

The impact of Ponto Caspian invaders on the parasite communities of multiple hosts within West and Central European river systems

Dissertation
zur
Erlangung des
Doktorgrades Dr. rer. nat.

der Fakultät für
Biologie
an der

Universität Duisburg-Essen

vorgelegt von
Michael Andreas Alfred Hohenadler

geboren in Troisdorf

3.10.2018

*I am a firm believer,
that without speculation there is no good
and original observation.*

Charles Darwin

Angaben zur Prüfung

Die der vorliegenden Arbeit zugrundeliegenden Experimente wurden in der Abteilung für Aquatische Ökologie der Universität Duisburg-Essen durchgeführt.

1. Gutachter: Prof. Dr. Bernd Sures
2. Gutachter: PD Dr. Franz Jirsa
3. Gutachter:

Vorsitzender des Prüfungsausschusses: Prof. Dr. Florian Leese

Tag der mündlichen Prüfung: 20.12.2018

DuEPublico

Duisburg-Essen Publications online

UNIVERSITÄT
DUISBURG
ESSEN

Offen im Denken

ub | universitäts
bibliothek

Diese Dissertation wird über DuEPublico, dem Dokumenten- und Publikationsserver der Universität Duisburg-Essen, zur Verfügung gestellt und liegt auch als Print-Version vor.

DOI: 10.17185/duepublico/47799

URN: urn:nbn:de:hbz:464-20191209-125038-9

Alle Rechte vorbehalten.

Acknowledgements

Foremost, I would like to express my sincere thanks to my advisor Bernd Sures for providing me with the opportunity to complete my PhD thesis at the University of Duisburg Essen, thanks for your continuous support, patience, motivation, immense knowledge, as well as for the many inspiring and humorous conversations. I could not have imagined a better mentor for my PhD study.

Special thanks to Franz Jirsa who shared his knowledge, lab and house with me.

Furthermore, I want to pronounce my gratitude to Milen Nachev who was very dedicated to his role as my secondary supervisor. Thanks for being there for me to exchange ideas and for the numerous motivating discussions. Thanks to Daniel Grabner for sharing your knowledge about molecular biology (and many other things) with me.

My sincere thanks also goes to all co-authors that are part of the published articles, for your support, contributions and help.

I also want to thank my current and former fellow PhD students and co-workers. Thanks to Christian Selbach, Michelle Pahl, and Marion Woermann for umpteenth entertaining hours and thoughtful conversations – you are simply the best and funniest office mates I can think of. Thanks to Katrin Honka, Thomas Meissner, and Jan Lemm for motivating me to leave work and to do sports instead – at least sometimes you were successful. Thanks to Jessica Schwelm for the many walking tours and deep conversations. Thanks to Jan Lemm and Louisa Rothe for your help with the graphic realization of some of my ideas. Thanks to Julian Enss, Katrin Honka, Diana Michler, and Jenia Kremers for directly supporting me by gathering and/or examining my samples. Thanks to Benjamin Kupilas, Caroline Winking, Maria Ceylan, Jessica Schwelm, Alexander Gieswein, Christian Feld, Mark Schumann, Henrike Hamer, Martin Sondermann, Maria Gies, Sonja Zimmermann, Thomas Knura, Birgit Rieth, Nina Kaiser, Daniel Hering, Kerstin Dangel, Armin Lorenz, everyone I mentioned before, and the rest of the Aquatic Ecology department for encouraging me in one or another way.

I would also express special thanks to the Deutsche Bundesstiftung Umwelt (DBU) and Volker Wachendörfer that made this work possible. Furthermore, I acknowledge funding by the Faculty of Biology at the University of Duisburg-Essen as well as travel grants by the German Academic Exchange Service (DAAD).

Finally, but by no means least, thanks to David John Yabis for your constant support, and believe in me, thanks for always being there for me. Thanks to Elke Hohenadler Manuela Hohenadler, Louise Jagemann, and Hans Hohenadler (I know you were always with me), and to all my friends for your love, believe, and support.

Dear family, I dedicate this thesis to all of you

Table of contents

List of abbreviations.....	8
List of figures.....	9
List of tables.....	11
1 Summaries.....	13
1.1 Summary.....	13
1.2 Zusammenfassung.....	15
2 General introduction.....	18
2.1 Background.....	18
2.2 Aims and hypotheses.....	23
3 General material and methods.....	25
3.1 Study area.....	25
3.2 Sampling.....	27
3.3 Parasitological analysis.....	30
Chapter I: Direct effects of Ponto-Caspian invasion on local fish parasite communities by the example of three native and one invasive host species.....	35
4 How Ponto-Caspian invaders affect local parasite communities of native fish.....	36
4.1 Summary.....	36
4.2 Introduction.....	37
4.3 Material and methods.....	40
4.4 Results.....	45
4.5 Discussion.....	58

Chapter II: Direct effects of Ponto-Caspian invasion on native parasites through the example of <i>Pomphorhynchus</i> sp. (Acanthocephala)	63
5 <i>Pomphorhynchus laevis</i> : An invasive species in the river Rhine?.....	64
5.1 Summary.....	64
5.2 Introduction.....	65
5.3 Material and methods.....	69
5.4 Results.....	71
5.5 Discussion.....	74
Chapter III: Indirect effects of Ponto-Caspian invasion for local hosts through the example of an interaction between <i>Pomphorhynchus laevis</i> (Acanthocephala) and the invasive <i>Anguillicola crassus</i> (Nematoda)	79
6 First evidence for a possible invasional meltdown among invasive fish parasites.....	80
6.1 Summary.....	80
6.2 Introduction.....	81
6.3 Material and methods.....	83
6.4 Results.....	85
6.5 Discussion.....	86
7 General discussion.....	89
8 References.....	94
Appendices.....	121
Appendix 1.....	121
Appendix 2.....	125
Contributions.....	129
Curriculum vitae.....	130
Erklärungen.....	133

List of abbreviations

bp	base pair
CH	Switzerland
CO1 (genes)	cytochrome c oxidase subunit I
DCF	data collection framework
dpi	days post infection
IMH	invasional meltdown hypothesis
IUCN	International Union for Conservation of Nature
ITS (genes)	internal transcribed spacer
L2 / L3	larvae stage 2 / 3
MDS	multidimensional scaling
MI	mean intensity
MQ	mean water discharge
N / n	total number
NIS	nonindigenous species
NL	the Netherlands
P	prevalence
PC	Ponto-Caspian
PCR	polymerase chain reaction
rpm	revolutions per minute
SD	standard deviation
sp. / spec.	species

List of figures

Figure 2.1:	Hypothetical example of three possible scenarios that might take place after an invasive host enters a new environment: parasite spillback, spill over, and dilution effect.....	22
Figure 3.1:	Map of all sampling locations indicating all different sampling periods.....	28
Figure 3.2:	Display of the final PCR-products after an agarose gel/ethidium bromide electrophoresis (including positive control of both <i>Pomphorhynchus</i> species; negative control). PCR- products with a length of 320 bp = <i>P. laevis</i> ; 350 bp = <i>P. tereticollis</i>	31
Figure 3.3:	Experimental setup of the artificial digestive process.....	32
Figure 4.1:	Sampling locations: A = Rhine River at the city of Grieth; B = Ems River at the city of Ditzum; C = Elbe River at the city of Winsen a. d. Luhe, D = Schwentine River, close to the Großer Plöner See....	41
Figure 4.2:	Distribution of species richness in the parasite infracommunities of the three different fish species (A) <i>G. cernua</i> ; B) <i>N. melanostomus</i> ; C) <i>A. anguilla</i>) within the four German rivers (blue = Rhine; orange = Ems; gray = Elbe; yellow = Schwentine).....	48
Figure 4.3:	Internal parasite community of A) <i>G. cernua</i> ; B) <i>N. melanostomus</i> ; C) <i>A. anguilla</i> within the tested rivers (Rhine; Ems; Elbe; Schwentine).....	51
Figure 5.1a:	Map of Europe, countries of interest highlighted in green.....	70

Figure 5.1b: Map of the sampling spots in the Rhine at Beneden Leeuwen (Netherlands), Karlsruhe (Germany) and Laufenburg (Switzerland).....	70
Figure 5.2: Sampling years (a = 2003, b = 2004, c = 2015), and <i>Pomphorhynchus</i> species distribution from eel at the three different sampling locations within the river Rhine [the Netherlands, Germany, Switzerland].....	72
Figure 6.1a: Cysts of encapsulated <i>P. laevis</i> individuals as detected and removed from the digestive tracts of <i>N. melanostomus</i>	85
Figure 6.1b: Encapsulated <i>P. laevis</i> irradiated with high light intensity.....	85
Figure 6.1c: Digested cyst with released <i>A. crassus</i> individuals.....	85
Figure 7.1: Graphical illustration of the three presented scenarios that follow a Ponto-Caspian invasion. Scenario: A) parasite spillback/spillover, B) extinction of native parasites, C) enhancement of local parasites through new transmission pathways.....	91

List of tables

Table 3.1:	Account of all sampling locations (GPS-Coordinates) including the general information (primary source, river mouth, river basin, and mean discharge) of each river.....	26
Table 3.2:	Sampled host species with corresponding sampling locations and number sampled for the sampling period between 2013-2016 (amphipods were only sampled in 2015 and 2016).....	29
Table 3.3:	List of variables that were used in order to calculate all mentioned ecological parameters.....	34
Table 4.1:	Mean sizes and weights of collected fish species at all sampling locations.....	41
Table 4.2:	Catch numbers of the three fish species (<i>G. cernua</i> , <i>A. anguilla</i> , <i>N. melanostomus</i>) for each sampling year (2014-2016) and waterbody (the rivers: Rhine, Ems, Elbe, Schwentine).....	42
Table 4.3:	Parasitological data of the parasite community of <i>G. cernua</i> , <i>N. melanostomus</i> and <i>A. anguilla</i> within the four sampled rivers (Rhine, Ems, Elbe, Schwentine).....	46
Table 4.4:	Component community and infracommunity diversity indices of all sampled fish species (<i>G. cernua</i> , <i>N. melanostomus</i> , <i>A. anguilla</i>) within the four German rivers (Rhine; Ems; Elbe; Schwentine).....	49
Table 4.5:	Beta diversity of all sampled fish species (<i>G. cernua</i> , <i>N. melanostomus</i> , <i>A. anguilla</i>) within the four German rivers (Rhine; Ems; Elbe; Schwentine).....	50

Table 4.6:	ANOSIM analyses (pairwise test) of the internal parasite communities of all sampled hosts (<i>G. cernua</i> , <i>N. melanostomus</i> , <i>A. anguilla</i>) within all rivers (Rhine; Ems; Elbe; Schwentine) individually.....	51
Table 4.7:	Parasitological parameters of the internal parasite community of <i>A. anguilla</i> and <i>G. cernua</i> from the rivers Rhine and Elbe obtained from parasitological studies performed before, during, and shortly after the two Ponto Caspian invasion intervals.....	53
Table 4.8:	Component community diversity indices of <i>A. anguilla</i> within the Rhine River obtained from parasitological studies performed before and after the first Ponto Caspian invasion interval.....	57
Table 5.1:	Comparative observation of the morphological features of <i>P. laevis</i> and <i>P. tereticollis</i>	67
Table 5.2:	Number of analyzed <i>Pomphorhynchus</i> - individuals with year and location of eel sampling.....	70
Table 5.3:	Number and species of amphipods, year and location of sampling (Rhine, Wupper), and number of analyzed <i>Pomphorhynchus</i> sp. .	71
Table 5.4:	Sequence-comparison of ITS 1 and ITS 2 genes of <i>P. laevis</i> samples from each sampling spot (2003 and 2004) and sequence dataset of acanthocephalans sampled worldwide.....	73

1 Summaries

1.1 Summary

Biological invasion has become one of the great threats to ecosystems worldwide. Frequently, invasion is caused by anthropogenic activities either intentionally due to an introduction of free-living organisms for agricultural or recreational purposes as well as unintentionally for instance if species are transported unperceived in cargo containers from one ecosystems to another.

However, only a small amount of these species will be able to establish itself after entering a new habitat. As soon as a species is successful they might become invasive and cause negative effects to local biocoenosis that might lead to loss of biodiversity or eventually initiate a meltdown of the entire system.

Biological invasion is a global problem, which is exacerbated inter alia by globalization, (wrong) environmental management and climate change. In that context Europe have become one of the hotspot for invasion where various Ponto-Caspian species that are considered very successful invaders established many self-sustaining communities. Ponto-Caspian (PC) species such as the zebra mussel, killer shrimp (*Dikerogammarus villosus*), round-, bighead-, and monkey goby usually adapt easily to environmental conditions and frequently become dominant species in newly invaded habitats. Additionally various PC species seem to favor the distribution of pathogens and parasites.

However, these relatively new discovery are scarcely studied, which represents a general problem in the research of invasion biology since the effects of invasive species on parasites are frequently neglected. The ignorance towards parasites is surprising because they are considered as important response variable for ecosystems health. Generally, parasites contribute to 40 % of the biomass of an ecosystem, govern host behavior, and potentially alter the structure, dynamics and function of food webs.

Therefore, the effects of invasion by the example of PC invasive species on local parasite communities were at the focus of the present doctoral thesis. Hence, their impact on parasite prevalence and infestation intensities within various hosts of habitats that were free of or invaded by PC species were compared and evaluated.

Additionally, possible interactions of co-introduced PC parasites with local parasites and the consequences for local species were investigated.

Thus, within a period of four years three local and one PC fish hosts as well as two different amphipod species were sampled in various rivers (in Germany, the Netherlands, Switzerland) and parasitological examined. The results were compared with each other and subsequently placed into context.

The results of various substudies showed that PC species affect the parasite communities of recently invaded habitats on many layers. In this context, the acanthocephalan *Pomphorhynchus laevis* and the nematode *Raphidascaris acus* have been identified as key species since an increase in their prevalence in local hosts seem to be related to PC invasion. Furthermore, the interaction between *P. laevis* and other PC species might have caused the replacement of the local acanthocephalan *P. tereticollis* in invaded habitats and eventually directly encouraged interactions between *P. laevis* and other local parasites.

The results of the present doctoral thesis have shown that effects of PC invasive species on parasites are multilayered and therefore suggest that not only PC species but invasive species in general might affect local parasite communities. Hence, parasites have to be integrated into proposed or existing conservation efforts, since invasive species might cause an increase of local parasites and/or a loss of parasite diversity, both with grave consequences for local environments.

1.2 Zusammenfassung

Die Ausbreitung oder biologische Invasion von gebietsfremden Arten in neue Lebensräume, die häufig durch anthropogene Beeinflussung verursacht bzw. begünstigt wird, hat sich zu einem der großen Probleme unserer Zeit entwickelt. Dieser Einfluss ist vielschichtig und kann intendiert, wie bei der Einfuhr von Nutz- und Haustieren sowie durch den Anbau von gebietsfremden Pflanzen in der Agrarindustrie, oder nicht vorsätzlich sein wie z.B. bei dem unbemerkten Einschleppen von Organismen die in Frachtcontainern bzw. Ballastwassertanks von Schiffen mitreisen und dadurch in neue Lebensräume vordringen.

In der Regel gelingt es jedoch nur einem kleinen Prozentsatz dieser Arten sich auch effektiv in einem neuen Gebiet zu etablieren. Sobald eine Art erfolgreich ist, kann sie Einfluss auf die Biozönose bzw. auf die Interaktionen zwischen verschiedenen Arten diese Ökosysteme nehmen oder sogar verschiedene Prozesse und Funktionen dieser Systeme stören oder beeinflussen. In der Folge kommt es gegebenenfalls zu Biodiversitätsverlust oder im Extremfall zu einem Zusammenbruch des ganzen Systems.

Biologische Invasion ist dabei ein globales Problem das unter anderem durch die Globalisierung, (falsches) Umweltmanagement und den Klimawandel verstärkt wird. In diesem Zusammenhang ist Europa zu einem der Hotspots für Invasion geworden, in dem sich in den vergangenen Dekaden besonders viele invasive Arten aus dem pontokaspischen Raum angesiedelt haben. Diese Region liegt auf dem eurasischen Kontinent und umfasst unter anderem das Schwarze und Kaspische Meer. Durch verschiedene (neue) Migrationsrouten konnten sich Arten wie die Zebrauschel, der große Höckerflohkrebs, die Schwarzmaul-, Kessler- und Flussgrundel ohne große Schwierigkeiten über den ganzen europäischen Kontinent ausbreiten. Alle diese Arten können sich leicht an neue Habitate anpassen und schaffen es in der Regel sich durch ihr aggressives Konkurrenzverhalten gegenüber lokalen Arten durchzusetzen und nach kurzer Zeit häufig zu den dominanten Arten in diesen Gebieten zu werden. Zusätzlich scheinen viele pontokaspische Invasoren eine Rolle bei der Verbreitung von Parasiten und Krankheiten zu haben.

Diese relativ neue Erkenntnis ist jedoch wenig erforscht, was ein generelles Problem in der Invasionsbiologieforschung offenlegt, da diese sich sehr selten mit den

Effekten oder Interaktionen von invasiven Arten auf oder mit Parasiten beschäftigt, obwohl Parasiten als wichtiger Indikator für die Gesundheit von Ökosystemen gelten. Sie machen nach neustem Erkenntnisstand rund 40 % der Biomasse eines Systems aus, gelten als Regulatoren ihrer Wirtsorganismen und sind wichtiger Bestandteil von Nahrungsnetzen deren Strukturen, Dynamiken und Funktionen sie auch beeinflussen.

Infolgedessen stand die Frage nach einem möglichen Effekt von Invasion, am Beispiel von verschiedenen pontokaspischen Arten, auf lokale Parasitengemeinschaften im Fokus der hier vorliegenden Doktorarbeit. Hierfür wurde der mögliche Einfluss von diesen Arten auf die Häufigkeit von Parasiten bzw. deren Befallsintensitäten bei verschiedener Wirtsarten, innerhalb von invadierten und nicht invadierten Habitaten, anhand von verschiedenen ökologischen Parametern evaluiert. Außerdem wurde untersucht, ob mögliche parallel eingeführte Parasitenarten mit lokalen Parasiten interagieren und was sich daraus für möglichen Konsequenzen für lokale Arten ergeben können.

In einem Zeitraum von 4 Jahren wurden hierzu in verschiedenen Flüssen (innerhalb Deutschlands, der Schweiz und den Niederlanden) insgesamt 3 einheimische und eine invasive Fischart, sowie verschiedene Amphipodenarten beprobt und parasitologisch untersucht. Die hieraus hervorgehenden Ergebnisse konnten in der Folge miteinander verglichen und anschließend in den entsprechenden Kontext eingeordnet werden.

Im Rahmen von verschiedenen Substudien wurde gezeigt, dass pontokaspische Arten vielschichtige Effekte auf die Parasitengemeinschaften von neuinvadierten Habitaten haben. In diesem Kontext wurden zwei Parasitenarten als Schlüsselarten identifiziert. Durch die Anwesenheit von pontokaspischen Wirtsarten scheinen sich besonders die Prävalenzen des ebenfalls pontokaspischen invasiven Kratzer *Pomphorhynchus laevis* und des einheimischen Nematoden *Raphidascaris acus*, in den untersuchten Wirten massiv erhöht zu haben. Des Weiteren wurden, wahrscheinlich durch das Zusammenwirken von *P. laevis* mit anderen pontokaspischen Wirtsarten, zumindest eine einheimische Parasitenart (*P. tereticollis*) aus dem Hauptstrom des untersuchten Gewässers verdrängt. Schließlich scheinen pontokaspische Wirtsarten direkte Interaktionen zwischen pontokaspischen und lokalen Parasiten zu fördern bzw. zu begünstigen.

Durch diese vielschichtigen Einflussnahmen lassen die Ergebnisse der vorliegenden Doktorarbeit den Schluss zu, dass nicht nur pontokaspische Arten,

sondern invasive Arten generell einen Einfluss auf lokale Parasitengemeinschaften haben. Daher schließt die Arbeit mit dem Appell, dass aufgrund der besonderen Bedeutung von Parasiten für die Biozönose verschiedener Ökosysteme und durch die Tatsache, dass Invasion zu höheren Befallsraten durch Parasiten für lokale Wirte und zu konkreten Nachteilen für einheimische Parasiten führt, Parasiten in Zukunft Teil von Managementplänen zum Naturschutz werden sollten bzw. müssen.

2 General Introduction

2.1 Background

Biological invasion has generally become a threat to ecosystems worldwide (Pimentel et al. 2001, Molnar et al. 2008, Pejchar and Mooney 2009). According to the International Union for Conservation of Nature (IUCN), invasive or alien species are any free-living organisms that are introduced by anthropogenic activities to places out of their natural range of distribution, where they become established and disperse, possibly generating a negative impact on the local ecosystem and species. Historically, humans introduced new species either intentionally or accidentally. Intentional reasons include the introduction of plants and livestock for nutritional and economical causes (Pimentel et al 2005), animals for recreational purposes such as sport fishing and hunting (Dextrase and Mandrak 2006, Genovesi et al. 2009, Kark et al. 2009), or as pets (Padilla and Williams 2004), as well as exotic plants for gardening (Pyšek et al. 2012). Furthermore, species were and still are introduced for population control of formerly introduced species (Messing and Wright 2006). Apart from that, wildlife often established itself accidentally in new areas as a result of global trade or anthropogenic induced changes of environments, e.g. building of channels and flood gates (Essl et al. 2011, Chapman et al. 2017).

Effects of invasive species on new environments can be wide-ranging since these species might affect populations, community interactions, ecosystem processes, and/or abiotic variables (Charles and Dukes 2007). Consequently, invasive species are able to evoke alterations in ecosystem functions, shifts in community dominances that can include a switch from a competition to a predation-dominated system, loss of biodiversity, and the meltdown of entire ecosystems (Löveit 1997, O'Dowd et al. 2003, Shochat et al. 2010).

Nevertheless, in accordance with several researchers, biological invasion is principally not always caused by anthropogenic activities nor do alien species always have a negative impact on new environments. Natural biological invasion might be caused by a change of natural conditions for instance as a consequence of extreme weather conditions, volcanic activities, earthquakes, or wild fires (Zalba et al. 2008,

Seebens et al. 2015, Han and Young 2016). A good example for natural invasion was presented by Lovette et al. (1999) who suggested that all colonization of the island Barbados by terrestrial organisms must have occurred naturally and relatively recently by birds from source populations of nearby islands.

However, different studies have demonstrated that just a small amount of species are able to establish self-sustaining populations after entering a new environment and only a small fraction of these species will become invasive (Williamson 1996, Zalba et al. 2008). In spite of this fact, these relatively few species potentially remain an immense and global disturbance for entire ecosystems.

Nowadays, globalization, (inconsiderate) environmental management, climate change, and other circumstances have transformed Europe into a hotspot for biological invasion, generally with severe ecological and economic consequences (Chiron et al. 2009, Hulme et al. 2009, Vilà et al. 2010). Invasive species like the giant hogweed (*Heracleum mantegazzianum*) or the Asian tiger mosquito (*Aedes albopictus*) can cause problems for human health due to allergic reactions or the transmission of diseases (Nielsen et al. 2005, Angelini et al. 2007). Native species such as the red squirrel (*Sciurus vulgaris*) are outcompeted or replaced by new species like the eastern gray squirrel (*Sciurus carolinensis*) due to their competition advantage regarding food and habitat resources (Gurnell et al. 2004). In addition to their impacts on living organisms, invaders might cause damages to the environment itself. The burrowing activities of the Chinese mitten crab (*Eriocheir sinensis*) for instance cause erosion of dikes and river embankments (Gilbey et al. 2008). According to the European Commission (COM/2008/0789 final), invasion-related impacts engender annual costs for Europe of 12 billion Euro, the removal of the toxic marine algae *Chrysochromulina polylepis* alone causes costs of 8.18 million Euro per year (Hopkins 2002).

As illustrated by these few examples, origins of invasive species that invade European ecosystems are globally distributed. However, in the course of the past decades, one specific region was identified as a source of various highly successful invaders that were able to establish themselves in nearly all European countries (Bij de Vaate et al. 2002, Ojaveer et al. 2002, MacNeil et al. 2010). This region, the so-called Ponto-Caspian steppe, is situated on the Eurasian continent and includes the Black, Azov and Caspian Seas (Cristescu et al. 2004). Mass range extension of species from the Pontic steppe to western and central Europe was enabled through the interconnection of river basins through manufactured canals (Bij de Vaate et al.

2002). The most relevant of these inland migration corridors is the Main-Danube Canal that was inaugurated in 1992, connecting the Danube with the Rhine Rivers (Müller et al. 2002, Molloy et al. 2007). Simultaneously, commercial shipping facilitated range extensions since individuals that either attached themselves to the ship's hull, or were passively accommodated in the vessels ballast tanks were transported over long distances (Bij de Vaate et al. 2002).

However, the availability of different invasion pathways contributes only partly to the invasion success of Ponto-Caspian species. Generally, these species are successful invaders that turn out as dominant species after they establish in a new environment (Ketelaars 2004, Gallardo and Aldridge 2015). The zebra mussel (*Dreissena polymorpha*) for instance is considered as one of the most aggressive freshwater invaders worldwide (Sinitsyna and Rotasov 1994, Karatayev et al. 2002). *Dreissena polymorpha* causes physical modification of aquatic environments by altering their biotic and abiotic characteristics (Fahnenstiel et al. 1995, Idrisi et al. 2001, Noonburg et al. 2003). Another example is the so-called killer shrimp *Dikerogammarus villosus* that has its origin in the Ponto-Caspian region as well (Bollache et al. 2004, Pöckl 2007). It easily adapts to new environmental conditions (Platvoet 2005) and usually has a high impact on other amphipods with the potential to reduce amphipod diversity in Europe by outcompeting and replacing indigenous, nonindigenous as well as other invasive species (Dick and Platvoet 2000, Caselatto et al. 2005, Taraschewski 2006, Pöckl 2007). Ponto-Caspian gobiid fish such as *Neogobius melanostomus*, *Ponticola kessleri*, and *Neogobius fluviatilis* are considered to be responsible for the decline and/or disappearance of local fish species due to their successful invasion strategies and their competitiveness for sustenance, energy resources, and spawning grounds (Dubs and Corkum 1996, Janssen and Jude 2001, Balshine et al. 2005, Karlson et al. 2007, Jakšić et al. 2016, Kessel et al. 2016).

Apart of the direct impacts of certain Ponto-Caspian species on free-living organisms in different European ecosystems, the effects of these invaders on parasite communities are still widely neglected. This is remarkable since parasites are considered important response variables for ecosystem health due to their role in food-web structures, as a contributor of biomass, or as a regulator of host populations (Dobson and Hudson 1986, Kuris et al. 2008, Lafferty et al. 2008, Sures et al. 2017). In fact, this does not only apply to Ponto-Caspian species in Europe, but generally to invasive species worldwide. According to Jeschke et al. (2012), there are several major

hypotheses in invasion biology that either focus on (1) ecosystems that experience invasion (biotic resistance hypothesis; Jeschke and Genovesi 2011), (2) the invasive species itself (tens rule; Jeschke 2008), or (3) invader-ecosystem interactions (invasional meltdown; Simberloff and von Holle 1999, enemy release hypothesis; Blumenthal et al. 2009). Although theoretically all of these hypotheses can also be applied to invasive parasites, only few studies have been carried out yet and usually concentrate on the enemy release hypothesis (Kvach and Stepien 2008, Ross et al. 2010, Prior et al. 2015). However, there are three additional theories discussed that are exclusively concerned with parasites and their host species (*cf* figure 1.1). The first hypothesis implies that if invasive parasites are co-introduced with their invasive hosts, they may spill over to naïve native host species, which can lead to high parasite abundances (Kelly et al. 2009). Secondly, local parasites spill back from a nonindigenous or invasive species if it acts as an appropriate host, which will finally result in higher infestation rates in native hosts as well as in an increase in the relative ratio of these parasites (Tompkins and Poulin 2006, Kelly et al. 2009, Slapansky et al. 2016). Thirdly, the “dilution effect” that occurs if a nonindigenous or invasive species acquires local parasites, but acts as an inappropriate or dead-end host. As a result, the infestation rate of this parasite decreases in native host populations (Gagne et al. 2016, Slapansky et al. 2016).

However, all theories described above are commonly single-sided since they address the impacts of free-living and parasitic invaders on a single parasite species rather than on entire parasite communities of a given group of hosts. Consequently, there is limited knowledge about the question if and how local parasite communities are impaired by invasive species and how they affect local host-parasite and / or parasite-parasite interactions.

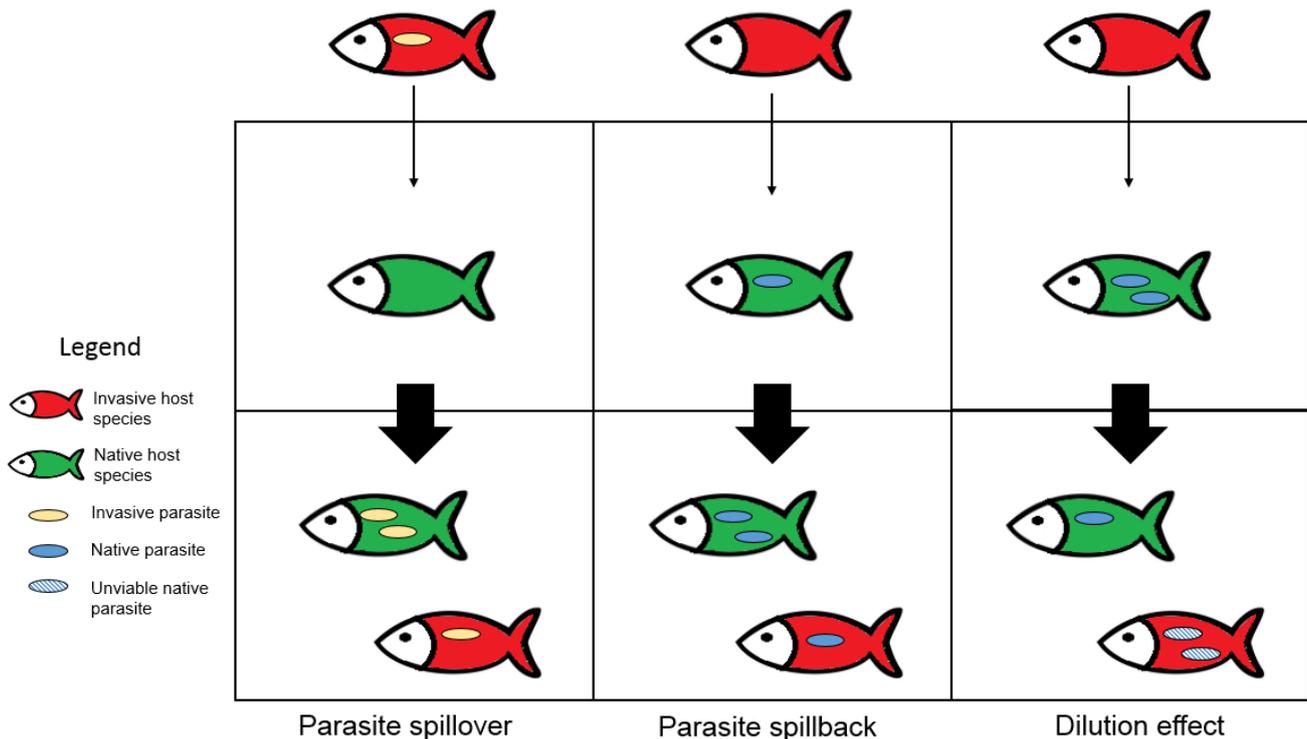


Figure 2.1: Hypothetical example of three possible scenarios that might take place following the introduction of an invasive host to a new environment

In order to illustrate possible invasion-related consequences on local parasite communities, attention will be paid once more to invasion from the Ponto-Caspian region. Over the last decades, different studies have determined the special importance of Ponto-Caspian species as (paratenic, intermediate) host species for local, nonindigenous or invasive parasites (Pronin et al. 1997, Ovcharenko et al. 2009, Francová et al. 2011, Rewicz et al. 2014). *Dikerogammarus villosus* demonstrably introduced the microsporidian parasite *Cucumispora dikerogammari* to its invaded environments, where it was able to infest local gammarid species (Ovcharenko et al. 2010). Kvach et al. (2015, 2017) have additionally shown that the parasite communities of Ponto-Caspian gobiids usually differ from the parasite communities of native fish species, especially in the first years after their introduction. Recent research has furtherly shown that Ponto-Caspian invaders seem to play also a role in the distribution of parasites and pathogens in their new environments (David et al. 2017, Herlevi et al. 2017). However, once again these results only demonstrate the effects of individual invasive species with a Ponto-Caspian origin on a single parasite or host species.

