

The somatic mass (M_S) was calculated as $M_S = M_T - (M_{\text{indexed organ}} + M_g)$ with M_g = gut content mass to compute the following indices: To test for differences in important body mass indices between specimens of a population, the hepato-somatic index ($\text{HSI} = 100\ M_{\text{liver}}\ M_S^{-1}$) and the gonado-somatic index ($\text{GSI} = 100\ M_{\text{gonads}}\ M_S^{-1}$) as a proxy of energetic investment into reproduction were calculated for both sexes (Marentette *et al.*, 2009). Fulton's condition factor K was calculated as $K = 100\ (M_T - M_g)\ L_T^{-3}$ to assess length-weight relationships between populations and specimens (Anderson & Neumann, 1996). To assess food uptake and to test for potential food limitation effects on feeding behaviour, the index of stomach fullness (I_{SF}) was calculated following Hyslop (1980) as $I_{SF} = 100\ M_g\ M_T^{-1}$.

Analogously to Brandner *et al.* (2013) the relative importance of a food item i among all items j for a population was calculated as the “index of food importance” (I_{FI}):

$$I_{FI}(i) = 100\, O(i)\, V(i) \left(\sum_{n=1}^{j} O(i)\, V(i) \right)^{-1}$$

with O = % occurrence of prey i

and V = % volume of prey i

I_{FI} varies from 0 to 100, with higher values corresponding to a larger contribution of one food item as compared to total gut content. Since benthic invertebrate samples were treated like gut content samples, importance of naturally available prey was also calculated following the above mentioned formula as “index of environmental importance” (I_{EI}) for each food item i.

Table 6.2: Population dynamics in *N. melanostomus* and bycatch at three areas (stages) of the invasion

The sampled rip-rap river stretches (upper Danube River, autumn 2009 to autumn 2011) were assigned to the three sampling areas “IF2010”, “IF2009”, “established area” using the time since invasion (year of first record), with the number of point abundance samples (PAS) and catch data of invasive round goby, *Barbus barbus* & *Squalius cephalus* (pooled) as most abundant autochthonous fish species and other fish species (rest). The catch (using electrofishing with continuous DC, duration 10s per PAS) is explained as the mean catch per unit effort (CPUE) [PAS^{-1}] and the mean frequency of occurrence (f_O) [%]. The abbreviation “nd” denotes “not detectable”. Data from the first time of occurrence are shown in bold.

				Round Goby		Barbel & Chub		Other Fish Species	
First Record	Sampling Area	Season	PAS [n]	CPUE [PAS^{-1}]	f_O [%]	CPUE [PAS^{-1}]	f_O [%]	CPUE [PAS^{-1}]	f_O [%]
September 2010	IF2010	late 2009	60	nd	nd	1.73	63.3	0.40	30.0
		early 2010	60	nd	nd	2.47	76.7	0.35	31.7
		late 2010	**60**	**0.08**	**8.3**	**1.28**	**68.3**	**0.90**	**53.3**
		early 2011	60	0.08	5.0	1.50	56.7	0.63	43.3
		late 2011	60	1.63	63.3	2.05	78.3	2.00	63.3
August 2009	IF2009	**late 2009**	**61**	**0.08**	**6.6**	**0.15**	**9.8**	**1.69**	**44.1**
		early 2010	60	0.03	3.3	0.20	16.7	0.57	35.0
		late 2010	60	2.18	78.3	0.23	20.0	3.43	83.3
		early 2011	60	4.60	96.7	0.20	8.3	0.23	16.7
		late 2011	60	3.47	86.7	0.13	10.0	0.38	31.7
before 2007	established area	late 2009	59	4.61	89.2	0.04	4.3	0.70	40.7
		early 2010	425	2.05	73.1	0.06	4.3	0.34	23.9
		late 2010	306	3.68	75.8	0.30	14.7	1.46	37.9
		early 2011	444	4.52	89.0	0.08	3.7	0.52	32.8
		late 2011	300	5.50	92.0	0.17	11.3	0.72	41.3

Dissimilarity-distances (squared Euclidian distance) between the 72 samplings from 10 river stretches were calculated using L_T, M_T and K of females, males and juveniles, the proportions of females (as a relative sex ratio) and catch data (mean CPUE and frequency of occurrence (f_O) of *N. melanostomus*, the most abundant autochthonous fish species *Barbus barbus* (L., 1758) and *Squalius cephalus* (L., 1758) pooled as an indicator for abundant potential prey, and other fish species) from the corresponding rip-rap sampling sites as variables. The results were plotted as a two-dimensional non-metric multi-dimensional scaling (NMDS). In order to assess the importance of catch data, L_T and M_T as well as sex-ratio, additional NMDS analyses considering these factors separately were carried out. As water temperature, discharge and seasonal effects within the sampling procedure may influence all these parameters, additionally paired Kruskal-Wallis tests were conducted to analyze potential trends and differences between the established populations. Only occasional and unsystematic differences without any trends in single parameters among single established populations were detected. Since no significant differences between the slopes of the established populations were found, these data were pooled by the time since invasion (year of first record, Table 6.2).

Specimens of the established area (n = 298) had a mean L_T of 9.82 cm (SD = 1.09 cm) with a slope of the length-weight-regression of b = 3.02 ($R^2 = 0.91$; $p < 0.001$). Specimens of the IF2009 (n = 36) had a mean L_T of 9.35 cm (SD = 1.25 cm) with a slope of the length-weight-regression of b = 3.09 ($R^2 = 0.96$; $p<0.001$). Specimens of the IF2010 (n = 31) had a mean L_T of 10.12 cm (SD = 1.41 cm) with a slope of the length-weight-regression of b = 3.11 ($R^2 = 0.98$; $p < 0.001$). ANCOVA comparisons of the slopes revealed no significant differences between these three groups (all $p > 0.05$). As L_T, M_T, K, I_{FI}, I_{EI}, I_{SF}, were not normally distributed (Shapiro-Wilk-test), multiple comparisons between populations and specimens were computed using non-parametric Kruskal-Wallis-tests followed by (post hoc) Mann-Whitney-U pairwise tests (Bonferroni corrected). Mann-Whitney U-tests were applied to analyze these metrics for potential sex-specific differences. Differences from an expected equilibrium in the distribution of males and females as well as potential differences in the distribution of males and females (sex ratio) between the sampling areas were tested using the chi-square test. Significance was accepted at $p \leq 0.05$ for all statistical tests. Statistical analyses and plots were computed using Statistica (version 6.1, StatSoft Inc., Tulsa, OK, USA).

6.4 Results

Fish community

A total of 30 fish species, comprising 9138 specimens (mean CPUE = 4.3 fish PAS^{-1}) were recorded at 2135 rip-rap PAS points in the upper River Danube between autumn 2009 and autumn 2011. Round goby contributed 73% (n = 6627) and a biomass of about 58% (62 kg) to the total catch. Round goby was found throughout the sampling area, except for the most upstream sampling stretch, where first invaders (four females and one male) were recorded in autumn 2010. With a proportion of 53.1% females to the total catch ($n_{females} = 3205$; $n_{males} = 2835$), the overall sex ratio (females : males = 1 : 0.88) was significantly (χ^2, $P < 0.001$) different from an expected equilibrium.

Other invasive Ponto-Caspian gobies like the bighead goby *Ponticola kessleri* (Günther, 1861) and the tubenose goby *Proterorhinus semilunaris* (Pallas, 1814) were found continuously but in much lower abundances (< 3% of total catch). One specimen of the racer goby *Babka gymnotrachelus* (Kessler, 1857) was found at river stretch #05 “Mariaposching” as a first record in Germany (Haertl *et al.*, 2012). Barbel *B. barbus* and chub *S. cephalus* were the most abundant autochthonous fish species detected in each of the three investigated areas in the upper Danube River comprising about 9% of the total catch. Other fish species mainly comprised ide *Leuciscus idus* (L., 1758), bleak *Alburnus alburnus* (L., 1758), common nase *Chondrostoma nasus* (L., 1758), European perch *Perca fluviatilis* (L., 1758) and pike-perch *Sander lucioperca* (L., 1758) as well as European eel *Anguilla anguilla* (L., 1758) and to some extent burbot *Lota lota* (L., 1758), Wels catfish *Silurus glanis* L., 1758 and northern pike *Esox lucius* L., 1758.

Species of high conservation priority such as zingel *Zingel zingel* (L., 1766) and the gudgeon species *Romanogobio vladikovy* (Fang, 1943) endemic to the Danube basin, as well as bullhead *Cottus gobio* L., 1758 and schneider *Alburnoides bipunctatus* (Bloch, 1782) were present in very low abundances and limited to the river stretches #06 and #01 .

Round goby population data

The CPUE of *N. melanostomus* differed significantly (Kruskal-Wallis, $P < 0.001$) between investigated areas (Table 6.2). In the IF2010 population, the CPUE (mean ± S.E.) of 0.4 ± 0.3 [PAS^{-1}] was about 10-fold (Mann-Whitney U, $P < 0.001$) lower compared to the area where the species has been established for at least 30 months (mean ± S.E. = 3.9 ± 0.4 [PAS^{-1}]). No significant differences in the mean CPUE were observed neither between IF2009 (mean = 2.1 [PAS^{-1}]; S.D. = 0.6 [PAS^{-1}]) and IF2010, nor between IF2009 and the established area. The proportion of point abundance samples containing *N. melanostomus* (f_O) significantly (Kruskal-Wallis, $P < 0.001$) differed between the investigated areas. In the established area, f_O was significantly (Mann-Whitney U; $P < 0.001$) higher (f_O = 82%; S.E. = 3%) compared with the IF2010 (f_O = 15 %; S.E. = 10%). No difference in f_O was observed between the established area and the IF2009 (f_O = 54 %; S.E. = 14%). In the established area, peak-abundances of 25 round goby PAS^{-1} (river stretch “07_Regensburg”; n = 1490; mean = 5.9 PAS^{-1}; S.D. = 3.6; f_O = 97.4%) and 26 round goby PAS^{-1} (river stretch “02_Vilshofen”; n = 1943; mean = 8.0 PAS^{-1}; S.D. = 5.3; f_O = 95.9%) were observed in autumn 2011. At the IF2010, round goby CPUE (20-fold) and f_O (8-fold) increased from the late season 2010 to 2011. Analogously, at the IF2009, CPUE (27-fold) and f_O (12-fold) increased from the late season 2009 to 2010. In both cases, one year later (i.e. the second year after the first record), round goby population density had doubled, reaching values similar to those from the established area (Table 6.2).

The mean CPUE of barbel and chub significantly (Kruskal-Wallis, $P < 0.001$) differed between the investigated areas being inversely related to goby abundance (Table 6.2). At the IF2010, the mean CPUE (mean = 1.8 [PAS^{-1}]; SE = 0.2 [PAS^{-1}]) of barbel and chub was significantly (Mann-Whitney U, $p < 0.001$) higher (about 20-fold) as compared with the established area (mean = 0.1 [PAS^{-1}]; S.E. = 0.03 [PAS^{-1}]) and (about 10-fold) the IF2009 (mean = 0.2 [PAS^{-1}]; S.E. = 0.02 [PAS^{-1}]). No significant difference in the mean CPUE was observed between the IF2009 and the established area. Also, the mean f_O of barbel and chub significantly (Kruskal-Wallis, $P < 0.001$) differed between the investigated areas. At the IF2010, the mean f_O was significantly (Mann-Whitney U; $P < 0.001$) higher (f_O = 69 %; S.E. = 5 %) as compared with the IF2009 (f_O = 13%; S.E. = 2%) and the established area. At the IF2009, mean f_O was significantly (Mann-Whitney U; $P < 0.05$) higher (f_O = 7 %; S.E. = 1%) as compared with the established area.

L_T of recorded *N. melanostomus* varied from 34 to 163 mm in females, and from 40 to 187 mm in males. The contribution of different length cohorts (Fig. 6.2) was not normally distributed (Lilliefors, $P < 0.05$) in each of the investigated populations. The largest individuals of each sex were captured in the established area as might be expected from the larger sample size (n = 5380; 99th percentile of L_T = 14.2 cm). However, mean L_T was highest (Kruskal-Wallis, $P < 0.001$) in the IF2010 (n =108; 99th percentile of L_T = 15.2 cm) in both sexes (Table 6.3, Fig. 6.2). Females and males from the IF2010 were both significantly (Mann Whitney U, $P < 0.001$) larger (both by about 20 %) than in the IF2009 and the established area (Mann-Whitney U, $P < 0.001$), by about 25 % and 16 %, respectively. Also, females from the IF2009 were significantly (Mann-Whitney U, $P < 0.01$) larger than in the established area (Table 6.3). Males from the IF2009 were not significantly larger than those from the established area. Females were larger than their male conspecifics at the IF2010 and at the IF2009, but not significantly. Males however, were significantly (Mann-Whitney U, $P < 0.001$) larger than females in the established area.