Therefore, the purpose of this doctoral thesis was to generate a deeper insight into the effects of invasive free-living species for entire local parasite communities covering different hosts. To maximize the outcome of this research, various

approaches were chosen. On one hand, the thesis addressed the question how free-living Ponto-Caspian species influence the densities and infestation rates of parasites within different local and invasive host species over a given period of several years. For this purpose various ecological parameters from host species sampled in habitats free of any Ponto-Caspian invaders and such invaded by Ponto-Caspian species were calculated and the results were compared with each other. On the other hand, this work aimed to fill the knowledge gaps regarding the interactions of invasive parasites with local parasites and the possible consequences that are caused on community level. The research combined different approaches including field sampling and various laboratory experiments and analyses.

2.2 Aims and hypotheses

This study aims at investigating the parasite communities from various native host species from waterbodies in Germany, the Netherlands and Switzerland in order to give an overview of the possible effects of invasion of free-living species on local parasite communities. Based on these aims, various hypotheses are derived leading to individual approaches:

1. The invasive free-living species induce changes in parasite infection patterns of native host species due to possible spillback-, spillover-, or dilution effects.

Spatial aspect: In order to evaluate the effect of free-living neozoans on the structure and composition of local parasite communities, parasitological investigations on *Anguilla anguilla* and *Gymnocephalus cernua* sampled from waterbodies with and without presence of invasive Ponto-Caspian species (e.g. *Neogobius melanostomus*, *Dikerogammarus villosus*) were performed and different parasitological and diversity parameters were compared.

2. Native and / or local parasite species might be replaced or outcompeted by invasive parasites, which leads to irreversible changes in local parasite fauna.

Timeline approach: The acanthocephalan species (mainly of the genus *Pomphorhynchus*) of the local *Anguilla anguilla* were analyzed twice over a period of three years (2003-2005 and 2013-2015) at three different sampling locations

within the Rhine River (Germany, the Netherlands, and Switzerland) in order to determine a change of different ecological parameters, and the absence / new appearance of different acanthocephalans. The first sampling period represented the time before, and / or while Ponto-Caspian invasion took place in the River Rhine, while the years 2013-2015 corresponded with the time where Ponto-Caspian species had already established.

3. Interspecific interactions between invasive Ponto-Caspian parasites and local parasite species will lead to higher infestation rates in hosts of invaded areas due to newly introduced transmission mechanisms.

Laboratory diagnostic approach: The role of Ponto-Caspian species for the transmission of local parasites was evaluated under laboratory conditions. Therefore, susceptible local fish species *Leuciscus cephalus* and *A. anguilla* were experimentally infected with encapsulated parasites found in body cavities of the invasive gobiid *N. melanostomus*. The infestation potential of these parasites (*Anguillicola crassus* and *Pomphorhynchus laevis*) was evaluated after checking the establishment rates in the selected fish species.

Taken together, the results will provide a more detailed insight into the role of invasion with regard to native parasite and host species.

3 Material and methods

This chapter gives a general outline of the material and methods that were used in order to study possible effects invasive Ponto-Caspian species might have on the local parasite communities within selected German rivers. However, detailed accounts of material and methods of the individual studies are provided in the chapters 3.1 Study area, 3.2 Sampling, and 3.3 Parasitological analysis.

3.1 Study area

In order to evaluate the possible effects that Ponto-Caspian host species have on local parasite communities after invading a new environment, various fish and amphipod species were sampled at different locations within certain German rivers (*cf* table 3.1 and figure 3.1). The rivers Rhine, Elbe, and Ems are part of the main dispersal routes for Ponto-Caspian species and are therefore already invaded by these species (Bij de Vaate et al. 2002). Sampling sites in the rivers Schwentine and Wupper were not invaded by any Ponto-Caspian species by the time the sampling took place. However, all sampling sites were part of natural water bodies that are used for commercial and/or recreational fishing. Furthermore, some of the rivers represent major routes for commercial shipping.

Table 3.1: Account of all sampling locations (GPS coordinates) including general information of each river system

River	Primary source in	Mouth	Basin	Discharge (mean)	Sampling location	GPS coordinates
Rhine	Switzerland	North Sea	185,000 km ²	2,900 m ³ /s	Laufenburg (Switzerland)	47°33'58.2 N 8°06'04.2 E
					Karlsruhe (Germany)	48°58'43.8 N 8°15'27.7 E
					Grieth (Germany)	51°47'31.0 N 6°19'07.3 E
					Beneden Leeuwen (Netherlands)	51°54'06.7 N 5°33'42.4 E
Elbe	Czech Republic	North Sea	148,268 km ²	870 m ³ /s	Winsen a. d. Luhe (Germany)	53°25'16.7 N 10°16'15.0 E
Ems	Germany	North Sea	17,934 km ²	80 m ³ /s	Ditzum (Germany)	51°14'34.6 N 7°06'12.6 E
Wupper	Germany	Rhine River	827 km ²	17 m ³ /s	Wuppertal (Germany)	54°07'27.7 N 10°24'40.9 E
Schwentine	Germany	Baltic Sea (Kiel Fjord)			Großer Plöner See (Germany)	54°07'27.7 N 10°24'40.9 E

3.2 Sampling

Different host species were sampled for parasitological examination. The sampling was divided into three different campaigns:

- 1) The spatial sampling was performed annually in four successive years (2013-2016) by professional fishermen. Fishes were sampled by bow nets, fish traps, and fishing rod. Each specimen was sacrificed immediately after catch and kept frozen at -20°C or was transported alive to the research facility. Here, fish were kept alive for a maximum of 4 days until parasitological examination (depending on sampling design). Table 3.2 lists each sampled host species with a detailed indication on sampling location and total number of individuals.
- 2) The timeline sampling took place annually within two independent periods, namely between the years 2003 and 2005 as well as between 2013 and 2015. Throughout both periods, sampling was performed at three different sampling locations within the Rhine River (*cf.* figure 3.1; I-III). Exclusively eels were sampled by professional fishermen by fish traps or electrofishing. The total number (n) for each sampling location and each period varied between 40 and 100 individuals.
- 3) The amphipod sampling was conducted by hand-nets or by kick-sampling in two consecutive years (2015-2016). Amphipods of the species *Dikerogammarus villosus* were collected from the river Rhine (*cf.* figure 3.1; A). *Gammarus pulex* specimens were sampled from the river Wupper (*cf.* figure 3.1; a).

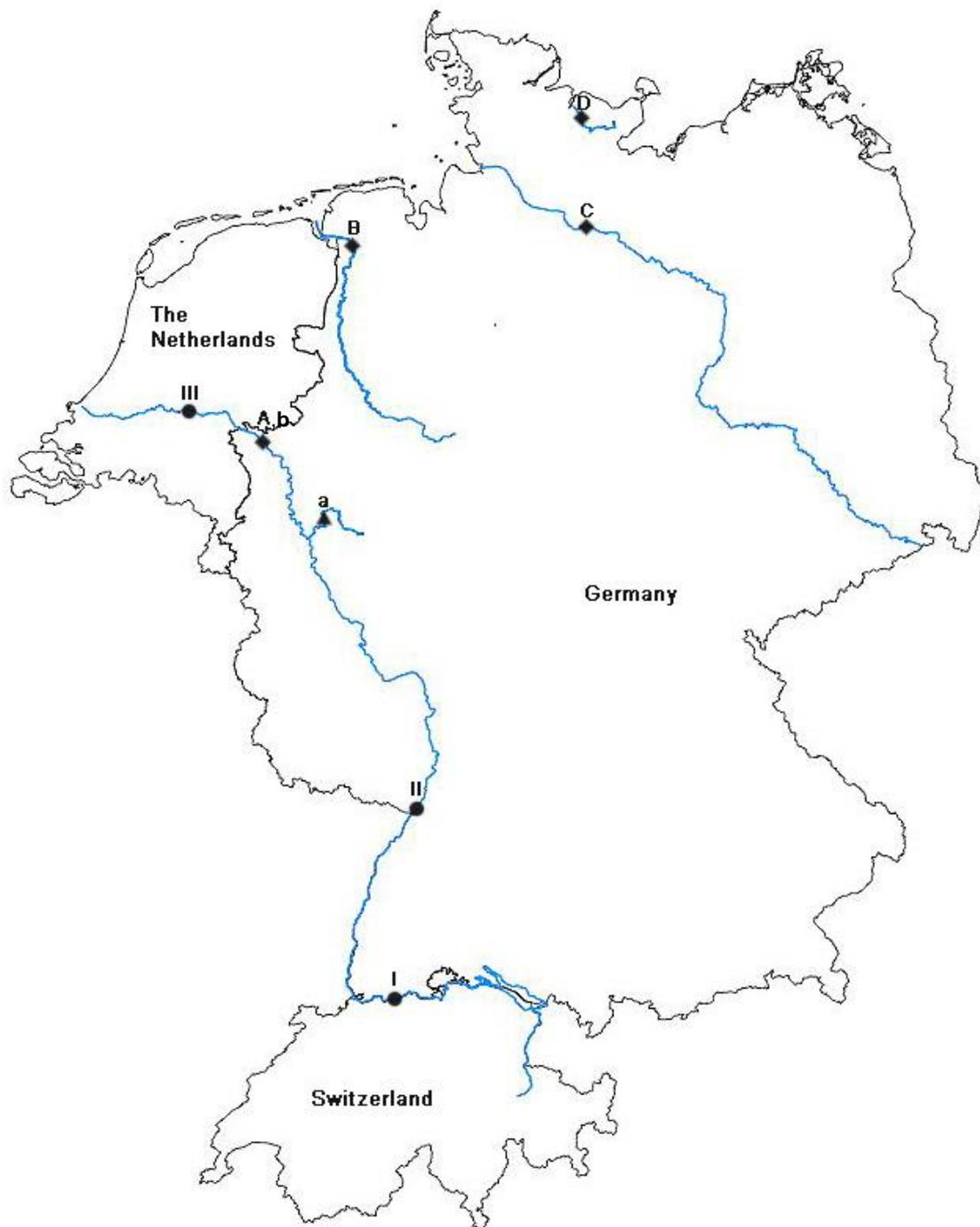


Figure 3.1: Map of all sampling locations indicating all different sampling periods: ● timeline sampling: 2003-2005 and 2013-2015, I-Laufenburg (Switzerland), II-Karlsruhe (Germany), III-Beneden Leeuwen (Netherlands); ◆ spatial sampling: 2013-2016, A-Grieth (Rhine River), B-Ditzum (Ems River), C-Winsen a.d. Luhe (Elbe River), D-Großer Plöner See (Schwentine River); ▲ amphipod sampling: 2015-2016, a-Wuppertal (Wupper River), b-Grieth (Rhine River)

Table 3.2: Host species of the fish sampling campaign (2013-2016) and the amphipod sampling campaign (2015 and 2016) with corresponding sampling locations and total number of individuals sampled

Fish species	Sampling location (river)	Number of sampled individuals
<i>Neogobius melanostomus</i>	Rhine	146
	Ems	55
	Elbe	91
<i>Anguilla anguilla</i>	Rhine	49
	Ems	79
	Weser	89
	Elbe	50
	Schwentine	62
<i>Gymnocephalus cernua</i>	Rhine	18
	Ems	33
	Elbe	6
	Schwentine	47
<hr/>		
Amphipod species	Sampling location (river)	Number sampled
<i>Dikerogammarus villosus</i>	Rhine	18
<i>Gammarus pulex</i>	Wupper	65

3.3 Parasitological analyses

Parasitological investigations

After sacrifice, each host individual was studied for parasites. Amphipods were crushed between two squeezing-plates and examined by means of a binocular (Olympus S7X9) with a 8 x magnification and visually checked for infestation with parasites. Alternatively, amphipods were twisted apart and carefully checked under the binocular.

Fish hosts were treated as follows: The fish's skin, fins, gills, eyes, liver (excluding *A. anguilla*), digestive tract, and swim bladder (if applicable) were examined under a binocular (10 x magnification) for the presence of parasites and the discovered parasites were removed and stored for further identification.

Parasite identification

Different identification keys (such as Brohmer et al. 1938, Moravec 1994, Moravec 2004) were used in order to identify detected parasites based on their morphology to species level or lowest possible taxon.

Due to a high possibility of morphological misidentification, parasites of the genus *Pomphorhynchus* were also analyzed by molecular identification methods.

Molecular identification of Pomphorhynchus species

DNA was extracted from tissue samples of acanthocephalans belonging to the genus *Pomphorhynchus* using "my-Budget DNA Mini Kit" (Bio Budget Technologies GmbH, Krefeld). Therefore, tissue samples of each parasite individual (~40 mg) were mixed with 400 µl lysis solution TLS and 25 µl Proteinase K. Subsequently, the mixture was incubated at 50 °C until the sample was completely lysed. Afterwards the sample was centrifuged at 12,000 rpm for 1 minute. Hereafter 400 µl binding solution TBS was added to the lysed sample before the mix was applied to a Spin Filter. Then the sample was centrifuged again at 12,000 rpm for 2 minutes. Subsequently, 500 µl washing solution HS was added and centrifuged again at the same speed for one minute. Then the sample was centrifuged again after 750 µl washing solution MS was added to it. In order to remove all traces of ethanol, samples were centrifuged again for 2 minutes at

maximum speed. Finally, 200 µl elution buffer was added and centrifuged again after one minute incubation time (room temperature) at 8,000 rpm for 1 minute. Subsequently, internal transcribed spacer 1 (ITS 1) rDNA genes were amplified by polymerase chain reaction (PCR) according to Franceschi et al. (2008). The length of the final PCR products (expressed in base pairs (bp)) allowed an explicit distinction between the sampled *Pomphorhynchus* species (cf figure 3.2).

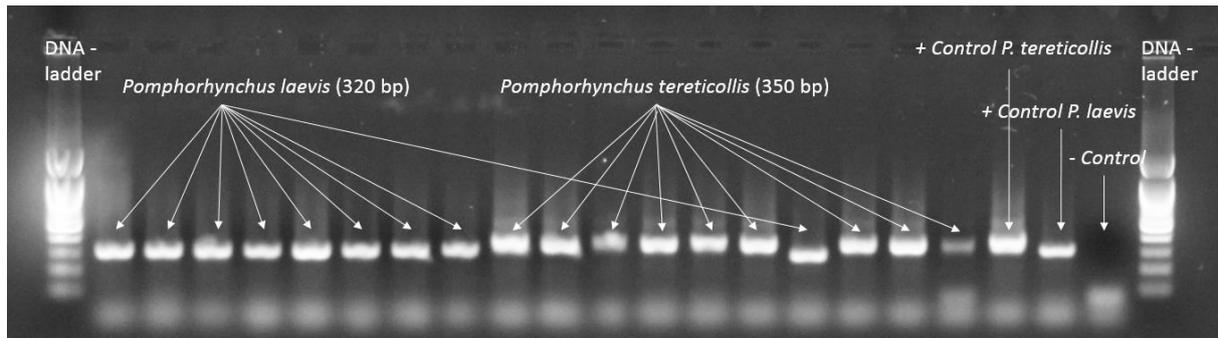


Figure 3.2: Molecular identification of acanthocephalans according to the size of PCR products including positive control of both *Pomphorhynchus* species; negative control (Mili-Q water) - 320 bp = *P. laevis*; 350 bp = *P. tereticollis*.

Additional molecular analysis were performed in order to locate the possible origin of the analyzed *Pomphorhynchus* specimens. Therefore five individuals from each sampling spot (sampling years 2003 and 2004), which were identified as *P. laevis*, have been randomly chosen. Subsequently, these samples were prepared for sequencing by using a “MicroElute® Cycle-Pure Kit” (Omega Bio-Tek, Inc. Norcross). Readily prepared samples were sent to GATC Biotech for sequencing. Final DNA sequences (ITS1 and ITS 2 genes) were inserted to GenBank (www.ncbi.nlm.nih.gov) in order to find a congruency between sequences that will allow conclusions about the geographical origin of the sampled individuals.

Laboratory experiments

In order to study the infestation potential and strategies of some parasites, namely *Anguillicola crassus* and *Pomphorhynchus laevis*, additional laboratory experiments were performed. The laboratory infestation experiments aimed to evaluate whether larvae of *A. crassus* are still able to infest the swim bladder of their final host and to

reproduce after entering cysts of encapsulated *P. laevis* individuals. First, cysts were removed from infested *N. melanostomus* individuals that were sampled in the Rhine River. Subsequently, cysts were stored in a 0.9 % sodium-chloride solution (Carl Roth GmbH + Co. KG, Karlsruhe, purity ≥ 99 %). Generally, all cysts contained individuals of *P. laevis*, but only a few of them were additionally infested by *A. crassus* larvae (L3). To determine the infestation rate with *A. crassus*, cysts were transferred from the saline solution to a well plate (cf. figure 3.3) that contained an artificial stomach acid solution, which was prepared according to Llarena-Reino et al. (2013) and was composed of 1% hydrochloric acid (Carl Roth GmbH + Co. KG, Karlsruhe, purity 35%) and pepsin (0.5 g per 100 ml) (Fluka Analytical, Bucharest). Cysts were placed in well plate compartments (one cyst per well). Afterwards each well-plate was incubated for 40 minutes at 37 °C.

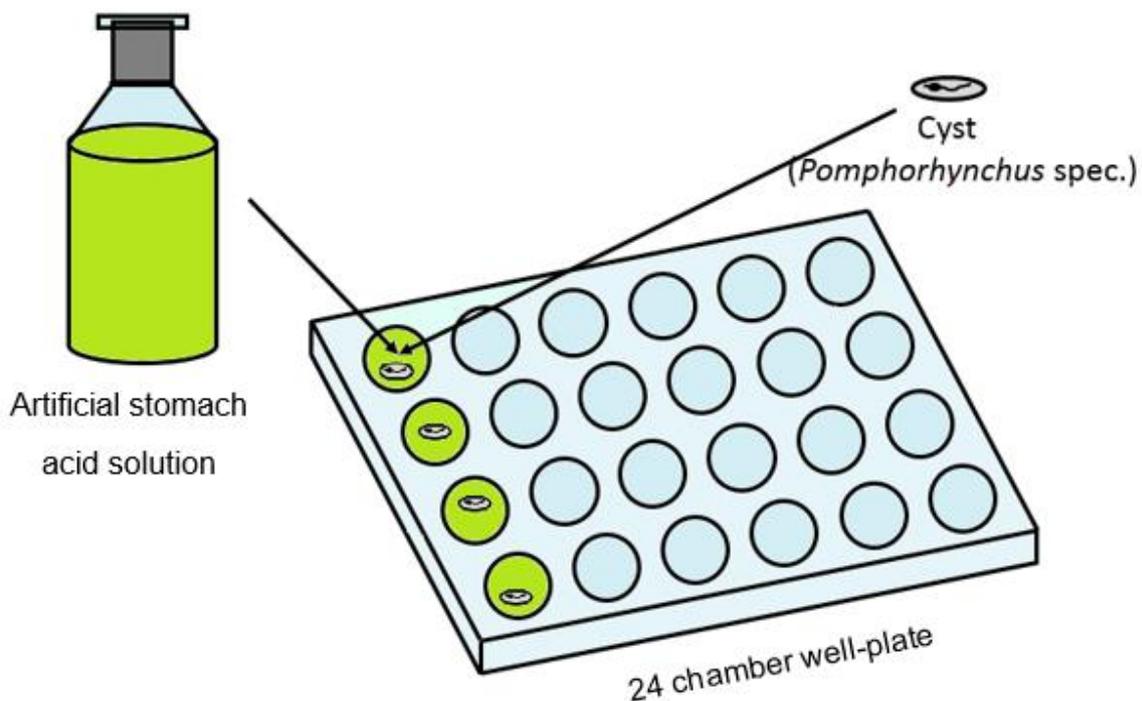


Figure 3.3: Experimental setup of the artificial digestive process

After the incubation, the cysts were dissolved while their content remained in the well plate chambers. Remains in each chamber were examined under a stereomicroscope (OLYMPUS SZX9) in order to access the infestation status with *A. crassus*. By means of the total number of infested cysts, the mean infestation rate was calculated.

Based on these results, a group of ten European eels (*A. anguilla*) with a mean size of 42.6 cm (SD = 6.4 cm) were infested with cysts of *P. laevis* that were collected from the body cavity of *N. melanostomus*. All eels of the experimental group were

provided by a commercial eel farm and were free of any infestation with *A. crassus*. The total number of cysts that were manually administered to each eel by a stomach tube ($\emptyset = 0.5$ mm) varied between 16 and 18. Subsequently to the infection, each eel was kept individually in water tanks with a volume of 30 liters at a water temperature between 10 and 13°C with permanent air supply. A parasite-free control group of five eels was kept simultaneously under the same experimental conditions.

After 154 days the eels were sacrificed and parasitologically examined. Inner organs were removed and digestive tracts as well as swim bladders were carefully examined under a stereomicroscope. Detected individuals of *A. crassus* were subsequently categorized according to larvae stage, sex, and size (measured in mm).

Mathematical analysis

In order to characterize the composition and diversity of the parasite communities different parasitological parameters and diversity measures at α - and β -diversity levels were calculated. All used variables are listed in table 3.3. The parameters that were used are:

- prevalence ($P = \frac{n_{inf.}}{N} \cdot 100[\%]$),
- mean intensity ($MI = \frac{\sum n_{Species}}{n_{inf.}}$),
- and abundance ($A = \frac{\sum n_{Species}}{N}$).

The alpha diversity was calculated by means of: a) Shannon Wiener Diversity Index, b) Shannon Wiener Evenness, c) Simpsons Diversity Index, and d) Brillouin Diversity Index (and standard deviation).

a)
$$H = -\sum_{i=1}^s (p_i \cdot \ln p_i)$$

b)
$$E = \frac{H}{H \max.} = \frac{H}{\ln s}$$

c)
$$D = \frac{1}{\sum p_i^2}$$

d)
$$HB = (\ln N! - s \ln n_i!) / N$$

In order to describe the similarity and to express the faunistic overlap of the parasite communities of the fish species and different river systems, the Sørensen index (Wolda 1981) was used.

Table 3.3: List of variables that were used in order to calculate all mentioned ecological parameters

Mathematical variable	Specific meaning
D	Simpsons Diversity Index
E	Shannon Wiener Evenness
H	Shannon Wiener Diversity Index
HB	Brillouin Diversity Index
H_{max}	Natural logarithm of the total number (of parasites) at sampling location
MA	Mean abundance
MI	Mean intensity
N	Total number of host individuals at sampling location
n_i	Number of individuals of parasite species i
n_{inf}	Number of infected host individuals
n_{species}	Total number of parasites
P	Prevalence
p_i	Relative frequency of parasite species i
S	Total number (of parasites) at sampling location

Multidimensional scaling (MDS) was applied for visualizing the similarities and dissimilarities of parasite communities of particular fish species between different rivers and for all fish species within each river. Additional ANOSIM analyses were used in order to further clarify the results of the MDS (if required). MDS as well as ANOSIM were performed with Primer 7 (©Primer-E Multivariate Statistics for Ecologists) by using the Bray-Curtis dissimilarity index.

Chapter I: Direct effects of Ponto-Caspian invasion on local fish parasite communities by the example of three native and one invasive host species

4 How Ponto-Caspian invaders affect local parasite communities of native fish

4 How Ponto-Caspian invaders affect local parasite communities of native fish

M. A. A. Hohenadler, M. Nachev, M. Freese, J. D. Pohlmann, R. Hanel, B. Sures

Submitted to: International Journal for Parasitology: Parasites and Wildlife (18-110)

4.1 Summary

Invasive species have become a major threat to ecosystems worldwide. Their effects on these systems are versatile and mostly well studied. However, not much is known about the impact that invasion might have on local parasite communities, although parasites are usually important response variables for ecosystem health. To improve the knowledge on how local fish parasite communities and their dynamics are affected by invasive species and how these processes change local host-parasite interactions over time, we studied different host-parasite systems in four German rivers. Three of these rivers (Rhine, Ems, and Elbe) are heavily invaded by different Ponto-Caspian species, while the fourth river (Schwentine) was free of any Ponto-Caspian invaders. Additionally, literature data on parasite communities before invasion were compared with the post invasion status for the rivers Rhine and Elbe. The results showed an explicit difference between the parasite communities of different host species from the three invaded rivers when compared to the Schwentine River. Among the local internal parasite communities, especially the acanthocephalan *Pomphorhynchus laevis* and the nematode *Raphidascaaris acus* have to be considered as key species associated to invasions from the Ponto-Caspian region. Our study revealed that the invasive Ponto-Caspian species interact with local parasite and host species potentially causing

an increase of parasite infestation rates in native host species due to a parasite spill back or spill over. The results were supported by the analysis of literature data on parasite communities. Consequences for local parasite communities include decreased prevalence of native parasites towards an extinction of entire parasite species.

4.2 Background

Anthropogenically induced invasion by neobiota has become a major threat to ecosystems worldwide (Pimentel et al. 2001, Molnar et al. 2008). Non-indigenous free-living species can disturb the balance in the new environment by reducing food and space resources for native species or by changing food web structures as well as energy flows in invaded ecosystems (Sapota 2004, Carman et al. 2006). Although many of these alterations have already been studied, the consequences of invasion on the transmission of local parasites as well as on the composition and dynamics of local parasite communities are often neglected. In contrast to their contribution to the general biodiversity (Poulin 2000, Bush et al. 2001), parasites may also have negative impacts on the fauna of the invaded ecosystem (Lafferty et al. 2008) and are therefore important response variables in impact evaluation (Sures et al. 2017). Changes in parasite communities can appear either if invasive host species interact with local parasites or if native host species are confronted with non-indigenous parasites. A good example of such a direct effect is the interaction between the nematode *Anguillicola crassus*, being invasive in Europe, and the native European eel (*Anguilla anguilla*). This parasite was presumably introduced to Europe with *Anguilla japonica* elvers from Taiwan for stocking programs in the 1980s (Wielgoss et al. 2008). As a result, the less adapted European eel shows both, higher prevalence and intensity of infection compared to the naturally co-evolved host, *A. japonica* (Münderle et al. 2006, Knopf 2006). As the European eel has experienced a massive decline in recruitment since the early 1970s, infection with *A. crassus* can be regarded as one of the potential causes together with other threats such as climate change, overfishing, obstruction of habitats, predation and pollution (Sures & Knopf 2004, Freese et al. 2016, 2017).

Nevertheless, invasion of parasites can also have indirect impacts on local free-living species. Parasites might be able to modify interactions between invasive and

native hosts or even mediate their interactions through their effects on host fitness (Dunn 2009). Examples could be a reduction of the host's competitive ability (Aliabadi and Juliano, 2002, Young et al. 2017), or a host-manipulation, which might lead to increased predation (Mouritsen and Poulin, 2005). With respect to free-living species, several scenarios show what might happen to an environment invaded by new species. For instance, the enemy release hypothesis states that invasive species usually benefit from a loss of natural enemies during the invasion process (Colautti et al. 2004). Subsequently, they also encounter fewer parasites in the new area compared to their native range, and therefore often show a lower parasite infestation rate when compared to native hosts (Torchin et al. 2002, Dang et al. 2009, Limbery et al. 2014). Although invasive species accumulate local parasites over time, their number generally reflects only a fraction of the ones they have escaped from (Torchin and Mitchell 2004). However, the non-indigenous species can also serve as an additional appropriate host that might amplify the transmission of local parasites. This so called "parasite spill back" results in an increased parasite infestation rate in native host species, which might lead to changes of relative ratios of certain parasite species within the community (Tomkins and Poulin 2006, Kelly et al. 2009, Slapansky et al. 2016). Furthermore, if non-native parasites are introduced together with their invasive hosts, they could "spill over" to the native host communities and become highly abundant (Kelly et al. 2009, Hohenadler et al. 2018). In this case, a replacement of native and sometimes closely related parasite species can occur due to a missing co-evolutionary history and missing resistance of local host species to newly introduced parasites (Diagne et al. 2016). However, the invasive free-living species might also decrease the infection risk in native host populations, i.e. the so called "dilution effect" (Slapansky et al. 2016, Gagne et al. 2016) if they acquire local parasites but turn out to be an inappropriate host for further parasite development or maturation (so called dead-end host).

The scenarios described above usually focus on effects of free-living or parasitic invaders on one or few parasite species but rarely on parasite communities of a given group of hosts. Therefore, the questions how local fish parasite communities and their dynamics are affected by invasive species and how these processes change local host-parasite interactions over time remain largely unanswered and are thus the focus of the present study. Some German river basins are a good basis to address this question as they provide access to the front of immigration of Ponto-Caspian species, where native free-living biota and their parasite communities are confronted with alien

species (Essink and Dekker 2002, Bernauer and Jansen 2006, Nehring 2006). Furthermore, there are published data sets of parasite communities of various hosts from different German rivers that were recorded before Ponto Caspian invasion had occurred, which allow additional analyses of possible impacts these invasions might have on local species and communities (e.g. Jakob et al. 2016). Usually, the invasion of Ponto-Caspian species, originating from the Black, Azov, and Caspian Seas (Micklin et al. 2014) is a consequence of anthropogenic impacts. For example, new passages and distribution pathways (e.g. cargo shipping, ship lifts, and canals) allowed former geographically isolated species to spread into new areas (Bij de Vaate et al. 2002, Boonstra et al. 2016). Examples are the so-called “killer shrimp”, *Dikerogammarus villosus*, and the freshwater mussel *Dreissena polymorpha*, both of which spread successfully from the Ponto-Caspian region to nearly all big water systems of Western and of Central Europe (Ketelaars 2004, Gallardo and Aldridge 2015). Additionally, migrating Ponto-Caspian gobiid fish were considered a potential risk for native fish species due to their high invasive potential and competitiveness for sustenance, energy resources, and spawning grounds (Jakšić et al. 2016). The most recognized species among these gobiids are the round goby (*Neogobius melanostomus*), the bighead goby (*Ponticola kessleri*), and the monkey goby (*Neogobius fluviatilis*), with *N. melanostomus* being the most successful invader among them. Despite the great attention that was given to Ponto-Caspian species lately, their role in the transmission of parasites as well as the potential risk of introducing new parasites as well as their specific impact on local parasite (infra-) communities of other (native) hosts remained partly unclear. It is already known that Ponto Caspian species can serve as intermediate and / or paratenic hosts for parasites (Ondračková et al. 2009), and therefore potentially evoke a parasite spillback or spillover (Hohenadler et al. 2018) when acquiring local parasites (Francová et al. 2011). However, Kvach et al. (2015, 2017) have shown that the parasite communities of Ponto-Caspian gobiids usually differ from the parasite communities of native fish species, especially in the first years after their introduction.

Consequently, in this study we focused on possible impacts of Ponto-Caspian invasive species (mainly *N. melanostomus*) on parasites and their transmission to native fish species. Therefore, we compared the internal parasite communities of three different fish species (including *N. melanostomus*) from habitats invaded by, and free of Ponto-Caspian species. Furthermore, we analyzed data of parasitological studies

performed before, during, and shortly after Ponto Caspian invasions had occurred in the same rivers in order to be able to place our results in a wider context.

4.3 Material and methods

Host and parasite sampling

Fish sampling was performed annually in three consecutive years (2014-2016) in the Rhine River, near the city of Grieth, (mean water discharge MQ < 3,000 m³/s; flows into the North Sea), the Ems River, near the City of Ditzum, (MQ < 150 m³/s; flows into the North Sea), the Elbe River, near the city of Winsen a.d. Luhr, (MQ < 900 m³/s; flows into the North Sea), and the Schwentine River (MQ < 50 m³/s; flows into the Baltic Sea), cf. figure 1. Each year, samplings were performed simultaneously in each river during summer season (June-September). The rivers Rhine, Ems, and Elbe were already invaded by *N. melanostomus* and other Ponto-Caspian species whereas the Schwentine was considered as a reference river system, which remained free of Ponto-Caspian invasion until the end of the sampling period.