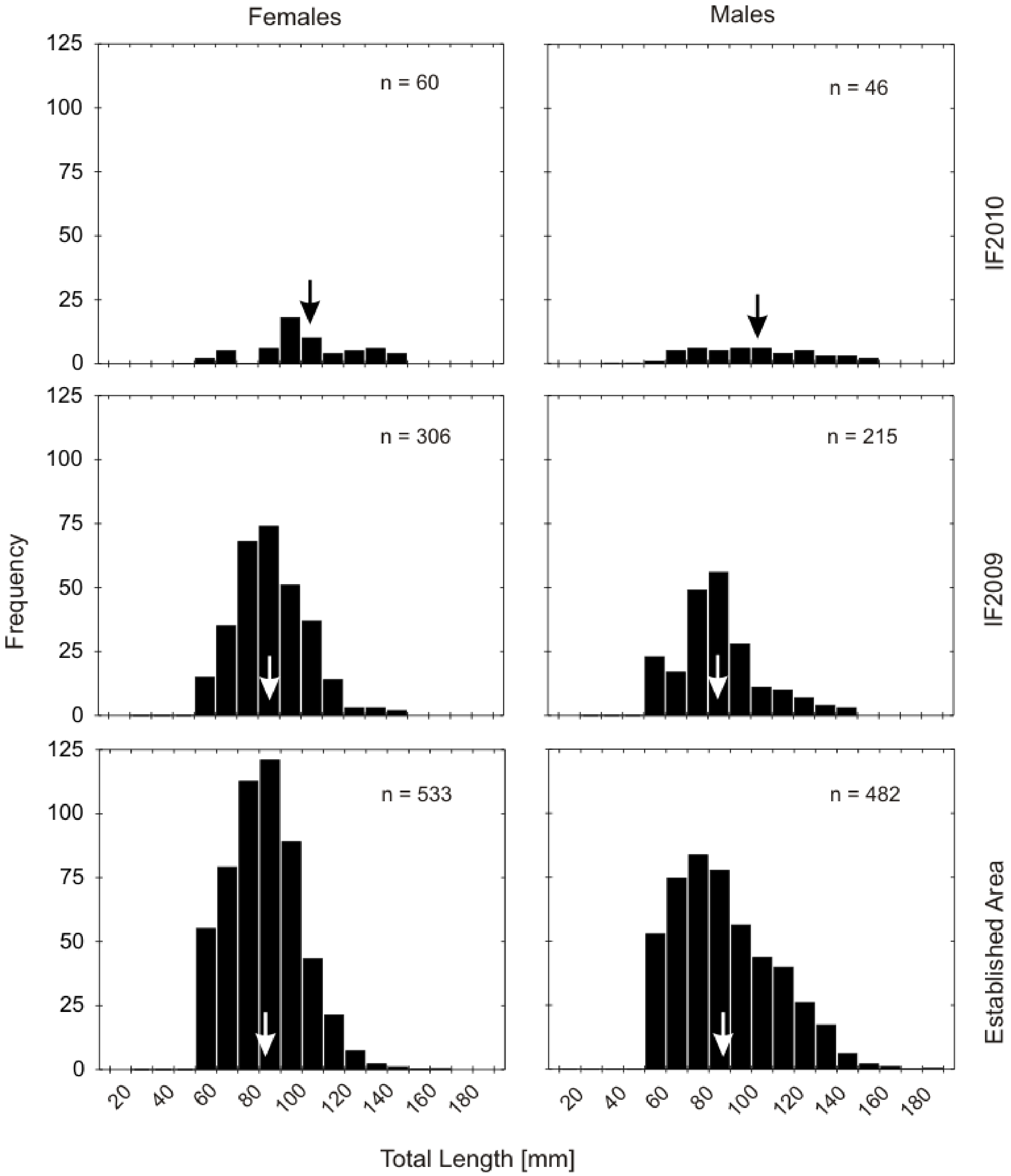

Fig. 6.2: Length-frequency distributions of newly colonizing and established populations

Length-frequency distributions (L_T in mm) of newly colonizing (invasion front 2010, “IF2010” and invasion front 2009, “IF2009”) and established (first record before 2007) *N. melanostomus* populations in the upper Danube River, Germany (left row: females; right row: males). The mean total lengths [mm] of each sex are indicated by arrows. The established area was normalised to 300 PAS-points to display identical catch effort in all populations. Note that juveniles (L_T < 5cm) were excluded from sex-specific comparison since definite assignment of sex remained doubtful in many cases for this size class without dissection.

Table 6.3: Comparison of performance indicators of *N. melanostomus* at population level

72 sub-populations from the upper Danube River were assigned to the categories “IF2010” (10 sub-populations), “IF2009” (10 sub-populations) and “established area” (52 sub-populations) using time since invasion (see Table 6.2). Numbers of fish analyzed, means and corresponding standard deviations (S.D.) of total length (L_T), weight (M_T) and Fulton´s condition factor (K) are displayed for both sexes and for juveniles (L_T < 5 cm). Percent females and males, as well as the overall sex-ratio were calculated from the total catch (excluding juveniles) of the sub-populations, respectively. Superscript letters denote significant differences (Kruskal-Wallis test) with p-values encoded by asterisks (*denotes $P \leq 0.05$; ** denotes $P < 0.01$; *** denotes $P < 0.001$). Values highlighted in bold denote significant (Mann-Whitney U-test) differences between sexes. Values in italics denote significant (χ^2 test) differences in the contribution of sexes between sampling areas.

Population-Level		IF2010			IF2009			Established Area		
Performance Indicators	*P*	n	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.
$L_{T\ \mathrm{females}}$ [cm]	***	60	10.4^{a}	2.3	306	8.6^{b}	1.7	2757	**8.3** ^{c}	1.8
$L_{T\ \mathrm{males}}$ [cm]	***	46	10.2^{a}	2.6	215	8.5^{b}	2.0	2478	**8.8** ^{b}	2.4
$L_{T\ \mathrm{juveniles}}$ [cm]	ns	2	4.6^{a}	0.1	95	4.3^{a}	0.4	633	4.3^{a}	0.5
$M_{T\ \mathrm{females}}$ [g]	***	60	20.2^{a}	13.0	306	10.4^{b}	7.3	2757	**9.0** ^{c}	6.5
$M_{T\ \mathrm{males}}$ [g]	***	46	18.5^{a}	14.4	215	10.3^{b}	8.6	2478	**11.7** ^{b}	10.6
$M_{T\ \mathrm{juveniles}}$ [g]	ns	2	1.4^{a}	0.3	95	1.0^{a}	0.3	633	1.0^{a}	0.4
K_{females}	***	60	**1.56** ^{a}	0.14	306	**1.44** ^{b}	0.18	2757	**1.38** ^{c}	0.18
K_{males}	***	46	**1.50** ^{a}	0.12	215	**1.41** ^{b}	0.16	2478	**1.36** ^{c}	0.17
$K_{\mathrm{juveniles}}$	ns	2	1.40^{a}	0.16	95	1.22^{a}	0.24	633	1.23^{a}	0.27
females [%]		60	56.6		**306**	58.7		**2757**	52.7	
Males [%]		46	43.4		**215**	41.3		**2478**	47.3	
Overall Sex Ratio (f : m)	***	106	1 : 0.77		571	*1 : 0.70*		5235	*1 : 0.90*	

M_T of *N. melanostomus* varied from 0.4 to 63.0 g in females, and from 0.4 to 98.4 g in males. Analogously to the trends observed in L_T, the heaviest individuals of each sex were captured in the established area. However, both in females and males, the mean M_T was highest (Kruskal-Wallis, $P < 0.001$) in the IF2010 population. In females, M_T from the IF2010 was both significantly (Mann-Whitney U, $P < 0.001$) higher than in the IF2009 and the established populations. M_T of females from the IF2009 was significantly (Mann-Whitney U, $P < 0.01$) higher than in the established area (Table 6.3). Also in males, M_T from the IF2010 was significantly (Mann-Whitney U, $P < 0.001$) higher than in both IF2009 and established area. Males from the IF2009 were not significantly heavier than those from the established area (Table 6.3). Females were heavier than their male conspecifics at the IF2010 and at the IF2009, however not significantly. Males however, were significantly (Mann-Whitney U, $P < 0.001$) heavier than females in the established area.

Both in females and males, K significantly (Kruskal-Wallis, $P < 0.001$) differed between the investigated populations. In females, the highest value (Mann-Whitney U, $P < 0.001$) was recorded at the IF2010, a medium value (Mann-Whitney U, $P < 0.001$) at the IF2009, and the lowest value (Mann-Whitney U, $P < 0.001$) in the established area. Also in males, K was highest (Mann-Whitney U, $P < 0.001$) at the IF2010, medium (Mann-Whitney U, $P < 0.001$) at the IF2009 and lowest (Mann-Whitney U, $P < 0.01$) at the established area (Table 6.3). In females, K was significantly higher than in males at the IF2010 (Mann-Whitney U, $P < 0.05$), at the IF2009 (Mann-Whitney U, $P < 0.05$) and at the established area (Mann-Whitney U, $P < 0.001$).

The proportion of fish smaller than 5 cm (juveniles) was 2% in the IF2010, 16% in the IF2009 and 9 % in the established area. No juveniles in this size-class were recorded at IF2010 and at IF2009 when round goby had been detected there for the first time.

All areas investigated were female dominated, with differences from equilibrium being significant at the IF2009 (χ^2, $p<0.001$) and the established area (χ^2, $P < 0.001$) (Table 6.3). Both at the IF2010 and the IF2009, relatively higher proportions of females were observed than in the established area. However, this female dominated pattern in the distribution of sexes was only significantly different (χ^2; $P < 0.01$) between IF2009 and the established area (Table 6.3).

An analysis of the 72 *N. melanostomus* samplings using NMDS revealed two clusters, with a separation of invasion front samples from established ones (Fig. 6.3 A), particularly for the 2010 data. The main factors underlying this pattern were found to be catch data, length and weight differences, whereas the sex ratio was not important (Fig. 6.3 B, C, D). This pattern remained stable, independent of using arithmetic mean or median values (data not shown) as input variables in the analyses. Overall, differences between established and invasion front populations were far more pronounced than differences among all established populations from different locations.

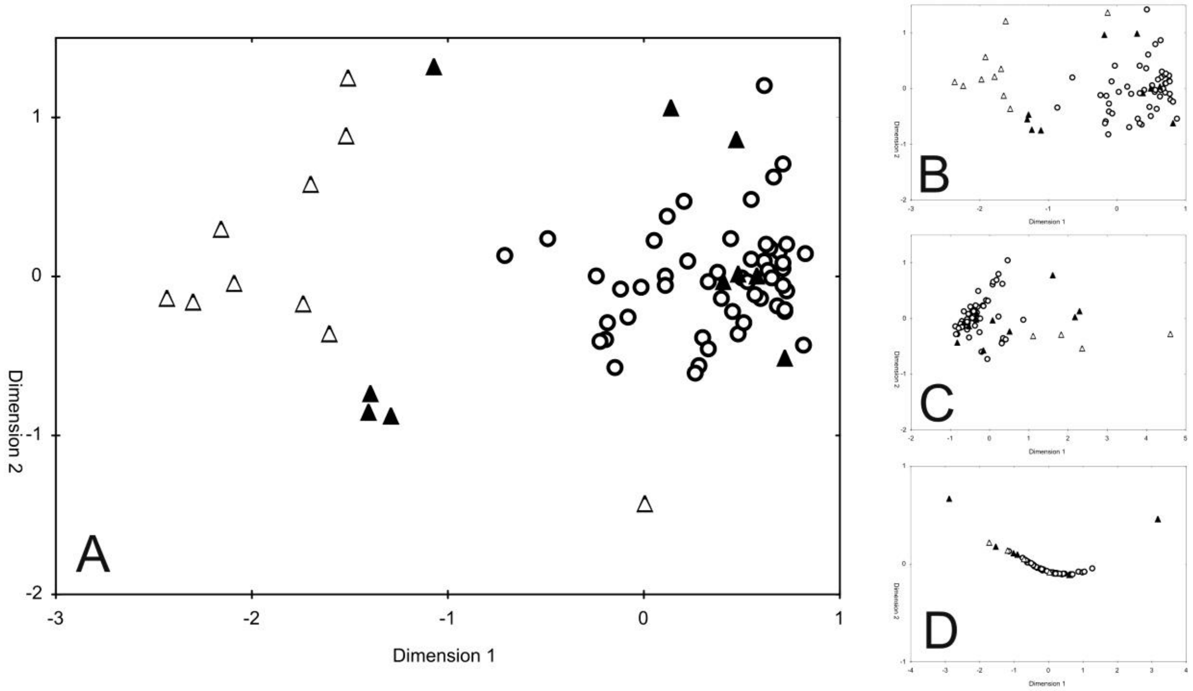

Fig. 6.3: Nonmetric multidimensional scaling of *N. melanostomus* performance metrics

Nonmetric multidimensional scaling (NMDS), of *N. melanostomus* population-specific performance metrics calculated from point-abundance sampling data (autumn 2009 – autumn 2011). Dissimilarity-distances between 72 samplings from 10 river stretches (rip-rap habitats) were calculated using the squared Euclidian distance and displayed by triangles (invasion front 2010, “IF2010”), filled triangles (invasion front 2009, “IF2009”) and circles (established populations). L_T(f), L_T(m), L_T(j), M_T(f), M_T(m), M_T(j), K(f), K(m), K(j), proportion of females and catch data (mean CPUE and frequency of occurrence of (i) *N. melanostomus*, (ii) *Barbus barbus* and *Squalius cephalus* (combined) and (iii) other fish species) from the corresponding sampling sites were used as variables in panel A (stress = 0.10). Catch data were analyzed in panel B (stress = 0.11). L_T(f), L_T(m), L_T(j), M_T(f), M_T(m), M_T(j), were analyzed in panel C (stress = 0.08) and the sex ratio (proportions of females and males) was analyzed in panel D (stress = 0.001).

Round goby specimen data

In females, the mean GSI differed between the investigated areas (Kruskal-Wallis; $P < 0.001$) with lowest values at the IF2010. At the IF2010, female GSI was significantly lower than in the IF2009 (Mann-Whitney U; $P < 0.001$) and the established populations (Mann-Whitney U; $P < 0.05$). Also in males, the GSI was lowest in the IF2010, however differences were not significant (Table 6.4).

Fulton´s Condition factor significantly differed between the investigated populations both in females (Kruskal-Wallis; $P < 0.01$) and males (Kruskal-Wallis; $P < 0.05$) with highest values at the invasion front. In females, K was significantly (Mann-Whitney U; $P < 0.01$) higher at the IF2010 compared with the established area. Also in males, K was both significantly (Mann-Whitney U; $P < 0.05$) higher at the IF2010 compared with the IF2009 and the established area. No significant sex-specific differences in K were observed within the populations analyzed.

Table 6.4: Comparison of performance indicators of *N. melanostomus* at specimen level

365 specimens (mean L_T = 9.8 cm; S.D. = 1.2 cm) originating from the investigated sub-populations “IF2010”, “IF2009” and “established area” along the upper Danube River (early season 2010 – late season 2011) were sampled for analyses. Numbers of fish dissected, means and corresponding standard deviations (S.D.) of fecundity and condition indices (gonado-somatic index GSI, Fulton´s Condition Factor *K*, hepato-somatic index HSI), stable isotope signatures ($\delta^{15}N$, $\delta^{13}C$), feeding indices (index of stomach fullness I_{SF}; index of food importance of Ephemeroptera, Trichoptera and Plecoptera $I_{FI\ (EPT)}$) and prey-specific indices (catch per unit effort CPUE $_{(EPT)}$ and index of environmental importance of Ephemeroptera, Trichoptera and Plecoptera $I_{EI\ (EPT)}$) and parasite infection indices were calculated for females and males. Values highlighted in bold denote significant differences (Mann-Whitney U-test) between sexes. Superscript letters denote significant differences (Kruskal-Wallis test) between populations with *P*-values encoded by asterisks (*denotes $P \leq 0.05$; ** denotes $P < 0.01$; *** denotes $P < 0.001$).

Specimen-Level		Invasion Front 2010			Invasion Front 2009			Established Area		
performance indicators	*P*	n	mean	S.D.	n	mean	S.D.	n	mean	S.D.
Fecundity and Condition										
GSI $_{females}$	***	18	2.0 ^{a}	3.3	18	3.6 ^{b}	4.1	145	4.3 ^{b}	5.0
GSI $_{males}$	ns	13	0.1	0.1	18	0.7	1.7	153	0.3	0.8
$K_{females}$	**	18	1.51 ^{a}	0.09	17	1.47 ^{b}	0.15	140	1.41 ^{b}	0.14
K_{males}	*	12	1.47 ^{a}	0.07	17	1.38 ^{b}	0.09	145	1.39 ^{b}	0.12
HSI $_{females}$	***	18	**6.5** ^{a}	1.3	6	3.2 ^{b}	0.90	76	4.6 ^{c}	1.3
HSI $_{males}$	**	12	**5.4** ^{a}	0.7	6	3.4 ^{b}	0.8	74	4.4 ^{b}	1.3
Stable Isotopes										
$\delta^{15}N_{females}$ [‰]	***	17	15.59 ^{a}	0.40	18	15.89 ^{a}	0.50	146	**14.91** ^{b}	0.60
$\delta^{15}N_{males}$ [‰]	***	13	15.38 ^{a}	0.35	18	15.93 ^{b}	0.54	152	**14.75** ^{c}	0.57
$\delta^{13}C_{females}$ [‰]	ns	17	-29.34	-0.42	18	**-29.11**	-0.55	146	-28.96	-0.86
$\delta^{13}C_{males}$ [‰]	ns	13	-29.37	-0.52	18	**-29.45**	-0.40	152	-29.08	-0.87
Feeding and Prey-Specific Indices										
I_{SF}	ns	31	2.8	0.8	34	3.4	1.6	285	3.1	1.4
$I_{FI\ (EPT)}$	**	33	7.7 ^{a}	22.9	46	5.3 ^{b}	19.5	500	0.6 ^{b}	5.1
CPUE $_{(EPT)}$ [min^{-1}]	***	24	1.4 ^{a}	1.22	30	0.3 ^{b}	0.45	161	0.2 ^{b}	0.56
$I_{EI\ (EPT)}$	***	24	3.1 ^{a}	8.5	30	1.1 ^{b}	2.9	161	0.1 ^{c}	0.1
Endoparasites (Acanthocephala)										
abundance [n] females	***	18	**108** ^{a}	54	18	20 ^{b}	17	146	49 ^{b}	68
abundance [n] males	***	13	**57** ^{a}	27	18	10 ^{b}	15	152	36 ^{c}	53
density [n/g] females	***	18	6.4 ^{a}	3.5	18	**1.4** ^{b}	1.2	146	**3.3** ^{b}	4.0
density [n/g] males	***	13	4.3 ^{a}	2.1	18	**0.7** ^{b}	0.7	152	**2.4** ^{c}	2.9
Ectoparasites (*Rossicotrema* spp.)										
abundance [0-3] females	ns	18	0.06	0.24	18	0.00	0.00	146	0.08	0.29
abundance [0-3] males	ns	13	0.15	0.38	18	0.00	0.00	152	0.07	0.30

The mean $\delta^{15}N$-values in both females and males significantly (Kruskal-Wallis; $P < 0.001$) differed between the investigated areas, with lowest values in the established area and highest values at the IF2009. Compared with the established area, in females, mean $\delta^{15}N$-values were significantly (Mann-Whitney U; $P < 0.001$) higher both at the IF2010 ($\Delta_{\delta 15N}$ = 0.7‰) and the IF2009 ($\Delta_{\delta 15N}$ = 1.0‰). No significant differences in female $\delta^{15}N$-values were observed between the IF2010 and the IF2009. Also in males, the mean $\delta^{15}N$-values were significantly (Mann-Whitney U; $P < 0.001$) higher at the IF2010 ($\Delta_{\delta 15N}$ = 0.6‰) and the IF2009 ($\Delta_{\delta 15N}$ = 1.2‰), compared with the established area. In case of males, mean $\delta^{15}N$-values significantly (Mann-Whitney U; $P < 0.001$) differed between the IF2010 and the IF2009. A significant (Mann-Whitney U; $P < 0.01$) sex-specific difference between females and males in the mean $\delta^{15}N$-value was observed in the established area only, where females had lower $\delta^{15}N$ values than males.

The $\delta^{15}N$ values of muscle tissue and gut contents of the additional *N. melanostomus* samples followed similar functions and were strongly dependent on L_T (Fig. 6.4). Both datasets from the established area (muscle tissue: $R^2 = 0.541$, $P < 0.001$; gut content: $R^2 = 0.306$, $P < 0.001$) and the IF2010 (muscle tissue: $R^2 = 0.213$, $P < 0.001$; gut content: $R^2 = 0.161$, $P < 0.001$) were highly significantly described by parabolic regressions with size and diet-tissue shifts of 3.1‰ (S.E. 0.3‰) in case of the established area and 4.7‰ (S.E. 0.2‰) in case of the IF2010. These mean diet-tissue shifts were significantly (Mann-Whitney U; $P < 0.001$) different and the residuals of the regressions indicated that diet and muscle were predicted equally well with a slight parabolic trend in the residuals. The $\delta^{15}N$ value of the gut content of *N. melanostomus* changed with L_T during the observed growth-phases. In the established population, $\delta^{15}N$ values increased by about 2.5‰ up to a L_T of 10 cm, and then decreased again, while $\delta^{15}N$ values increased by about 0.8‰ up to a L_T of 12.5 cm, and then slightly decreased in the IF2010. Notably, the mean $\delta^{15}N$ value of the gut contents was calculated from the mean $\delta^{15}N$ values of the detected species and thus reflects the change in the composition of the prey species but not an isotopic change within the individual prey species.

The mean $\delta^{13}C$ values did not significantly differ between the investigated populations in both sexes; however a significant (Mann-Whitney U; $P < 0.01$) sex-specific difference was observed in the IF2009.

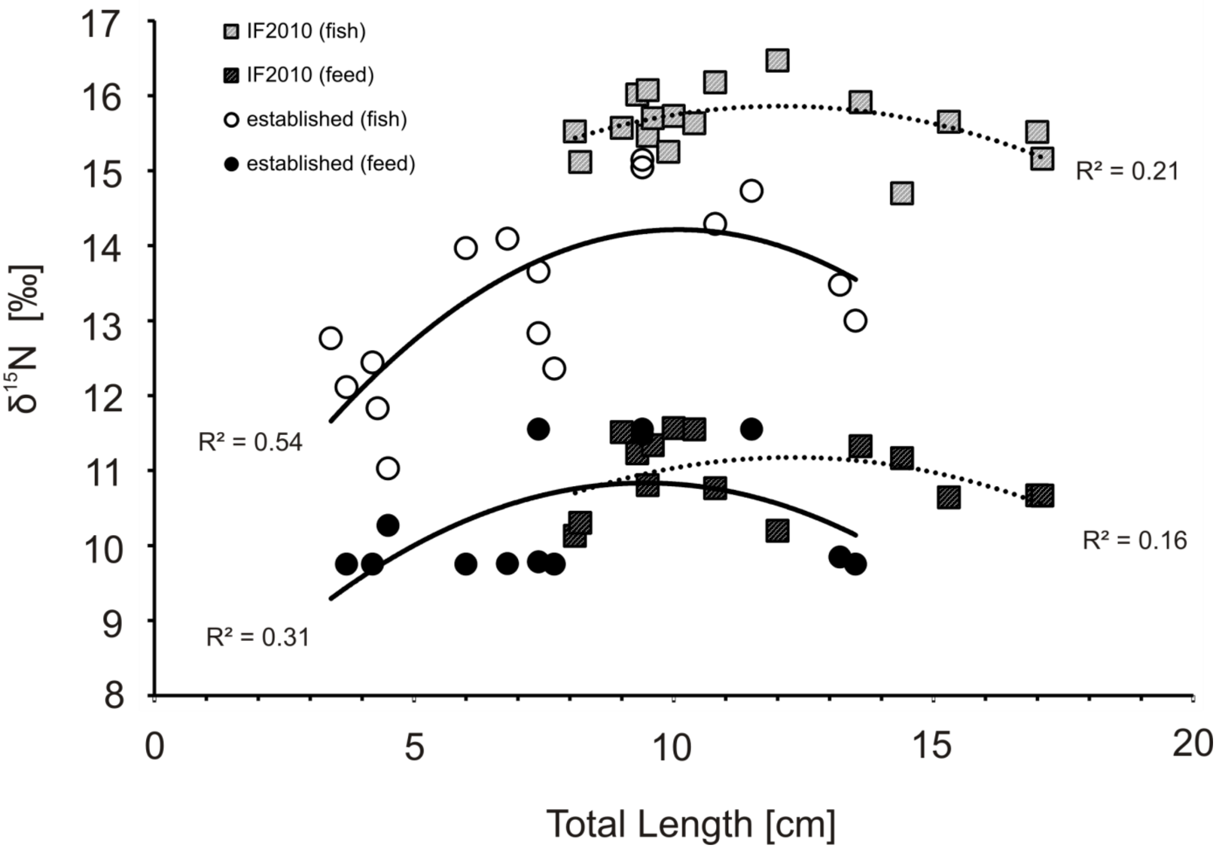

Fig. 6.4: Diet-tissue shift and ontogenetic dietary shift in *N. melanostomus*

Changes (diet-tissue shift, ontogenetic dietary shift) in the relative nitrogen isotope ratio of gut contents ("Feed", filled symbols) and muscle tissue of *N. melanostomus* ("Fish", open symbols) from the invasion front 2010 ("IF2010", squares, dashed lines) and the "established area" (circles, continuous lines) are displayed in relation to the total length. Lines are parabolic regressions ($P < 0.001$) based on total length and the type of tissue (with R² given in the diagram).

The mean HSI significantly differed between the investigated populations both in females (Kruskal-Wallis; $P < 0.001$) and males (Kruskal-Wallis; $P < 0.01$), with highest values at the IF2010. Compared with the IF2009, mean HSI in females was significantly higher in the IF2010 (Mann-Whitney U; $P < 0.001$) and the established area (Mann-Whitney U; $P < 0.05$). In females, the mean HSI was significantly (Mann-Whitney U; $P < 0.001$) higher in the IF2010 than in the established area. In males, the mean HSI was significantly higher at the IF2010 (Mann-Whitney U; $P < 0.05$) and the established area (Mann-Whitney U; $P < 0.01$) compared with the IF2009, whereas no significant difference was observed between the IF2010 and the established area. In females, the mean HSI was significantly (Mann-Whitney U; $P < 0.05$) higher (about 20%) compared to males in the IF2010.