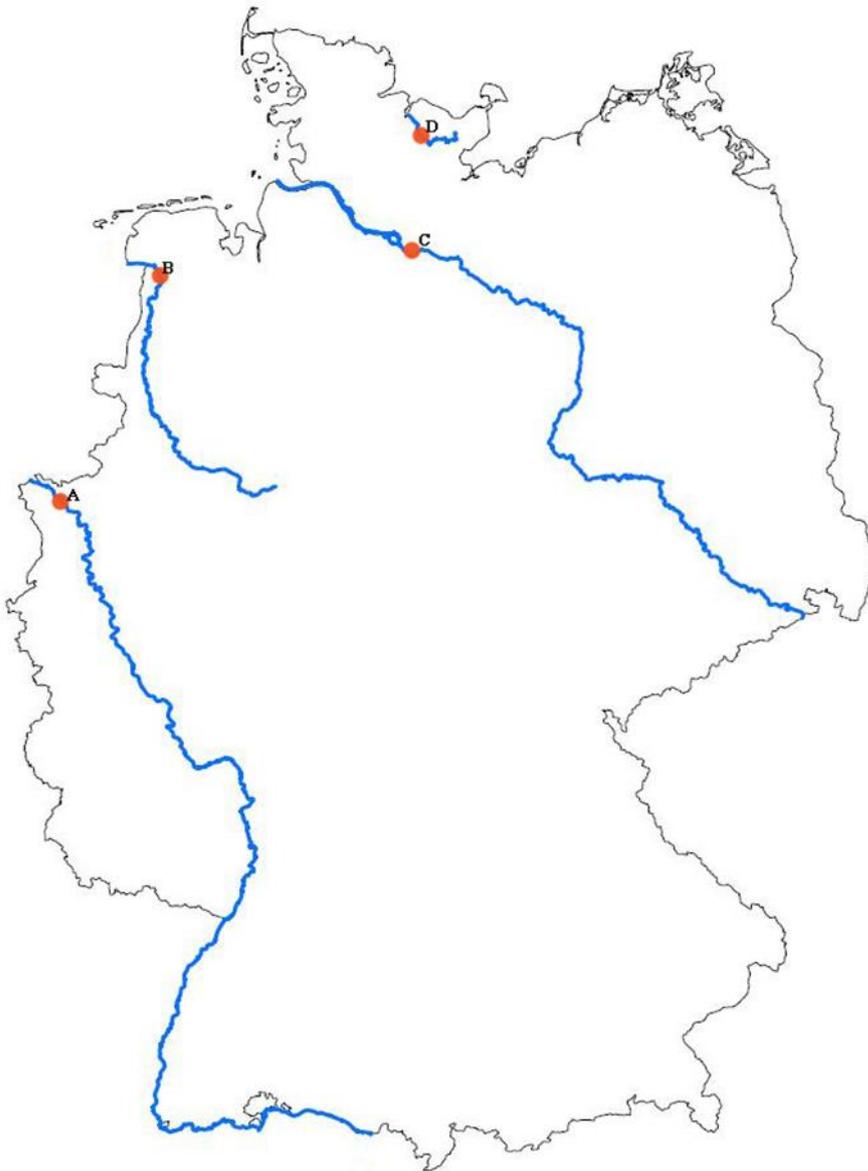


Figure 4.1: Sampling locations: A = Rhine River at the of city Grieth; B = Ems River at the city of Ditzum; C = Elbe River at the city of Winsen a. d. Luhe, D = Schwentine River, close to the Großer Plöner See

Target fish species have been sampled during this study, namely, the invasive *N. melanostomus*, as well as the native species, ruffe (*Gymnocephalus cernua*), and European eel (*Anguilla anguilla*) (cf. table 2). During each sampling, collected individuals corresponded to the same size class (cf. table 1).

Table 4.1: Mean sizes and weights of collected fish species at all sampling locations

Host species	Mean size (mm)	Mean weight (g)
<i>N. melanostomus</i>	11.9 (9.7 / 13.4)	22.8 (14.3 / 34.1)
<i>G. cernua</i>	11.1 (9.8 / 12.3)	16.4 (10.4 / 28.7)
<i>A. anguilla</i>	56.8 (44.9 / 74.0)	326.0 (157.9 / 587.2)

The native fish species were selected due to their rather broad distribution within the selected river systems. All of them are spawn predating, bottom dwelling fishes, and share partly the same habitats (Gebhardt and Ness 1997, fishbase.org). Furthermore, both selected native species interact with the invasive round goby, another bottom dwelling fish and spawn predator, for either sustenance (*A. anguilla*, *G. cernua*), spawning grounds (*G. cernua*), or form a prey-predator relationship (*A. anguilla*) (e.g. Jůza et al. 2018, Hohenadler et al. 2018).

Table 4.2: Catch numbers of the three fish species (*G. cernua*, *A. anguilla*, *N. melanostomus*) for each sampling year (2014-2016) and waterbody (the rivers: Rhine, Ems, Elbe, Schwentine)

Waterbody	Year	Fish species	Number individuals (n)
Rhine River	2014	<i>Anguilla anguilla</i>	20
		<i>Neogobius melanostomus</i>	43
		<i>Gymnocephalus cernua</i>	18
	2015	<i>A. anguilla</i>	9
		<i>N. melanostomus</i>	22
	2016	<i>A. anguilla</i>	20
<i>N. melanostomus</i>		28	
Ems River	2014	<i>A. anguilla</i>	8
		<i>N. melanostomus</i>	35
		<i>G. cernua</i>	12
	2016	<i>A. anguilla</i>	44
		<i>N. melanostomus</i>	22
		<i>G. cernua</i>	21
Elbe River	2015	<i>N. melanostomus</i>	33
		<i>A. anguilla</i>	46
	2016	<i>N. melanostomus</i>	40
		<i>G. cernua</i>	6
Schwentine River	2014	<i>A. anguilla</i>	27
		<i>G. cernua</i>	21
	2015	<i>A. anguilla</i>	21
		<i>G. cernua</i>	21
	2016	<i>A. anguilla</i>	17
		<i>G. cernua</i>	26

Due to an unequal sampling size (different number of individuals per year, locality, and species), rarefaction analysis was performed in order to ensure that results are representative for each year.

In line with sampling for the Data Collection Framework (DCF) of the European

Commission, eel and forage fish samples were obtained from commercial fishermen, fishing with fyke nets at the given locations. Immediately after sampling, the fish were sacrificed and kept frozen at -20°C or transported alive to the research facility where they were kept in designated tanks for a maximum of 4 days until parasitological examination (latter only applied for individuals of *N. melanostomus* for the year 2014 that were sampled in the Rhine River). The respective fish treatment in 2014 was depended on fish handling by local fishermen. However, frozen and fresh fish provide comparable results if dissected within four days after sampling (Kvach et al. 2016). Therefore, freezing is an accurate alternative to fresh samples as it provides adequate data on parasite species richness, although the results might not reflect the parasites abundance sufficiently (Kvach et al. 2018). The fish's liver, digestive tract, and swim bladders (with exception of gobies) were checked for presence of endoparasites using a binocular and the recovered parasites were removed and identified to species level or alternatively to the lowest possible taxonomic level by morphological identification. Morphological parasite identification was based on keys provided in Moravec (1994 & 2004) and Brohmer and Ehrmann (1966).

As acanthocephalans of the genus *Pomphorhynchus* usually have a very similar morphology, all individuals were identified using molecular approaches. Internal transcribed spacer 1 (ITS 1) rDNA genes were amplified by PCR according to Franceschi et al. (2008), which ensures a differentiation between all eligible *Pomphorhynchus* species by the length of their PCR-product (Perrot-Minnot 2004; Nachev et al. 2010).

Evaluation and processing of parasitological data

In order to describe the internal parasite community within each habitat and for each examined fish species, different parasitological and diversity parameters were calculated. Parasitological parameters such as prevalence, mean intensity and abundance were determined according to Bush et al. (1997). Parameters were determined for each year in which sampling was performed with no distinct differences among the years, consequently only their mean values are presented in this study. The diversity indices considered in this study were Shannon Wiener Diversity Index, Shannon Wiener Evenness, Simpsons Diversity Index, and Brillouin Diversity Index. Latter were calculated according to Magurran (1988). In order to describe the similarity and to express the faunistic overlap of the parasite communities of the fish species within the different rivers, the Sørensen index (Wolda 1981) was used. All described

parameters are commonly used in parasitological research (e.g. Sures et al., 1999, Thielen 2005, Ondračková et al. 2015, Jacob et al. 2016), and can therefore be placed in a wider context.

Multidimensional scaling (MDS) was applied for visualizing the similarities and dissimilarities of parasite communities of particular fish species between different rivers, and for all fish species within each river. Additional ANOSIM analyses were used in order to further clarify the results of the MDS (if required). MDS as well as ANOSIM analyses were performed with Primer 7 (©*Primer-E Multivariate Statistics for Ecologists*) by using the Bray-Curtis dissimilarity index.

In order to discuss our results and to be able to determine possible changes between the parasite communities before and after Ponto-Caspian invasion had occurred, additional analyses were performed, using data of the past three decades of the parasite communities of *A. anguilla* and *G. cernua* from the rivers Rhine and Elbe.

4.4 Results

Parasitological data such as prevalence, mean intensity, and abundance of the detected parasites are summarized for all investigated rivers (*cf.* table 3). Generally, the acanthocephalan *Pomphorhynchus laevis* was a common or sometimes the dominant parasite species in the rivers Rhine, Ems, and Elbe while no records were made of it in any hosts sampled from the Schwentine River. The nematode *Anguillicola crassus* was the dominant parasite species in all sampled eels (with no distinct variations in prevalence) within all rivers. Comparison of the internal parasite community composition between the rivers showed clear differences for the parasite communities of *G. cernua* sampled from the Schwentine River when compared to the rivers Rhine and Elbe with none of the ruffe sampled from the Schwentine River being infected with any parasite.

Table 4.3: Parasitological data of the parasite community of *G. cernua*, *N. melanostomus* and *A. anguilla* within the four sampled rivers (Rhine, Ems, Elbe, Schwentine)

River	Host species	Nematoda									Acanthocephala					
		<i>A. crassus</i>			<i>R. acus</i>			<i>P. laevis</i>			<i>A. lucii</i>			<i>P. ambiguus</i>		
		Prevalence	Abundance	Mean Intensity (Standard deviation)	Prevalence	Abundance	Mean Intensity (Standard deviation)	Prevalence	Abundance	Mean Intensity (Standard deviation)	Prevalence	Abundance	Mean Intensity (Standard deviation)	Prevalence	Abundance	Mean Intensity (Standard deviation)
Rhine	<i>G. cernua</i>	-	-	-	5.0	0.1	1.0 (0.0)	5.0	0.1	1.0 (0.0)	-	-	-	-	-	-
	<i>N. melanostomus</i>	-	-	-	12.6	0.4	2.2 (3.6)	83.4	13.6	18.6 (24.1)	-	-	-	-	-	-
	<i>A. anguilla</i>	73.5	3.2	4.2 (5.3)	13.6	0.5	3.1 (2.5)	27.3	4.5	12.1 (17.5)	10.7	0.5	3.3 (12.9)	11.1	7.3	66.0 (0.0)
Ems	<i>G. cernua</i>	-	-	-	12.5	0.4	1.7 (0.9)	8.3	0.2	2.5 (1.5)	2.4	>0.0	1.0 (0.0)	-	-	-
	<i>N. melanostomus</i>	-	-	-	4.6	0.1	1.0 (0.0)	8.0	1.7	1.1 (1.2)	-	-	-	-	-	-
	<i>A. anguilla</i>	61.9	3.1	3.1 (2.2)	15.9	3.7	14.4 (7.2)	2.3	0.1	1.0 (0.0)	10.8	8.7	36.8 (52.5)	3.4	3.6	65.3 (46.8)
Elbe	<i>G. cernua</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<i>N. melanostomus</i>	-	-	-	3.3	>0.0	1.0 (0.0)	12.2	0.2	1.4 (0.8)	-	-	-	-	-	-
	<i>A. anguilla</i>	80.0	10.3	10.3 (9.9)	15.7	0.3	1.6 (0.7)	13.3	0.3	1.8 (1.2)	33.3	9.0	21.0 (36.4)	11.1	2.4	16.8 (20.3)
Schwentine	<i>G. cernua</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<i>A. anguilla</i>	72.7	5.7	8.9 (12.1)	6.8	3.2	1.0 (0.0)	-	-	-	8.1	0.4	4.3 (2.3)	5.9	0.3	2.3 (1.9)

Table 4.3: Continuation

River	Host species	Cestoda						Microsporidia		
		<i>B. claviceps</i>			<i>Proteocephalus spec.</i>			<i>Microsporidia</i>		
		Prevalence	Abundance	Mean Intensity (Standard deviation)	Prevalence	Abundance	Mean Intensity (Standard deviation)	Prevalence	Abundance	Mean Intensity (Standard deviation)
Rhine	<i>G. cernua</i>	-	-	-	-	-	-	-	-	-
	<i>N. melanostomus</i>	-	-	-	-	-	-	8.4	0.2	4.8 (3.0)
	<i>A. anguilla</i>	14.1	0.7	4.5 (4.2)	10.7	1.0	7.2 (0.5)	-	-	-
Ems	<i>G. cernua</i>	-	-	-	-	-	-	28.6	2.5	8.8 (6.1)
	<i>N. melanostomus</i>	-	-	-	-	-	-	35.8	2.0	4.6 (3.0)
	<i>A. anguilla</i>	9.1	0.2	1.4 (0.5)	-	-	-	-	-	-
Elbe	<i>G. cernua</i>	-	-	-	-	-	-	-	-	-
	<i>N. melanostomus</i>	-	-	-	-	-	-	24.0	2.0	9.1 (7.7)
	<i>A. anguilla</i>	8.9	0.2	1.8 (1.3)	-	-	-	-	-	-
Schwentine	<i>G. cernua</i>	-	-	-	-	-	-	-	-	-
	<i>A. anguilla</i>	6.4	0.2	1.5 (0.5)	-	-	-	-	-	-

All three fish species were infested by parasite species in different prevalence and abundances. In all rivers hosts were usually infested by 1 to 2 parasite species (when infested) (*cf.* figure 2). The highest infracommunity richness within a single host individual was found in the Rhine River. However, the results from the Elbe River provided the most striking results, with single host individuals being infested with up to six different parasite species (*cf.* figure 2 C).

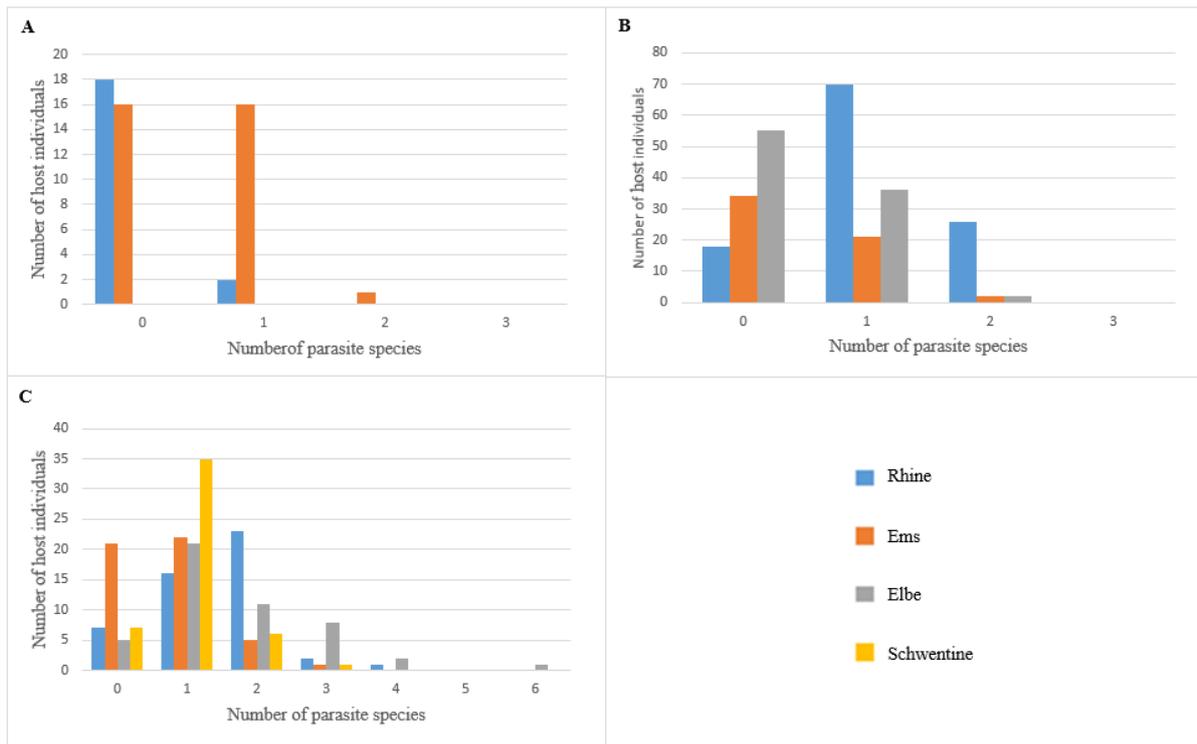


Figure 4.2: Distribution of species richness in the parasite infracommunities of the three different fish species (A) *G. cernua*; B) *N. melanostomus*; C) *A. anguilla*) within the four German rivers (blue = Rhine; orange = Ems; gray = Elbe; yellow = Schwentine)

Diversity measures of the component parasite communities at sampling locations showed that the internal parasite communities in the Rhine River exhibited always the highest diversity, except for *N. melanostomus* whose internal parasite community showed the highest diversity in the Elbe River. The parasite communities within the Schwentine River showed the lowest diversity when compared to any of the other three rivers (table 4). An exception here is the internal parasite community of *G. cernua*, since no parasites were detected in any sampled individual from the rivers Elbe and Schwentine.

Table 4.4: Component community and infracommunity diversity indices of all sampled fish species (*G. cernua*; *N. melanostomus*; *A. anguilla*) within the four German rivers (Rhine; Ems; Elbe; Schwentine)

Fish species	Waterbody	Species richness	Shannon Wiener Diversity Index	Shannon Wiener Evenness	Simpsons Diversity Index	Brillouin Diversity Index (mean / SD)
<i>G. cernua</i>	Rhine	2	0.28	0.40	2.16	0.13 ± 0.10
	Ems	4	0.83	0.46	2.03	0.03 ± 0.31
	Elbe	0	-	-	-	-
	Schwentine	0	-	-	-	-
<i>N. melanostomus</i>	Rhine	3	0.32	0.30	1.26	0.05 ± 0.13
	Ems	3	0.37	0.33	1.22	0.01 ± 0.04
	Elbe	3	0.54	0.49	3.61	0.01 ± 0.05
<i>A. anguilla</i>	Rhine	7	1.44	0.74	3.58	0.21 ± 0.28
	Ems	6	1.26	0.70	2.87	0.09 ± 0.21
	Elbe	6	1.12	0.62	2.61	0.12 ± 0.15
	Schwentine	6	0.35	0.19	1.15	0.04 ± 0.10

The beta diversity for the parasite communities of *A. anguilla* showed a high similarity among the rivers Rhine, Ems, and Elbe, whereas latter rivers had a lower similarity with the Schwentine River (table 5). The parasite beta diversity of all endoparasites of *G. cernua* was less similar between the rivers Rhine and Ems, while no endoparasites were found within the rivers Elbe and Schwentine. The parasite beta diversity of *N. melanostomus* did not show any differences in any of the sampled rivers.

Table 4.5: Beta diversity of all sampled fish species (*G. cernua*; *N. melanostomus*; *A. anguilla*) within the four German rivers (Rhine; Ems; Elbe; Schwentine)

Fish species	Waterbody	Rhine	Ems	Elbe
	Waterbody			
<i>G. cernua</i>	Rhine		66.7	
	Ems	66.7		
	Elbe	0.0	0.0	
	Schwentine	0.0	0.0	
<i>N. melanostomus</i>	Rhine		100.0	100.0
	Ems	100.0		100.0
	Elbe	100.0	100.0	
<i>A. anguilla</i>	Rhine		92.3	92.3
	Ems	92.3		100.0
	Elbe	92.3	100.0	
	Schwentine	76.9	83.3	83.3

Comparative analyses (MDS, ANOSIM) between the parasite infracommunities of each fish species within all tested rivers confirmed that the communities of *G. cernua* did not show any distinct differences between the rivers Rhine and Ems. The results of the MDS analysis for *A. anguilla* revealed patterns with no visible differences for either of the four rivers. The same applied for the analysis of the different parasite communities of the three different rivers in which *N. melanostomus* was sampled (*cf.* figure 3). The additional ANOSIM analyses for *A. anguilla* and *G. cernua* showed no further effect of the sampling locations on the parasite communities (R value between 0.03 and 1.15) while the results for *N. melanostomus* show that the endoparasite communities of the rivers Rhine, Ems, and Elbe do not show a distinct dissimilarity (*cf.* table 6).

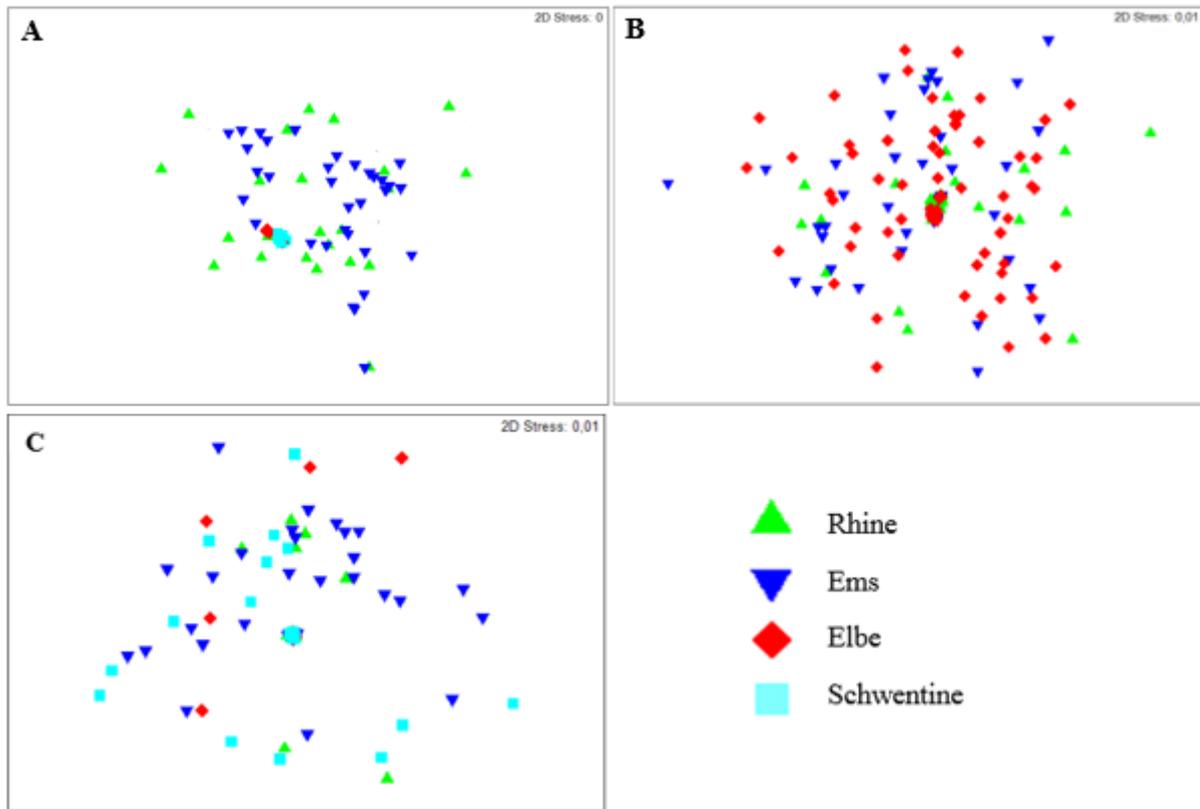


Figure 4.3: Internal parasite community of A) *G. cernua*; B) *N. melanostomus*; C) *A. anguilla* within the tested rivers (Rhine; Ems; Elbe; Schwentine).

Table 4.6: ANOSIM analyses (pairwise test) of the internal parasite communities of all sampled hosts (*G. cernua*, *N. melanostomus*, *A. anguilla*) within all rivers (Rhine; Ems; Elbe; Schwentine) individually

<i>G. cernua</i>	Sample Statistics (Global R): 0.153	Significance level of sample statistic: 0.1%	
	Sample Statistics (Global R): 0.340	Significance level of sample statistic: 0.1%	
<i>N. melanostomus</i>	Group	R Statistics	Significance Level (%)
	Rhine – Ems	0.50	0.1
	Rhine – Elbe	0.38	0.1
	Ems – Elbe	-0.01	89
<i>A. anguilla</i>	Sample Statistics (Global R): 0.032	Significance level of sample statistic: 0.8%	

Data analyses of the internal parasite community of *A. anguilla* from the rivers Rhine and Elbe from times before, during, and after Ponto Caspian invasion took place

showed that the parasitological parameters of the internal parasites varied over the last three decades (*cf.* table 7). The prevalence of *A. crassus* steadily increased from 76.7 % to over 90 % in the Rhine and from 78 to 100 % in the Elbe River. Simultaneously, the prevalence of the acanthocephalan *A. lucii* in the Rhine River varied between 1.6 and 15.8 %, while its prevalence in the Elbe River was at a similar level during the entire period with only one outlier in 1994. The prevalence of *P. ambiguus* in the river Rhine did not show much variation neither, although the prevalence of the parasite in *A. anguilla* slightly decreased between 1995 and 2004. Due to a lack of datasets, only one sampling period is represented for *P. ambiguus* in the river Elbe with a mean intensity of 373 individuals per eel. The prevalence of *P. laevis* in *A. anguilla* accounted for around 6.5 % in the Rhine River shortly before the first invasion interval occurred, it increased to around 16 % between both intervals and up to 60-90 % during the second interval. Similar developments occurred within the Elbe River (*cf.* table 7). During the presented sampling period the prevalence of the nematode *R. acus* in the Rhine River increased from 6.6 % to 30.0 %. A similar increase can be recognized for the Elbe River. However, while the prevalence of *B. claviceps* in the Elbe River differs over the years following no specific pattern, its prevalence in the river Rhine decreased from 21.7 % in 1995 to 4.0 % in 2001.

The parasitological parameters of the internal parasites of *G. cernua* are fragmentary, however the prevalence of *P. laevis* was 72.7 % in 2001, while at the same time the prevalence of *R. acus* was 13.6 %.

Table 4.7: Parasitological parameters of the internal parasite community of *A. anguilla* and *G. cernua* from the rivers Rhine and Elbe obtained from parasitological studies performed before, during, and shortly after the two Ponto Caspian invasion intervals

Host	Parasite species	Locality	N	Prevalence	Mean intensity	Mean Abundance	Year of sampling	Temporal classification	Reference
<i>A. anguilla</i>	<i>A. crassus</i>	Rhine	61	76.7 %	5.0	3.9	1995	Shortly before first invasion interval	Sures et al. 1999
		Rhine	19	85.0 %	6.4	5.5	1999	Several years after first invasion interval	Sures and Streit 2001
		Rhine	26	92.3 %	3.0	2.5	2000	Between first and second invasion interval	Sures 2002
		Rhine	25	91.3 %	8.4	7.7	2001		
		Elbe	13	78 %	21	-	1995	Before first invasion interval	Movarec and Scholz 2015
		Elbe	10	100 %	1	-	2001	During first invasion interval	
	<i>R. acus</i>	Rhine	61	6.6 %	3.3	0.2 / 0.9	1995	Shortly before first invasion interval	Sures et al. 1999
		Rhine	19	26.3 %	4.8	1.3 / 3.1	1999	Several years after first invasion interval	Sures and Streit 2001
		Rhine	26	23.1 %	5.5	1.3	2000	Between first and second invasion interval	Sures 2002

	Rhine	29	30.0 %	2.3	0.7	2005	Beginning of second invasion interval	Thielen 2005
	Elbe	30	13.3 %	3.0	0.3	2006	Before second invasion interval	Jakob et al. 2016
	Elbe	30	23.3 %	246.4	56.67	2007	Beginning second invasion interval	Jakob et al. 2016
<i>P. laevis</i>	Rhine	61	6.6 %	14.0	0.9	1995	Shortly before first invasion interval	Sures et al. 1999
	Rhine	19	15.8 %	1.0	0.2	1999	Several years after first invasion interval	Sures and Streit 2001
	Rhine	20	60.0 %	2.2	1.3	2004	Beginning of second invasion interval	Thielen 2005
	Rhine	20	90.0 %	7.6	6.9	2005	Beginning of second invasion interval	Thielen 2005
	Elbe	16	Parasitological examinations did not			1988	Before first invasion interval	Movarec and Scholz 2015
	Elbe	13	show any individuals of <i>P. laevis</i> within			1991		
	Elbe	31	<i>A. anguilla</i>			1994		
	Elbe	30	6.6 %	1	0.1	2007	Beginning second invasion interval	Jakob et al. 2016
<i>A. lucii</i>	Rhine	61	1.6 %	5.0	0.1 / 0.6	1995	Shortly before first invasion interval	Sures et al. 1999
	Rhine	19	15.8 %	4.0	0.6 / 1.7	1999	Several years after first invasion interval	Sures and Streit 2001

	Rhine	29	10.3 %	1.3	0.1	2004	Beginning of second invasion interval	Thielen 2005
	Elbe	10	63.0 %	2	-	1982	Before first invasion interval	Movarec and Scholz 2015
	Elbe	10	67.0 %	19	-	1990		
	Elbe	31	13.0 %	2	-	1994		
	Elbe	10	57.0 %	5	-	2000	During and shortly after first invasion interval	
	Elbe	10	67.0 %	5	-	2003		
<i>P. ambiguus</i>	Rhine	61	39.3 %	41.6	16.4	1995	Shortly before first invasion interval	Sures et al. 1999
	Rhine	19	36.8 %	16.3	6.0 / 17.2	1999	Several years after first invasion interval	Sures and Streit 2001
	Rhine	29	31.0 %	33.9	10.5	2004	Beginning of second invasion interval	Thielen 2005
	Elbe	30	6.6 %	373	22.4	2007	Beginning second invasion interval	Jakob et al. 2016
<i>B. claviceps</i>	Rhine	61	21.7 %	4.7	1.0 / 2.5	1995	Shortly before first invasion interval	Sures et al. 1999
	Rhine	19	20.0 %	2.3	0.5 / 1.0	1999	Several years after first invasion interval	Sures and Streit 2001
	Rhine	26	11.5 %	1.7	0.2 / 0.6	2000	Between first and second invasion interval	Sures 2002
	Rhine	25	4.0 %	0.0	0.1 / 0.4	2001		

		Elbe	31	38 %	2	-	1994	Before first invasion interval	Movarec and Scholz 2015
		Elbe	13	18 %	4	-	1995		
		Elbe	10	33 %	1	-	2008		
<i>Proteocephalus spec</i>		Rhine	61	-	-	-	1995	Shortly before first invasion interval	Sures et al. 1999
		Rhine	19	15.8 %	4.7	0.7 / 1.9	1999	Several years after first invasion interval	Sures and Streit 2001
		Rhine	26	-	-	-	2000	Between first and second invasion interval	Sures 2002
		Rhine	25	8.0 %	2.0	0.2 / 0.6	2001		
		Rhine	29	3.4 %	4.0	0.1	2004	Beginning of second invasion interval	Thielen 2005
		Elbe	30	50 %	4.9	2.45	2007	Beginning second invasion interval	Jakob et al. 2016
	G. cernua	<i>P. laevis</i>	Rhine	22	72.7 %	27.5	20.0	2001	Between first and second invasion interval
	<i>R. acus</i>	Rhine	22	13.6 %	2.7	0.4	2001	Between first and second invasion interval	Thielen 2005

Diversity measures of the component parasite communities within the river Rhine showed that the internal parasite communities of *A. anguilla* are remarkably less diverse before than several years after the first Ponto Caspian inversion interval (cf. table 8).

Table 4.8: Component community diversity indices of *A. anguilla* within the Rhine River obtained from parasitological studies performed before and after the first Ponto Caspian invasion interval

Host species	Water body	Sampling year	Species richness	Shannon Wiener Diversity Index	Shannon Wiener Evenness	Simpsons Diversity Index	Calculations based on
<i>A. anguilla</i>	Rhine River	1995	6	0.36	0.2	1.18	Sures et al. 1999
		1999	6	0.58	0.32	1.33	Sures and Streit 2001
		2000	6	1.36	0.76	3.05	Sures
		2001	6	0.91	0.51	1.77	2002
		2004	6	1.22	0.68	2.92	Thielen 2005

4.5 Discussion

Our study revealed notable differences between the internal parasite communities of various fish species from the Schwentine River when compared to the rivers Rhine, Elbe, and Ems (*cf.* table 3, 6; figure 3). These different results might be related to the invasion of Ponto-Caspian species into the three rivers, which occurred in two intervals. Ponto-Caspian gammarids such as *Dikerogammarus villosus* entered West German rivers around 1995, whereas the Ponto-Caspian gobiids were first recorded in the early 2000s (Kinzler et al. 2009, Borchering et al. 2011). These results are further strengthened by data analyses of the internal parasite communities of *Anguilla anguilla* and *Gymnocephalus cernua* from the rivers Rhine and Elbe from times before, during and after the two invasion intervals. In that context analyses both of our results as well as of historical data revealed that the results for the acanthocephalan *Pomphorhynchus laevis* and the nematode *Raphidascaris acus* are particularly striking since both parasites might be considered as key species associated to invasions from the Ponto Caspian region. Simultaneously, the results for the other internal parasites did not show an unambiguous link between changes in the parasites prevalence and the arrival of Ponto Caspian species (*cf.* table 7).