The I_{SF} did not significantly differ between the investigated populations. No significant sex-specific differences were observed within the mean I_{SF} within the populations, indicating a similar feeding status.

Benthic invertebrate availability

The benthic invertebrate community mainly consisted of highly abundant amphipods (*Dikerogammarus spp.*, *Chelicorophium spp.*, *Jaera spp.),* molluscs (*Dreissena spp.*, *Corbicula spp., Potamopyrgus spp.*) and other exotic species*,* primarily originating from the Ponto-Caspian area. Overall, alien species comprised more than 50% of all taxa and about 90 % of I_{FI}. However no differences in I_{FI} could be observed between the analyzed areas. In contrast to molluscs and amphipods, all Ephemeroptera, Trichoptera and Plecoptera (EPT) were indigenous and part of the typical and original fauna. The mean I_{FI} of EPT significantly (Kruskal-Wallis; p<0.01) differed between the investigated areas. The mean $I_{FI\ (EPT)}$ was significantly (Mann-Whitney U; $P < 0.01$) higher in the IF2010 (about 13-fold) and the IF2009 (about 9-fold, but not significantly) compared with the established area. Except for the cumulative category EPT, no significant differences in both CPUE and I_{EI} of all other benthic invertebrate taxa were observed between the analyzed river stretches. The mean $CPUE_{(EPT)}$ significantly (Kruskal-Wallis; $P < 0.001$) differed between the investigated areas. At the IF2010, the mean CPUE was both significantly (Mann-Whitney U; $P < 0.001$) higher than in the IF2009 (about 5-fold) and the established area (about 8-fold). No significant difference in the mean $CPUE_{(EPT)}$ was observed between the IF2009 and the established area. The mean $I_{EI\ (EPT)}$ significantly (Kruskal-Wallis; $P < 0.001$) differed between the investigated areas, with highest values at the IF2010 and lowest values in the established area. The mean $I_{EI\ (EPT)}$ of the IF2010 was significantly (Mann-Whitney U; $P < 0.001$) higher (30-fold) compared with the established area and the IF2009 (3-fold), but not significantly. The mean $I_{EI\ (EPT)}$ did not significantly differ between IF2009 and the established area.

Parasitic load

Indicators for (endoparasitic) acanthocephala infection, abundance and density significantly (Kruskal-Wallis; $P < 0.001$) differed between the investigated areas in females and males, with highest values at the IF2010. At the IF2010, the mean acanthocephalan abundance in females was both significantly (Mann-Whitney U; $P < 0.001$) higher compared with in the IF2009 (about 5-fold) and the established area (about 2-fold). The mean abundance of acanthocephalans in males from the IF2010 was significantly (Mann-Whitney U; $P < 0.001$) higher (about 6-fold) compared to the IF2009 and significantly (Mann-Whitney U; $P < 0.01$) higher compared to the established area (about 2-fold). Males from the IF2009 had a significantly (Mann-Whitney U; $P < 0.01$) lower (3-fold) mean abundance of acanthocephalans than males from the established population. The only sex-specific difference was observed in the IF2010, where females had a significantly (Mann-Whitney U; $P < 0.01$) higher mean abundance of acanthocephalans than their male conspecifics.

At the IF2010, the mean density of acanthocephalans in females was both significantly (Mann-Whitney U; $P < 0.001$) higher compared with in the IF2009 (about 5-fold) and the established area (about 2-fold). Also in males, the mean density of acanthocephalans from the IF2010 was significantly (Mann-Whitney U; $P < 0.001$) higher (about 6-fold) compared with the IF2009 and significantly (Mann-Whitney U; $P < 0.01$) higher compared to the established area (about 2-fold).

Males from the IF2009 had a significantly (Mann-Whitney U; $P < 0.01$) lower (3-fold) mean density of acanthocephalans than males from the established area. Females had significantly (Mann-Whitney U; $P < 0.05$) higher densities of acanthocephalans than males in both the IF2009 (2-fold) and the established area (1.4-fold). The mean abundance of ectoparasites of the genus *Rossicotrema spp.* (Plathyhelminthes) was generally low and did neither significantly differ between specimens of the populations analyzed nor between sexes (Table 6.4).

6.5 Discussion

This study provides evidence of differences in demography and sex ratio, morphology, feeding behaviour and parasitic load of invasive round gobies among specimens sampled at an invasion front and those from the established area. These results support the previously suggested plasticity of this species based on comparisons of population data from the native range with those from invaded areas (Gutowsky & Fox, 2011; Brownscombe & Fox, 2012; Gutowsky & Fox, 2012). Pioneering populations from the invasion front were female dominated, comprising large sized, heavier individuals with highest condition and lowest gonado-somatic index. At the established area, *N. melanostomus* revealed an ontogenetic diet shift with a switch from preying upon insects and crustaceans to a mainly mollusc dominated diet at a L_T of 10.0 cm. In contrast, the pioneering population (IF2010) exhibited a less pronounced, more continuous diet shift with a deferred and weaker diet switch at a larger size of about 12.5 cm. According to the "*enemy release*"-hypothesis, lower abundance and density of endoparasites would have been expected at the invasion front. Instead, opposite results of higher acanthocephalan loads were observed in both sexes at the invasion front (IF2010) and seemingly did not hamper invasion success. Compared with the established area, CPUE and I_{FI} of indigenous Ephemeroptera, Trichoptera and Plecoptera was higher at the invasion front, whereas no differences in both CPUE and I_{EI} of all other benthic invertebrate taxa were observed between all analyzed river stretches. Generally, the IF2009 behaved intermediately, with characteristics of both the IF2010 and the established population, underlining the high pace of the observed invasion processes. Overall, the pronounced changes in fish and invertebrate communities with a dominance of alien species suggest an invasional meltdown and a shift of the upper Danube River towards a novel ecosystem with species that have greater resistance to goby predation. This seems to contribute to overcoming biological resistance and improve rapidity of dispersal.

Increased competitive ability

In line with our initial hypothesis, female and male round gobies from the invasion front were bigger (larger and heavier), revealing higher condition factors than those from established areas. These characteristics probably increase their performance and competitive ability, also reducing predation risk at the early stages of the invasion process. In turn, this can contribute to better chances for establishment and further spread. The greater availability of prey and a smaller degree of intraspecific competition in novel areas of distribution may also contribute to this pattern. Generally, females had a higher condition than males, but did not differ in body-size and weight. In line with our findings, Gutowsky and Fox (2011) also caught the largest individuals of each sex at initially invaded areas, but found significantly larger males than females at the edges of upstream expansion areas. This size-specific difference may result from using angling as a sampling method by these authors since angling was found to be selective for larger males (Brandner *et al.*, 2013b). In contrast to our findings, Brownscombe and Fox (2012) caught smaller round gobies at recently invaded areas compared to longer established sites, indicating that local habitat conditions and community structure can strongly influence trait selection.

According to the results of our study, five to seven years after introduction, males from the established area seem to grow larger and become heavier than females, reflecting observed sexual dimorphism and indicating major changes within invasive populations of round goby over time. In contrast to the increased somatic growth in initially invaded areas, the lower GSI of females at the invasion front compared to established areas suggests that somatic performance seems to be more important than investment in reproduction during the early stages of the invasion process. It needs to be noted, however, that egg size may also play a role since individuals with the same GSI but different egg sizes can produce different numbers of offspring. Such relationships between fecundity, egg size and juvenile performance are well known in many fish species (Ponce de León, 2011), however, there is no clear association between egg size and maximum body length of newborn gobiids (Miller, 1984). Males had a similar reproductive power along the invasion pathway (constant by time), while fecundity of females increased over time since invasion. A similar pattern had been reported from the Trent-Severn-Waterway, where GSI in female round gobies increased by time, too (Gutowsky & Fox, 2012). A possible explanation may be that the age structure of the studied populations changed over time, with increased fecundity in larger and older females which occur at higher frequency later in the year.

In case of plants, an "*evolution of increased competitive ability*" has been proposed, suggesting that specimens produce more seeds or grow more vigorous and taller in environments outside their native ranges (Blossey & Nötzold, 1995; Crawley, 1987). This concept also seems applicable to round goby and may explain the invasive success of this species, particularly in the early stages of the invasion process. In the case of gobies, stronger emphasis seems to be put on growth-related traits instead of reproductive traits to increase competitive ability. Also, the fact that they are dispersing into a highly altered environment containing alien but familiar food resources could play a role.

Effects on the food web

Different feeding strategies of invasive round goby were detected between established and pioneering populations. According to the stable isotope analyses, only females and males from the IF2010 utilized similar food resources, as evident from both similar $\delta^{15}N$ and $\delta^{13}C$ signatures indicating the same trophic niche. A clear sex-specific difference was observed both in the established population, indicated by different $\delta^{15}N$ -values (with no difference in $\delta^{13}C$-values), and in the IF2009, indicated by different $\delta^{13}C$-values (with no difference in $\delta^{15}N$ -values). Such sex-specific signatures could result from selective feeding or competition between males and females under food-resource limitation (the latter seems unlikely because no differences in the distribution and abundance of benthic invertebrates had been found except for EPT). These sex-specific differences in SIA could also derive from different habitat utilisation, thus indicating beginning habitat saturation.

The $\delta^{15}N$ signatures of the recently invaded IF2010 and IF2009 exceeded the values of the established area indicating a slightly higher trophic level there, which may result from a targeting of more valuable larger-sized, energetically enriched prey.

Food web baseline variation between the river stretches, i.e. the mean decrease / increase in $\delta^{15}N$-values of the primary consumers *D. polymorpha* and *C. fluminalis* (Van Riel, 2006), which was not corrected for, only played a minor role, as $\delta^{15}N$-values (mean = 9.91, SD = 0.31) had a very narrow range.

At the established area, *N. melanostomus* exhibits a pronounced and continuous ontogenetic diet shift, which determines a broad dietary niche at the population level. At a total length of about 10 cm, it switches from preying upon insects and crustaceans (increasing limb, Fig. 6.4) to a mainly mollusc dominated diet (decreasing limb, Fig. 6.4), which can also be interpreted as an increasing specialization at the individual level (Brandner *et al.*, 2013a). The IF2010 population exhibits a less pronounced, more continuous diet shift, indicating a narrower dietary niche. At a total length of about 12.5 cm, *N. melanostomus* tend to switch from an amphipod-based diet (increasing limb, Fig. 6.4) to some preying upon molluscs (decreasing limb, Fig. 6.4), which mirrors a high preference towards amphipods under conditions of low intraspecific competition. This plasticity within an ontogenetic determined behaviour may contribute to the high invasion success.

The decreasing trend in CPUE and I_{EI} in EPT by time, with highest values at the IF2010 clearly highlights the impact of round goby on native biodiversity, similar to the one described by Kipp and Ricciardi (2012) for North American rivers. Underlining the extraordinary preference of round goby for these native taxa, the $I_{\mathrm{FI\ (EPT)}}$ by far exceeded the $I_{\mathrm{EI\ (EPT)}}$ in all round goby populations (Table 6.4).

Along the 200 river-km invasion pathway of round goby within the upper Danube River, a benthic-invertebrate community, highly dominated by non-native species (similar in abundance and distribution) had already been established before round goby invaded. This highly altered benthic invertebrate community with an aquatic fauna typical for lower sections of streams, found for the whole investigated stretch of the upper Danube River, corroborates analogous findings from the River Rhine (Van Riel, 2006). Round goby and other Ponto-Caspian neogobiids rather seem to complete a faunistic homogenization of such homogenized large rivers (Moyle & Mount, 2007) than they would represent an independent single species invasion phenomenon. The observed impact on EPT may underline this ongoing process, described as an "*invasional meltdown*" (Simberloff & Von Holle, 1999), which already seemed to happen in an advanced stage at the upper Danube River. Such an invasional meltdown scenario was also discussed for the Laurentian Great Lakes area (Ricciardi, 2001), indicating similar conditions and developments there.

Sex ratio

Although males revealed a more exploratory behavior and greater moving distances in recent studies (Marentette *et al.*, 2011), round goby populations at invasion fronts appear to be female-biased (Brownscombe & Fox, 2012; Groen *et al.*, 2012; this study), while established populations seem to be typically male-dominated (Trent River: Gutowsky & Fox, 2011; Bronnenhuber *et al.*, 2011; Lake Ontario: Young *et al.*, 2010; Gulf of Gdansk, Baltic Sea: Corkum *et al.*, 2004). This observation is in line with this study, as four out of five first recorded invaders in autumn 2010 (right shoreline) and seven out of thirteen pioneers in autumn 2011 (right shoreline) were female at the IF2010, suggesting that a higher proportion of females may contribute to range expansion in round goby. Despite the sex-selectivity of the different sampling techniques used, which is higher in hook-and-line based sampling than in electrofishing as applied in this study (see Brandner *et al.* 2013b), migrating adult females appear to be a main driver of range expansion. Among various reasons, inbreeding depression avoidance, asymmetry in the costs of dispersal and mating system characteristics (Gros *et al.*, 2009) can cause sex-biased dispersal in invasions. Since male round gobies invest more energy in parental care and territorial defense than females, sex-biased dispersal by females could also be a possible strategy to first, reduce intraspecific competition for mates among females and second, to benefit from a lower predation risk at the invasion front (Brownscombe & Fox, 2013), which might be especially true for larger individuals.

Parasitic load

Fish parasites of the genus Acanthocephala, which are specific entoparasites with a complex life-cycle, were surprisingly found in highest densities and abundance in goby-specimens from IF2010, while unspecific ectoparasites (*Rossicotrema spp.*) were equally distributed in very low abundance and densities among gobies along the whole invasion pathway. Finding highest abundance and density of Acanthocephala in round goby from the invasion front compared to the other investigated areas contradicts our hypothesis and the "*enemy release*"-hypothesis". In the complex life-cycle of acanthocephalans, amphipods serve as highly species-specific intermediate hosts (Yamaguti, 1963). A high proportion of amphipods in the diets can lead to high infection rates of Acanthocephala in *N. melanostomus*, but simultaneously also to high values of lipid storage due to the nutritive value of the consumed prey. Thus even heavy acanthocephalan infections are unlikely to have a large pathogenic effect in gobies (Ondračková *et al.*, 2010). Consequently, the highest values of HSI, observed in the specimens from the IF2010 and IF2009 can probably be explained by better feeding conditions in these areas. Both, the higher HSI as a short-term indicator and the higher abundance of Acanthocephala as a long-term indicator mirror a better energetic status and probably higher fitness displayed by higher *K* in gobies from the invasion front compared with established populations. This seems especially to be true for females compared to their male conspecifics (Table 6.4). Since no difference had been observed in the degree of stomach fullness between all populations, no symptom for food limitation was observed using this metric.

Acanthocephala are also known to possess the ability to induce behavioural changes in their intermediate hosts, which increase the likelihood of becoming a prey for a fish (Bakker *et al.*, 1997). Since abundance of amphipods was equally distributed among the areas investigated, at the invasion front a smaller number of gobies can therefore chose among a relatively higher number of amphipods, possibly effectively selecting for infected intermediate hosts. In case of equally distributed infected intermediate hosts, a small number of gobies will acquire a higher number of acanthocephalans in areas with low goby abundance. Consequently, this effect also indicates an unlimited, “free-to-choose” availability of high valuable food-resources at an invasion front.

Time trends

The invasion in the upper Danube River can be considered a fast process. During study initiation, the most upstream located sampling stretch (#10 “Kelheim”) was intended to serve as a negative control area, free of round goby. However, *N. melanostomus* was established at the invasion front of the year 2009 (#09 “Bad Abbach”) within two years, and successfully invaded the projected negative control by upstream migration within one year. Due to their benthic morphology and their small home range, round gobies would be expected to have a poor natural dispersal ability, especially in upstream direction (Wolfe & Marsden 1998; Ray & Corkum, 2001). This study corroborates recently reported fast spread-rates with estimates ranging from 500 m year^{-1}on average (Bronnenhuber *et al.*, 2011) to up to 1–4 km year^{-1} in selected areas (Kornis *et al.*, 2012). Since this study indicated a spread rate, being up to four-times higher by covering even a distance of about 17 river-km in about one year, the high pace of round goby invasion might have been underestimated. Similarly, Brownscombe *et al.* (2012) calculated dispersal rates of 5 to 27 km year^{-1} using gamma distribution models. Generally, round goby riverine colonization appears to be driven by “stratified dispersal”, a strategy combined of contiguous diffusion over short distances by most individuals and long-distance colonization (jump events) by migrant individuals (Kornis *et al.*, 2012; Bronnenhuber *et al.*, 2011).

Given a low sampling bias (Brandner *et al.*, 2013b) and a minimum population doubling time (estimates based on empirical models) of 1.4 to 4.4 years (Froese & Pauly, 2013), the danubian invasion of *N. melanostomus* seems to be mainly driven by a high upstream directed propagule pressure from densely populated established areas with strong, large sized individuals, rather than by an increased reproductive success at the invasion front. The anti-cyclical trend between round goby density increase and population decrease of the two most abundant autochthonous fish species (barbel and chub) and EPT observed in this study (Table 6.2, Table 6.4), suggest alterations of the food web. It needs to be noted however, that the causality of these relationships needs further testing since no evidence for preying on eggs and larvae of other fishes was detectable.

The increasing trend in the CPUE at the established area (Table 6.2) indicates that the population density is still increasing there (73% to the total catch in the analyzed rip-rap mesohabitat), suggesting that the carrying capacity has not being reached yet. Round goby populations in Hamilton Harbor (North-American Great Lakes area) reached saturation densities approximately one decade after arrival, with densities being about 50% greater than the expected carrying capacity (Vélez-Espino *et al.*, 2010).

Conclusions

In this study, an upstream-directed colonization of *N. melanostomus* along a fluvial gradient with a distinct invasion front was observed, from total absence until establishment. Competitive ability and invasion success of the gobies at the invasion front seems to be largely determined by somatic investment ("bigger is better") instead of reproductive investment. The larger size and higher condition factor of gobies at the invasion front compared to those at established areas can be explained by less limited food resources in newly invaded areas. The finding of higher parasitic load at the invasion front was surprising and in contrast to expectations according to the "*enemy release*"-hypothesis, indicating that this factor is less important. The resulting pronounced changes in fish and invertebrate communities induced by the goby invasion suggest the occurrence of an invasional meltdown and a shift of the upper Danube River towards a novel ecosystem with communities and species that have greater resistance to goby predation. This seems to contribute to overcoming biological resistance and improve rapidity of dispersal. Such a complex change is also along the lines of what is happening to other aquatic systems in the world, i.e. the creation of novel ecosystems through the combination of environmental change and the impact of invasive species (Hobbs *et al.*, 2006; Hobbs *et al.*, 2009). As a result, novel ecosystems may provide different functional properties and ecosystem services, even though their persistence and values remain largely unknown (Hobbs *et al.*, 2009). This also appears to be true for the Danube River, where we observed a rapid ongoing shift from indigenous biodiversity towards a ubiquitous faunistic complex of potentially co-evolved exotic species which are adapted to human-altered aquatic systems. Consequently, especially the success of Ponto-Caspian invaders reflects fundamental ecological changes in the large European freshwater ecosystems (Brandner *et al.*, 2013a), which make a return to original communities almost impossible. This also questions the use of historical reference conditions and communities as a conservation target, e.g. in the context of the European Water Framework Directive and the development of any other conservation target.

7. First record of *Babka gymnotrachelus* from Germany

A similar version of this chapter was published as:

Haertl, M., A. F. Cerwenka, J. Brandner, J. Borcherding, J. Geist & U. K. Schliewen, 2012. First record of *Babka gymnotrachelus* (Kessler. 1857) from Germany (Teleostei, Gobiidae, Benthophilinae). *Spixiana* **35**: 155-159.

7.1 Abstract

The Ponto-Caspian racer goby *Babka gymnotrachelus* (Kessler, 1857) is recorded for the first time in Germany from a Danube backwater close to the city of Regensburg, and from the Danube main channel close to the village of Mariaposching. Several specimens were collected and photographed in May and September 2011, and one kept until April 2012 in captivity. Previously reported records of this species from the German River Rhine are male *Neogobius fluviatilis* (Pallas, 1841), or possibly hybrids between different benthophiline goby species.

7.2 Introduction

Ponto-Caspian gobies of the subfamily Benthophilinae Beling & Iljin, 1927 (Teleostei, Gobiidae) are globally invasive and pose serious ecological threats to invaded waters (e.g. Neilson & Stepien, 2009). In Germany, the Rhine system and the Danube were connected by the Main-Danube junction (MD canal) in 1992, and since then have become one of the main dispersal routes for invasive Ponto-Caspian species in Central Europe (Leuven *et al.*, 2009). Until recently four invasive benthophiline freshwater gobies had been reported from the Lower Rhine and/or the Upper Danube in Germany and Austria, i.e. *Proterorhinus semilunaris* (Heckel, 1837), *Ponticola kessleri* (Günther, 1861), *Neogobius melanostomus* (Pallas, 1814) and *Neogobius fluviatilis* (Pallas, 1814) (Copp *et al.*, 2005). A fifth species, the racer goby *Babka gymnotrachelus* (Kessler, 1857), may have been discovered outside of its native range in the middle section of the Danube before 1991 (Hegedis *et al.*, 1991, but see Jurajda *et al.*, 2005), and reached Vienna (Austria) in 1999 (Zweimüller *et al.*, 2000, Ahnelt *et al.*, 2001). Eleven years later it was reported from Germany in the River Rhine (Borcherding *et al.*, 2011b). Racer gobies were now discovered and photographed from a backwater of the Upper Danube River at Regensburg, and from a groyne-head habitat in the Danube main channel at Mariaposching, Germany (Fig. 7.1).

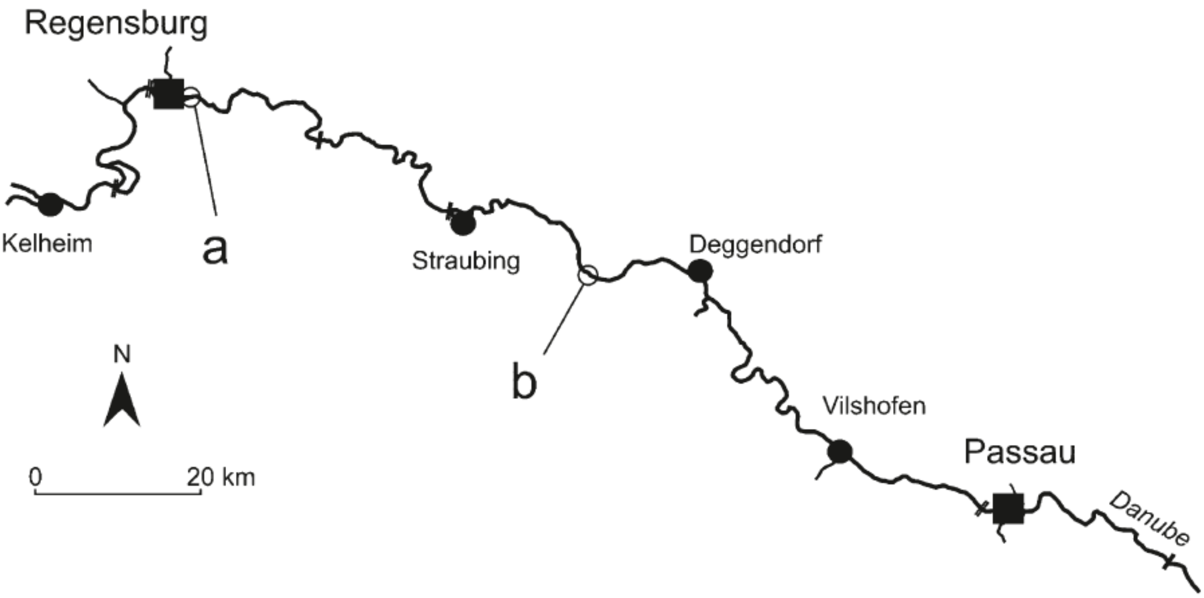

Fig. 7.1: Collection points of *Babka gymnotrachelus* in the upper Danube River

Collection points of *Babka gymnotrachelus* in the upper Danube River (Germany) with (a) Danube backwater “Almer Grube” opposite the east harbor of Regensburg and (b) Danube main channel at Mariaposching.

A closer examination confirmed their preliminary identification and prompted a re-examination of the specimen that was reported as the first record of *B. gymnotrachelus* in Germany (see Borcherding *et al.*, 2011a).

7.3 Material and Methods

Several specimens identified as *B. gymnotrachelus* were collected from below stones in shallow waters (< 1 m) from a backwater in the River Danube in May 2011 and a single one by electrofishing at a groyne head near Mariaposching in September 2011. One male specimen from Regensburg was kept in an aquarium until April 2012, upon it was anaesthetized, preserved and deposited at ZSM. This and the Mariaposching specimen, as well as the one collected and previously identified as *B. gymnotrachelus* in the River Rhine (Borcherding *et al.*, 2011b) were (re-)identified using published keys (Miller & Vasil’eva, 2003, Kottelat & Freyhof, 2007). If not otherwise mentioned, measurements, counts and other characters are taken as described in Schliewen & Kovacib (2008) and compared with literature data (Ahnelt *et al.*, 2001, Pinchuk *et al.*, 2003b,c, Kottelat & Freyhof, 2007) and with comparative material from as well as with additional *N. fluviatilis* material from the River Rhine.

Babka gymnotrachelus **(Kessler, 1857):**

Fig. 7.2: Racer goby (*Babka gymnotrachelus)*

Babka gymnotrachelus (ZSM 41739), photographed shortly after collection (Photo: M. Haertl).

ZSM 41739 (1 male, 92.2 mm SL), Germany, backwater “Almer Grube” of River Danube close to Regensburg close to an artificial rocky outcrop (49.0127° N, 12.1802° E), M. Haertl, collected May 2011, preserved 19 April 2012. ZSM 41336 (1 female, 78.5 mm SL), Germany, River Danube downriver of Mariaposching at a groyne-head (48.8255° N, 12.8194° E). J. Brandner, G. Nassl, D. Köck, 30 September 2011; partly dissected. ZSM 26420 (2 specimens, 53.9-62.0 mm SL), Turkey, Lake Sapanca, M. Winter, June 4th, 1984. ZSM 23288 (2 specimens, 52.7-56.8 mm SL), Romania, Lake Crapina, floodplain of the River Danube near Macin, P. Banarescu, November 16th, 1964.