Pomphorhynchus laevis, which occurred as (sub-) adults in all studied hosts, presents itself as a common parasite species in samples from most rivers in this study, which reflects the outcome of previous studies (Sures et al 1999, Emde et al 2014). However, this only applies to the rivers Rhine, Elbe, and Ems since the parasite did not appear even as a single finding in any sampled host species from the Schwentine River. In fact, molecular analysis revealed that *P. laevis* is native to the Ponto-Caspian region and was recently co-introduced to European waterbodies together with different host species such as *D. villosus* and *Neogobius melanostomus* (Hohenadler et al. 2018). These hosts promoted the fast dispersal of the parasite within the new habitats due to a parasite spill-over (Kvach et al. 2006). Subsequently, the infestation rates with acanthocephalans of the genus *Pomphorhynchus* within the rivers Rhine, Ems, and Elbe increased noticeably (Sures et al. 1999, Jakob et al. 2009). After the first Ponto-Caspian invasion interval two different *Pomphorhynchus* species, the invasive *P. laevis* and the native *P. tereticollis*, were recorded in all three rivers. Due to a very similar morphology, the two species were most probably often misidentified and thus confused

with each other (O'Mahony et al. 2004; Kennedy 2006; Tain et al. 2006; Emde et al. 2012). Therefore, no reliable statements about the dispersal of the two species and their interaction with each other can be made. However, after several years of coexistence, *P. laevis* outcompeted the native *P. tereticollis* within the rivers Rhine, Ems and Elbe.

As *P. laevis* could only be found in rivers invaded by Ponto-Caspian species, it can be concluded that there is strong evidence that the appearance of *P. laevis* and the consequences for local ecosystems such as extinction of native parasites as well as high infestation rates in native hosts are related to the Ponto-Caspian invasion. The absence of the parasite in the Schwentine River supported this assumption.

However, when discussing possible reasons for the differences in the fish parasite communities it must be considered that the Schwentine River shows the lowest mean flow velocity of all rivers, and is furthermore the only river that flows through a system of different sized lakes on its way from spring to estuary. Furthermore, the Schwentine is the only river in this study that discharges into the Baltic Sea while the rivers Ems, Elbe and Rhine all discharge in the North Sea. Environmental conditions of both seas differ substantially in terms of salinity, especially in the coastal areas (Heath et al. 1991, Carlsson 1997). Appropriately, previous research on European perch (*Perca fluviatilis*) showed that parasite infestation rates differ between rivers that discharge in the Baltic and rivers flowing in the North Sea (Morozińska-Gogol 2013), which could also be partly responsible for the outcome of this study. Further analyses of (beta) diversity indices demonstrated similar results for each host species studied within the rivers Rhine, Elbe (exclusive *G. cernua*), and Ems, whereas results for *A. anguilla* showed a considerably lower parasite diversity (including the non-appearance of species such as *P. laevis*) for the Schwentine (cf. table 4 and 5).

Nevertheless, the infestation rates with other acanthocephalans suggest that the Schwentine is a comparable sampling site. While *P. laevis* could not be found in samples from the Schwentine River, infestation rates with other acanthocephalans such as *Acanthocephalus lucii* and *Paratenuisentis ambiguus* correspond to the results from the other rivers, suggesting suitable ecological conditions for acanthocephalans. Distribution patterns and infestation rates of both acanthocephalans do not show evidence that they can be related to Ponto-Caspian invasion. On the contrary, *P. ambiguus* usually shows a high host specificity for its intermediate as well as its final

host, and is strongly affected by environmental conditions such as salinity (Hiepe et al 2006). However, the Schwentine system also bears certain advantages, as it has one terminal dam at a hydropowerplant with a fish pass, that is monitored on regular bases. This and the fact that the authors of this study have been in direct contact with all local commercial fishermen during the sampling period made it easy to exclude a possible invasion of the Ponto Caspian species from the Baltic Sea.

Although these observations and assumptions do not rule out doubts about the Schwentine River as a suitable control site, an additional data evaluation of the internal parasite communities of *A. anguilla* and *G. cernua* in the rivers Elbe and Rhine from various periods (before, during and shortly after the two Ponto Caspian invasion intervals occurred; cf. table 7) further strengthen the conclusion that Ponto Caspian species have had a notable impact on the internal parasite communities of local host species. Analysis of the internal component parasite communities from hosts (*A. anguilla*) from the Rhine River sampled during the mentioned time period showed that Ponto Caspian invaders might have caused a notable change in the diversity of internal parasites (cf. table 8). The possible link between Ponto Caspian invasion and a change in the (internal) component parasite community is even more pronounced through comparison analyses with these results and the results gained from this study (cf. table 4). Further analyses have shown that changes in prevalence of *P. laevis* occurred parallel to the spreading of Ponto Caspian invaders in both studied rivers. The results showed that the prevalence of *P. laevis* increased from a low single digit value before the first invasion interval up to 90 % at the end of the second (cf. table 7). Between 2014 and 2016 the prevalence of *P. laevis* in *A. anguilla* in the Rhine reached a value of around 27 % (cf. table 3), indicating that after the invasive species evoked an exponential density growth of the parasite that lead to a short peak phase, its prevalence decreased and stabilized at a lower value, which also corresponds to the density development of Ponto Caspian species within the studied rivers.

For *G. cernua* no reliable data is available for the period before Ponto-Caspian invasion at any of our sampling locations and / or rivers. However, samples of *G. cernua* from the Rhine River that were taken shortly after the second invasion interval showed a prevalence of 73 % indicating a similar situation than for *P. laevis* obtained from *A. anguilla* (Thielen 2005).

Simultaneously, the infestation rates with the nematode *R. acus* also showed notable differences between the Schwentine River and the other three rivers. While

the prevalence of *R. acus* in the three fish species within the rivers Rhine, Elbe (excluding *G. cernua*), and Ems was between 3.5 and 24.1% (depending on sampling location and host species), it only appeared in the Schwentine River in *Anguilla anguilla* with a significantly lower prevalence (cf. table 3). The parasite occurred as 3rd stage larvae (encapsulated) in *N. melanostomus* and *G. cernua*, and as 4th stage larvae and adult in *A. anguilla*. Thus, our results as well as previous studies have shown that all fish species sampled in the present study are suitable hosts (intermediate, paratenic, definitive or pardefinitive) for *R. acus* (Moravec 1994). Therefore, the lack of *R. acus* in *G. cernua* as well as its very low prevalence in *A. anguilla* from the Schwentine River might be an indication of less infection pressure due to a lower general occurrence of *R. acus*. Accordingly, Ponto-Caspian species might be an important factor in the distribution of *R. acus* in Western European water systems. Invasive Ponto-Caspian species such as *N. melanostomus* as well as the amphipod *D. villosus* were described as suitable host species for *R. acus* (Ondračová et al 2005, Emde et al. 2014). It therefore appears to be likely that both species are able to cause higher prevalences of *R. acus* due to a parasite spillback in the rivers Rhine, Ems, and Elbe (Kvach et al. 2017). Prior to the arrival of the Ponto-Caspian amphipod *D. villosus*, the native amphipod *Gammarus pulex* was a frequent species in the rivers Rhine and Elbe where it was outcompeted after several years (Taraschewski 2006). Apparently, *G. pulex* did not support a fast distribution of *R. acus* although it is considered a suitable host (Moravec 1996). As *G. pulex* was the dominant amphipod species in the Schwentine River during the sampling period (state authority for nature and environment of the Land Schleswig-Holstein; personal correspondence), this might explain that the occurrence of *R. acus* was much lower compared to the other three rivers.

Further data analyses of the parasitological parameters of *R. acus* in eels and ruffes sampled in the rivers Rhine and Elbe did also emphasize a possible correlation between Ponto Caspian invasion and the distribution of *R. acus*. Various research have shown that the prevalence of *R. acus* in *A. anguilla* in the Rhine River increased from 6.6 % before the first to 30 % during the second invasion interval. There is no reliable data for the river Elbe from the time before Ponto Caspian species invaded the river but the prevalence increased noticeable (by 10 %) within a short period during the second interval. Similar to the distribution process of *P. laevis*, nowadays the prevalence of *R. acus* is lower than immediately during the second invasion interval but appreciably higher than before the first invasion interval. Furthermore, no

sufficiently reliable data for *G. cernua* was available.

Furthermore, it can be noted that various sampling sizes (different number of individuals per year, locality, and species) seem not to have had an effect on the outcome of the presented study. However, the results for *G. cernua* that were sampled in the river Elbe are striking since no single internal parasite was detected during the study most probably because of the very small sampling size of 6 individuals.

Finally, after evaluating our results, as well as parasitological data of the same hosts before, during, and shortly after the two different invasion intervals in Rhine and Elbe, we can conclude that the invasion of Ponto-Caspian species seem to have an impact on local parasite communities. Parasite infestation rates increased in native host species due to a parasite spill back or spill over caused by Ponto-Caspian species, possibly with negative consequences for other parasite (towards an extinction of entire species) and potential host species. Therefore, we highly recommend that more attention has to be paid on local parasite communities when studying biological invasions.

Chapter II: Direct effects of Ponto-Caspian invasion on native parasites through the example of *Pomphorhynchus* sp. (Acanthocephala)

Biol Invasions (2018) 20:207–217
https://doi.org/10.1007/s10530-017-1527-9



ORIGINAL PAPER

Pomphorhynchus laevis: An invasive species in the river Rhine?

M. A. A. Hohenadler · M. Nachev · F. Thielen · H. Taraschewski · D. Grabner · B. Sures

Received: 14 April 2017 / Accepted: 23 July 2017 / Published online: 27 July 2017
© Springer International Publishing AG 2017

Abstract Acanthocephalans of the globally distributed genus *Pomphorhynchus* have been intensively studied during the past decades. In Europe, *Pomphorhynchus* species have shown a certain degree of variability in their morphological characteristics and behavior. Nowadays two different *Pomphorhynchus* species, namely *P. laevis* and *P. tereticollis*, have been described for different waterbodies in Western and Central Europe. However, until now it appears that both species do not co-occur at the same time in the same habitat. We assume that due to their very similar morphology some of the individuals that were identified based on their morphological characteristics as

either of the two species might have been misidentified in previous studies. To avoid this possible source of error, we used molecular marker (ITS 1, COX genes) to identify *Pomphorhynchus* individuals from European eels that were sampled within a time period of 12 years from the Rhine River to elucidate the distribution of the two species in Western and Central Europe. Our results together with a re-examination of former studies provide evidence that *P. laevis* might have been introduced together with potential host species (intermediate, paratenic, and/or definitive) from the Ponto-Caspian region, and that it outcompeted and repelled the endemic *P. tereticollis* from the mainstream of the river. Our results also provide first evidence on the former coexistence of both *Pomphorhynchus* species in the river Rhine.

M. A. A. Hohenadler (✉) · M. Nachev · D. Grabner · B. Sures
Aquatic Ecology and Centre for Water and Environmental Research, University of Duisburg-Essen, Universitätsstr. 5, 45141 Essen, Germany
e-mail: michael.hohenadler@uni-due.de

F. Thielen
Natur & Umwelt, Kierchestrooss 2, 9753 Heinerscheid, Luxembourg

H. Taraschewski
Department of Ecology and Parasitology, Zoological Institute, Karlsruhe Institute of Technology, Kornblumenstrasse 13, 76131 Karlsruhe, Germany

B. Sures
Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park, Johannesburg 2006, South Africa

Keywords *Pomphorhynchus laevis* · *Pomphorhynchus tereticollis* · Ponto-Caspian invaders

Background

Acanthocephalans are endoparasites with a complex life cycle that are usually found as adults in the digestive tracts of fishes, birds, mammals and less frequently in amphibians, and reptiles (Taraschewski 2000; Sures 2014). Acanthocephalans develop from one larval stage to the next in one or several

Springer

5 *Pomphorhynchus laevis*: An invasive species in the river Rhine?

5 *Pomphorhynchus laevis*: An invasive species in the river Rhine?

M. A. A. Hohenadler, M. Nachev, F. Thielen, H. Taraschewski, D. Grabner & B. Sures

Published in: Biological Invasions (2018) DOI 10.1007/s10530-017-1527-9

5.1 Summary

Acanthocephalans of the globally distributed genus *Pomphorhynchus* have been intensively studied during the past decades. In Europe, *Pomphorhynchus* species have shown a certain degree of variability in their morphological characteristics and behavior. Nowadays two different *Pomphorhynchus* species, namely *P. laevis* and *P. tereticollis*, have been described for different waterbodies in Western and Central Europe. However, until now it appears that both species do not co-occur at the same time in the same habitat. We assume that due to their very similar morphology some of the individuals that were identified based on their morphological characteristics as either of the two species might have been misidentified in previous studies. To avoid this possible source of error, we used molecular marker (ITS 1, COX genes) to identify *Pomphorhynchus* individuals from European eels that were sampled within a period of 12 years from the Rhine River to elucidate the distribution of the two species in Western and Central Europe. Our results together with a re-examination of former studies provide evidence that *P. laevis* might have been introduced together with potential host species (intermediate, paratenic, and/or definitive) from the Ponto-Caspian region, and that it outcompeted and repelled the endemic *P. tereticollis* from the mainstream of the

river. Our results also provide first evidence on the former coexistence of both *Pomphorhynchus* species in the river Rhine.

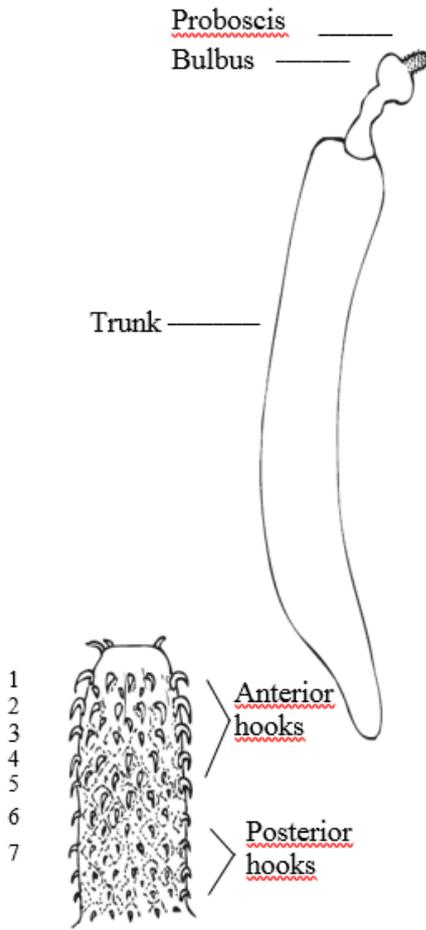
5.2 Background

Acanthocephalans are endoparasites with a complex life cycle that are usually found as adults in the digestive tracts of fishes, birds, mammals and less frequently in amphibians, and reptiles (Taraschewski 2000, Sures 2014). Acanthocephalans develop from one larval stage to the next in one or several intermediate host(s) and complete their life cycle either after the ingestion of the intermediate or the paratenic hosts by definitive hosts where they reach adulthood and start reproducing, or by post-cyclic transmission (Schmidt and Nickol 1985, Taraschewski 2000, Kennedy 2006). Transmission to the definitive host is facilitated by behavioral changes of the intermediate hosts induced by larval acanthocephalans (summarized in Sures 2014). According to Dezfuli et al. (2003) uninfested gammarids (e.g. *Echinogammarus stammeri*) usually reduce their activity when potential predators are present in their surrounding while gammarids that are infested with acanthocephalans stay active, making them easier accessible and more attractive for their predators that may serve as the parasites' definitive hosts (Baldauf et al. 2007). Additionally, some gammarid species show preferences to swim to the open water due to manipulated light responses when infested by acanthocephalans while uninfested conspecifics usually stay in dark and shaded areas where they are protected from predation (Brown and Thompson 1986). On the level of parasite community ecology, acanthocephalans usually do not contribute substantially to parasite species richness in mammals and birds. In fish helminth communities however, acanthocephalans are of enormous importance since they usually are among the most dominant parasite species (Kennedy 2006, Nachev and Sures 2009, Sures 2014). This also applies to the genus *Pomphorhynchus*, which is globally distributed with a high frequency of occurrence in Europe (Kennedy 2006).

In Europe, the genus *Pomphorhynchus* has been intensively studied (Lundström 1942, Engelbrecht 1957) with an emphasis on its distribution in European waterbodies since the late 1970s and early 1980s (Kennedy et al. 1977, Kennedy 1984). Early publications on *Pomphorhynchus* species refer to two different species,

i.e. *P. laevis* (Zoega in Müller 1776) and *P. tereticollis* (Rudolphi 1809). Subsequently, both species were considered to be subspecies of *P. proteus* (Meyer 1932). Thereafter, *P. tereticollis* appeared to be a synonym of *P. laevis* and therefore individuals of *Pomphorhynchus* were commonly designated to be *P. laevis* since the middle of the twentieth century (Kennedy 2006). Intensive research in and around the British Isles have identified two freshwater strains (Irish and English), and one marine strain (Munro et al. 1989, 1990). All these strains use different Gammarus species as intermediate hosts as well as different fish species as suitable definitive hosts (Kennedy 1989). Moreover, all strains show minor differences in their morphology, and their utilization of microhabitats (Kennedy 1984, Munro et al. 1989, 1990). Based on these differences, molecular techniques were used to show if either strain shows variabilities within a population (Brown 1987) or if they are sibling species within the genus *Pomphorhynchus* (Munro et al. 1990). The results gave evidence for possible strainspecific polymorphisms. Although these analyses were done at a time when molecular techniques were limited, it is a matter of fact that individuals that belong to the same species may show a certain degree of variability in their morphological characteristics and behavior. While the only species of the genus *Pomphorhynchus* that was found in European waterbodies was *P. laevis* (Králová-Hromadová et al. 2003, O'Mahony et al. 2004) the appearance of a second species, namely *P. tereticollis*, in the early 2000s (Perrot-Minnot 2004, Tain et al. 2006) had led to confusion and scientific dispute several years later. The identification of both *Pomphorhynchus* species was carried out by means of morphological features like the proboscis characteristics as well as special features of the anterior and posterior hooks (Brown et al. 1986, O'Mahony et al. 2004). However, phenotypical variations as well as a very similar morphology (cf. table 5.1) make it rather difficult to separate *P. laevis* and *P. tereticollis*, therefore a reliable distinction between the two species which is based only on morphological characteristics was considered hard or even impossible (Franceschi et al. 2008). Nonetheless, from the time of the discovery of the second species until the early 2010s morphological features were still used in order to identify and distinguish the two species (Kennedy 2006, Emde et al. 2012).

Table 5.1: Comparative observation of the morphological features of *P. laevis* and *P. tereticollis*

	Morphological characteristics	<i>P. tereticollis</i>	<i>P. laevis</i>
	Ratio between anterior and posterior proboscis hooks ^(*)	Anterior hooks bigger than posterior hooks	Anterior hooks bigger than posterior hooks
	Special feature of basal parts of the proboscis hooks ^(**)	Possess proximal projection	No proximal projection
	Total number of hooks per row ^(***)	11	8-13
	Total number of longitudinal rows ^(***)	18	14-18
	Position of longest anterior hook ^(*)	4	Usually 2 or 3 / High variations possible
	Position of shortest anterior hooks ^(*)	6	Usually 4 or 5 / High variations possible
	Possible differences between male and female ^(****)	-	Significant differences in length of 5 th hook Significant differences in width of hooks (position 5 and 7)

Remark: Both species might show phenotypical variations depending on host species and environmental habitat^(I,II,III)

(*) O'Mahony et al. 2004; (**) Špakulová et al. 2011; (***) Brown et al. 1986; (****) own observation, not published yet; (I) Molloy et al. 1995; (II) Kennedy 1984; (III) Buckner & Nickol 1975

Based on morphological features and simultaneous molecular analysis of *P. tereticollis*, Špakulová et al. (2011) have recently redescribed this species. Consequently, the method for morphological identification should be reconsidered

because although some morphological features like hook number, and-length can be used in order to distinguish both species (fixed material), several characteristics like the alignment of the basal parts of the proboscis hooks, which differ between the two mentioned *Pomphorhynchus* species, are visible exclusively in living worms (Brown et al. 1986, O'Mahony et al. 2004, Špakulová et al. 2011).

Furthermore, it was shown that *P. tereticollis* must have occurred in Europe earlier than mentioned previously since individuals that were previously identified as *P. laevis* were conspecific with *P. tereticollis*. It appears that misidentification can be dated back until the late 1950s when Engelbrecht (1957) misidentified species from the Baltic coast in Germany, which are demonstrably *P. tereticollis* (Špakulová et al. 2011). In summary, identifications of *Pomphorhynchus* species from Western and Central European waterbodies in the past can be considered at least questionable since *Pomphorhynchus* individuals that showed certain morphological features were generally identified as *P. laevis* (e.g. Sures et al. 1994). Accordingly, many results have to be reconsidered, especially since the intraspecific morphological and genetic variability of the two species are still a matter of debate (Nachev et al. 2010, Emde et al. 2012, 2014, Smrzlić et al. 2015).

Moreover, it remains unclear if both *Pomphorhynchus* species were endemic in the Western parts of Europe (Kennedy 2006, Špakulová et al. 2011) or if one has been introduced, e.g. from the Ponto-Caspian area together with the massive invasion of amphipods and *gobiids* following the inauguration of the Main–Danube-Canal in the year 1992.

In order to answer the question if the invasion of potential host species (intermediate, paratenic, and/or definitive) from the Ponto-Caspian region had an effect on different *Pomphorhynchus* species within large rivers molecular techniques are used in the present paper. Due to a long history of eel parasite studies (Sures et al. 1999, Sures and Streit 2001, Thielen et al. 2007) several individuals of *Pomphorhynchus* were stored at -80 °C for possible later investigations. Accordingly, the internal transcribed spacer 1 (ITS 1) gene of *Pomphorhynchus* individuals was analyzed in *Pomphorhynchus* individuals from former studies as well as in individuals collected recently. All individuals originated from European eels (*Anguilla anguilla*) which were sampled at different sites along the river Rhine, as well as intermediate hosts (*Dikerogammarus villosus*) that were sampled in the Rhine. The river Rhine was chosen as a study site since both *Pomphorhynchus* species have been reported there frequently in different fish species (Sures et al. 1999, van Riel et al. 2003, Emde et al.

2012). Moreover, the river Rhine represents a connection between the Ponto-Caspian region and the North Sea as a major waterway for commercial shipping. Thus, it might play a role in the invasion processes from the Black Sea to West- and Central-Europe (Bij de Vaate et al. 2002). The River Wupper, which is currently not affected by invasive species, was used as a reference site.

5.3 Material and methods

Host and parasite sampling

All acanthocephalans were collected from European eels (*A. anguilla*) from the Rhine River. In the years 2003, 2004, and 2015, eels from three different locations (*cf.* figure 5.1) along the river Rhine were sampled for parasitological analyses (*cf.* table 5.2). The *Pomphorhynchus*-samples of 2003 and 2004 originated from the study of Thielen et al. (2007). All samples taken prior to 2015 were kept frozen at -80 °C.

Eels were collected by professional fishermen in the main stream of the river Rhine. After sampling, all eels were transported to the laboratory where they were kept alive for a maximum of 4 days. Within this period all fish were killed and digestive tracts (including the stomach) were carefully removed, opened and checked under a binocular for parasites. Acanthocephalans were removed and determined morphologically to genus level.

Amphipods were collected with hand-nets or by kick-sampling. Infested individuals were dissected and acanthocephalan cystacanths removed. *D. villosus* was collected from the river Rhine near the town of Grieth (Rhine km 844) and *Gammarus pulex* from the river Wupper (near the city of Wuppertal, North Rhine-Westphalia, Germany, *50 km from the Rhine river mouth, one of the Rhine's tributaries) (*cf.* table 5.3).

The Wupper as part of the Rhine's catchment area was chosen as a reference site since no invasive Ponto-Caspian species have been found more than 10 km upstream the Rhine estuary as weirs and barrages prevent the dispersal of these species within the river. All *Pomphorhynchus*-individuals were stored at -80 °C until further analysis.

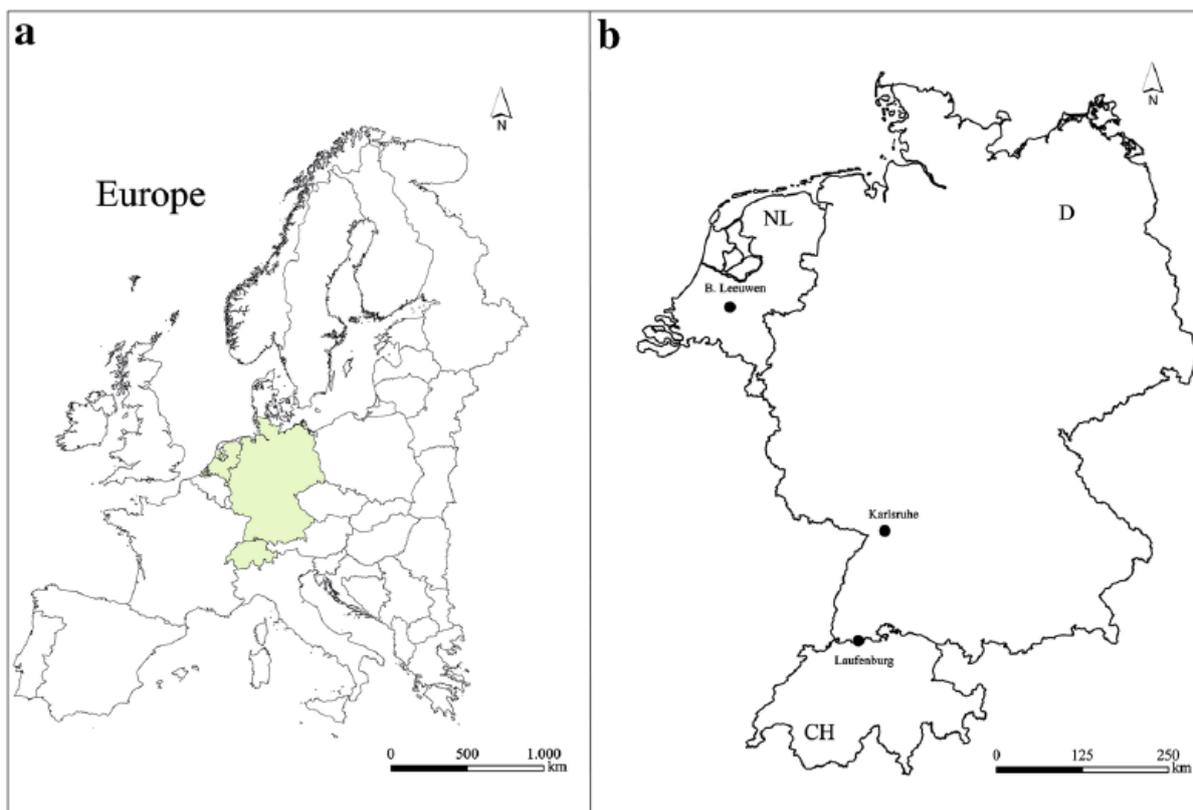


Figure 5.1: a Map of Europe, countries of interest (the Netherlands, Germany, Switzerland) highlighted in green, b map of the sampling spots in the Rhine at Beneden Leeuwen (the Netherlands, NL), Karlsruhe (Germany, D) and Laufenburg (Switzerland, CH)

Table 5.2: Number of analyzed *Pomphorhynchus*- individuals with year and location of eel sampling

Sampling year	Country	Sampling location	Number sampled <i>Pomphorhynchus</i> individuals
2003	Switzerland	High Rhine in Laufenburg (Rhine km 120)	6
	Germany	Upper Rhine in Karlsruhe (Rhine km 361)	28
	Netherlands	Lower Rhine in Beneden Leeuwen (Rhine km 910)	5
2004	Switzerland	High Rhine in Laufenburg	23
	Germany	Upper Rhine in Karlsruhe	6
2015	Switzerland	High Rhine in Laufenburg	155
	Germany	Upper Rhine in Karlsruhe	24
			Total: 247

Table 5.3: Number and species of amphipods, year and location of sampling (Rhine, Wupper), and number of analyzed *Pomphorhynchus* sp.

Year	River	Species	Number <i>Pomphorhynchus</i> individuals
2015	Rhine	<i>D. villosus</i>	7
2015	Rhine	<i>D. villosus</i>	6
2016	Rhine	<i>D. villosus</i>	5
2015	Wupper	<i>G. pulex</i>	28
2016	Wupper	<i>G. pulex</i>	37

Molecular analyses

In order to ensure a reliable identification of the *Pomphorhynchus* species molecular methods have been used. Internal transcribed spacer 1 (ITS 1) rDNA genes were amplified by PCR according to Franceschi et al. (2008), which allows a differentiation between *P. laevis* and *P. tereticollis* according to the size of the PCR-product (Perrot-Minnot 2004). The product for *P. laevis* had a length of 320 base pairs (bp) and 350 bp for *P. tereticollis* (Franceschi et al. 2008).

In order to locate the possible origin of the analyzed *Pomphorhynchus* specimens, five samples of each sampling spot (2003/2004) have been randomly chosen, sequenced, and compared with gene sequences from *P. laevis* individuals from the Danube River in Bulgaria (Ponto-Caspian origin) and isolated strains from Western Europe (France).

5.4 Results

Pomphorhynchus sp. from European eels

All *Pomphorhynchus* individuals from European eels sampled in 2003 in the Netherlands were identified as *P. laevis*. At the same time, a co-existence of *P. laevis* and *P. tereticollis* was detected at the sampling site in Germany, while at the site in Switzerland eels were only infested by *P. tereticollis* (cf. figure 5.2). In 2004, all *Pomphorhynchus* from eels at the German sampling spot were identified as *P. laevis* while in Switzerland both species co-existed. In 2015, *P. tereticollis* also disappeared

from the eels sampled in Switzerland. Thus, in 2015 *P. laevis* was the only *Pomphorhynchus* species at each sampling site.

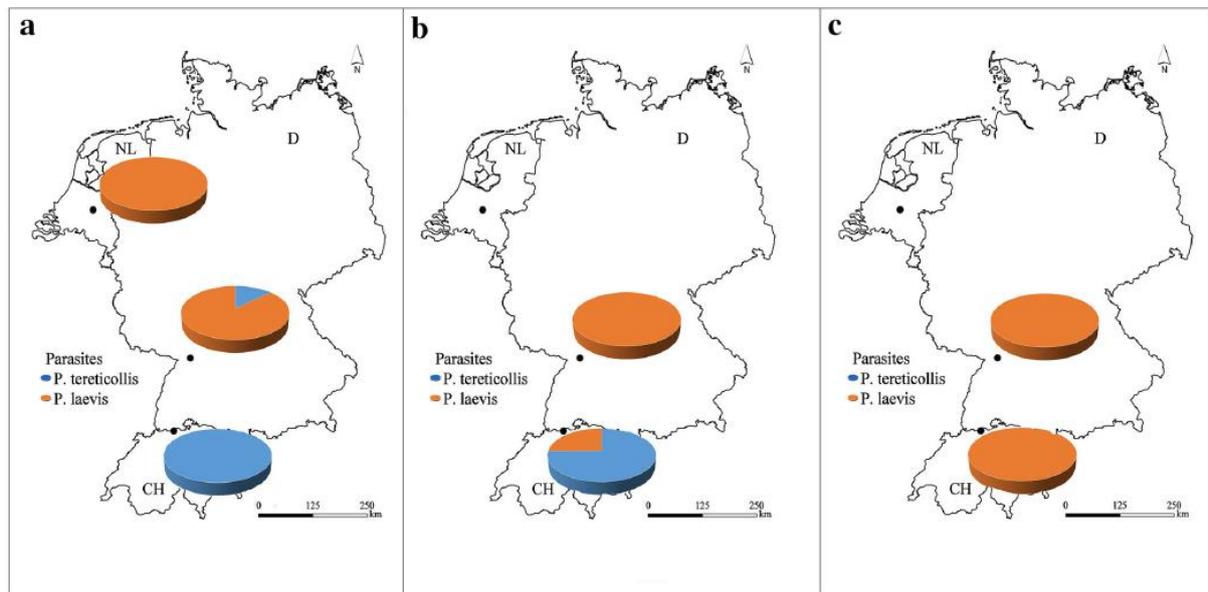


Figure 5.2: Sampling years (a = 2003, b = 2004, c = 2015), and *Pomphorhynchus* species distribution from eel at the three different sampling locations (filled circle) within the river Rhine [the Netherlands (NL), Germany (D), Switzerland (CH)]

Pomphorhynchus sp. from gammarids

Gammarids sampled in the Rhine in 2015 and 2016 were determined as *D. villosus*. In 2015, a total of 13 individuals (0.94% prevalence), and 5 individuals (0.83% prevalence) in 2016, were infested with *P. laevis*, whereas *P. tereticollis* was not found. Collection of gammarids in the Wupper during the same years showed contrasting results concerning host and parasite species. In the Wupper, all gammarids were determined as *G. pulex*, and the cystacanths were exclusively identified as *P. tereticollis*. With 28 individuals (4.58% prevalence) in 2015, and 37 individuals (3.32% prevalence) in 2016, infestation rates of *Pomphorhynchus* sp. in gammarids were between 4 and 5 times higher in the Wupper than in the Rhine.

Comparison of *P. laevis* sequences

The comparison of sequences (ITS 1 and ITS 2 genes) of *P. laevis* that were collected from eels in 2003 and 2004 at each sampling spot and sequences from GenBank (www.ncbi.nlm.nih.gov) showed a congruency of 99% between our samples and samples that were collected in the Danube River in different Central- and East

European countries (Table 4). Furthermore, our samples showed a similarity of 91% and less with samples from isolated lakes in the Slovak Rep. (Accession number: AY135417.1) and waterbodies of Southern Europe (Italy). The results of a comparison of sequences of *P. laevis* individuals that were sampled in the Danube River in Bulgaria (Danube km 685) in September 2006 show a high similarity as well (congruency 99%). Analysis of the CO1 gene showed the same results. Gene sequences of *P. tereticollis* have not been analyzed in detail.