Neogobius fluviatilis **(Günther, 1861):**

Fig. 7.3: Monkey goby (*Neogobius fluviatilis*)

Neogobius fluviatilis (ZSM 41740), photographed shortly after collection (Photo: J. Borcherding).

ZSM 41740 (1 male, 95.8 mm SL), Germany, River Rhine near city of Rees (Rhine-km 843). J. Borcherding, S. Gertzen, S. Staas, 21 September 2010. ZSM 23289 (6 specimens, 83.4-88.0 mm SL), Romania, Lake Crapina, floodplain of River Danube near Macin, P. Banarescu, 24-25 August 1966. ZSM 23863 (12 specimens, 52.6-92.5 mm SL), Romania, Danube estuary at Sulina, P. Banarescu, September 15th, 1968. ZSM 41579 (4 specimens, 73.9-80.5 mm SL), Germany, River Rhine near city of Rees (51.7621° N, 6.3408° E), A. Cerwenka, S. Gertzen, J. Brandner *et al.*, August 8th, 2011.

7.4 Results and Conclusions

Measurements and counts of the three benthophiline specimens are reported in Table 7.1. The two Danube specimens ZSM 41739 (Fig. 7.2) and ZSM 41336 key out as *B. gymnotrachelus* in keys provided by Kottelat & Freyhof (2007) and Miller & Vasil'eva (2003), and exhibit almost all applicable diagnostic character states of *B. gymnotrachelus* as reported in the most thorough recent review of the species (Pinchuk *et al.*, 2003b: 266): midline of nape naked in front of preoperculum, otherwise scales cycloid or ctenoid; interorbit one third eye diameter (0.33/0.31); upper lip of rather uniform width and 0.68 and 0.61 times in lateral preorbital area as measured between lip and eye; anterior membrane of pelvic disc without lateral lobes; D1 moderately high, rounded in profile; coloration with oblique dark bands across body; lateral line scales count 59 and 65. The pelvic disc does not reach the anus in specimen ZSM 41739 and is slightly smaller than the diagnostic value given by Pinchuk *et al.* (2003b). i.e. 0.9 or more than the abdomen length (0.84); in specimen ZSM 41336 it does reach the anus and therefore fits the diagnostic value (0.96); further, the caudal peduncle depth is slightly larger than 0.5-0.6 of its own length (0.76 and 0.71). We conclude, that both specimens are conspecific with *B. gymnotrachelus*, however, with a slightly smaller pelvic disc in one specimen as compared to the character state reported by Pinchuk *et al.* (2003b). Using the same keys, the Rhine specimen ZSM 41740 (Fig. 7.3) keys out as *N. fluviatilis*, except that it does not conform to the character state of Kottelat & Freyhof (2007) "first branched ray of second dorsal fin about twice as long as penultimate ray: no. but a bit shorter than first", because the first branched ray is only about 1.5 × as long as the penultimate ray (14.2/11.2 mm).

Table 7.1: Measurements and meristic counts of *Babka gymnotrachelus* specimens

Measurements (mm) and meristic counts of *B. gymnotrachelus* specimens (ZSM 41739, ZSM 41336) and the *N. fluviatilis* specimen previously misidentified as *B. gymnotrachelus* (ZSM 41740).

	Babka gymnotrachelus		*Neogobius fluviatilis*
	ZSM 41739	ZSM 41336	ZSM 41266
Distance measurements			
Sl, standard length	92.2	78.5	95.8
Tl, total length	114.1	98.4	120.1
Ab, anal fin base	31.2	24.6	31.5
Ad, body depth at anal fin origin	15.4	13.1	14.5
Aw, body width at anal fin origin	10.2	8.8	n/a
Bd, body depth	18.6	14.1	16.0
Cl, caudal fin length	21.9	19.9	24.3
CP, caudal peduncle length	12.4	10.6	12.2
CPd, caudal peduncle depth	9.5	7.6	7.6
D1b, first dorsal fin base	11.5	11.8	14.9
D2b, second dorsal fin base	36.1	31.5	39.1
Hl, head length	26.1	23.2	28.4
HwO, head width between opercles	19.2	13.6	13.2
Hd, head depth	13.4	9.5	12.9
E, eye diameter	5.6	5.2	5.2
SN, snout length	7.5	6.3	8.4
ULl, upper lip length	10.6	8.3	10.6
AULw, anterior upper lip width	2.4	1.9	1.8
LPd, lateral preorbital depth	3.9	3.3	5.0
Chd, cheek depth	8.0	5.2	8.0
PO, postorbital head length	15.3	11.7	16.2
I, interoribital width	2.0	1.6	3.8
IDs, interdorsal space	4.7	3.4	1.0
Pl, pectoral fin length	21.1	16.9	23.9
SN/A, snout to A	51.5	45.1	55.8
SN/AN, snout to anus	46.2	39.0	46.0
SN/D1, snout to D1	28.0	24.8	32.9
SN/D2, snout to D2	46.0	38.6	47.5
SN/V, snout to V	26.9	23.6	31.2
V/AN, pelvic to anus	19.8	17.0	16.5
Vd, body depth at pelvic fin origin	18.6	13.9	14.8
Vl, pelvic fin length	16.6	16.4	18.7
Vw, body width at pelvic fin origin	18.2	13.5	14.0
Counts			
LL, lateral line scale count	59+3	65+4	55+4
D1, spines in the first dorsal fin	VI	VI	VII
D2, spines and branched rays in the second dorsal fin	I/17	I/17	II/17
A, spines and branched rays in the anal fin	I/15	I/14	I/14

Nevertheless, it exhibits all applicable diagnostic characters of *N. fluviatilis* as reported in the most thorough recent review of the species (Pinchuk *et al.*, 2003c): Nape scaled completely, scales ctenoid; head depth at eyes about equal to width as measured between upper origin of opercles (12.9/13.2 mm); interorbit no more than 0.75 of eye diameter (0.72: 3.8/5.3 mm); angle of jaw below snout between eye and posterior nostril; snout 1.47 times larger than eye (7.8/5.3 mm); upper lip not swollen at angle, 0.4 times in lateral preorbital area as measured between lip and eye (1.8/4.5 mm); pelvic disc 0.94 of abdomen length (18.7/19.8 mm); anterior membrane of pelvic disc with small rounded, lateral lobes, less than 0.2 width of rear edge; D1 high, with acute anterior profile; median fins edged yellowish in breeding males. Lateral line scales count 55. We conclude that specimen ZSM 41740 is a male of *N. fluviatilis* in pre- or postbreeding coloration. We conclude so despite the missing character state in the key of Kottelat & Freyhof (2007), because this character state is not even evident from the photograph of a large *N. fluviatilis* male (page 579). We hypothesize that adult *B. gymnotrachelus* males develop enlarged median fins as a secondary sexual character, as fin shape differences are a kind of sexual dimorphism typical for many adult gobiid species (Horsthemke, 1995).

7.5 Remarks

In its native range *B. gymnotrachelus* is a typical inhabitant of mud, sand, gravel or muddy-sandy bottoms (Pinchuk *et al.*, 2003c), and it is abundant in backwaters (Kottelat & Freyhof, 2007). Two of the upper Danube records, the one from Austria (Ahnelt *et al.*, 2001) and the one from Regensburg, Germany, are from large backwaters. The specimen from Mariaposching was collected near a groyne-head, which is adjacent to a soft bottom area with comparatively calm water, and this is the single specimen that has been recorded from main channel habitats despite intensive shallow water electrofishing efforts along the Danube in Germany in 2010 and 2011, which yielded thousands of benthophiline goby specimens (pers. obs. A. Cerwenka and J. Brandner). This preliminary result suggests that monitoring of invasive freshwater fishes should target soft-bottom backwaters and soft bottom main river habitats more intensively, and that the invasive range of *B. gymnotrachelus* may already be larger than previously assumed.

8. General Discussion - Synthesis

This project analyzed strategies facilitating the invasion success of the two sympatric invasive neogobiids *N. melanostomus* and *P. kessleri*, which have recently colonized the headwater reach of the upper Danube River in Germany. Here to my knowledge for the first time, a distinct invasion front was detected, with a fast invasion process from total absence to first arrival until establishment. Notably, time from invasion until establishment only took about two years as shown in chapter 6.

After establishment, *N. melanostomus* formed up to 80% of the fish abundance and about 60% of the fish biomass in rip-rap bank habitats, indicating “superdominance” of this species in the upper Danube River. Similar abundance data, with *N. melanostomus* reaching higher numerical densities than native fishes have been reported from the Laurentian Great Lakes (Johnson *et al.*, 2005a; Brush *et al.*, 2012; Kornis *et al.*, 2012), the Baltic Sea (Karlson *et al.*, 2009) and many large European streams like the lower River Rhine (Borcherding *et al.*, 2012) and the middle Danube River (Erös *et al.*, 2008).

Within the last two decades, abundance data of invasive Ponto-Caspian gobies have been collected from many aquatic systems worldwide (reviewed in Kornis *et al.*, 2012 and Roche *et al.*, 2013). However, many of these studies used different sampling strategies and fishing methods, which hampers the comparability of catch data (see chapter 4). Thus, a sampling design applying accurate and precise methods, suitable for the sampling of neogobiids, their prey, and the associated fish assemblage in nearshore habitats of large rivers was established in this thesis. PAS-electrofishing was the most effective and least selective method in terms of size, feeding status and species composition, while angling had the second highest effectiveness, but was more size selective and resulted in a higher proportion of males compared to electrofishing. Since low selectivity is particularly crucial for population characterizations in terms of length-frequency or age distribution, sex ratio and analyses of feeding patterns (e.g. DeLury, 1947; Casselman *et al.*, 1990), standardized PAS-electrofishing was applied as method of choice in all case studies of this thesis.

Accordingly, goby-specimens were systematically collected to determine the trophic niches of both sympatric invaders using a combination of stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$), gut content analyses and morphometric analyses of the digestive tract. As against, in many studies aiming these objectives, specimens were collected using different fishing methods, hereby overlooking specific characteristics and not taking the influence of sampling bias on the comparability of catch data (as analyzed in chapter 4) into account (e.g. Borza *et al.*, 2009; Borcherding *et al.*, 2011; Brush *et al.*, 2012; Števove & Kováč, 2013).

In line with literature from recently invaded similar habitats (Borcherding *et al.*, 2011; Kornis et al., 2012), both species were identified as predacious omnivores with high dietary overlap and a generalist feeding strategy as well as a preference towards amphipods (especially invasive *Dikerogammarus* spp.). Trophic niches in both species expanded during the growth period with increasing intraguild predation and cannibalism in *P. kessleri* and increasing molluscivory in *N. melanostomus*. *Ponticola kessleri* showed a higher degree of specialization and more stable feeding patterns across seasons, whereas *N. melanostomus* adapted its diet according to the natural prey availability. Such ecological differentiation is a necessary condition for coexistence and may prevent both sympatric invaders from competitive exclusion. Based on life history traits however, *P. kessleri* is supposed to win the competition in the long run (Kováč *et al.*, 2009). Whereas, based on trophic interactions, *N. melanostomus* is likely to have advantages under changing food resource availabilities. A future monitoring of the success of both species using the herein established methodology in the danubian headwaters may provide valuable insights into the relative importance of both factors.

Herein for the first time in freshwater habitats, SIA of invasive goby muscle tissue provided a valuable complement to stomach content analyses as suggested by Brush *et al.* (2012). Both means combined can display new insights into food web relationships and consumption patterns at different spatio-temporal scales (Vander Zanden *et al.*, 1997; Post, 2002; Perga & Gerdeaux, 2005), however, it is important to exercise caution in interpretation (Brush *et al.*, 2012). Notably, the δ15N values (chapter 5) indicated a significantly lower trophic position (of about half a trophic level) of *P. kessleri* compared to *N. melanostomus* despite the fact that the gut content analyses would have predicted the opposite results since more fish (mainly *N. melanostomus*) was consumed by *P. kessleri* and more bivalves were consumed by *N. melanostomus*. In this case, further investigation is necessary to fully understand this antagonistic phenomenon (see chapter 5).

Founder populations from the invasion front were different from longer established *N. melanostomus* populations in demography, morphology, feeding behaviour, sex ratio and parasitic load, indicating that plasticity in these traits determines invasion success. This finding is in line with the previously suggested plasticity of this species based on comparisons of population data from the native range with those from invaded Great Lakes waterbodies (Gutowsky & Fox, 2011; Brownscombe & Fox, 2012; Gutowsky & Fox, 2012). Overall, adaptive phenotypic plasticity seems to be an important trait governing invasion success of non-native species in newly occupied areas (see Cerwenka *et al.*, 2013). The pronounced ontogenetic diet shift in *N. melanostomus*, which was observed at established populations and which has also been reported from other waterbodies (Great Lakes: French & Jude, 2001; Barton *et al.*, 2005; Baltic Sea: Karlson *et al.*, 2007; River Rhine: Borcherding *et al.*, 2012) appeared to be weak and retarded at the invasion front. This high flexibility even within an ontogenetic determined behaviour corroborates the relevance of adaptive phenotypic plasticity in the light of invasion success. In line with findings from a Great Lakes tributary (Gutowsky & Fox, 2011; Brownscombe & Fox, 2013), pioneering invaders from the invasion front were bigger (larger and heavier), revealing higher condition factors than their conspecifics from established areas, which probably increases their performance and competitive ability. In case of plants, an "*evolution of increased competitive ability"* has been proposed, suggesting that specimens can grow more vigorous and taller in environments outside their native ranges (Crawley, 1987; Blossey & Nötzold, 1995). This concept also seems applicable to invasive animals (here: Pisces: Perciformes: Neogobiidae) and might explain their invasion success, particularly in the early stages of the invasion process.