Table 5.4: Sequence-comparison of ITS 1 and ITS 2 genes of *P. laevis* samples from each sampling spot (2003 and 2004) and sequence dataset of acanthocephalans sampled worldwide

Sample location	Sample size (n)	Year	Similarity (in %)	Accession number	Matching location
Netherlands	4	2003	99	AY135415.1	Rokytná River (Czech Republic), Danube River basin; river course
			99	KF559307.1	Sava River (Danube River basin); river course
Germany	3	2003	99	AY135415.1	Rokytná River (Czech Republic), Danube River basin; river course
			99	KF559307.1	Sava River (Danube River basin); river course
			99	KF559307.1	Sava River (Danube River basin); river course
Germany	1	2004	99	KF559307.1	Sava River (Danube River basin); river course
Switzerland	4	2004	99	AY135415.1	Rokytná River (Czech Republic), Danube River basin; river course
				KF559307.1	Sava River (Danube River basin); river course

5.5 Discussion

Our data show the shift from *P. tereticollis* to *P. laevis* in eel from the Rhine (Germany, Switzerland) in about a decade and provides first evidence on the former coexistence of both *Pomphorhynchus* species in the Rhine. This might be due to the recent redescription of *P. tereticollis* by Špakulová et al. (2011) who clearly defined *P. laevis* and *P. tereticollis* as separate species. In earlier studies, *P. tereticollis* might have been assigned erroneously to *P. laevis*, as scientists were not aware of the two species. Therefore, it has not been questioned that *P. laevis* was the only endemic *Pomphorhynchus* species in Central and Western European waterbodies for many years.

Recently, the occurrence of *P. tereticollis*, in the Rhine was reported but never together with *P. laevis* (Emde et al. 2012, 2014). However, the results of the present study suggest that *P. laevis* must be considered the species introduced into the river Rhine while *P. tereticollis* appears to be the endemic species. We assume that due to the similar morphology of *P. laevis* and *P. tereticollis*, *Pomphorhynchus* individuals have long been misidentified (e.g. Sures et al. 1994). The results of the present study indicate that *P. tereticollis* and *P. laevis* co-occurred for some years (evidence for the years 2003 and 2004) after the introduction of *P. laevis* to the river Rhine in the early 2000s, which finally lead to a replacement of *P. tereticollis* by *P. laevis* in the investigated area. At a first glance, the results obtained from the sampling in the Netherlands do not support this conclusion. At the first sampling in 2003, all analyzed *Pomphorhynchus* specimens from this site were exclusively identified as *P. laevis*. This might indicate that *P. laevis* has been either endemic in the sampled area or that a replacement of the endemic *P. tereticollis* by *P. laevis* had occurred before 2003. The latter possibility is supported by comparisons between gene sequences of *P. laevis* that show a high conformity between our samples and strains from the Danube River. This aspect will be discussed in more detailed below.

A possible explanation on how *P. laevis* could invade the Rhine and replace the native *P. tereticollis* within just a couple of years might arise in the context of the biological invasions from the Ponto-Caspian (PC) region. This region is situated in Eurasia and includes the Black, Azov, and Caspian Sea and is known for its numerous native species that are considered successful invaders in many parts of Western Europe, and in other parts of the world (Copp et al. 2005, Alexandrov et al. 2007,

Gallardo and Aldridge 2013). With the inauguration of the Main–Danube–Canal in 1992, several species from the PC region invaded the Rhine River using the new dispersal corridor from the Danube River (Bij de Vaate et al. 2002). One of the very effective invaders is the so called “killer shrimp” *D. villosus*, which is considered a suitable first intermediate host for *Pomphorhynchus* sp. (Rewicz et al. 2014). *D. villosus* was reported for the first time in Germany in 1992 and spread up to the estuary of the Rhine in just 3 years, overcoming a distance of around 900 km (Kinzler et al. 2009).

In addition to *D. villosus* and other invasive amphipods also different species of the family Gobiidae migrated upstream the Danube and invaded the river Rhine (Borcherding et al. 2011, Kalchhauser et al. 2013). The most successful invaders that belong to this genus are the round goby, *Neogobius melanostomus* and the bighead goby *Ponticola kessleri*. Both species have been described as common paratenic hosts for *P. laevis*, both in their native and non-native range (Ondračková et al. 2006; Kvach and Skóra 2007). *P. laevis* usually occurs in high abundances in a larval or pre-adult stage encysted in the abdominal cavity of these invasive *gobiid* species (Ondračková et al. 2009). However, PC species have not only introduced *P. laevis* to the Rhine River, but also favored its dispersal since nonindigenous species (NIS) usually serve as intermediate or reservoir hosts for invasive or already established parasites. Therefore, parasite infestation rates might increase in native host communities resulting in a spillover (Prenter et al. 2004, Tompkins and Poulin 2006, Kelly et al. 2009, Peeler et al. 2011, Ślapanský et al. 2016). If the invasive species additionally serves as a suitable host for a native or already established parasite, the number of dispersal pathways and host species for parasites increase, leading to higher infestation risks among native communities (spillback) (Kelly et al. 2009). In addition to its fast dispersal, the current distribution pattern of *P. laevis* in the river Rhine supports the hypothesis that it was introduced from the PC region. While the invasion downstream of the estuary of Rhine and Main occurred fast and with high success, the one upstream took longer (*cf.* figure 5.2). Usually species move easier downstream of a river (flow direction) than upstream (striving against the stream), showing that in the present case *P. laevis* has most likely entered the Rhine through the Main (van Riel et al. 2011). This can also be supported by the fact that *D. villosus* was reported for the first time in Switzerland in 2003, several years after its first appearance in the Rhine in Germany (Bollache et al. 2004).

However, the removal of bio-geographical barriers and the emergence of new dispersal corridors through which a species can spread naturally to new environments is not the only possibility for species to overcome huge distances to invade new habitats. Commercial shipping as part of world trade plays a major part in the dispersal of invasive species as well (Ruiz et al. 2000, Ricciardi 2006). Since all big cargo ships use water from one location to fill up their ballast water tanks to stabilize the vessel (Pereira et al. 2016). Together with this ballast water living organisms (fish larvae and small fish, crustaceans, algae, invertebrates) are transported and released to a new environment when the ship reaches the next harbor and discharges its ballast to load cargo (Ruiz et al. 1997). This approach could also explain why *P. laevis* was found in the Netherlands in 2003 since our sampling site was located within a radius of 60 km within major ports such as Rotterdam and Nijmegen. *N. melanostomus* and other PC species were reported in the Netherlands several years before they have been reported in Germany suggesting a possible dispersal via ballast water (van Beek 2006, Borchering et al. 2011). Therefore, *P. laevis* was co-introduced to these sites before it invaded the downstream sections of the river Rhine.

Finally, the results from the sampling in the river Wupper, one of the Rhine's tributaries, also support the assumption that *P. laevis* was introduced from the PC region to the river Rhine. All *Pomphorhynchus* individuals that were sampled in the Wupper (n = 65) were exclusively identified as *P. tereticollis*. Invasive amphipod species as well as other NIS from the PC region are not able to spread within the river Wupper since man-made weirs and barrages prohibit their dispersal. Furthermore, these species usually stay in the main stream and do not migrate far upstream in the rivers' tributaries (Chen et al. 2012). Therefore, we can assume that the native amphipods are repelled and/or isolated from the estuary regions of the river Rhine to further areas of its tributaries due to natural or manmade barriers as well as competition with the invasive amphipod species. Assuming that the same applies for the native parasites, our observations strengthen the hypothesis that the invasive *P. laevis* has caused the displacement of *P. tereticollis* from its former habitat (the mainstream of the Rhine) to the tributaries, where it was naturally adapted to the most common *Gammarus* species, *G. pulex* (Westram et al. 2011).

As previously mentioned some acanthocephalans show preference for specific host species. The Rhine River as a hot spot of "invertebrate-xenodiversity" has shown many examples of host specificity. In the late 1980s *Gammarus tigrinus*, an invasive amphipod from North America invaded the river Rhine and replaced *G. pulex* as the

dominant species occasionally. The likewise invasive acanthocephalan *Paratenuisentis ambiguus* showed a host specificity for *G. tigrinus* as intermediate host and for the European eel as final host. It became the dominant parasite within the parasite community in eels (Sures et al. 1999). With the arrival of *D. villosus* in the mid-1990s, *G. tigrinus* populations declined and finally this species was completely extinguished. Simultaneously, *P. ambiguus* vanished from the Rhine River (Taraschewski 2006). As assumed earlier, *P. laevis* might have been introduced to the river Rhine together with *D. villosus*. In its native habitat, *P. laevis* shows host specificity for *D. villosus* (Djikanovic et al. 2010), therefore the high prevalence of *D. villosus* might be an additional reason for the likewise high frequency of *P. laevis* in the Rhine River. *G. pulex*, which occurred simultaneously but with a lower frequency than *G. tigrinus* within the mainstream of the Rhine River was also outcompeted and repelled by *D. villosus* from the mainstream to its tributaries. Since there is evidence that *P. tereticollis* prefer *G. pulex* as its first intermediate host, the fact that *G. pulex* got repelled to the upstream areas of the river Rhine's tributaries might be the reason why *P. tereticollis* nowadays is only present in the same areas as its preferred intermediate host. Hence, a certain degree of host specificity appears to be highly influential for the dispersal of acanthocephalans in an environment after the invasion of potential host species.

In summary, results of the present study support the assumption that *P. laevis* was introduced to the river Rhine from the PC region. With its arrival to a new habitat and a wide range of potential host species, *P. laevis* spread quickly showing an example of parasite spillover.

Since invasive PC amphipods like *D. villosus* might have introduced *P. laevis* to the Rhine, and other PC species such as gobiids might favor its fast and successful spreading, the importance of species such as *N. melanostomus* and *P. kessleri* in the distribution mechanisms of *P. laevis* need to be examined more closely. As mentioned before, *P. laevis* is found in high prevalences and abundances in an encysted and premature stage in these non-native fishes (Ondračková et al. 2006). Furthermore, it is known that after entering new habitats, invasive species like *N. melanostomus* usually become an important part of the food-webs (Dietrich et al. 2006, Johnson et al. 2010). These opportunistic fishes can adapt fast to changing prey supply (Kornis et al. 2012), and might serve as prey for local common and apex predators (Campbell et al. 2009, Almqvist et al. 2010). Examinations of stomach content of several fishes that were sampled in the Rhine showed sporadic findings of *N. melanostomus* or *P. kessleri*

(not published yet) and there is evidence that they favor post-cyclic transmission of *P. laevis*.

Therefore, we assume that PC species that serve as paratenic hosts for *Pomphorhynchus* sp. take an important role in their distribution processes. However, valid data to support this assumption are still lacking. Furthermore, more research on the biology of the two *Pomphorhynchus*-species is essential since it is not known if they do co-infest the same host individuals. Further studies are required to clarify these questions.

Chapter III: Indirect effects of Ponto-Caspian invasion for local hosts through the example of an interaction between *Pomphorhynchus laevis* (Acanthocephala) and the invasive *Anguillicola crassus* (Nematoda)

6 First evidence for a possible invasional meltdown among invasive fish parasites

6 First evidence for a possible invasional meltdown among invasive fish parasites

M. A. A. Hohenadler, K.I. Honka, S. Emde, S. Klimpel, B. Sures

Accepted in: Scientific Reports (SREP-18-12009A)

6.1 Summary

Biological invasions are frequently studied topics in ecological research. Unfortunately, within invasion ecology parasite-associated aspects such as parasite impacts on new environments and on local host populations are less well-studied. Round gobies migrating from the Ponto-Caspian region into the Rhine River system are heavily infested with the Ponto-Caspian acanthocephalan parasite *Pomphorhynchus laevis*. As shown by experimental infestations the acanthocephalans occur as preadults in host-encapsulated cysts within the internal organs of the migrating gobies, but remain infective for their definitive host chub. Recently, we described the occurrence of larvae of another parasite, the invasive eel swim bladder nematode *Anguillicola crassus*, in these *Pomphorhynchus* cysts. In the present study, we could prove the infectivity of the nematode larvae for European eels for the first time. After experimental inoculation of *Pomphorhynchus* cysts occasionally infested with *A. crassus* larvae, the nematodes grow to maturity and reproduce whereas all *P. laevis* were unviable. We therefore postulate that the nematode larvae behave like immunological hitchhikers that follow a “Trojan horse strategy” in order to avoid the paratenic host’s immune response. Accordingly, the interaction between both invasive parasites, gives first evidence that the invasional meltdown hypothesis may also apply to parasites.

6.2 Background

Invasion of free-living organisms and their effects on new habitats has emerged as a major threat for ecosystems around the globe, partly with irreversible consequences for the local biota. Invasive species might cause habitat modification, extinctions of endemic species, affect human health, and therefore engender enormous economic costs (Perrings et al 2000, Simberloff et al. 2013, Jeschke et al. 2014). However, not every newly introduced species will be able to establish itself in a new habitat (Sol et al. 2012). Success rates depend on different biotic and abiotic conditions such as absence/presence of enemies, competition with local species for resources, and climatic conditions (Stachowicz et al. 2002, Vignon and Sasal 2010, Blakeslee et al. 2013). Besides these aspects, the occurrence of other invasive species is one of the most substantial factors for invasion success. The so-called invasional meltdown hypothesis (IMH) states that if several new species invade the same habitat, they usually facilitate each other's establishment since one species might serve e.g. as food or energy resource for another, which initiate its invasion process (Simberloff and Holle 1999, Green et al. 2011). This might result in an increased rate of invasion, leading to crucial impacts within the new habitat (Gallardo and Aldridge 2015). In this context, it seems surprising that alien parasites, although generally co-introduced to new environments with invasive host species (Sasal et al. 2008, Ondrackova et al. 2011, Pizzatto et al. 2012, Amundsen et al. 2013), are often not taken into account when evaluating the effects and mechanisms of invasion. This is even more surprising as parasites are considered an important response variable for ecosystem health (Lymbery et al. 2014, Ortega et al. 2015, Goedknecht et al. 2016). Although the IMH did not show any significant differences among taxonomic groups, that have been studied yet (Jeschke et al. 2012). It remains unclear if it also applies to nonindigenous parasites.

In order to be able to invade a new habitat parasites usually depend on free-living alien hosts (Vignon et al. 2009a, 2009b). Therefore, the presence of a sufficient number of free-living invasive species is an obligate prerequisite for the establishment of non-indigenous parasites. Nevertheless, the question whether a certain parasite species also benefits from the occurrence of other invasive parasites remains to be unanswered. The Rhine River, a Western European river is considered a hot spot for

biological invasion, and thus might be an ideal system to study the relevance of the IMH for invasive parasites (Galil et al. 2007, van Kessel et al. 2016). Although many nonindigenous species were able to establish in the Rhine River over the past decades, invaders from water bodies of the Ponto-Caspian steppe were among the most successful (van Kessel et al. 2016). Species such as the amphipod *Dikerogammarus villosus* or the fish species *Neogobius melanostomus* or *Ponticola kessleri* usually become dominant species in newly invaded areas due to their invasion strategy that provide them with competition advantages against local species (Kinzler et al. 2009, Borchering et al. 2011). Recent research has shown that both the amphipods as well as the fish species introduce the acanthocephalan *Pomphorhynchus laevis* to the river Rhine since the mid 1990's after the inauguration of the Main-Danube-Canal (cf. chapter II). Subsequently, the parasite spread rapidly and successfully established itself along the river Rhine, showing a high prevalence in cyprinid fishes as well as in predators that feed on infected intermediate host species (Dudinak and Snabel 2001, Tieri et al. 2006). After a potential paratenic host ingests pre-adult individuals of *P. laevis*, a cyst will be formed by both the hosts' immune response and the parasite itself. Such parasite stages thus occur encapsulated in the hosts' internal organs as well as in its body cavity (Dezfuli 1992, Sures and Siddall 2001, Cornet et al. 2010). Infection experiments with chub, *Squalius cephalus*, have demonstrated that encapsulation does not have any apparent effect on the parasite since it remains infective for its definitive host (unpublished data). Recent research has also shown that cysts of *P. laevis* in *N. melanostomus* may contain larvae of another invasive parasite species, *Anguillicola crassus* (Emde et al. 2014). This nematode causes severe health impacts for the native eel species in Europe (Molnar et al. 1991, Würtz et al. 1996). Initially it was co-introduced with Japanese eels (*Anguilla japonica*) to European waterbodies in the early 1980s (Knopf and Mahnke 2004). Shortly after its arrival, *A. crassus* adapted to local environmental conditions and accepted the European eel (*Anguilla anguilla*) as its suitable final host. Within a short period, the infestation rates of *A. crassus* in *A. anguilla* increased to more than 90 % in large parts of Western and Central Europe (e.g. Taraschewski et al. 1987, Würtz et al. 1996, Sures and Streit 2001, Jakob et al. 2009). The nematode parasitizes the swim bladder of its final host after undergoing different development stages by using a wide variety of species as intermediate and paratenic hosts (Emde and Klimpel 2015). Accordingly, the eel's swim bladder is frequently affected to a significant extent, leading to a reduced

functionality, which might result in the host's death during its spawning migration from the European coast to the Sargasso Sea (Pelster 2015). In fact, *A. crassus* is also held partly responsible for the massive decline of the overall stock of European eel that resulted in its occurrence on the list of critically endangered species by the International Union for Conservation of Nature (Sures and Knopf 2004, Jacoby and Gollock 2014).

The fact that individuals of *A. crassus* utilize cysts of encapsulated *P. laevis* individuals provides evidence that establishment of a parasite species might have been facilitated by the arrival of another invasive parasite within the Rhine River. Hyperparasitized cysts – what in detail describes acanthocephalan cysts that were simultaneously infested by *P. laevis* and *A. crassus* - which were gathered from *N. melanostomus* individuals from the Rhine River demonstrated that *A. crassus* frequently enters the cyst most likely to avoid immune responses of the paratenic host. Generally, third-stage larvae (L3) of *A. crassus* evoke an immune response of their paratenic hosts, with diversified intensities among the various host species, which might cause the parasites' death (Emde et al. 2014). Recently, it was suggested that *A. crassus* might use the cyst as a “hideout” to evade the immune response of the round goby, which might serve as prey for *A. anguilla*, one of the parasites' definitive host. Therefore, the nematode larvae are protected from host defenses while being in the goby. Theoretically, with such a “Trojan horse” strategy the parasite could be able to infest the hosts' swim bladder more readily. However, it is still unknown whether *A. crassus* is still infectious for the definitive hosts after entering the acanthocephalan cyst. If yes, this could be seen as support that the IMH also applies to nonindigenous parasites. In order to test the viability and infectivity of encapsulated *A. crassus* larvae, we therefore conducted an experiment where European eels were infested with cysts collected from Ponto-Caspian gobies.

6.3 Material and methods

A total of 22 individuals of the invasive goby *Neogobius melanostomus* were collected by professional fishermen with bow nets in the River Rhine close to the city of Grieth at Rhine km 844 (North Rhine Westfalia, Germany). Within two days after sampling, all fish were sacrificed and examined for the presence of acanthocephalans of the

genus *Pomphorhynchus*, which were discovered encapsulated in the abdominal cavities of the fishes. All encapsulated *P. laevis* individuals (n = 364) were stored in a 0.9 % sodium-chloride (saline) solution at 5 °C.

200 isolated cysts were transferred one by one into a well-plate chamber to check whether cysts were infested by *Anguillicola crassus*. Wells were filled up with artificial stomach acid-solution, composed of 1 % hydrochloric acid and Pepsin (0.5 g per 100 ml) (Llarena-Reino et al. 2013). Filled-up well plates were incubated for 40 minutes at 37°C to induce cysts to break open and allow parasites to be released and eventually found free in the solution (*cf.* figure 6.1C). After the incubation time, the content of each chamber was carefully examined in order to determine whether cysts were infested by *A. crassus* and if so to what extent. A mean infestation rate (number of *A. crassus* per cyst of *P. laevis*) was calculated, which was then used as a basis for subsequent infection experiments with European eels. We infested ten European eels (mean size of 426 mm) that were provided by a commercial eel farm known to be free of any infestation with *A. crassus* and / or *Pomphorhynchus* sp. with the remaining cysts (n = 164). Apart from a longstanding cooperation with the eel farm, eels are regularly checked by parasitological examinations to verify absence of *A. crassus* as well as of any other endoparasites. A total of 16 to 18 cysts were manually administered to each eel by a stomach tube (diameter of 0.5 mm). Following infection, the eels were kept individually in a single water tank (30 l) at a water temperature between 10 and 13°C with permanent air supply. An uninfected control group of five eels (mean size of 464 mm) was kept under the same experimental conditions to verify that the eels were free of parasites. The eels were killed and examined for parasites 154 days post infection (dpi). Internal organs were removed and digestive tracts and swim bladders were carefully examined under a stereomicroscope for the presence of *A. crassus* and *P. laevis*. Individuals of *A. crassus* were subsequently categorized according to their developmental stage and sex.

All experimental protocols were approved by the Ethics Council (Landesamt für Natur, Umwelt und Verbraucherschutz, Nordrhein-Westfalen, permit number: 84-02.04.2017.A245) and were carried out in accordance with the relevant guidelines and regulations.

6.4 Results

The initial screening of cysts removed from *N. melanostomus* (cf. figure 6.1) showed a prevalence of 12 % of *A. crassus* larvae within the cysts. In all 200 cysts 96 larvae of *A. crassus* were detected, thus the mean intensity was four nematodes per cyst (ranging between one to twelve larvae per cyst). Individuals of *P. laevis* found in the cysts were alive and showed a normal activity level.



Figure 6.1: A) Cysts of encapsulated *P. laevis* individuals as detected and removed from the digestive tracts of *N. melanostomus* B) Encapsulated *P. laevis* irradiated with high light intensity C) Digested cyst with released *A. crassus* individuals

Eels administered with intact cysts showed a prevalence with *A. crassus* of 40 % 154 days post infection (dpi). While two eels were found to be infested by an individual *A. crassus* (either male or female) each, two eels showed a double infestation. In one eel two females occurred, whereas a pair of both sexes containing eggs with L2 larvae was detected in the second eel. In sum, 164 cysts were administered to the eels, which corresponds to a total of 79 *A. crassus* when considering the results of the initial cyst screening. Based on these results, the recovery rate can be determined as 7.6 %. The size of the *A. crassus* individuals found in the eels corresponds with the developmental period of 154 days when compared with previous infection experiments (Knopf et al. 1998, Keppel et al. 2014). Further parasitological examination of the eels did not show any infection with *P. laevis* in the experimental group. Eels of the uninfected control did not contain any individual of either parasite species.

6.5 Discussion

The present study demonstrates for the first time that larvae of *A. crassus*, enclosed in the cysts of encapsulated *P. laevis*, remain able to infest their definitive host, the European eel. The experiment showed that *A. crassus* is still able to complete its life-cycle and produce offspring after entering the cysts in a potential paratenic host. Moreover, as the invasive nematode larvae use the cyst of an invasive acanthocephalan parasite species, the invasional meltdown hypothesis is supported.

Parasitological examination of the eels revealed a prevalence of 40 % of *A. crassus* with a recovery rate of 7.6 %. Previous experiments with eels using isolated L3 of *A. crassus* under similar conditions showed generally higher recovery rates of up to 40 % (Knopf et al. 1998, Keppel et al. 2014). Apart from the fact that the number of introduced *A. crassus* larvae can only be estimated as an average value and not using exact data, the relatively low infestation rate might also be related to this, so far unknown, way of transmission of *A. crassus*. It was demonstrated that encapsulation might be a barrier for some parasites in order to establish themselves after being transmitted to a new host (Kennedy 1999). As implied by the relatively low prevalence and recovery such a barrier effect might also apply for *A. crassus*. Nonetheless, the use of cysts containing encapsulated *P. laevis* in fish lacking a swim bladder represents an additional way of transmission to the preferred final host for *A. crassus*.

The results demonstrate the infectivity of *A. crassus* individuals from cysts co-infected with *P. laevis*. Interestingly, *A. crassus* has no obvious disadvantage of the co-infection of the cysts whereas no individual of *P. laevis* was detected inside the eels at the end of the experiment although *P. laevis* is regularly found in eels from the Rhine River (*cf.* chapter II). This is striking since encapsulated *P. laevis* that were ingested by their preferred definitive hosts such as *S. cephalus* and *Barbus barbus* are able to mature (Dezfuli 1992, Sures et al. 1994, Kennedy 1999, Dezfuli et al. 2012), which was also confirmed by additional infection experiments in which encapsulated *Pomphorhynchus* individuals developed to full maturity after being infested to individuals of *S. cephalus* (unpublished data). The lack of any *P. laevis* in the examined eels after 154 dpi might therefore be related to the following reasons. On the one hand, European eel as a non preferred host was used for laboratory infestation experiments. Even if *P. laevis* can regularly be found in eels in the field (*cf.* chapter II) this might be

a result of eels ingesting cystacanths from the first intermediate host, i.e. different species of amphipods and not by feeding on paratenic hosts. On the other hand, it is also conceivable that the lifetime of *P. laevis* in its non-preferred hosts is shorter than the time of seven to eight months estimated for this species in their preferred definitive hosts (Nachev and Sures 2016). In the latter case, the acanthocephalans might have already been shed from the eels after 154 dpi. However, during daily inspections, no acanthocephalans were recovered in the tanks.

Both parasites have been described as successful invaders in European waterbodies and have been intensively studied during the past decades (Norton et al. 2005, Knopf 2006, Keppel et al. 2014). Nonetheless, a relation or possible interaction between the two invasive parasites was only discovered recently (Emde et al. 2014). The reason might be that usually *P. laevis* is carefully removed from the cysts and then further examined while the tissue of the cyst is treated as waste material. Simultaneously, the larvae of *A. crassus* are not recognized since they are hardly seen by bare eye. Accordingly, the parasite has always been overlooked prior to the preliminary field study by Emde et al. 2014. Furthermore, we assume that if individuals of *A. crassus* have already been detected in gobies before, their exact localization (in the cysts) was not recognized. However, in the context of these findings and the results of the present study we assume that *P. laevis* might facilitate *A. crassus*' establishment and distribution in a new environment. This corresponds to the invasional meltdown hypothesis (IMH), which has never been described for invasive parasites before, although interactions of free-living invasive species are already referred to as a major aspect of biological invasion (Green et al. 2011, Jeschke 2012, O'Loughlin et al. 2017). The IMH states that the arrival of nonindigenous species in an environment facilitates the establishment of other invasive species (Simberloff and Holle 1999). The fact that both parasites were able to establish themselves successfully in environments that are recognized as hotspots for invasion, such as the river Rhine, and the fact that *A. crassus* seem to benefit from the presence of encapsulated invasive parasites supports the assumption that the IMH also applies to invasive parasites.

Although *A. crassus* larvae utilize cysts and thereby eventually avoid the paratenic host's immune response (of e.g. *N. melanostomus*) this could also be a side effect associated to the fact that gobies lack a swim bladder. It is already known that *A. crassus* larvae can be found in many different tissues of paratenic hosts (De Charleroy et al. 1990, Moravec and Konecny 1994, Li et al. 2015). The idea that the

parasite uses a “Trojan horse strategy” was firstly mentioned in 2014 (Emde et al. 2014). Although the present results do not directly support a trojan horse strategy as no immunological responses were analysed, they show that *A. crassus* benefits from the presence of the cysts of encapsulated *P. laevis* individuals. Obviously, the distribution and establishment of *A. crassus* is (at least partly) facilitated by another invasive parasite that consequently turned a possible dead-end host into a paratenic host in order to increase the nematodes’ infestation success. As there are not many other fish species described in which *P. laevis* occurs in cysts (Moravec and Scholz 1991, Sures and Siddall 2001), the particular type of co-occurrence of both parasites that is described here is only known for gobies.

The fact that both parasite species have been studied intensively over the past decades but their interaction was only discovered recently demonstrates the necessity of future research on possible interactions between (invasive) parasites in order to evaluate the effects of parasites invasion on local biota.

7 General Discussion

The present doctoral thesis comprises several surveys addressing the effects of Ponto-Caspian invasive species such as *Dikerogammarus villosus* and *Neogobius melanostomus* on local parasite communities in invaded habitats (cf. figure 7.1).

The spatial approach (cf. chapter I) revealed significant differences between the parasite communities of specific host species from habitats that experienced Ponto-Caspian invasion and habitats that were free from any Ponto-Caspian invaders. The analysis highlighted that the nematode *Raphidascaris acus* and the likewise invasive Ponto-Caspian acanthocephalan *Pomphorhynchus laevis* distinguished themselves as key species associated to invaders from the Ponto-Caspian region. Generally, both parasites were common species in rivers that were invaded by invasive Ponto-Caspian (paratenic / intermediate) hosts presumably facilitating the distribution of the likewise invasive *P. laevis* and the local and widely distributed *R. acus*. Eventually, Ponto-Caspian hosts evoked an increase of the infestation rates within local hosts due to a parasite spillover (*P. laevis*) and spillback (*P. laevis* and *R. acus*), respectively (cf. figure 7.1 A). These results disclosed a core problem in invasion biology research, which rather focuses on the absence of natural enemies in a new habitat (enemy release hypothesis (ERH)) than on the presence of additional and / or new species as a possible interacting counterpart when covering the drivers of invasion success. This is especially true for invaders from the Ponto-Caspian region, for which also the ERH has been frequently tested (e.g. Kvach and Stepien 2007, Kvach et al. 2014). On the downside, not much attention has been paid to what happens to or with these invaders after entering a new habitat. New approaches such as parasite spillback and spillover or the dilution effect have only been rarely taken into account (Kvach et al. 2017). However, the spatial approach has shown that although the absence of natural enemies might have accelerated and simplified the establishment of Ponto-Caspian species in new environments, interactions between invasive and local potential host species might also facilitate the mass distribution of additional Ponto-Caspian parasites such as the co-introduced *P. laevis*. Furthermore, these interactions might have encouraged an increase of the prevalence of local parasites in various German rivers. Therefore, the study explicitly demonstrated that the approaches of a parasite spillback and / or spillover are important factors when evaluating the consequences

and results of invasion.

Into the bargain, the second study (*cf.* chapter II) showed that Ponto-Caspian species might have further impacts on local parasites. The timeline approach demonstrated that the acanthocephalan *P. laevis*, which most probably was introduced with the other Ponto-Caspian invaders, might have caused the extinction of the local and closely related *P. tereticollis* population after both parasites have co-occurred for several years at different locations along the Rhine River. The results suggested that the local *P. tereticollis* was outcompeted by *P. laevis* through enhanced infection abundance as a result of the invasion of Ponto-Caspian amphipods such as *Dikerogammarus villosus*, which in turn outcompeted local amphipods such as *Gammarus pulex*, which is the preferred first intermediate host of *P. tereticollis*.

Additionally, the third study (*cf.* chapter III), namely the lab diagnostic approach showed that Ponto-Caspian species could contribute and intensify the transmission of local parasites. Generally, the immune response of various host species to different local parasites might be evaded since the presence of Ponto-Caspian parasites and the host responses to these invaders ensure a 'hide out' condition for local (potentially also invasive) parasites. Furthermore, the lab diagnostic approach provides evidence that the presence of different invasive host and parasite species facilitated the establishment of additional invasive parasites, which corresponds to the invasional meltdown hypothesis (IMH). According to Jeschke et al. (2012), the IMH represents one of the major hypotheses of invasion biology that conclusively explain a successful establishment of new species in non-native habitats. However, a possible application of the IMH to parasites has only rarely been discussed prior to the present study. The results showed that interspecific interactions between invasive Ponto-Caspian parasites and local (but also invasive) parasites such as the nematode *Anguillicola crassus* might promote higher infestation rates in the available hosts of invaded areas (*cf.* figure 7.1 C).

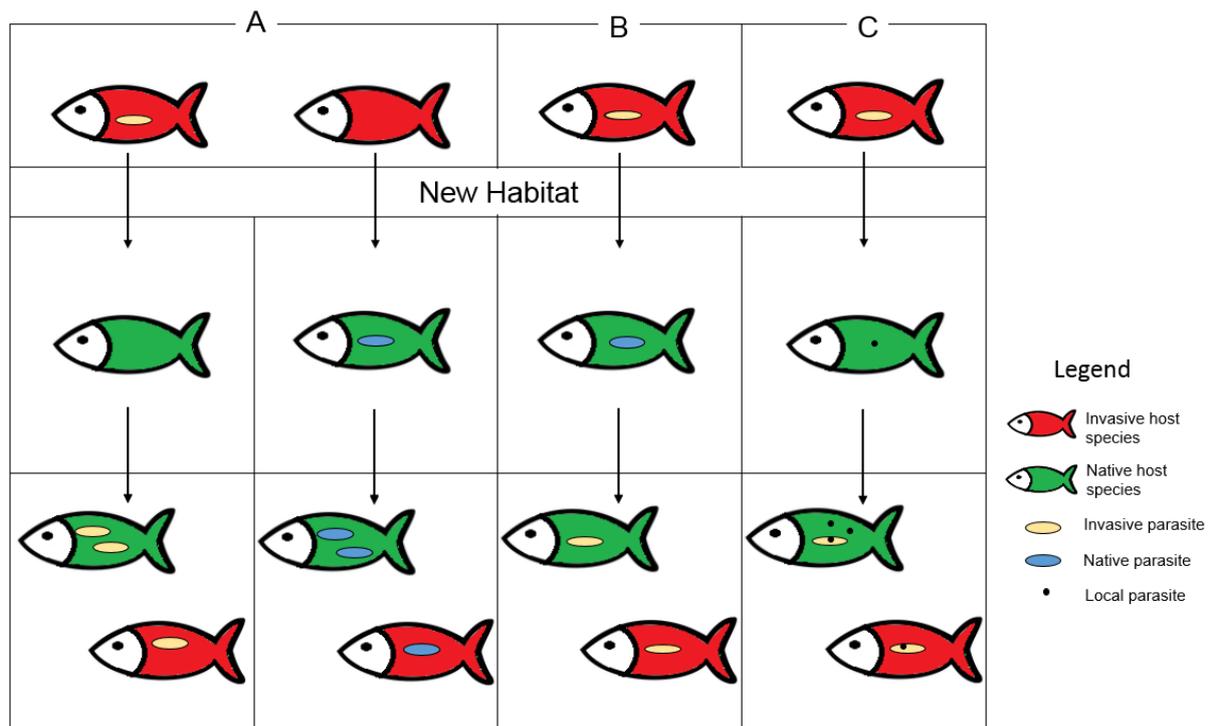


Figure 7.1: Graphical illustration of the three presented scenarios that potentially follow a Ponto-Caspian invasion. Scenario: A) parasite spillback / spillover, B) extinction of native parasites, C) enhancement of local parasites through new transmission pathways

In summary, each of the studies within the present doctoral thesis confirmed that Ponto-Caspian invasive species are able to potentially affect local parasite communities on different levels, which was often underestimated or completely neglected in the context of invasion biology (Kelly et al. 2009, Dunn et al. 2012).

The evaluation of the results additionally implied that not only Ponto-Caspian species, but presumably most invasive (or nonindigenous) species that are suitable hosts for local parasites potentially cause severe effects for local parasite communities. Occasional research with other invasive hosts (including amphibians, reptiles, fishes, mammals, insects, and birds) corroborate this assumption (Rauque et al. 2003, Sepulveda et al. 2004, Colautti et al. 2005, Thieltges et al. 2006, Burke et al. 2007, Kelly et al. 2009, Wells et al. 2015). However, these approaches usually addressed the impacts on a few species rather than on entire communities. Nevertheless, analyses show that the increase of parasite infestation rates in an environment might have grave consequences for local species that might result in the extinction of individual species (White et al. 2006, Tompkins et al. 2011), which potentially cause (severe) disturbances for entire ecosystems (Kuris et al. 2008, Hatcher and Dunn 2011, Dunn et al. 2012).

Generally, the loss of biodiversity induced by invasion and the resulting consequences for local biota have become a major threat to ecosystem health (Vitousek et al. 1996, Naeem 2002, Didham et al. 2005, Worm et al. 2006, Cardinale et al. 2012). Therefore, the management of invasive species and the adaptation of measures to avoid severe effects for local systems have been part of various conservation strategies in order to maintain global biodiversity (e.g. Dorrough et al. 2004, Tschamntke et al. 2005, Dudgeon et al. 2006). Ordinarily, these considerations do not include the preservation and / or management of the parasitic biodiversity (Dunn et al. 2009, Griffith 2012, Gómez and Nichols 2013), although parasites are recognized to drive and sustain biodiversity (Meffe et al. 2006, Derso et al. 2016). Remarkably, according to Dobson et al. (2008), and Weinstein and Kuris (2016), around 40 % of all species in an ecosystem are parasitic, at least at one stage of their life cycle and are involved in 75 % of the species interactions within the system (Preston and Johnson 2010). Inter alia, parasites are drivers of community compositions, govern host behavior, and potentially alter the structure, dynamics and function of food webs (Arim et al. 2007, Lafferty et al. 2008, Lefevre et al. 2009, Dunne et al. 2013, Derso et al. 2016).

Nevertheless, these attributes did not yet initiate the implementing of parasites as a component of management plans for biodiversity conservation. However, the results of the present doctoral thesis should further contribute to a change of this policy. So far conservation biologists frequently regard parasites as a threat that generally enable, encourage, and / or accelerate the loss of biodiversity (Daszak et al. 2000, Lafferty and Gerber 2002, Altizer et al. 2003). Naturally, the results of this thesis partly support this assumption since introduced (intermediate / paratenic) hosts have most likely caused an increase of specific parasites (*P. laevis* and *R. acus*) in newly invaded habitats with possible negative effects for local hosts. However, beyond that, the results of this thesis also showed that local parasites and their communities are affected by invasion as well. Invasive parasites might thus replace native and local key species and the diversity of the parasite communities might be negatively influenced due to a massive increase of a few species. Consequently, the parasite community might be more homogeneous and vulnerable to disturbances leading to a meltdown of local parasite communities with negative impacts on ecosystem level (Gompper and Williams 1998, Dunn et al. 2009, Derso et al. 2016).

Conclusively, effects of invasive species on new environments are widely

studied showing various consequences for global biota (Didham et al. 2005, Charles and Dukes 2007, Reich et al. 2012). As addressed and criticized several times, parasites usually not taken into consideration when evaluating the effects of invasion and in approaches to manage these effects, respectively. The present doctoral thesis demonstrated that henceforth parasites have to be integrated into proposed or existing conservation efforts, since invasive species might cause an increase of local parasites and / or a loss of parasite diversity, both with grave consequences for local environments.

Nonetheless, although the results of this doctoral thesis are striking, they give only a coarse overview about the various possibilities how invasion might affect the parasite-parasite and / or parasite-host interactions of a system. However, the results open the doors to additional aspects in ecological parasitology and may induce a revision of evaluations of previous researches. Therefore, further research is highly recommended, which should include other parasites, hosts, as well as aquatic and terrestrial habitats.

References

- Alexandrov B, Boltachev A, Kharchenko T, Lyashenko A, Son M, Tsarenko P, Zhukinsky V (2007) Trends of aquatic alien species invasions in Ukraine. *Aquatic Invasions* 2:215–242
- Aliabadi BK, Juliano SA (2002) Escape from gregarine parasites affects the competitive impact of an invasive mosquito. *Biological Invasions* 4:283-297
- Almqvist G, Strandmark AK, Appelber M (2010) Has the invasive round goby caused new links in Baltic food webs? *Environmental Biology of Fishes* 89:79–93
- Altizer S, Harvell D, Friedle E (2003) Rapid evolutionary dynamics and disease threats to biodiversity. *Trends in Ecology* 18:589-596
- Amundsen PA, Lafferty KD, Knudsen R, Primicerio R, Kristoffersen R, Klemetsen A, Kuris AM (2013) New parasites and predators follow the introduction of two fish species to a subarctic lake: implications for food-web structure and functioning. *Oecologia* 171: 993–1002
- Angelini R, Finarelli AC, Angelini P, Po C, Petropulacos K, Macini P, Fiorentini C, Fortuna C, Venturi G, Romi R, Majori G, Nicoletti L, Rezza G, Cassone A (2007) An outbreak of chikungunya fever in the province of Ravenna, Italy. *Euro Surveill.* 12:pii=3260
- Arim M, Marquet PA, Jaksic FM (2007) On the relationship between productivity and food chain length at different ecological levels. *The American Naturalist* 169: 62–72
- Baldauf SA, Thünnen T, Frommen JG, Bakker TCM, Heupel O, Kullmann H (2007) Infection with an acanthocephalan manipulates an amphipod's reaction to a fish predator's odors. *International Journal of Parasitology* 37:61–65
- Balshine S, Verma A, Chant V, Theysmeyer T (2005) Competitive interactions between round gobies and logperch. *Journal of Great Lakes Research* 31:68–77
- Bernauer D and Jansen W (2006) Recent invasions of alien macroinvertebrates and loss of native species in the upper Rhine River, Germany. *Aquatic Invasions* 2:55-71

- Bij de Vaate A, Jazdzewski K, Ketelaars HAM, Gollasch S, Van der Velde G (2002) Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Canadian Journal of Fisheries and Aquatic Science* 59:1159–1174
- Blakeslee AMH, Fowler AE, Keogh CL (2013) Marine Invasions and Parasite Escape: Updates and New Perspectives. *Advances in Marine Biology* 66:87–169
- Blumenthal D, Mitchell CE, Pyšek P, Jarošík V (2009) Synergy between pathogen release and resource availability in plant invasion. *Proceedings of the National Academy of Sciences USA* 106:7899-7904
- Bollache L, Devin S, Wattier R, Chovet M, Beisel JN, Moreteau JC, Rigaud T (2004) Rapid range extension of the Ponto-Caspian amphipod *Dikerogammarus villosus* in France: potential consequences. *Archiv für Hydrobiologie* 160:57–66
- Boonstra H, Wiggers R, Swarte M (2016) First record of the Ponto-Caspian amphipod *Obesogammarus obesus* (Sars, 1894) (Amphipoda: Pontogammaridae) from the Netherlands. *Biological Invasions* 3:155–158
- Borcherding J, Staas S, Krüger S, Ondrackova M, Slapansky L, Jurajda P (2011) 3 Non-native Gobiid species in the lower River Rhine (Germany): recent range extensions and densities. *Journal of Applied Ichthyology* 27:153–155
- Brohmer P, Ehrmann P, Ulmer G (1938) Die Tierwelt Mitteleuropas. Urtiere-Hohltiere-Würmer. Acanthocephala. Leipzig, Quelle and Meyer
- Brown AF (1987) Anatomical variability and secondary sexual characteristics in *Pomphorhynchus laevis* (Müller, 1776) (Acanthocephala). *Systematic Parasitology* 9:213–219
- Brown AF, Thompson DBA (1986) Parasite manipulation of host behavior, acanthocephalans and shrimps in the laboratory. *Journal of Biological Education* 20:121–127
- Brown AF, Chubb JC, Veltkamp CJ (1986) A key to the species of Acanthocephala parasitic in British freshwater fishes. *Journal of Fish Biology* 28:327–334

- Buckner SC, Nickol BB (1975) Morphological variation of *Moniliformis moniliformis* (Bremser, 1811) Travassos 1915 and *Moniliformis clarki* (Ward, 1917) Chandler 1921. *Journal of Parasitology* 61:996–998
- Burke RL, Goldberg SR, Bursley CR, Perkins SL, Andreadis PT (2007) Depauperate parasite faunas in introduced populations of *Podarcis* (Squamata : Lacertidae) lizards in North America. *Journal of Herpetology* 41:755–757
- Bush AO, Lafferty KD, Lotz JM, Shstak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology* 83:575-83
- Bush AO, Fernandez JC, Esch GW, Seed JR (2001) Parasitism. The diversity and ecology of animal parasites. Cambridge University Press, Cambridge.
- Campbell LM, Thacker R, Barton D, Muir DCG, Greenwood D, Hecky RE (2009) Re-engineering the eastern Lake Erie littoral food web: the trophic function of non-indigenous Ponto-Caspian species. *Journal of Great Lakes Research* 35:224–231
- Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, Narwani A, Mace GM, Tilman D, Wardle DA, Kinzig AP, Daily GC, Loreau M, Grace JB, Larigauderie A, Srivastava DS, Naeem S (2012) Biodiversity loss and its impact on humanity. *Nature* 486:59–67
- Carlsson M (1997) Sea Level and Salinity Variations in the Baltic Sea - an Oceanographic Study using Historical Data. Thesis. Department of Oceanography Goteborg University. ISSN 1400-3813
- Carman SM, Janssen J, Jude DJ, Berg MB (2006) Diel interactions between prey behaviour and feeding in an invasive fish, the round goby, in a North American river. *Freshwater Biology* 51:742–755
- Casellato S, La Piana G, Latella L, Ruffo S (2005) *Dikerogammarus villosus* (Sowinsky, 1894), a new invasive species in the Garda Lake (Northern Italy). Abstracts for Biological Invasions in Inland Waters (IN-WAT) Workshop, Firenze, Italy, May 5–7, 2005, p 26
- Chapman D, Purse BV, Roy HE, Bullock JM (2017) Global trade networks determine the distribution of invasive non-native species. *Global Biology and Biography* 26:907-917

- Charles H and Dukes JS (2007) Impacts of Invasive Species on Ecosystem Services. Ecological Studies, Vol. 193. Biological Invasions. Springer-Verlag Berlin Heidelberg
- Chen W, Bierbach D, Plath M, Streit B, Klaus S (2012) Distribution of amphipod communities in the Middle to Upper Rhine and five of its tributaries. Biological Invasions Records 1:263–271
- Chiron F, Shirley S, Kark S (2009) Human-related processes drive the richness of exotic birds in Europe. Proceedings of the Royal Society B: Biological Science 276:47–53
- Colautti RI, Muirhead JR, Biswas RN, Maclsaac HJ (2005) Realized vs apparent reduction in enemies of the European starling. Biological Invasions 7:723–732
- Colautti RI, Ricciardi A, Grigorovich IA, Maclsaac HJ (2004) Is invasion success explained by the enemy release hypothesis? Ecology Letters 7:721–733
- Commission of the European Communities (2008) Communication from the Commission to the Council, the European Parliament, the European Economic and Social Committee and the Committee of the Regions - Towards an EU strategy on invasive species [SEC(2008) 2887 et SEC(2008) 2886] /* COM/2008/0789 final */
- Copp GH, Bianco PG, Bogutskaya NG, Eros T, Falka I, Ferreira MT, Fox MG, Freyhof J, Gozlan RE, Grabowska J, Kovac V, Moreno-Amich R, Naseka AM, Penaz M, Povz M, Przybylski M, Robillard M, Russell IC, Stakenas S, Sumer S, Vila-Gispert A, Wiesner C (2005) To be, or not to be, a non-native freshwater fish? Journal of Applied Ichthyology 21:242–262
- Cornet S, Sorci G, Moret Y (2010) Biological invasion and parasitism: invaders do not suffer from physiological alterations of the acanthocephalan *Pomphorhynchus laevis*. Parasitology 137:137–47
- Cristescu MEA, Witt JDS, Grigorovich IA, Hebert PDN, Maclsaac HJ (2004) Dispersal of the Ponto-Caspian amphipod *Echinogammarus ischnus*: invasion waves from the Pleistocene to the present. Heredity 92:197–203
- Dang C, Montaudouin X de, Bald D, Jude F, Raymond N, Lanceleur L, Paul-Pont I, Caill-Milly N (2009) Testing the enemy release hypothesis: trematode parasites

- in the non-indigenous Manila clam *Ruditapes philippinarum*. *Hydrobiologia* 630:139-148
- Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife – threats to biodiversity and human health. *Science* 287:443–449
- David GM, Staentzel C, Schlumberger O, Perrot-Minnot MJ, Beisel JN, Hardion L (2017) A minimalist macroparasite diversity in the round goby of the Upper Rhine reduced to an exotic acanthocephalan lineage. Cambridge University Press, Cambridge
- De Charleroy D, Grisez L, Thomas K, Belpaire C, Ollevier F (1990) The life cycle of *Anguillicola crassus*. *Diseases of Aquatic Organisms* 8:77–84
- Derso A, Nibret E, Munshea A (2016) Prevalence of intestinal parasitic infections and associated risk factors among pregnant women attending antenatal care center at Felege Hiwot Referral Hospital, northwest Ethiopia. *BMC Infectious Diseases* 16:530
- Dextrase AJ & Mandrak NE (2006) Impacts of alien invasive species on freshwater fauna at risk in Canada. *Biological Invasions* 8:13-24
- Dezfuli BS (1992) Occurrence of *Pomphorhynchus laevis* Müller 1776 (Acanthocephala) in *Silurus glanis* (L.) from the River Po. *Parassitologia* 34:71–82
- Dezfuli BS, Lui A, Squerzanti S, Lorenzoni M, Shinn AP (2012) Confirmation of the hosts involved in the life cycle of an acanthocephalan parasite of *Anguilla anguilla* (L.) from Lake Piediluco and its effect on the reproductive potential of its amphipod intermediate host. *Parasitology Research* 110:2137–2143
- Dezfuli BS, Maynard BJ, Wellnitz TA (2003) Activity levels and predator detection by amphipods infected with an acanthocephalan parasite, *Pomphorhynchus laevis*. *Folia Parasitologica* 50:129–134
- Diagne C, Ribas A, Charbonnel N, Dalecky A, Tataro C, Gauthier P, Haukisalmi V, Fossati-Gaschignard O, Bâ K, Kane M, Niang Y, Diallo M, Sow A, Piry S, Sembène M, Brouat C (2016) Parasites and invasions: changes in gastrointestinal helminth assemblages in invasive and native rodents in Senegal. *International Journal of Parasitology* 46:857-869

- Dick JTA, Platvoet D (2000) Invading predatory crustacean *Dikerogammarus villosus* eliminates both native and exotic species. *Proceedings of the Royal Society, Series B* 267:977–983
- Didham RK, Tylianakis JM, Hutchison MA, Ewers RM, Gemmill NJ (2005) Are invasive species the drivers of ecological change? *Trends in Ecology* 20:470-474
- Dietrich JP, Morrison BJ, Hoyle JA (2006) Alternative ecological pathways in the eastern Lake Ontario food web—round goby in the diet of lake trout. *Journal of Great Lakes Research* 32:395–400
- Djikanovic V, Gacic Z, Cakic P (2010) Endohelminth fauna of barbel *Barbus barbus* (L. 1758) in the Serbian section of the Danube River, with dominance of acanthocephalan *Pomphorhynchus laevis*. *Bulletin of the European Association of Fish Pathologists* 30:229–236
- Dobson AP, Hudson PJ (1986) Parasites, disease and the structure of ecological communities. *Trends in Ecology and Evolution* 1:11-15
- Dobson A, Lafferty KD, Kuris AM, Hechinger RF, Jetz W (2008) Homage to Linnaeus: How many parasites? How many hosts? *Proceedings of the National Academy of Sciences* 105:11482-11489
- Dorrough J, Yen A, Turner V, Clark SG, Crosthwaite J, Hirth JR (2004) Livestock grazing management and biodiversity conservation in Australian temperate grassy landscapes. *Australian Journal of Agricultural Research* 55:279-295
- Dubs DOL, Corkum LD (1996) Behavioural interactions between round gobies *Neogobius melanostomus* and mottled sculpins *Cottus bairdi*. *Journal of Great Lakes Research* 22:838–844
- Dudgeon D, Arthington AH, Gessner MO, Kawabata Z-I, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard AH, Soto D, Stiassny MLJ, Sullivan CA (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews*, 81:163-182
- Dudinak V, Snabel V (2001) Comparative analysis of Slovak and Czech population of *Pomphorhynchus laevis* (Acanthocephala) using morphological and isoenzyme analysis." *Acta Zoologica Universitatis Comenianae* 44:41–50

- Dunn AM (2009) Parasites and Biological Invasions. *Advances in Parasitology* 68: 161-184
- Dunn AM, Torchin ME, Hatcher MJ, Kotanen PM, Blumenthal DM, Byers JE, Coon CAC, Frankel VM, Holt RD, Hufbauer RA, Kanarek AR, Schierenbeck KA, Wolfe LM, Perkins SE (2012) Invasions and Infections: Indirect effects of parasites in invasions. *Functional Ecology* 26:1262–1274
- Dunne J.A., Lafferty K.D., Dobson A.P., Hechinger R.F., Kuris A.M., Martinez N.D., McLaughlin J.P., Mouritsen K.N., Poulin R., Reise K., Stouffer D.B., Thieltges D.W., Williams R.J., Zander K.D. (2013) Parasites affect food web structure primarily through increased diversity and complexity. *PLoS Biology* 11:1-17
- Emde S, Klimpel S (2015) *Anguillicola crassus*. In *Encyclopedia of Parasitology*, edited by Heinz Mehlhorn. Springer, Berlin, Heidelberg
- Emde S, Rueckert S, Palm HW, Klimpel S (2012) Invasive Ponto-Caspian amphipods and fish increase the distribution range of the acanthocephalan *Pomphorhynchus tereticollis* in the river Rhine. *PLoS ONE* 7(12):e53218
- Emde S, Rueckert S, Kochmann J, Knopf K, Sures B, Klimpel S (2014) Nematode eel parasite found inside acanthocephalan cysts—a “Trojan horse” strategy? *Parasites and Vectors* 7:504
- Engelbrecht H (1957) Einige Bemerkungen zu *Pomphorhynchus laevis* (Zoega in Müller, 1776) als Parasit in *Pleuronectes flesus* und *Pleuronectes platessa*. *Zentralblatt für Bakteriologie und Parasitenkunde Infektionskrankheiten* 168:474–479
- Essl F, Dullinger S, Rabitsch W, Hulme PE, Hulber K, Jarosík V, Nentwig W (2011) Socioeconomic legacy yields an invasion debt. *Proceedings of the National Academy of Sciences USA*, 108:203–207
- Essink K, Dekker R (2002) General Patterns in Invasion Ecology Tested in the Dutch Wadden Sea: The Case of a Brackish-Marine *Polychaetous* Worm. *Biological Invasions* 4:359–368
- Fahnenstiel GL, Bridgeman TB, Lang GA, McCormick MJ, Nalepa TF (1995) Phytoplankton productivity in Saginaw Bay, Lake Huron: Effects of zebra

- mussels (*Dreissena polymorpha*) colonization. *Journal of Great Lakes Research* 21:465-475
- Franceschi N, Bauer A, Bollache L, Rigaud T (2008) The effects of parasite age and intensity on variability in acanthocephalan-induced behavioural manipulation. *International Journal of Parasitology* 38:1161–1170
- Francová K, Ondrackova M, Polacik M, Jurajda P (2011) Parasite fauna of native and non-native populations of *Neogobius melanostomus* (Pallas, 1814) (Gobiidae) in the longitudinal profile of the Danube River. *Journal of Applied Ichthyology* 27:879–886
- Freese M, Sühling R, Pohlmann JD, Wolschke H, Magath V, Ebinghaus R, Hanel R (2016) A question of origin: dioxin-like PCBs and their relevance in stock management of European eels. *Toxicology* 25:41-55
- Freese M, Sühling R, Marohn L, Pohlmann JD, Wolschke H, Byer J, Alae M, Ebinghaus R, Hanel R (2017) Maternal transfer of dioxin-like compounds in artificially matured European eels. *Environmental Pollution* 227:348-356
- Gagne RB, Heins DC, McIntyre PB, Gilliam JF, Blum MJ (2016) Mutual dilution of infection by an introduced parasite in native and invasive stream fishes across Hawaii. *Parasitology* 143:1605-1614
- Galil BS, Nehring S, Panov V (2007) Waterways as invasion highways – impact of climate change and globalization. In *Biological Invasions*, 59–74, Springer, Berlin, Heidelberg
- Gallardo B, Aldridge DC (2013) Priority setting for invasive species management: risk assessment of Ponto-Caspian invasive species into Great Britain. *Ecological Applications* 23:352–364
- Gallardo B, Aldridge DC (2015) Is Great Britain heading for a Ponto–Caspian invasional meltdown? *Journal of Applied Ecology* 52:41–49
- Gebhardt H, Ness A (1997) *Fische: die heimischen Süßwasserfische sowie Arten der Nord- und Ostsee*. 3., durchgesehene Neuauflage. BLV Verlag, München, Wien, Zürich

- Genovesi P, Bacher S, Kobelt M, Pascal M, Scalera R (2009) Alien mammals of Europe. Chapter 9. DAISIE, Handbook of Alien Species in Europe. Invading nature: Springer series in invasion ecology, Volume 3. Springer edition
- Gilbey V, Attrill MJ, Coleman RA (2008) Juvenile Chinese mitten crabs (*Eriocheir sinensis*) in the Thames estuary: distribution, movement and possible interactions with the native crab *Carcinus maenas*. *Biological Invasions* 10:67-77
- Goedknecht MA, Feis ME, Wegner KM, Luttikhuisen PC, Buschbaum C, Camphuysen K, van der Meer J, Thielges DW (2016) Parasites and Marine Invasions: Ecological and Evolutionary Perspectives. *Journal of Sea Research* 113:11–27
- Gómez A, Nichols E (2013) Neglected wild life: Parasitic biodiversity as a conservation target. *International Journal of Parasitology: Parasites and Wildlife* 2:222–227
- Gompper ME, Williams ES (1998) Parasite conservation and the black-footed ferret recovery program. *Conservation Biology* 12:730-732
- Green PT, O 'Dowd DJ, Abbott KL, Jeffery M, Retallick K, Mac Nally R (2011) Invasional meltdown: invader—invader mutualism facilitates a secondary invasion. *Ecology* 92:1758–1768
- Griffith GW (2012) Do we need a global strategy for microbial conservation? *Trends Ecol. Evol.* 27:1–2
- Gurnell J, Wauters LA, Lurz PWW, Tosi G (2004) Alien species and interspecific competition: effects of introduced eastern grey squirrels on red squirrel population dynamics. *Journal of Animal Ecology* 73:26–35
- Han JC Young SL (2016) Invasion during Extreme Weather: Success and Failure in a Temperate Perennial Grassland. West Central Research and Extension Center, North Platte -Paper 95
- Hatcher MJ, Dunn AM (2011) Parasites in Ecological Communities. From Interactions to Ecosystems. Cambridge University Press, Cambridge
- Heath MR, Henderson EW, Slesser C, Woodward EMS (1991). High salinity in the North Sea. *Nature* 352:116

- Herlevi H, Puntila R, Kuosa H, Fagerholm HP (2017) Infection rates and prevalence of metazoan parasites of the non-native round goby (*Neogobius melanostomus*) in the Baltic Sea. *Hydrobiologia* 792:265–282
- Hiepe T, Lucius R, Gottstein B (2006) *Allgemeine Parasitologie – Mit den Grundzügen der Immunologie, Diagnostik und Bekämpfung*. MVS Medizinverlage, Stuttgart
- Hohenadler MAA, Nachev M, Thielen F, Taraschewski H, Grabner D, Sures B (2018) *Pomphorhynchus laevis*: An invasive species in the river Rhine? *Biological Invasions* 20:207–217
- Hopkins CCE (2002) Introduced marine organisms in Norwegian waters, including Svalbard. In: Leppäkoski E, Gollasch S, and Olenin S (Eds). *Invasive aquatic species of Europe: distribution, impacts and management*. Dordrecht, Netherlands: Kluwer Academic Publishers
- Hulme PE, Pyšek P, Nentwig W, Vilà M (2009) Will threat of biological invasions unite the European Union? *Science* 324:40–41
- Idrisi N, Mills EL, Rudstam LG, Stewart DJ (2001) Impact of zebra mussels (*Dreissena polymorpha*) on the pelagic lower trophic levels of Oneida Lake, New York. *Canadian Journal of Fisheries and Aquatic Science* 58:1430-1441
- Jacoby D, Gollock M (2014) *Anguilla anguilla*. The IUCN Red List of Threatened Species 2014 823
- Jakob E, Walter T, Hanel R (2009) A checklist of the protozoan and metazoan parasites of European eel (*Anguilla anguilla*): checklist of *Anguilla anguilla* parasites. *Journal of Applied Ichthyology* 32:1-49
- Jakšić G, Jadan M, Piria M (2016) The Review of Ecological and Genetic Research of Ponto-Caspian Gobies (Pisces, Gobiidae) in Europe. *Croatian Journal of Fisheries* 74:110-123
- Janssen J, Jude DJ (2001) Recruitment failure of mottled sculpin *Cottus bairdi* in Calumet Harbor, southern Lake Michigan, induced by the newly introduced round goby *Neogobius melanostomus*. *Journal of Great Lakes Research* 27:319–328

- Jeschke JM (2008) Across islands and continents, mammals are more successful invaders than birds. *Diversity and Distributions* 14: 913-916
- Jeschke JM, Bacher S, Blackburn TM, Dick JTA, Essl F, Evans T, Gaertner M, Hulme PE, Kühn I, Mrugała A, Pergl J, Pyšek P, Rabitsch W, Ricciardi A, Richardson DM, Sendek A, Vilà M, Winter M, Kumschick S (2014) Defining the impact of non-native species. *Conservation Biology* 28:1188–1194
- Jeschke JM, Genovesi P (2011) Do biodiversity and human impact influence the introduction or establishment of alien mammals? *Oikos* 120:57–64
- Jeschke JM, Gómez Aparicio L, Haider S, Heger T, Lortie CJ, Pyšek P, Strayer DL (2012) Support for major hypotheses in invasion biology is uneven and declining. *NeoBiota* 14:1-20
- Johnson JH, Ross RM, McCullough RD, Mathers A (2010) Diet shift of double-crested cormorants in eastern Lake Ontario associated with the expansion of the invasive round goby. *Journal of Great Lakes Research* 36:242–247
- Jůza T, Blabolil P, Baran RD, Barton DC, Cech M, Draščík V, Frouzová J, Holubová M, Ketelaars HAM, Kočvara L, Kubecka J, Muška M, Prchalová M, Říha M, Sajdlova Z, Šmejkal M, Tušer M, Vašek M, Vejřík L, Vejříková I, Wagenvoort AJ, Zak J, Peterka J (2018) Collapse of the native ruffe (*Gymnocephalus cernua*) population in the Biesbosch lakes (the Netherlands) owing to round goby (*Neogobius melanostomus*) invasion. *Biological Invasions* 20:1523–1535
- Kalchhauser I, Mutzner P, Hirsch PE, Burkhardt-Holm P (2013) Arrival of round goby *Neogobius melanostomus* (Pallas, 1814) and bighead goby *Ponticola kessleri* (Günther, 1861) in the High Rhine (Switzerland). *Biological Invasions Records* 2:79–83
- Karatayev AY, Burlakova LE, Padilla DK (2002) Impacts of Zebra Mussels on Aquatic Communities and their Role as Ecosystem Engineers. In: Leppäkoski E., Gollasch S., Olenin S. (eds) *Invasive Aquatic Species of Europe. Distribution, Impacts and Management*. Springer, Dordrecht
- Kark S, Solarz W, Chiron F, Clergeau P, Shirley S (2009) Alien birds, Amphibians and Rptiles of Europe. Chapter 8. DAISIE, Handbook of Alien Species in Europe. *Invading nature: Springer series in invasion ecology, Volume 3*. Springer edition

- Karlson AML, Almqvist G, Skóra KE, Appelberg M (2007) Indications of competition between non-indigenous round goby and native flounder in the Baltic Sea. *ICES Journal of Marine Science* 64:479–486
- Kelly DW, Paterson RA, Townsend CR, Poulin R, Tompkins DM (2009) Parasite spill back: a neglected concept in invasion ecology? *Ecology* 90:2047–2056
- Kennedy CR (1984) The status of flounders, *Platichthys flesus* L., as hosts of the Acanthocephalan *Pomphorhynchus laevis* (Müller) and its survival in marine conditions. *Journal of Fish Biology* 24:135–149
- Kennedy CR (1989) Discontinuous distributions of the fish acanthocephalans *Pomphorhynchus laevis* and *Acanthocephalus anguillae* in Britain and Ireland: an hypothesis. *Journal of Fish Biology* 34:607–619
- Kennedy CR (1999) Post-Cyclic Transmission in *Pomphorhynchus laevis* (Acanthocephala). *Folia Parasitologica* 46:111–116
- Kennedy CR (2006) *Ecology of the Acanthocephala*. Cambridge University Press, Cambridge, pp 35–41
- Kennedy CR, Broughton PF, Hine PM (1977) The status of brown and rainbow trout, *Salmo trutta* and *S. gairdneri* as hosts of acanthocephalan, *Pomphorhynchus laevis*. *Journal of Fish Biology* 13:265–275
- Keppel M, Dangel KC, Sures B (2014) Comparison of infection success, development and swim bladder pathogenicity of two congeneric *Anguillicola* species in experimentally infected *Anguilla anguilla* and *A. japonica*. *Parasitology Research* 113: 3727–35
- Kessel van N, Dorenbosch M, Kranenbarg J, van der Velde G and Leuwe RSEW (2016) Invasive Ponto-Caspian gobies rapidly reduce the abundance of protected native bullhead. *Aquatic Invasions* 11:179–188
- Ketelaars HM (2004) Range extensions of Ponto-Caspian aquatic invertebrates in Continental Europe. *Aquatic Invasions in the Black, Caspian, and Mediterranean Seas*. Springer, Dordrecht, 209–236