Upstream-directed range expansion is seemingly not driven by out-migrating weak or juvenile individuals that were forced to leave high density areas due to high competition but by pioneering invaders with increased exploratory behavior, a highly adaptive phenotypic plasticity and an increased competitive ability (see chapters 5 and 6). In contrast, downstream range expansion is mainly governed by the drift of juveniles (Janáč *et al.*, 2013).

Greater abundance and densities of acanthocephalan endoparasites were observed at the invasion front, which contradicts the expectation that invasion success is determined by lower parasitic pressure in newly invaded areas ("*enemy release*"-hypothesis; see e.g. Williamson, 1996; Keane & Crawley, 2002). This is in contrast to what was observed in the Great Lakes-St. Lawrence River ecosystem, where recently established *N. melanostomus* populations were parasitized by a depauperate community of generalist helminthes (Gendron *et al.*, 2012). In fact, *N. melanostomus* is even able to carry new parasite species into invaded ecosystems, since this species recently introduced the non-indigenous acanthocephalan *Pomphorhynchus tereticollis* (Rudolphi, 1809) into the River Rhine (Emde *et al.*, 2012).

Since both goby species consumed mainly other non-native species (~92% of gut contents) and seem to benefit from previous invasions of exotic prey species, their invasion success also mirrors fundamental ecological changes in large European freshwater ecosystems.

Overall, the pronounced changes in fish and invertebrate communities with a dominance of alien species indicate an invasional meltdown and a pronounced shift of the upper Danube River towards a novel ecosystem with species that have greater resistance to goby predation. Such a complex change, i.e. the creation of novel ecosystems through the combination of environmental change and the impact of invasive species (Hobbs *et al.*, 2006; Moyle *et al.*, 2012a), is also along the lines of what is happening to the the River Rhine (Tockner *et al.*, 2011), the north American Sacramento-San Joaquin Delta (Moyle *et al.*, 2012b) and other aquatic systems worldwide (Hobbs *et al.*, 2009).

Searching for possibly undetected benthophiline gobiid species, the Ponto-Caspian racer goby *Babka gymnotrachelus* (Kessler, 1857) was recorded for the first time in Germany in the context of this study (chapter 7). Previously reported records of this species from the German River Rhine (Borcherding et al., 2011b) turned out to be misidentified *Neogobius fluviatilis* (Pallas, 1841), or possibly hybrids between different benthophiline goby species (see chapter 7). Collecting an additional outgroup-sample in the lower River Rhine area, such hybrids (*N. melanostomus* × *N. fluviatilis*) were found during this study (Lindner *et al.*, 2013). Thus, the study design and the sampling technique applied in this project proved to be a suitable strategy to assess both neogobiids and the associated fish fauna. The ability to detect also rare species in low densities and even single hybrid specimens corroborates this general methodological aspect and recommends the applied methods for future goby research.

This study revealed new insights into factors which affect invasion success in Ponto-Caspian gobies, whilst these findings might be of general relevance to any other biological invasion.

8.1 Comparability of catch data

Decisions on sampling strategies and techniques are the first and most crucial steps in any fish biological investigation. A variety of fishing methods has been successfully applied to collect abundance data of invasive Ponto-Caspian gobies in many aquatic systems worldwide (reviewed in Kornis *et al.*, 2012 and Roche *et al.*, 2013). Due to a lack of method inter-calibration, catch data derived from different sampling strategies and fishing methods (see chapter 4) are hardly comparable. Especially in large rivers, the relative efficiency of many sampling methods is largely unknown. Thus, a sampling design applying accurate and precise methods suitable for the sampling of neogobiids, their prey, and the associated fish assemblage in nearshore habitats of large rivers was established herein. The analysis of the effects of sampling techniques on population assessment (as performed in chapter 4) had been the first attempt to inter-calibrate PAS-electrofishing, hook-and-line fishing and minnow traps. These methods had been applied in many studies on invasive gobies in the past, however not taking the influence of specific bias on the comparability of catch data (e.g. Borza *et al.*, 2009; Borcherding *et al.*, 2011; Brush *et al.*, 2012; Števove & Kováč, 2013) into account. Thus, research in suitable sampling techniques was an important pre-condition to systematically test the hypotheses objected in this study.

PAS-electrofishing was the most effective and least selective method in terms of size, feeding status and species composition, while angling had the second highest effectiveness, but was more size selective and resulted in a higher proportion of males compared to electrofishing. With a probability of 97.5%, PAS-electrofishing had the highest effectiveness to detect gobies on the distinctive sampling sites. Since low selectivity is particularly crucial for population characterizations in terms of length-frequency or age distribution, sex ratio and analyses of feeding patterns (e.g. DeLury, 1947; Casselman *et al.*, 1990), standardized PAS-electrofishing was applied as method of choice in this thesis.

Using this method, goby-specimens were systematically collected to determine the trophic niches of both sympatric invaders using a combination of SIA ($\delta^{13}C$ and $\delta^{15}N$), gut content analyses and morphometric analyses of the digestive tract.

After establishment, *N. melanostomus* formed up to 80% of the fish abundance and about 60% of the fish biomass in rip-rap bank habitats, indicating superdominance of this species in the upper Danube River. Similar abundance data, with *N. melanostomus* reaching higher numerical densities than native fishes have been reported from many habitats worldwide (Johnson *et al.*, 2005a; Erös *et al.*, 2008; Karlson *et al.*, 2009; Borcherding *et al.*, 2012; Brush *et al.*, 2012; Kornis *et al.*, 2012).

Standardized angling yielded the second highest catch-numbers of *N. melanostomus*. As differences in catch efficiency between two angling techniques with different hook sizes were not observed, angling results from different studies (Gutowsky & Fox, 2011; Gutowsky & Fox, 2012; Brownscombe & Fox, 2012; Brownscombe & Fox, 2013) appear comparable within the herein applied range of gear specification. Angling can be an effective sampling method in a wide range of environmental conditions, where electrofishing is less effective. However, angling is limited for comprehensive population assessments, since this method was selective for size and sex. Since suitable sampling procedures to adequately and systematically catch gobies in deep water areas of fluvial ecosystems are still lacking, the herein established methodology can provide an important basis for the development of new methods. A combination of angling and PAS-electrofishing might be a promising suitable method to sample even deep water areas, however must be validated in a similar procedure as applied in chapter 4.

Baited minnow traps by far had the lowest efficiency among all methods tested. Except for the very poor catch efficiency of minnow traps, the performance of this method was more similar to those observed from PAS-electrofishing than angling. Due to low CPUE and low frequency-of-occurrence, fish traps appear unsuitable for many of the applied scientific research questions and require further research in methodological standardisation.

Generally, the established methods can be applied and possibly adapted to further research to achieve a better comparability of goby and bycatch population data from different habitats.

8.2 Impact on autochthonous species

Due to the suspected serious regime shifts in freshwater ecosystems and the still continuing spread of *N. melanostomus* and *P. kessleri* throughout North-American and European waterbodies (reviewed in Kornis *et al.*, 2012 and Roche *et al.*, 2013), it is important to analyze population dynamics and their potential impact on autochthonous species.

Since pioneering *N. melanostomus* preferred autochthonous Ephemeroptera, Plecoptera and Trichoptera with highest proportions in gut contents at the most upstream edge of the invasion front, invasive gobies can affect native endangered species. A decline in abundance of these goby prey-species was observed depending on time since invasion. Such a decline in abundance depending on time since invasion was also observed in barbel and chub, indicating an impact on autochthonous fish species, too.

However, but analogously to latest studies from the middle Danube (e.g. Števove & Kováč, 2013), no single piece of fish as prey was found in the gut contents of *N. melanostomus* in this thesis. Both findings together are in line with the significant fish species decline in the German waterway network, which has been observed long before the first goby invaded, and which was mainly mediated by habitat loss and river regulation (Vilcinskas & Wolter, 1994; Lelek, 1996; Wolter & Vilcinskas, 1997). In contrast to most of the indigenous species, invasive gobies seem to benefit from first, the large-scale habitat destruction in large rivers (Postel *et al.*, 1998; Moyle & Mount 2007; Wolter & Röhr, 2010) and second, from earlier invasions of their preferred prey species (chapter 5: Brandner *et al.*, 2013a), which seemingly enables them to out-compete native fauna.

All results combined suggest an indirect but strong impact, potentially caused by competition for food and habitat resources, since *N. melanostomus* is well known for its aggressive nature and its high competitive behaviour (e.g., Phillips *et al.*, 2003; Brownscombe & Fox, 2012; Groen *et al.*, 2012).

Therefore, at least *N. melanostomus* was identified as highly invasive, since this species is able to out-compete native fauna, supporting an invasional meltdown. Similar life history and niche utilization in *P. kessleri* may also contribute to the invasion success in this species and indicate a high invasive potential, too. The abundance data presented in the case studies (chapters 4-6) mirror the higher invasion success of *N. melanostomus* (i.e. a factor 25 greater abundance) compared to *P. kessleri*, especially in the first years after invasion. However, being the goby species with lower abundance in this sympatric invasion, *P. kessleri* is suspected to succeed over longer time periods because of a better capability to cope with long-term unstable and less predictable environmental changes (Kováč, 2009). Competition as one of the most important factors in ecology is therefore of particular interest for this sympatric fish invasion, since the invaders may not only out-compete native species but also each other. Observation of developments and dynamics in *N. melanostomus* and *P. kessleri* populations at aged conditions of the invasion process using the established methods will help answering the question which species may win the competition.

This thesis introduced the importance to a better understanding of basic processes and consequences of biological invasions. Combining all case study results, a new integrated model of a biological invasion can be suggested here (Fig. 8.1).

This comprehensive conceptual model considers the “classical” stages of the invasion process including introduction, establishment, spread and impact, but also integrates propagule pressure (frequency of introductions, inoculation size) and time (since invasion) as demanded by Strayer *et al.* (2006). Relevance and character of these newly considered determinants are discussed in chapters 6 and 7. Environmental regime shifts can influence biological invasions over time. Thus, changes in biotic and abiotic parameters, such as hydromorphological modifications, changes in water temperatures and new species interactions might be important variables with the potential to trigger biological invasions.

According to the integrated conceptual model, introductions with below-threshold propagule pressure fail to establish as the number of individuals released to a new environment being too low (frequency of release events and inoculation size being too small). Introductions with threshold propagule, which encounter optimum environmental conditions (threshold level 1) can lead to successful establishment of a founder population (threshold level 2). This step of a biological invasion might be observed in case of the *B. gymnotrachelus* first record in Germany (see chapter 7) below an important harbour near Regensburg. A lag-phase separates establishment from the spread-phase with a trigger ending this “sleeping” mode. This trigger might be external (environmental changes) or population mediated (e.g., adaptation to new environment), starting an exponential mass development within the spread-phase leading to a superdominance of the invader.

Although such a trigger could not be detected in this study (and was not objected by the way), an upstream-directed colonization of *N. melanostomus* along a fluvial gradient with a distinct invasion front was observed. At this invasion front, population dynamics were observed from total absence until establishment (see chapter 6). In downstream areas, where gobies arrived about five years earlier, *N. melanostomus* formed up to 80% of the fish abundance and about 60% of the fish biomass in rip-rap habitats during the spread phase of the invasion process, with still increasing population densities (see chapters 4 and 5). This impact phase with a clear superdominance of *N. melanostomus* may feed-back environmental changes as a consequence of the increasing population size. Since the extensive utilization of resources (see chapter 5) may limit further spread, a boom-and-bust cycle seems an inevitable consequence. This also seems a realistic scenario for the upper Danube River, where declines in goby populations are supposedly just a matter of time.