- Kinzler W, Kley A, Mayer G, Waloszek D, Maier G (2009) Mutual predation between and cannibalism within several freshwater gammarids: *Dikerogammarus villosus* versus one native and three invasives. *Aquatic Ecology* 43:457–464
- Knopf K (2006) The swimbladder nematode *Anguillicola crassus* in the European eel *Anguilla anguilla* and the Japanese eel *Anguilla japonica*: differences in susceptibility and immunity between a recently colonized host and the original host. *Journal of Helminthology* 80:129–136
- Knopf K, Mahnke M (2004) Differences in susceptibility of the European eel (*Anguilla anguilla*) and the Japanese eel (*Anguilla japonica*) to the swim-bladder nematode *Anguillicola crassus*. *Parasitology* 129:491–496
- Knopf K, Würtz J, Sures B, Taraschewski H (1998) Impact of low water temperature on the development of *Anguillicola crassus* in the final host *Anguilla anguilla*. *Diseases of Aquatic Organisms* 33:143–49
- Kornis M, van der Zanden J, Mercado-Silva N (2012) Twenty years of invasion: a review of round goby *Neogobius melanostomus* biology, spread and ecological implications. *Journal of Fish Biology* 80:235–285
- Králová-Hromadová I, Tietz DF, Shinn AP, Špakulová M (2003) ITS rDNA sequences of *Pomphorhynchus laevis* (Zoega in Müller, 1776) and *P. lucyi* William & Rogers, 1984 (Acanthocephala: Palaeacanthocephala). *Systematic Parasitology* 56:141–145
- Kuris AM, Hechinger RF, Shaw JC, Whitney KL, Aguirre-Macedo L, Boch CA, Dobson AP, Dunham EJ, Fredensborg BL, Huspeni TC, Lorda J, Mababa L, Mancini FT, Mora AB, Pickering M, Talhouk NL, Torchin ME, Lafferty KD (2008) Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature* 454:515-518
- Kvach Y, Boldyrev V, Lohner R, Stepien CA (2015) The parasite community of gobiid fishes (Actinopterygii: Gobiidae) from the Lower Volga River region. *Biologia* 70:948—957
- Kvach Y, Ondračková M, Janáč M, Jurajda P (2018) Methodological issues affecting the study of fish parasites. III. Effect of fish preservation method. *Diseases of Aquatic Organisms* 127:213–224

- Kvach Y, Ondračková M, Janáč M, Jurajda P (2017) The parasite community of round goby *Neogobius melanostomus* (Pallas, 1814) (Actinopterygii: Gobiidae) newly introduced into the upper Elbe. *Knowledge and Management of Aquatic Ecosystems*: 418-419
- Kvach Y, Ondračková M, Janáč M, Jurajda P (2016) Methodological issues affecting the study of fish parasites. I. Duration of live fish storage prior to dissection. *Diseases of Aquatic Organisms* 119:107–115
- Kvach Y, Skóra KE (2006) The comparative analysis of infection parameters of the round goby *Neogobius melanostomus* (Pallas) (Gobiidae: Osteichthyes) in the Gulf of Gdańsk, Baltic Sea, Poland, and the Northwestern Black Sea, Ukraine. *Acta Ichthyologica Romanica* 1:141–152
- Kvach Y, Skóra KE (2007) Metazoa parasites of the invasive round goby *Apollonia melanostoma* (*Neogobius melanostomus*) (Pallas) (Gobiidae: Osteichthyes) in the Gulf of Gdansk, Baltic Sea, Poland: a comparison with the Black Sea. *Parasitology Research* 100:767–774
- Kvach Y, Stepien CA (2008) The invasive round goby *Apollonia melanostoma* (Actinopterygii: Gobiidae) – a new intermediate host of the trematode *Neochasmus umbellus* (Trematoda: Cryptogonimidae) in Lake Erie, Ohio, USA. *Journal of Applied Ichthyology* 24:103–105
- Lafferty KD, Allesina S, Arim M, Briggs CJ, De Leo D, Dobson AP, Dunne JA, Johnson PTJ, Kuris AM, Marcogliese DJ, Martinez ND, Memmott J, Marquet PA, McLaughlin JP, Mordecai EA, Pascual M, Poulin R, Thieltges DW (2008) Parasites in food webs: the ultimate missing links. *Ecology Letters* 11:533–546
- Lafferty K, Gerber L (2002) Good medicine for conservation biology: the intersection of epidemiology and conservation theory. *Conservation Biology* 16:593–604
- Lefevre T, Lebarbenchon C, Gauthier-Clerc M, Misse D, Poulin R, Thomas F (2009) The ecological significance of manipulative parasites. *Trends in Ecology and Evolution* 24:41–48
- Li WX, Arnott SA, Jones KMM, Braicovich PE, de Buron I, Wang GT, Marcogliese DJ (2015) First record of paratenic hosts of the swimbladder nematode *Anguillicola crassus* in North America. *Journal of Parasitology* 101:529–35

- Llarena-Reino M, Pineiro C, Antonio J, Outerino L, Vello C, González AF, Pascual S (2013) Optimization of the pepsin digestion method for anisakids inspection in the fishing industry. *Veterinary Parasitology* 191:276–283
- Lovette IJ, Seutin G, Ricklefs RE, Bermingham E (1999) The assembly of an island fauna by natural invasion: Sources and temporal patterns in the avian colonization of Barbados. *Biological Invasions* 1:33–41
- Löveit GL (1997) Biodiversity: Global change through invasion. *Nature* 388:627–628
- Lundström A (1942) Die Acanthocephalan Schwedens. [The Swedish Acanthocephala.] Dissertation. University of Lund
- Lymbery AJ, Morine M, Gholipour Kanani H, Beatty SJ, Morgana DL (2014) Co-invaders: The effects of alien parasites on native hosts. *International Journal for Parasitology: Parasites and Wildlife* 2:171–177
- MacNeil C, Platvoet D, Dick JDA, Fielding N, Constable AJ, Hall N, Aldridge D, Diamond M (2010) The Ponto-Caspian ‘killer shrimp’, *Dikerogammarus villosus* (Sowinsky, 1894), invades the British Isles. *Aquatic Invasions* 5:441-445
- Magurran AE (1988) *Ecological diversity and its measurement*. Springer, Dordrecht
- Meffe GK, Carroll RC, Groom MJ (2006) What is conservation biology? In: Groom MJ, Meffe GK, Carroll RC (Eds.), *Principles of Conservation Biology*. Sinauer Associates, Inc., Sunderland, MA, USA
- Messink RH, Wright MG (2006) Biological control of invasive species: solution or pollution? *Frontiers in Ecology and the Environment* 4:132-140
- Meyer A (1932) Acanthocephala. I. In: Bronn HG (ed) *Klassen und Ordnungen des Tierreichs*, 4th edn. Akademische Verlagsgesellschaft, Leipzig:1–332
- Micklin P, Aladin N, Plotnikov I (2014) *The Aral Sea - The Devastation and Partial Rehabilitation of a Great Lake*. Springer, Dordrecht
- Molloy DP, Bij de Vaate A, Wilke T, Giamberini L (2007) *Discovery of Dreissena rostriformis bugensis* (Andrusov 1897) in Western Europe. *Biological Invasions* 9:871–874
- Molloy S, Holland C, O’Regan M (1995) Population biology of *Pomphorhynchus laevis* in brown trout from two lakes in the west of Ireland. *J Helminthol* 69:229–235

- Molnar K, Szekely C, Baska F (1991) Mass mortality of eel in Lake Balaton due to *Anguillicola crassus* infection. Bulletin of the European Association of Fish Parasitologist 11: 211
- Molnar JL, Gamboa RL, Revenga C, Spalding MD (2008) Assessing the global threat of invasive species to marine biodiversity. Frontiers in Ecology and the Environment 6:485–492
- Mouritsen KN, Poulin R (2005) Parasites boosts biodiversity and changes animal community structure by trait-mediated indirect effects. Oikos 108:344-350
- Moravec F (1994) Parasitic Nematodes of Freshwater Fishes of Europe. Springer, Dordrecht
- Moravec F (1996) The amphipod *Gammarus fossarum* as a natural true intermediate host of the nematode *Raphidascaris acus*. Journal of Parasitology 82:668-669
- Moravec F (2004) Metazoan Parasites of Salmonid Fishes of Europe. Academia Czech Republic
- Moravec F, Konecny R (1994) Some new data on the intermediate and paratenic hosts of the nematode *Anguillicola crassus* (Dracunculoidea), a swimbladder parasite of eels. Folia Parasitologica 41:65–70
- Moravec F, Scholz T (2015) Macroparasites and their communities of the European eel *Anguilla anguilla* (Linnaeus) in the Czech Republic. Folia Parasitologica 62:033
- Moravec F, Scholz T (1991) Observations on the biology of *Pomphorhynchus laevis* (Zoega in Müller, 1776) (Acanthocephala) in the Rokytná River, Czech and Slovak Federative Republic. Helminthologia 28:23–29
- Morozińska-Gogol J (2013) Parasite communities of European perch, *Perca fluviatilis* L. (Actinopterygii: Perciformes: Percidae) from lake Łebsko (Central Coast, Poland). Annals of Parasitology 59:89–98
- Müller JC, Hidde D, Seitz A (2002) Canal construction destroys the barrier between major European invasion lineages of the zebra mussel. Proceedings of the Royal Society of London. Series B 269:1139–1142

- Münderle M, Taraschewski H, Klar B, Chang CW, Shiao JC, Shen KN, He JT, Lin SH, Tzeng WN (2006) Occurrence of *Anguillicola crassus* (Nematoda: Dracunculoidea) in Japanese eels *Anguilla japonica* from a river and an aquaculture unit in SW Taiwan. *Diseases of Aquatic Organisms* 71:101–108
- Munro MA, Whitfield PJ, Diffley R (1989) *Pomphorhynchus laevis* (Müller) in the flounder, *Platichthys flesus* L., in the tidal River Thames: population structure, microhabitat utilization and reproductive status in the field and under conditions of controlled salinity. *Journal of Fish Biology* 35:719–735
- Munro MA, Reid A, Whitfield PJ (1990) Genomic divergence in the ecologically differentiated English freshwater and marine strains of *Pomphorhynchus laevis* (Acanthocephala: Palaeacanthocephala): a preliminary investigation. *Parasitology* 101:451–454
- Nachev M, Sures B (2009) The endohelminth fauna of barbell (*Barbus barbus*) correlates with water quality of the Danube River in Bulgaria. *Parasitology* 136:545–552
- Nachev M, Sures B (2016) Seasonal profile of metal accumulation in the acanthocephalan *Pomphorhynchus laevis*: a valuable tool to study infection dynamics and implications for metal monitoring. *Parasites and Vectors* 9:300
- Nachev M, Zimmermann S, Rigaud T, Sures B (2010) Is metal accumulation in *Pomphorhynchus laevis* dependent on parasite sex or infrapopulation size? *Parasitology* 137:1239–1248
- Naeem S (2002) Ecosystem Consequences of Biodiversity Loss: The Evolution of a Paradigm. *Ecology* 83:1537–1552
- Nehring S (2006) Four arguments why so many alien species settle into estuaries, with special reference to the German river Elbe. *Helgoland Marine Research* 60:127
- Nielsen C, Ravn HP, Nentwig W, Wade M (2005) The Giant Hogweed Best Practice Manual. Guidelines for the management and control of an invasive weed in Europe. Forest & Landscape Denmark, Hoersholm.
- Noonburg EG, Shuter BJ, Abrams PA (2003) Indirect Effects of Zebra Mussels (*Dreissena polymorpha*) on the Planktonic Food Web. *Canadian Journal of Fisheries and Aquatic Science* 60:1353–1368

- O'Dowd, D.J., Green, P.T. & Lake, P.S. (2003) Invasional 'meltdown' on an oceanic island. *Ecological Letters*, 6:812–817
- O'Loughlin LS, Green PT (2017) Secondary invasion: when invasion success is contingent on other invaders altering the properties of recipient ecosystems. *Ecology and Evolution* 7:7628–37
- O'Mahony EM, Bradley DG, Kennedy CR, Holland CV (2004) Evidence for the hypothesis of strain formation in *Pomphorhynchus laevis* (Acanthocephala): an investigation using mitochondrial DNA sequences. *Parasitology* 29:341–347
- Ojaveer H, Leppäkoski E, Olenin S, Ricciardi A (2002) Ecological Impact of Ponto-Caspian Invaders in the Baltic Sea, European Inland Waters and the Great Lakes: An Inter-Ecosystem Comparison. In: Leppäkoski E, Gollasch S, Olenin S (eds) *Invasive Aquatic Species of Europe. Distribution, Impacts and Management*. Springer, Dordrecht
- Ondračová M, Dávidová M, Pečínková M, Blažek R, Gelnar M, et al. (2005) Metazoan parasites of *Neogobius* fishes in the Slovak section of the River Danube. *Journal of Applied Ichthyology* 21:345–349
- Ondračková M, Dávidová M, Blažek R, Gelnar M, Jurajda P (2009) The interaction between an introduced fish host and local parasite fauna: *Neogobius kessleri* in the middle Danube River. *Parasitological Research* 105:201–208
- Ondračková M, Dávidová M, Prikrylová I, Pečínková M (2011) Monogenean Parasites of Introduced Pumpkinseed *Lepomis gibbosus* (Centrarchidae) in the Danube River Basin. *Journal of Helminthology* 85:435–41
- Ondračková M, Trichkova T, Jurajda P (2006) Present and historical occurrence of metazoan parasites in *Neogobius kessleri* (Gobiidae) in the Bulgarian section of the Danube River. *Acta Zoologica Bulgarica* 58:399–406
- Ondračková M, Valová Z, Hudcová I, Michálková V, Andrea Šimková A, Borcharding J, Jurajda P (2015) Temporal effects on host-parasite associations in four naturalized goby species living in sympatry. *Hydrobiologia* 746:233–243
- Ortega N, Price W, Campbell T, Rohr J (2015) Acquired and introduced macroparasites of the invasive cuban treefrog, *Osteopilus septentrionalis*. *International Journal for Parasitology: Parasites and Wildlife* 4:379–84

- Ovcharenko M, Codreanu-Balcescu D, Grabowski M, Konopacka A, Wita I, Czaplinska U (2009) Gregarines (Apicomplexa) and microsporidians (Microsporidia) of native and invasive gammarids (Amphipoda, Gammaroidea), occurring in Poland. *Wiadomosci Parazytologiczne* 55: 237–247
- Ovcharenko M, Bacela K, Wilkinson T, Ironside J, Rigaud T, Wattier RA (2010) *Cucumispora dikerogammarin*. gen. (Fungi:Microsporidia) infecting the invasive amphipod *Dikerogammarus villosus*: a potential emerging disease in European rivers. *Parasitology* 137:191–204
- Padilla DK, Williams SL (2004) Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. *Frontiers in Ecology and the Environment* 2:131-138
- Peeler EJ, Oidtmann BC, Midtlyng PJ, Miossec L, Gozlan RE (2011) Non-native aquatic animals introductions have driven disease emergence in Europe. *Biological Invasions* 13:1291–1303
- Pejchar L, Mooney HA (2009) Invasive species, ecosystem services and human well-being. *Trends in Ecology and Evolution* 24:497-504
- Pelster B (2015) Swimbladder function and the spawning migration of the European eel *Anguilla anguilla*. *Frontiers in Physiology* 5:486
- Pereira NN, Colomboc FB, Chávezc MIA, Brinatib HL, Carrenõc MNP (2016) Challenges to implementing a ballast water remote monitoring system. *Ocean Coast Management* 131:25–38
- Perrings C, Williamson MH, Dalmazzone S (2000) The economics of biological invasions. Edited by Charles Perrings, Mark Williamson, and Silvana Dalmazzone. Cheltenham: Edward Elgar Publishing Limited.
- Perrot-Minnot MJ (2004) Larval morphology, genetic divergence, and contrasting levels of host manipulation between forms of *Pomphorhynchus laevis* (Acanthocephala). *International Journal of Parasitology* 34:45–54
- Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52:273–288

- Pimentel D, McNair S, Janecka J, Wightman J, Simmonds C, O'Connell C, Wong E, Russel L, Zern J, Aquino T, Tsomondo T (2001) Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystem & Environment* 84:1–20
- Pizzatto L, Kelehear C, Dubey S, Barton D, Shine R (2012) Host-parasite relationships during a biologic invasion: 75 years postinvasion, cane toads and sympatric Australian frogs retain separate lungworm faunas. *Journal of Wildlife Diseases* 48:951–961
- Platvoet D. (2005) Temperature dependent feeding in *Dikerogammarus villosus* Sowinsky. Abstracts for Biological Invasions in Inland Waters (INWAT) Workshop, Firenze, Italy, p. 60
- Pöckl M (2007) Strategies of a successful new invader in European fresh waters: fecundity and reproductive potential of the Ponto-Caspian amphipod *Dikerogammarus villosus* in the Austrian Danube, compared with the indigenous *Gammarus fossarum* and *G. roeseli*. *Freshwater Biology* 52:50–63
- Poulin R (2000) The diversity of parasites. *The Quarterly Review of Biology* 75:277–293
- Poulin R (2007) *The Evolutionary Ecology of Parasites*. Princeton University Press, Princeton
- Prenter J, MacNeil C, Dick JTA, Dunn AM (2004) Roles of parasites in animal invasion. *Trends in Ecology and Evolution* 19:385–390
- Preston D, Johnson PTJ (2010) Ecological consequences of parasitism. *Nature Education Knowledge* 1:39
- Prior KM, Powell THQ, Joseph AL, Hellmann JJ (2015) Insights from community ecology into the role of enemy release in causing invasion success: the importance of native enemy effects. *Biological Invasions* 17:1283–1297
- Pronin NM, Fleischer GW, Baldanova DR, Pronina SV (1997) Parasites of the recently established round goby (*Neogobius melanostomus*) and tubenose goby (*Proterorhinus marmoratus*) (Cottidae) from the St. Claire River and Lake St. Claire, Michigan, USA. *Folia Parasitologica* 44:1–6

- Pyšek P, Chytrý M, Pergl J, Sádlo J, Wild J (2012) Plant invasions in the Czech Republic: current state, introduction dynamics, invasive species and invaded habitats. *Preslia* 84: 575–629
- Rauque CA, Viozzi GP, Semenas LG (2003) Component population study of *Acanthocephalus tumescens* (Acanthocephala) in fishes from Lake Moreno, Argentina. *Folia Parasitologica* 50:72–78
- Reich PB, Tilman D, Isbell F, Mueller K, Hobbie SE, Flynn DFB, Eisenhauer N (2012) Impacts of Biodiversity Loss Escalate Through Time as Redundancy Fades. *Science* 336:589-592
- Rewicz T, Grabowski M, MacNeil C, Bacela-Spychalska K (2014) The profile of a 'perfect' invader—the case of killer shrimp, *Dikerogammarus villosus*. *Aquatic Invasions* 9:267–288
- Ricciardi A (2006) Patterns of invasion in the Laurentian Great Lakes in relation to changes in vector activity. *Diversity and Distribution* 12:425–433
- Ross JL, Ivanova ES, Severns PM, Wilson MJ (2010) The role of parasite release in invasion of the USA by European slugs. *Biological Invasions* 12:603–610
- Ruiz GM, Carlton JT, Grosholz ED, Hines AH (1997) Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. *Integrative and Comparative Biology* 37:621–632
- Ruiz GM, Fofonoff PW, Carlton JT, Wonham MJ, Hines AH (2000) Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. *Annual Review of Ecology and Systematics* 31:481–531
- Sapota MR (2004) The round goby (*Neogobius melanostomus*) in the Gulf of Gdańsk – a species introduction into the Baltic Sea. *Hydrobiologia* 514:219-224
- Sasal P, Taraschewski H, Valade P, Grondin H, Wielgoss S, Moravec F (2008) Parasite communities in eels of the Island of Reunion (Indian Ocean): a lesson in parasite introduction. *Parasitology Research* 102:1343-1350
- Schmidt GD, Nickol BB (1985) *Biology of the Acanthocephala*. Cambridge University Press, Cambridge, pp 273–305

- Seebens H, Essl F, Dawson W, Fuentes N, Moser D, Pergl J, Pysek P, Kleunen M, Weber E, Winter M, Blasius B (2015) Global trade will accelerate plant invasions in emerging economies under climate change. *Global Change Biology* 21:4128–4140
- Sepulveda F, Marin SL, Carvajal J (2004) Metazoan parasites in wild fish and farmed salmon from aquaculture sites in southern Chile. *Aquaculture* 235:89–100
- Shochat E, Lerman SB, Anderies JM, Warren PS, Faeth SH, Nilon CH (2010) Invasion, Competition, and Biodiversity Loss in Urban Ecosystems. *BioScience* 60:199–208
- Simberloff D, von Holle B (1999) Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions* 1: 21–32
- Simberloff D, Martin JL, Genovesi P, Maris V, Wardle DA, Aronson J, Courchamp F, Galil B, García-Berthou E, Pascal M, Pyšek P, Sousa R, Tabacchi E, Vilà M (2013) Impacts of biological invasions: what's what and the way forward. *Trends in Ecology and Evolution* 28:58–66
- Sinitsyna OO, Rotasov AAP (1994) Structural and functional characteristics of benthic communities as criteria for the setting of thermal standards for cooling ponds. *Journal of Hydrobiological Journal* 30:23-33
- Šlapanský L, Jurajda P, Janáč M (2016) Early life stages of exotic gobiids as new hosts for unionid glochidia. *Freshwater Biology* 61:979–990
- Smrzlić VI, Valić D, Kapetanović D, Filipović Marijić V, Gjurčević E, Teskeredžić E (2015) *Pomphorhynchus laevis* (Acanthocephala) from the Sava River basin: new insights into strain formation, mtDNA-like sequences and dynamics of infection. *Parasitology International* 64:243–250
- Sol D, Maspons J, Vall-Llosera M, Bartomeus I, García-Peña GE, Piñol J, Freckleton RP (2012) Unraveling the life history of successful invaders. *Science* 337:580–83
- Špakulová M, Perrot-Minnot MJ, Neuhaus B (2011) Resurrection of *Pomphorhynchus tereticollis* (Rudolphi, 1809) (Acanthocephala: Pomphorhynchidae) based on new morphological and molecular data. *Journal of Helminthology* 48:268–277

- Stachowicz JJ, Fried H, Osman RW, Whitlatch RN (2002) Biodiversity, invasive resistance, and marine ecosystem function: reconciling pattern and process. *Ecology* 83:2575–2590
- State authority for nature and environment of the Land Schleswig-Holstein (2008) *Seender Schwentine*
- Sures B (2014) Ecology of the acanthocephala. In: Schmidt-Rhaesa A (ed) *Handbook of zoology. Gastrotricha, Cycloneuralia and Gnathifera*, 3rd edn. de Gruyter, Berlin, pp 337–344
- Sures B (2002) *Charakterisierung aquatischer Wirt-Parasit-Interaktionen aus ökologischer und (öko-)toxikologischer Sicht. Habilitation treatise, University of Karlsruhe.*
- Sures, B, Knopf, K (2004) Parasites as a threat to freshwater eels? *Science* 304:208-209
- Sures B, Knopf K, Taraschewski H (1999a) Development of *Anguillicola crassus* (Dracunculoidea, Anguillicolidae) in experimentally infected Balearic congers *Ariosoma balearicum* (Anguilloidea, Congridae). *Diseases of Aquatic Organisms* 39:75-78
- Sures B, Knopf K, Würtz J, Hirt J (1999b) Richness and diversity of parasite communities in European eels *Anguilla anguilla* of the River Rhine, Germany, with special reference to helminth parasites. *Parasitology* 119:323-330
- Sures B, Nachev M, Keppel M, Grabner D, Selbach C, (2017) Parasites as drivers of key processes in ecosystems: facts and future perspectives. *Experimental Parasitology* 180:141-147
- Sures B, Siddall R (2001) Comparison between lead accumulation of *Pomphorhynchus laevis* (Palaeacanthocephala) in the intestine of chub (*Leuciscus cephalus*) and in the body cavity of goldfish (*Carassius auratus auratus*). *International Journal for Parasitology* 31:669–673
- Sures B, Streit B (2001) Eel parasite diversity and intermediate host abundance in the River Rhine, Germany. *Parasitology* 123:185–191

- Tain L, Perrot-Minnot MJ, Ce'zilly F (2006) Altered host behaviour and brain serotonergic activity caused by acanthocephalans: evidence for specificity. *Proceedings. Biological Sciences* 273:3039–3045
- Taraschewski H (2000) Host-parasite interactions in Acanthocephala: a morphological approach. *Advanced Parasitology* 46:1–179
- Taraschewski H (2006) Hosts and parasites as aliens. *Journal of Helminthology* 80:99–128
- Taraschewski H, Moravec F, Lamah T, Anders K (1987) Distribution and morphology of two helminths recently introduced into European eel populations: *Anguillicola crassus* (Nematoda, Dracunculoidea) and *Paratenuisentis ambiguus* (Acanthocephala, Tenuisentidae). *Diseases of Aquatic Organisms* 3:167–76
- Thielen F (2005) Der Einfluss einwandernder Amphipodenarten auf die Parasitose des Europäischen Aals (*Anguilla anguilla*). Dissertation, University Karlsruhe.
- Thielen F, Münderle M, Taraschewski H, Sures B (2007) Do eel parasites reflect the local crustacean community? A case study from the Rhine river system. *Journal of Helminthology* 81:179–189
- Thieltges DW, Krakau M, Andresen H, Fottner S, Reise K (2006) Macroparasite community in molluscs of a tidal basin in the Wadden Sea. *Helgoland Marine Research* 60:307–316
- Tieri ELM, Ortis M, Berti M, Battistini ML (2006) Endoparasites of chub (*Leuciscus cephalus*) in two rivers of the Abruzzo Region of Italy. *Veterinaria Italiana* 42:271–279
- Tompkins D, Dunn AM, Smith MJ, Telfer S (2011) Wildlife diseases: from individuals to ecosystems. *Journal of Animal Ecology*, 80:19–38
- Tompkins DM, Poulin R (2006) Parasites and biological invasions. *Biological Invasion in New Zealand* 186:67–84
- Torchin ME, Lafferty KD, Kuris AM (2002) Parasites and marine invasions. *Parasitology* 124:137-151

- Torchin ME, Mitchell CE (2004) Parasites, pathogens, and invasions by plants and animals. *Frontiers in Ecology and the Environment* 2:183-190
- Tscharntke T, Klein AM, Kruess A, Steffan-Dewenter I, Thies C (2005) Landscape perspectives on agricultural intensification and biodiversity – ecosystem service management. *Ecological Letters* 8:857–874
- van Beek GCW (2006) The round goby *Neogobius melanostomus* first recorded in the Netherlands. *Aquatic Invasions* 1:42–43
- van Kessel N, Dorenbosch M, Kranenborg J, van der Velde G, Leuven RSEW (2016) Invasive Ponto-Caspian gobies rapidly reduce the abundance of protected native bullhead. *Aquatic Invasions* 11:179–188
- van Riel MC, van Velde G, Bij de Vaate A (2003) *Pomphorhynchus* spec. (Acanthocephala) uses the invasive amphipod *Chelicorophium curvispinum* (G. O. Sars, 1895) as an intermediate host in the river Rhine. *Crustaceana* 76: 241–246
- van Riel MC, van Velde G, Bij de Vaate A (2011) Dispersal of invasive species by drifting. *Current Zoology* 57:818–827
- Vignon M, Sasal P (2010) Multiscale determinants of parasite abundance: A quantitative hierarchical approach for coral reef fishes. *International Journal of Parasitology* 40:443–451
- Vignon M, Sasal P, Galzin R (2009a) Host introduction and parasites: a case study on the parasite community of the peacock grouper *Cephalopholis argus* (Serranidae) in the Hawaiian Islands. *Parasitology Research* 104:775–782
- Vignon M, Sasal P, Rigby M, Galzin R (2009b) Multiple parasite introduction and host management plan: case study of lutjanid fish in the Hawaiian Archipelago. *Diseases of Aquatic Organisms* 85:133–145
- Vilà M, Basnou C, Pysek P, Josefsson M, Genovesi P, Gollasch S, Nentwig W, Olenin S, Roques A, Roy D, Hulme PE, DAISIE partners (2010) How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. *Frontiers in Ecology and the Environment* 8:135–144

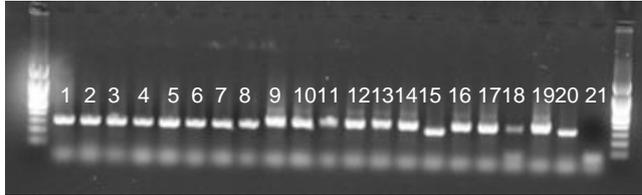
- Vitousek P, D'Antonio C, Loope L, Westbrooks R (1996) Biological invasions as global environmental change. *American Scientist* 84:468-478
- Weinstein SB, Kuris AM (2016) Independent origins of parasitism in Animalia. *Biology Letters* 12:20160324
- Wells K, O'Hara RB, Morand S, Lessard JP, Ribas A (2015) The importance of parasite geography and spillover effects for global patterns of host–parasite associations in two invasive species. *Diversity and Distributions* 21:477–486
- Westram AM, Baumgartner C, Keller I, Jokela J (2011) Are cryptic host species also cryptic to parasites? Host specificity and geographical distribution of acanthocephalan parasites infecting freshwater *Gammarus*. *Infections, Genetics and Evolution* 11:1083–1090
- White EM, Wilson JC, Clarke AR (2006) Biotic indirect effects: a neglected concept in invasion biology. *Diversity and Distributions*, 12:443–455
- Wielgoss S, Taraschewski H, Meyer A, Wirth T (2008) Population structure of the parasitic nematode *Anguillicola crassus*, an invader of declining North Atlantic eel stocks. *Molecular Biology* 17:3478-3496
- Williamson M (1996) *Biological Invasions*. Chapman & Hall, London
- Wolda H (1981) Similarity Indices, Sample Size and Diversity. *Oecologia (Berl)* 50: 296-302.
- Worm B, Barbier EB, Beaumont N, Duffy JE, Folke C, Halpern BS, Jackson JBC, Lotze HK, Micheli F, Palumbi SR, Sala E, Selkoe KA, Stachowicz JJ, Watson R (2006) Impacts of Biodiversity Loss on Ocean Ecosystem Services. *Science* 314:787-790
- Würtz J, Taraschewski H, Pelster B (1996) Changes in gas composition in the swimbladder of the European eel (*Anguilla anguilla*) infected with *Anguillicola crassus* (Nematoda). *Parasitology* 112:233–238
- Young HS, Parker IM, Gilbert GS, Guerra AS, Nunn CL (2017) Introduced Species, Disease Ecology, and Biodiversity–Disease Relationships. *Trends in Ecology and Evolution* 32:41–54

Zalba SM, Cuevas YA, Boo RM (2008) Invasion of *Pinus halepensis* Mill. following a wildfire in an Argentine grassland nature reserve. *Journal of Environmental Management* 88:539–546

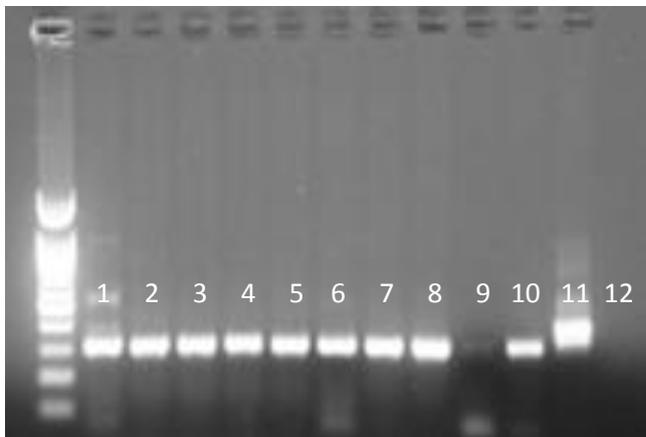
Appendix 1

Agarose gel electrophoresis of the PCR product of ITS 1/2 genes for DNA extracted from analyzed *Pomphorhynchus* individuals (*P. laevis*, *P. tereticollis*), extracted from European eels sampled in the river Rhine in Germany (KA-R), Switzerland (CH-R), and the Netherlands (NL-R) between 2003 and 2005

Visual illustration of PCR-products	Amplified gene sample /sample location, year , ID	Identified as
	1 Germany (2003) KA-R-03 A	<i>P. laevis</i>
	2 Germany (2003) KA-R-03 B	<i>P. laevis</i>
	3 Germany (2003) KA-R-03 C	<i>P. laevis</i>
	4 Germany (2003) KA-R-03 D	<i>P. laevis</i>
	5 Germany (2003) KA-R-03 E	<i>P. laevis</i>
	6 Germany (2003) KA-R-03 F	<i>P. laevis</i>
	7 Positive control <i>P. tereticollis</i>	
	8 Positive control <i>P. laevis</i>	
	9 Blank	
	1 Switzerland (2003) CH-R-03 A	<i>P. tereticollis</i>
	2 Switzerland (2003) CH-R-03 B	<i>P. tereticollis</i>
	3 Switzerland (2003) CH-R-03 C	<i>P. tereticollis</i>
	4 Switzerland (2003) CH-R-03 D	<i>P. tereticollis</i>
	5 Germany (2003) KA-R-03 G	<i>P. laevis</i>
	6 Germany (2003) KA-R-03 H	<i>P. laevis</i>
	7 Germany (2003) KA-R-03 I	<i>P. laevis</i>
	8 Switzerland (2003) CH-R-03 E	<i>P. tereticollis</i>
	9 Positive control <i>P. tereticollis</i>	
	10 Positive control <i>P. laevis</i>	
	11 Blank	
	1 Germany (2003) KA-R-03 H	<i>P. laevis</i>
	2 Germany (2003) KA-R-03 I	<i>P. laevis</i>