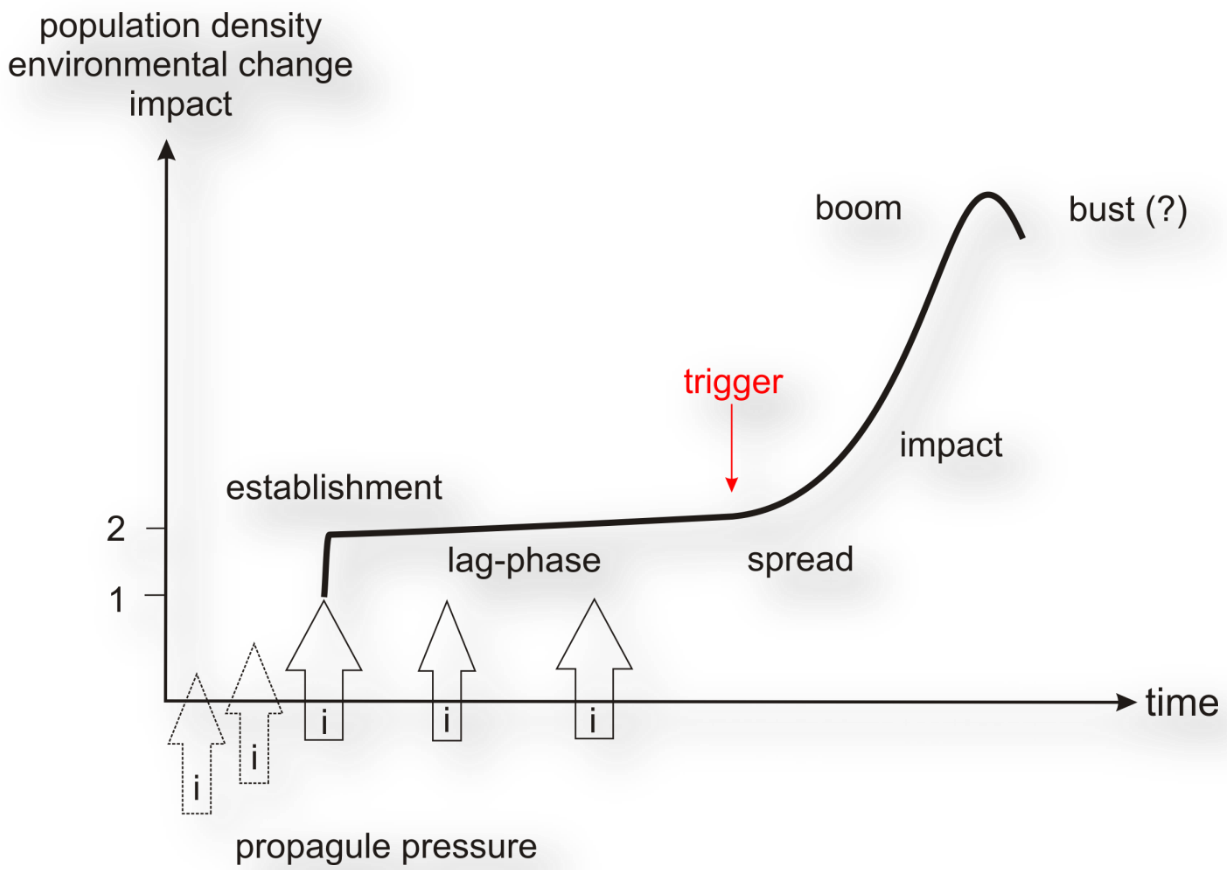

Fig. 8.1: Integrated conceptual model of a biological invasion

Integrated conceptual model of a biological invasion (i = introduction; 1=threshold level suitable for establishment; 2= threshold level for spread phase) integrating propagule pressure and time since invasion. Environmental changes, such as hydromorphological modifications, changes in water temperatures and new species interactions may trigger a switch from the lag-phase to spread, leading to a monodominance of the invader.

This integrated conceptual model of a biological invasion should be validated, at best using the invasion of *B. gymnotrachelus* which just arrived in the study area (see chapter 7). Thus, the upper Danube River generally offers a great possibility to study further ecosystem changes in general and further waves of biological invasions of closely related sympatric fish species in a low-noise gradient in special.

8.3 Drivers of range expansion

Movement among habitats has been recognized as the norm rather than the exception in many freshwater fish species (Lucas & Baras, 2001). Natural range-expansion in invasive species is directly linked to the out-migration of pioneering invaders from their home-ranging populations in numbers (inoculation size) and introduction events (propagule pressure) (Hanski, 2001). Immigration and emigration are therefore two sides of the same coin. Besides survival and reproduction, immigration and emigration may be numerically important and vital to population processes (Hanski, 2001). Thus, a thorough understanding of the spatio-temporal population dynamics (large scale) requires an understanding of mechanistic processes determining habitat selection and emigration at the individual level (small-scale), and vice versa. However, it still remains difficult to measure emigration rates and to clearly distinguish out-migration from foraging movements or local exploratory behavior of individuals. Thus, interaction between individual behaviour and population dynamics has been critically questioned in fish ecology, and linking the two has proven difficult (McMahon & Matter, 2006).

In the case study “Bigger is better: Characteristics of round gobies at the invasion front” (chapter 6) population and specimen-specific analyses revealed a higher competitive ability of pioneering invaders compared with their established conspecifics. Competitive ability and invasion success of gobies at the invasion front seem to be largely determined by somatic instead of reproductive investment (“bigger is better”). The larger size and higher condition factor of gobies at the invasion front compared to those at established areas might be explained by less limited food resources in newly invaded areas. The finding of higher parasitic load at the invasion front was surprising and in contrast to expectations according to the “*enemy release*”-hypothesis, indicating that this factor is less important.

Sale (1969) provided an early conceptual model, suggesting that habitat selection is a continually active running process, being governed by the intensity of exploratory (appetitive or searching) behaviour, with exploratory behaviour being regulated by the interaction of internal drives (motivation) for needed resources. Based upon this theory, McMahon & Matter (2006) developed the “*habitat selection-emigration”*-model, linking resource availability and individual habitat selection to exploratory behaviour, emigration and population-level responses. According to this model, the rapid range expansion of neogobiids in the upper Danube River could be driven by two important, interconnected key-processes: First, the availability of (highly preferred) superior food resources and second, an increased intraspecific competition as a consequence of the fast density increase in established populations. This seems especially true for the upstream directed migration by pioneering invaders with increased exploratory behavior and a high adaptive phenotypic plasticity (see chapters 5 and 6). In contrast, downstream range expansion (spread) is mainly governed by the drift of juveniles (Janáč *et al.*, 2013).

8.4 Fisheries management and conservation

With peak densities up to 100 fish per m² in some of the invaded areas, *N. melanostomus* is too abundant and widespread in large systems for eradication efforts to succeed (Kornis *et al.*, 2012). Moreover, climate change and habitat destruction might facilitate further range expansion of invasive species.

Since large boulders and rip-rap structures are preferred substrates of gobies in their native range (Sindilariu *et al.*, 2002) and the majority of the European inland waterways were highly modified water bodies embanked with rip-rap, gobies can potentially utilize these well suited structures and will further disperse along these optimal habitat corridors as long as superior food-resources like amphipods are available.

In the face of climate change, fish species preferring warm water may benefit from increasing water temperatures, whereas cold-water species may lose suitable habitats. Thus, and considering their invasion patterns, further Ponto-Caspian neogobiids as well as the amur sleeper *Percottus glenii* Dybowski 1877 are most relevant candidates (Table 1.1) for near future invasions both into the upper Danube River (Wolter & Röhr, 2010; Rabitsch *et al.*, 2013) and similar habitats worldwide. This prognosis is strongly underlined by the recent records of newly establishing populations of the Ponto-Caspian racer goby in the German section of the upper Danube River (Haertl *et al.*, 2012; Brandner *et al.*, 2013b), as demonstrated in chapter 7.

Invasion biology often faces situations, where invasive species act as hybrid partners of an indigenous species, thereby compromising the native congener's genotypes and consequently its autochthonous genetic integrity (Arnold, 1997; Petit *et al.*, 2004). The fishing effort during this thesis in the Danube River as well as in the River Rhine, which yielded more than 7500 carefully screened goby individuals, revealed first evidence for interspecific hybridization between the invasive neogobiids *N. fluviatilis* and *N. melanostomus* (Lindner *et al.*, 2013). This new finding is remarkable since fish hybrid lineages, e.g. of the sculpin genus *Cottus* spp., have also recently invaded the River Rhine and their hybrid genome may have contributed to their invasion success (Nolte *et al.*, 2005). In general, any hybrid genotype expressing a novel phenotype might occupy a novel niche (Fitzpatrick *et al.*, 2012). Since hybrids themselves (given they are fertile) may again found novel invasive lineages with unique adaptions, facilitating the invasion of novel areas (Seehausen, 2004; Nolte *et al.*, 2005), these neogobiid hybrids may become a new quality of threat to the autochthonous biodiversity. In order to detect such hybrids, which could easily be overseen or even misidentified (e.g. Borcherding *et al.*, 2011b), all invasive goby specimens should be monitored carefully. The established sampling strategy and the extensive sampling programme (introduced in chapter 4) proofed as an accurate and precise methodology to detect such rare hybrid specimens (chapter 7) and can thus be used for potential future samplings of neogobiids in rip-rap and gravel habitats of large rivers.

The high pace of global change in fish assemblages mirrors new challenges for alien fish management and scientific research, not only in the Danube River. The results and implications of this study are of general relevance for similar habitats worldwide. Thus, three perspectives have to be deduced from this project:

First, this rapid ongoing shift from indigenous biodiversity towards a ubiquitous faunistic complex of potentially co-evolved exotic species which are adapted to human-altered aquatic systems also questions the use of historical reference conditions and communities as a conservation target, e.g. in the context of the European Water Framework Directive and the development of any other conservation target.

Second, especially the success of Ponto-Caspian invaders reflects fundamental ecological changes in the large European freshwater ecosystems (Brandner *et al.*, 2013a), which make a return to original communities almost impossible. Resiliation by restoration of heavily modified water-bodies has to be implemented into EC-WFD river management procedures in order to counter further changes caused by rising water temperatures in the face of climate change.

Third, given an ongoing invasional meltdown, further invasions are just an inevitable matter of time. Because of the difficulty of eradication of already established alien species (Vander Zanden, 2005), prevention from introduction might be the only promising key approach in future fisheries management.

Overall, considering the recent rapid environmental and biotic changes in similar ecosystems worldwide, the results of this thesis underline that invasion ecology also needs to be linked to other scientific disciplines, such as conservation biology, global change biology, restoration ecology, resource economics, human geography and policy (see also Richardson, 2011).

8.5 Outlook

The observed multispecies driven upstream graded invasional meltdown and the possibility to observe characteristics of invaders at a distinct invasion front generally define the upper Danube River a unique study system worldwide.

Here, within this thesis a sampling design with adequate methods was established to examine the invasion biology of two sympatric bottom dwelling gobies (as model species) and their prey in nearshore habitats of a large river. Since suitable sampling procedures to adequately and systematically catch gobies in deep water areas of such fluvial ecosystems are still lacking, the established methodology can provide an important basis for the development of new methods. Moreover, the upper River Danube is a well arranged ideal system to analyse an ongoing invasional meltdown in a novel ecosystem (i.e., the further impact of further invasive species), since other independent model organisms are available to study invasion processes right from introduction. In addition, the recent invasion of *Babka gymnotrachelus* and *Pomphorhynchus tereticollis* recommends a critical review of the herein presented ecological and parasitological aspects, but could generally improve knowledge on newly establishing invasive host-parasite systems. These conditions also recommend an observation of future developments and dynamics in *N. melanostomus* and *P. kessleri* populations, their prey and their parasites at aged conditions of the invasion process to validate the presented conclusions. A future monitoring of the success of both species using the same methodology in the danubian headwaters may furthermore provide valuable insights into the relative importance of adaptive phenotypic plasticity in the light of invasion success and the question which of both invasive gobies will win the competition.

Overall, further systematic investigations on novel ecosystems themselves and their underlying ongoing changes are necessary since the impact of neogobiids and their exotic prey on food web interactions is not yet fully understood and estimates where or when invasional meltdown may halt or possibly could be halted are urgently needed by authorities and science.

9. Publications

The following publications derived from this integrated study:

Haertl, M., A. F. Cerwenka, J. Brandner, J. Borcherding, J. Geist & U. K. Schliewen (2012): First record of *Babka gymnotrachelus* (Kessler. 1857) from Germany (Teleostei, Gobiidae, Benthophilinae). *Spixiana* **35**: 155-159.

Brandner, J., K. Auerswald, A. F. Cerwenka, U. K. Schliewen & J. Geist (2013): Comparative feeding ecology of invasive Ponto-Caspian gobies. *Hydrobiologia* **703**: 113-131.

Brandner, J., J. Pander, M. Mueller, A. F. Cerwenka & J. Geist (2013): Effects of sampling techniques on population assessment of invasive round goby. *Journal of Fish Biology* ***82**: 2063-2079.*

Brandner J., A. F. Cerwenka, U. K. Schliewen & J. Geist (2013): Bigger is better: Characteristics of round gobies forming an invasion front in the Danube River. *PLoS ONE* 8(9): e73036. doi:10.1371/journal.pone.0073036.

Lindner, K., A. F. Cerwenka, J. Brandner, S. Gertzen, J. Borcherding, J. Geist & U. K. Schliewen (2013): First evidence for interspecific hybridization between invasive goby species *Neogobius fluviatilis* and *Neogobius melanostomus* (Teleostei: Gobiidae: Benthophilinae). *Journal of Fish Biology* **82**: 2128-2134.

Cerwenka, A. F., P. Alibert, J. Brandner, J. Geist & U. K. Schliewen (2013): Phenotypic differentiation of Ponto-Caspian gobies during a contemporary invasion of the upper Danube River. *Hydrobiologia:* DOI 10.1007/s10750-013-1668-5.

Cerwenka, A. F., J. Brandner, J. Geist & U. K. Schliewen (2014): Strong versus weak population genetic differentiation after a recent invasion of gobiid fishes (*Neogobius melanostomus* and *Ponticola kessleri*) in the upper Danube River. *Aquatic Invasions* **9**: in press.

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