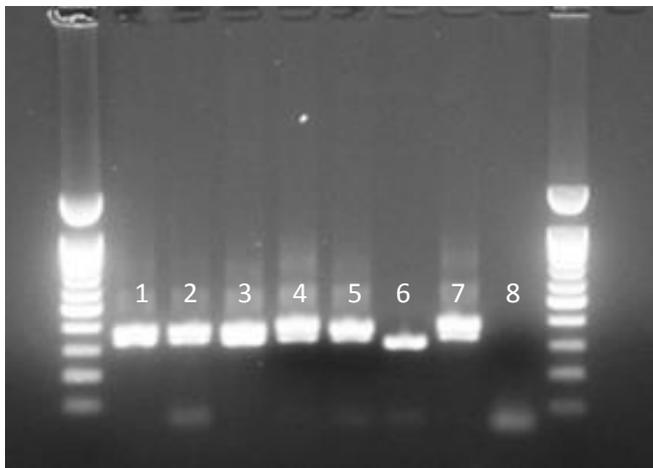
3	Germany (2003) KA-R-03 J	<i>P. laevis</i>
4	Germany (2003) KA-R-03 K	<i>P. laevis</i>
5	Germany (2003) KA-R-03 L	<i>P. laevis</i>
6	Germany (2003) KA-R-03 M	<i>P. laevis</i>
7	Germany (2003) KA-R-03 N	<i>P. laevis</i>
8	Germany (2003) KA-R-03 O	<i>P. laevis</i>
9	Switzerland (2004) CH-R-04 A	<i>P. tereticollis</i>
10	Switzerland (2004) CH-R-04 B	<i>P. tereticollis</i>
11	Switzerland (2004) CH-R-04 C	<i>P. tereticollis</i>
12	Switzerland (2004) CH-R-04 D	<i>P. tereticollis</i>
13	Switzerland (2004) CH-R-04 E	<i>P. tereticollis</i>
14	Switzerland (2004) CH-R-04 F	<i>P. tereticollis</i>
15	Switzerland (2004) CH-R-04 G	<i>P. laevis</i>
16	Switzerland (2004) CH-R-04 H	<i>P. tereticollis</i>
17	Switzerland (2004) CH-R-04 I	<i>P. tereticollis</i>
18	Switzerland (2004) CH-R-04 J	<i>P. tereticollis</i>
19	Positive control <i>P. tereticollis</i>	
20	Positive control <i>P. laevis</i>	
21	Blank	



1	Germany (2004) KA-R-04 A	<i>P. laevis</i>
2	Germany (2004) KA-R-04 B	<i>P. laevis</i>
3	Germany (2003) KA-R-03 P	<i>P. laevis</i>
4	Germany (2003) KA-R-03 Q	<i>P. laevis</i>
5	Germany (2004) KA-R-04 C	<i>P. laevis</i>
6	Germany (2004) KA-R-04 D	<i>P. laevis</i>
7	Germany (2004) KA-R-04 E	<i>P. laevis</i>
8	Germany (2004) KA-R-04 F	<i>P. laevis</i>
9	Germany (2004) KA-R-04 G	<i>P. laevis</i>
10	Positive control <i>P. tereticollis</i>	
11	Positive control <i>P. laevis</i>	
12	Blank	

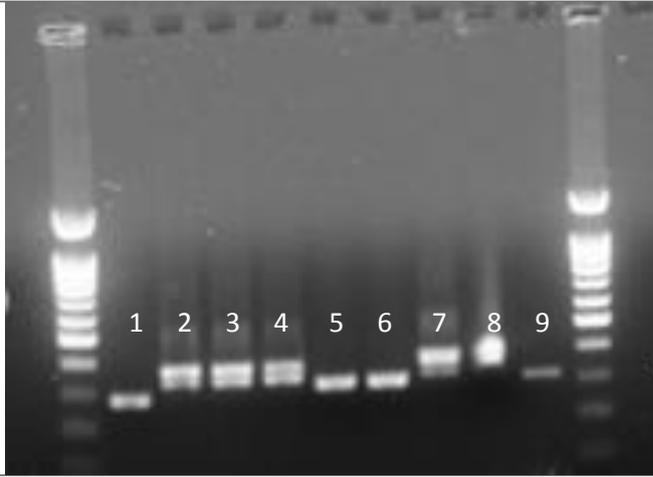


1	Switzerland (2004) CH-R-04 K	<i>P. tereticollis</i>
2	Switzerland (2004) CH-R-04 L	<i>P. tereticollis</i>
3	Switzerland (2004) CH-R-04 M	<i>P. tereticollis</i>
4	Switzerland (2004) CH-R-04 N	<i>P. tereticollis</i>
5	Switzerland (2004) CH-R-04 O	<i>P. tereticollis</i>
6	Germany (2004) KA-R-04 H	<i>P. laevis</i>
7	Germany (2003) KA-R-03 R	<i>P. tereticollis</i>
8	Germany (2003) KA-R-03 S	<i>P. laevis</i>
9	Germany (2003) KA-R-03 T	<i>P. tereticollis</i>
10	Germany (2003) KA-R-03 U	<i>P. laevis</i>
11	Germany (2003) KA-R-03 V	<i>P. tereticollis</i>
12	Blank	
13	Blank	
14	Positive control <i>P. tereticollis</i>	
15	Positive control <i>P. laevis</i>	

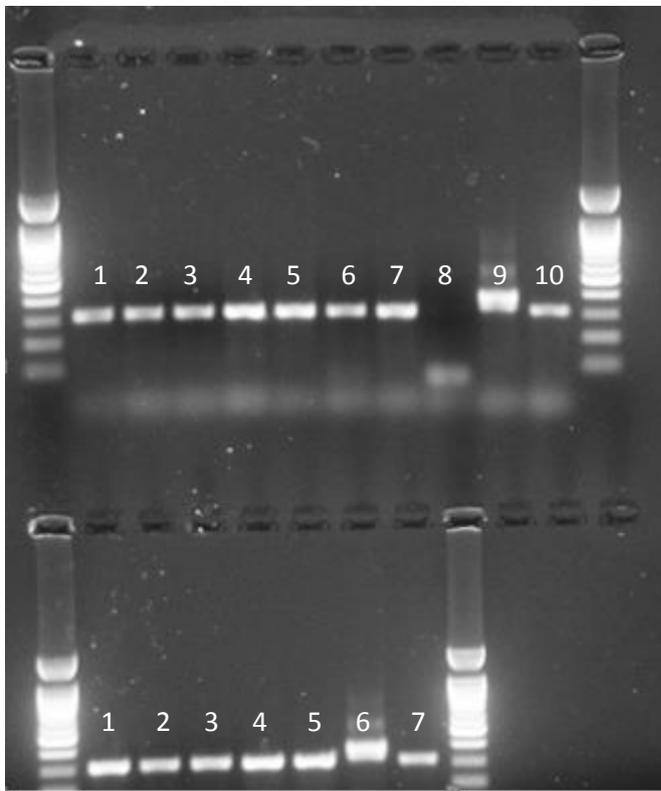


1	Switzerland (2004) CH-R-04 P	<i>P. tereticollis</i>
2	Switzerland (2004) CH-R-04 Q	<i>P. tereticollis</i>
3	Switzerland (2004) CH-R-04 R	<i>P. tereticollis</i>
4	Switzerland (2004) CH-R-04 S	<i>P. tereticollis</i>
5	Switzerland (2003) CH-R-03 F	<i>P. tereticollis</i>
6	Positive control <i>P. laevis</i>	
7	Positive control <i>P. tereticollis</i>	
8	Blank	

1	Switzerland (2004) CH-R-04 T	<i>P. laevis</i>
2	Switzerland (2004) CH-R-04 U	<i>P. tereticollis</i>
3	Switzerland (2004) CH-R-04 V	<i>P. tereticollis</i>
4	Switzerland (2004) CH-R-04 W	<i>P. tereticollis</i>
5	The Netherlands (2003) NL-R-03 A	<i>P. laevis</i>
6	The Netherlands (2003) NL-R-03 B	<i>P. laevis</i>
7	Positive control <i>P. tereticollis</i>	



-
- 8 Positive control *P. laevis*
-
- 9 Blank
-



-
- 1 Germany (2005) KA-R-05 A *P. laevis*
-
- 2 Germany (2005) KA-R-05 B *P. laevis*
-
- 3 Germany (2005) KA-R-05 C *P. laevis*
-
- 4 Germany (2005) KA-R-05 D *P. laevis*
-
- 5 Germany (2005) KA-R-05 E *P. laevis*
-
- 6 The Netherlands (2003) NL-R-03 C *P. laevis*
-
- 7 The Netherlands (2003) NL-R-03 D *P. laevis*
-
- 8 Blank
-
- 9 Positive control *P. tereticollis*
-
- 10 Positive control *P. laevis*
-
- 11 Germany (2003) KA-R-03 W *P. laevis*
-
- 12 Switzerland (2005) CH-R-05 A *P. laevis*
-
- 13 Switzerland (2005) CH-R-05 B *P. laevis*
-
- 14 Switzerland (2005) CH-R-05 C *P. laevis*
-
- 15 Switzerland (2005) CH-R-05 D *P. laevis*
-
- 16 Switzerland (2005) CH-R-05 E *P. laevis*
-
- 17 Positive control *P. tereticollis*
-
- 18 Positive control *P. laevis*
-

Appendix 2

Detailed sequencing information of ITS 1 /2 and CO1 genes of *Pomphorhynchus laevis* individuals sampled in the rivers Rhine (Germany, the Netherlands, Switzerland) and Danube (Bulgaria) that were used for the sequence-comparison as presented in chapter II (cf. table 5.4)

Seq. ID	Habitat / sampling year	Chromatogram / sequence information				r(DNA) gene sequences	
38FG21	Rhine in the Netherlands, 2003	81	AAAGATCAGG	GTTACAAGAT	AGAGTGGAGA	TCGGGTAGCA	ITS
		121	TGATCTACTA	TATATATTCA	CTGATTACAA	GTGCTCGCCT	
		161	GCATCAGGCG	TTTATCGTTA	CCGAAGTTGT	TTAATAAATA	
		201	GTCTGCTCTT	GCACTGTTAA	TATATTCATA	TTAATCGACC	
		241	TCTGGTAGAT	AAGCTATAAA	TTAGACCTCA	CAGCTGTCGT	
		281	TTGCCCTCAA	AAACTATCTG	TTCCGTGTGT	CCGGCAGTTC	
		321	ACAGAAATTA	TCACATATGG	CGACGTTCTT	GCTCAAATTG	
		361	CGATTCAACT	GATTTCGATGT	CTGCCGAGGC	TCATTTCTTG	
		401	TTTTATCTGC	CATCGGCAAA	TAAATAATAA	TTCTTGCCTA	
		441	GCTCTAGAAC	AGAATATTAT	TGACACAAAG	TCAGTTTCAA	
		481	TAAACACCTG	TTGACAGTCA	GCGCATGTCA	TATTCGCCTG	
38FG24		Sequencing was not successful!					
38FG25	Rhine in Switzerland, 2004	81	GGGCTTGCTC	TACACTATAT	ACTACGCTCC	TCCCTGCCAC	CO1
		121	CCAAGTAAAC	AAAAATATTAA	TTGAACCTAA	AATCGATGAC	
		161	AACCCCACTA	CATGCAACCC	CAAAAATTATT	AAGTCAACAG	
		201	CTACCCCAAG	CCTGTAATCC	CTCAACATGA	GGGGTGGGTA	
		241	CATTGTTCAA	CCGGCCCCAC	CTCCACCCAA	CAGTAATGAT	
		281	ACCCCAATAA	TCCCTAACCT	AGCAAGTAGT	AAAAATCAACC	
		321	TCAAATTATT	TAAACGGGGT	AGGGCCATGT	CCCTCAACCC	
		361	TAATATAACA	GGTATTAGTC	AATTACCAAA	CCCCCCCATA	
		401	AAAACTGGTA	TAACATAAAA	GAATACTATC	ATAACTGCAT	
		441	GCCTAGTCAC	CAATACGTTA	TAGATGGCCT	CTCTACCCAT	
		481	CCAAACTCCC	CCTCTCCCTA	ACTCTAAGCC	AATTAATATA	
38FG27	Rhine in Germany, 2003	81	CGCCTGTTCT	ACTCTATACA	CTACCCCTTCT	TCCAGCTACC	CO1
		121	CAGGTAATCA	GGATATTGAT	TGAGCCTAGA	ATAGAAGAAA	
		161	GACCTACTAC	ATGCAACCTC	AGGATCATT	GATCAACAGC	
		201	TACACAGATC	CTATAGTCCC	CTAACATCAG	TGGGGGATAT	
		241	ATTGTCCAAC	CAGCACCCACC	CCCTCCTAAA	AGCAAAGATA	
		281	CTCCTATAAT	ACCCAGCCTA	GCGAGAAGCA	GGATAAGCCT	
		321	TAAATTGTTT	AATCGGGGAA	GGGCCATATC	CCTTAACCCC	
		361	AACATCACTG	GGATGAGCCA	ATTACCGAAT	CCCCCAATAA	
		401	ATACCGGTAT	TACTAGAAAA	AAGACCATTA	TAACAGCGTG	
		441	TCTAGTTACT	AACACATTAT	ACACAGCCTC	CCTCCCTAAC	
		481	AAACCCCTCC	TCTCCCNCT	CTAATCCTAT	TTANACNATA	
38FG31	Danube in Bulgaria, 2006	81	CCTGTTCTAC	TCTATACACT	ACCCTTCTTG	GAGCTACCCA	CO1
		121	GGTAAATCAGG	ATATTGATTG	AGCCTAGAAT	AGAAGAAAAG	
		161	CCTACTACAT	GCAACCTCAG	GATCATTAGA	TCAACAGCTA	
		201	CTCCGGACCT	ATAGTCCOCT	AACATCAGTG	GGGGATATAT	
		241	TGTCCAACCA	GCACCACCCC	CGCCTAAAAG	CAAAGATACT	
		281	CCTATAATAC	CCAGCCTAGC	GAGAAGCAGG	ATAAGCCTTA	
		321	AATTGTTCAA	TCGGGGAAGG	GCCATATCCC	TTAACCCCAA	
		361	CATCACTGGG	ATGAGCCAAT	TACCGAATCC	CCCCAGAAAT	
		401	ACCGGTATTA	CTAGAAAAAA	GACCATTATA	ACAGGCGTGT	
		441	CTAGTTACTA	ACACATTATA	CACAGTCTCC	CTCCCTATTC	
		481	AAACCCCTCC	TCTCCGCAAA	TTCTGGTCGA	ATTAATCGCC	

38FG32	Rhine in Switzerland, 2004					ITS
		81	TATCGTCCGG	GTGTCATGAT	CTAATGGGGC	TCATCTAGTC
		121	TGTTCTACTA	TAAATAGTCA	CTGATTACTA	TTGCTCGCCT
		161	GCATTAGGCA	CTTATCGTTA	GCGGCGTTAT	TTAATATGTA
		201	TACTGCACTA	ACACTGTAA	TATATTCATA	TTAATCGACC
		241	TCAAACGGAT	GTAGCTATAA	ATTTGACCTC	GCAGCTGTCC
		281	TTTGC GTTCA	AAAAC TATCT	GTTCCG GTG	TCCTGCAGTT
		321	CACAGAA TTT	ATCACATATG	GCAACG TTTCT	TCCTCGAATG
		361	GCTAGCCAAG	TGATACGCTG	TACGCCGACG	TTCATTTCTT
		401	GTTTCATCTA	CCATCGGCAA	GTA AATGAGA	ATTCATGCCT
		441	AGCTCAAGAG	CATTATACTA	TTGCCACAAA	TTCGGTTTCA
		481	GTAAGCAACT	GTTGACAGCC	GAGGCTACTC	ATATTCGCCT
38FG33	Danube in Bulgaria, 2006					ITS
		81	CGTCCGTGTT	TCATGATATA	GTGGAGTTCG	GGTAGCATGT
		121	TCTACTATAT	ATAGGCACTG	ATTACAATTG	CTCGCCTGCA
		161	TTATGCACTT	ATCGTTACCG	GCGTTATTTA	ATATGTATTC
		201	AGCACTAACA	CTGTTAATAT	ATTCATATTA	ATCGACCTCA
		241	AACGGATGTA	GCTATGAGTT	TGACCTCACA	GCTGTCA TTT
		281	GCGTTC AAAAA	ACTATCTGTT	CCGTGTGTCC	TGCAGTTCAC
		321	AGAAATTATC	ACATATGGCA	ACGTTCTTCC	TCGATTTGCG
		361	AGCCAAGTGA	TTCGCTGTAC	GCCGACG TTC	ATTTCTTGTT
		401	TTATCTACCA	TCAGCCAGTA	AATGATAGTT	CTTGCCTAGC
		441	TCAAGAACAT	TATATTATTG	CCACAAATTC	GGTTTCAATA
		481	AACGACAGTT	TACAGTCAAC	GAATGTCATA	TTCGCATGAC
38FG34	Rhine in Germany, 2003					CO1
		81	CCTGTTCTGC	TCTATACACT	ACCCTTCTTC	CAGCTACCCA
		121	GGTAATCAGG	ATATTGATTG	AGCCTAGAAT	AGAAGAAAAG
		161	CCTACTACAT	GCAACCTCAG	GATCATTAGA	TCAACAGCTA
		201	CACCAGACCT	ATAGTCCCCT	AACATCAGTG	GGGGATATAT
		241	TGTCCAACCA	GCACCACCCC	CTCCTAAAAAG	CAAAAGATACT
		281	CCTATAA TAC	CCAGCCTAGC	GAGAAGCAGG	ATAAGCCTTA
		321	AATTGTTCAA	TCGGGGAAAG	GCCATATCCC	TTAACCCCAA
		361	CATCACTGGG	ATGAGCCAAT	TACCGAATCC	CCCC TAAAT
		401	ACCGGGATTA	CTAGAAAAAA	GACCATTATA	ACAGCGTGTG
		441	TAGTTACTAA	CACATTATAC	ACAGACTCCC	TCCCTATCCA
		481	AAACCCCCCT	CTCCCCAAGT	CTAAGCCAAT	TATTA AACTT
38FG35	Rhine in the Netherlands, 2003					ITS
		81	AATCGGGGTT	TAATGATATA	GTGCAGAACG	TGTAGTTTGT
		121	TTTATTATTG	TTGGCACAGA	TGACTTGTGC	TCGCCTGCTT
		161	CAGGCGCTTA	TAGGTACCGG	CGTTTTTTAA	TATATATAAA
		201	GCACTAGAGC	TAGTAATATA	CTCATATTTA	TCGACCTCAA
		241	ACGGATGTAA	CTATGAATTT	GACCTCGCGT	CCGTCA TTTG
		281	CGTTC AAAAA	CTATCTGAGC	AGGAA GTCCG	GCGGTTCTCA
		321	GAAATTATCA	CAATGGCGGC	GTTCTTCCTC	AA TTTGCTTG
		361	CCAACGATAT	GCCGGATGCC	GACGCTCACT	TCTAGTTGCA
		401	TCTGCCATCG	ACAAA TAAAT	GAGAATTCTT	GCCTGCTTTC
		441	GAA CAGTCTA	CTATTGCAAC	AAATTCGGTT	TCGGTAAAACG
		481	CCAGATGACC	GTGGGGAGAA	GTCATATTCA	CATGACAATA
38FG36	Rhine in Germany, 2003					ITS
		81	CGTCCGGGTT	TCATGATATA	GTGGAGTTCG	GCTAGCATGT
		121	TCTACTATAT	ATAGTCACTG	ATTACAATTG	CTCGCCGGCA
		161	TTAGGCACTT	ATCGTTAGCG	GCGTTATTTA	ATATGTAGTC
		201	AGCACTAACA	CTGTTAATAT	ATTCATATTA	ATCGACCTCA
		241	AACGGATGTA	GCTATGAGTT	TGACCTCACA	GCTGTCA TTT
		281	GCGTTC AAAAA	ACTATCTGTT	CCGTGTGTCC	TGCAGTTCAC
		321	AGAAATTATC	ACATATGGCA	ACGTTCTTCC	TCGATTTGCG
		361	AGCCAAGTGA	TTCGATGTAC	ACCGACG TTC	ATTTCTTGTT
		401	TTATCTACCA	TCAGCAAGTA	AATGATAGTT	CTTGCCTAGC
		441	TCAAGAACAT	TATATTATTG	CCACAAATTC	GGTTTCAATA
		481	AACAACAGTT	TACAATCGAG	GCAAGTCATA	TTCGCATGAC

38FG37	Rhine in the Netherlands, 2003	81	GTTCTACTCT	ATACACTACC	CTTCTTCGAG	CTACCCAGGT	CO1	
		121	AATCAGGATA	TTGATTGAGC	CTAGAATAGA	AGAAAAGACCT		
		161	ACTACATGCA	ACCTCAGGAT	CATTAGATCA	ACAGCTACAC		
		201	CAGACCTATA	GTCCCCTAAC	ATCAGTGGGG	GGTATATTGT		
		241	CCAACCAGCA	CCACCCCCTC	CTAAAAGCAA	AGATACTCCT		
		281	ATAATACCCA	GCCTAGCGAG	AAGCAGGATA	AGCCTTAAAT		
		321	TGTTCAATCG	GGGAAGGGCC	ATATCCCTTA	ACCCCAACAT		
		361	CACTGGCATG	AGCCAATTAC	CGAATCCCCC	CATAAATACC		
		401	GGTATTACTA	GAAAAAAGAC	CATTATAACA	GCCTGTCTAG		
		441	TTACTAACAC	ATTATACACA	GCCTCCCTCC	CTATCAAACC		
		481	CCCCTCTCCC	CACTCTAATC	GAATTAATAG	CCTTATAGAA		
38FG38	Danube in Bulgaria, 2006	81	TGTTCTACTC	TATACACTAC	CCTTCTTCCG	GCTACCCAGG	CO1	
		121	TAATCAGGAT	GTTGATTGAA	CCTAGAATAG	AGGAAAAGACC		
		161	TACTACATGC	AACCTCAAGA	TCATTAGATC	AACAGCTACC		
		201	CCGGACCTAT	AGTCCCCTAA	CATCAGGGGG	GGATATATTG		
		241	TCCAACCAGC	ACCACCCCCT	CCTAAAAGCA	AAGATACTCC		
		281	TATAATACCC	AGCCTAGCGA	GAAGTAGGAT	AAGCCTTAAA		
		321	TTGTTCAATC	GGGGAAGAGC	CATATCCCTT	AACCTTAACA		
		361	TCACTGGGAT	GAGACAATTA	CCGAATCCTC	CCATAAATAC		
		401	CGGTATTACT	AGAAAAAATA	CCATTATAAC	AGCGTGTCTT		
		441	GTTACTAAAA	CATTATATAC	AGGCTCTCTT	CCTATCCAAA		
		481	ACCCTCCTCT	CCCTATTTCT	GATCGAATTA	ATAGCCTTTA		
38FG39	Rhine in Switzerland, 2004	81	CTGTTCTACT	CTATACTCTA	CCCTTCTTCC	AGCTACCCAG	CO1	
		121	GTAATCAGGA	TATTGATTGA	GCCTAGAATA	GAAGAAAAGAC		
		161	CTACTACATG	CAACCTCAGG	ATCATTAGAT	CAACAGCTAC		
		201	ACCAGACCTA	TAGTCCCCTA	ACATCAGTGG	GGGATATATT		
		241	GTCCAACCAG	AACCACCCCC	TCCTAAAAGC	AAAAGATACTC		
		281	CTATAATACC	CAGCCTAGCG	AGAAGCAGGA	TAAGCCTTAA		
		321	ATTGTTCAAT	CNGGNAAGGG	CCATATCCCT	TAACCCCAAC		
		361	ATCACTGGGA	TGAGCCAATT	ACCGAATCCC	CCCCGAAAATA		
		401	COGGTATTAC	TAGAAAAAAG	ACCATTATAA	CAGCGTGTCT		
		441	AGTTACTAAC	ACATTATACA	CAGCCTCCCT	CCCTATANAA		
		481	TCCCTCTCTC	CCCCNCNTT	GCTAATTTTA	TCCTTATAAA		
38FG40	Danube in Bulgaria, 2006	81	TCGTCCGGGT	TTCATGATAT	AGTGGAGTTC	GGCTAGCATG	ITS	
		121	TTCTACTATA	TATAGTCACT	GATTAGAATT	GCTCGCCTGC		
		161	ATTAGGCACT	TATCGTTAGC	GGCGTTGTTT	AATATGTATT		
		201	CATCACTAAC	ACTGTTAATA	TATTCATATT	AATCGACCTC		
		241	AAACGGATGT	AGCTATGAGT	TTGACCTCAC	AGCTGTCATT		
		281	TGCGTCAAAA	AACTATCTGT	TCCGTGTGTC	CTGCAGTTCA		
		321	CAGAAATTTAT	CACATATGGC	AACGTTCTTC	CTCGATTTGC		
		361	GAGCCAAGTG	ATTCACTGTA	CACCGACGTT	CATTTCTTGT		
		401	TTTATCTACC	ATCAACAAGT	AAATGATAAT	TCTTGCCTAG		
		441	CTCAAGAACA	TTATATTATT	GCCACAAAAT	CGTTTTCAAT		
		481	AAACAACAGT	TGACAATCGA	GGCAAGTCAT	ATTGCGATGA		
38FG41			Sequencing was not successful!					
38FG42	Rhine in Switzerland, 2004	81	CGTCAGTTTT	TCTTGATATA	GTGCAGGTTT	TGCAGGCATA	ITS	
		121	TTCTACATAT	ATAGTCACTG	ATTAGAATGG	CTAGCCTGCA		
		161	TTTTGCACTT	ATCGATGACA	GCGTGGTTTA	GTATGCAATC		
		201	ATCATTAAACA	CTGTTAATAT	ATTCATATAT	TTATCGACCT		
		241	CAAACGGATG	TAGCTATGAG	TTTGACCTCA	CAGCTGTCAT		
		281	TTGCGTTCAA	AAACTATCTG	TTCCGTGTGT	CCTGCAGTTC		
		321	ACAGAATTTA	TCACATATGG	CAACGTTCTT	CCTCGAATTTG		
		361	CGAGCCAAGT	GATTCACTGT	ACACCGACGT	TCATTTCTTG		
		401	TTTTAGCTAC	CATCAACAAG	TAAATGACAT	CTCTTGCCTA		
		441	GCTCAAGAAC	ATTCAGATAT	TATTGCCACA	AACCAGTATA		
		481	TTGCCACAAA	ACCACCTTCT	GTCCGTAAAT	NCANTTGCNN		

38FG43	Rhine in Germany, 2003					CO1	
		81	GTTCTACTCT	ATACACTACC	CTTCTTCCAG	CTACCCAGGT	
		121	AATCAGGATA	TTGATTGAGC	CTAGAATAGA	AGAAAAGACCT	
		161	ACTACATGCA	ACCTCAGGAT	CATTAGATCA	ACAGCTACAC	
		201	CAGACCTATA	GTCCCCTAAC	ATCAGTGGGG	GATATATTGT	
		241	CCAACCAGCA	CCACCCCTC	CTAAAAGCAA	AGATACTCCT	
		281	ATAATACCCA	GCCTAGCGAG	AAGCAGGATA	AGCCTTAAAT	
		321	TGTTCAATCG	GGGAAGGGCC	ATATCCCTTA	ACCCCAACAT	
		361	CACTGGGATG	AGCCAATTAC	CGAATCCCCC	CATAAATACC	
		401	GGTATTACTA	GAaaaaaAGAC	CATTATAACA	GCGTGTCTAG	
		441	TACTAACAC	ATTATACACA	GCCTCCCTCC	CTATCAAACC	
		481	CCCCTCTCNC	CACTCTCATC	TAATTTATCC	NTTATAAAAA	
38FG44			Sequencing was not successful!				
38FG47	Rhine in Germany, 2003					ITS	
		81	GTATCGTCCG	GGTTACAAGA	TATAGTGGAG	TTCAGGTAGC	
		121	ATGTTCTACT	ATTTATAGTC	ACTGATTACA	ATTGCTCGCC	
		161	TGCATTATGC	ACTTATCGTT	AGCGGCGTTA	TTTAATATGT	
		201	ATTCAGCACT	AACACTGTTA	ATATATTCAT	ATTAATCGAC	
		241	CTCTAACGGA	TGTAGCTATG	AGTTTGACCT	CACAGCTGTC	
		281	ATTTGCGTTC	AAAAACTATC	TGTTCCGTGT	GTCCTGCATT	
		321	TCACAGAAAT	TATCACATAT	GGCAACGTTT	TTCCTCAAAT	
		361	TGCGAGCCAA	GTGATTCGCT	GTACACCGAC	GTTCAATTCT	
		401	TGTTTCATCT	ACCATCAACC	AGTAAATGAT	AGTTCTTGCC	
		441	TAGCTCAAGA	ACATTATATT	ATTGCCACAA	ATTCGGTTTC	
		481	AATAAACAAAC	AGATGACAAT	CAAGGCATGT	CATATTCGCA	

Contributions

MAA Hohenadler, M Nachev, F Thielen, H Taraschewski, D Grabner and B Sures (2018) *Pomphorhynchus laevis*: An invasive species in the river Rhine? *Biological Invasions* 20:207–217.

Personal contributions: Together with FT and MN, I obtained the samples for this study. I undertook the morphological species identifications, together with DG we did the molecular analysis and I drafted the manuscript. BS conceived and together with MN helped to coordinate the study. MN, DG, BS, HT, and FT discussed the results and helped draft the manuscript. All authors reviewed the final manuscript before submitting.

MAA Hohenadler, M Nachev, M Freese, JD Pohlmann, R Hanel, B Sures (2018) How Ponto-Caspian invaders affect local parasite communities of native fish. Submitted to *International Journal for Parasitology: Parasites and Wildlife*

Personal contributions: MN, MF, JDP, and I obtained the samples for this study. I conducted the parasitological examination and together with MN we undertook the morphological species identification. I performed the statistical analysis of the results. BS conceived the study. I prepared the first draft of the manuscript. MN, MF, JDP, RH and BS discussed the results and helped draft the manuscript. All authors reviewed the final manuscript before submitting.

MAA Hohenadler, KI Honka, S Emde, S Klimpel, B Sures (2018) First evidence for a possible invasional meltdown among invasive fish parasites. Accepted in *Scientific Reports* (SREP-18-12009A)

Personal contributions: BS and SK conceived the study and supervised the project. KIH and I conducted the experiments and wrote the manuscript (shared first authorship). SE collected infested gobies. BS, SK and SE oversaw the writing and reviewed the manuscript.

Der **Lebenslauf** ist in der Online-Version aus Gründen des Datenschutzes
nicht enthalten

Erklärung:

Hiermit erkläre ich, gem. § 7 Abs. (2) d) + f) der Promotionsordnung der Fakultät für Biologie zur Erlangung des Dr. rer. nat., dass ich die vorliegende Dissertation selbständig verfasst und mich keiner anderen als der angegebenen Hilfsmittel bedient, bei der Abfassung der Dissertation nur die angegebenen Hilfsmittel benutzt und alle wörtlich oder inhaltlich übernommenen Stellen als solche gekennzeichnet habe.

Essen, den _____

Unterschrift des/r Doktoranden/in

Erklärung:

Hiermit erkläre ich, gem. § 7 Abs. (2) e) + g) der Promotionsordnung der Fakultät für Biologie zur Erlangung des Dr. rer. nat., dass ich keine anderen Promotionen bzw. Promotionsversuche in der Vergangenheit durchgeführt habe und dass diese Arbeit von keiner anderen Fakultät/Fachbereich abgelehnt worden ist.

Essen, den _____

Unterschrift des/r Doktoranden/in

Erklärung:

Hiermit erkläre ich, gem. § 6 Abs. (2) g) der Promotionsordnung der Fakultät für Biologie zur Erlangung der Dr. rer. nat., dass ich das Arbeitsgebiet, dem das Thema „The impact of Ponto Caspian invaders on the parasite communities of multiple hosts within West and Central European river systems“ zuzuordnen ist, in Forschung und Lehre vertrete und den Antrag von Herrn Michael Andreas Alfred Hohenadler befürworte und die Betreuung auch im Falle eines Weggangs, wenn nicht wichtige Gründe dem entgegenstehen, weiterführen werde.

Essen, den _____
Unterschrift eines Mitglieds der Universität Duisburg-Essen