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Role of abiotic factors and biotic interactions in biological invasions: a comparison of natural and human-induced invasions in freshwater ecosystems

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SUMMARY

Invasive non-native species are key components of human-induced global environmental change and lead to a loss of biodiversity, alterations of species interactions and changes of ecosystem services. Freshwater ecosystems in particular are strongly affected by biological invasions, since they are spatially restricted environments and often already heavily impacted by anthropogenic activities. Recent human-induced species invasions are often characterized by long-distance dispersal, with many species having extended their native distribution range within a very short time frame. However, a long term view into the past shows that biological invasions are common phenomena in nature — representing the arrival of a species into a location in which it did not originally evolve — as a result of climatic changes, geotectonic activity or other natural events. Once a species arrives in a new habitat, it may experience an array of novel selection pressures resulting from abiotic and biotic environmental factors and simultaneously act as a novel selective agent on the native fauna. Consequences of species invasions are manifold. My thesis, which combines seven studies on different aspects of biological invasions, aims to explore the influence of abiotic stressors and biotic interactions during species introductions and range expansions, as well as the consequences of biological invasions on evolutionary and ecosystem processes.

The first part of my thesis examines human-induced biological invasions. It starts with the characterization of a thermally altered stream ecosystem in Germany and provides basic ecological characteristics of the community structure that consist of a unique mixture of native and invasive species (in this case teleost fishes). Next, I investigated the impact of introduced fish predators on native prey organisms with complex life cycles, in this case biting mosquitoes (*Culex pipiens*). The results highlight the importance of a shared (co-evolutionary) history of predator and prey species for the ability of prey to detect and respond to a (novel) predator type. Furthermore, it illustrates the strength of selection on both native and introduced species to adapt quickly to novel predator-prey interactions after biological invasions. While a growing body of literature focuses on the adverse effects of biological invasions, studies on the effects of invasions on components of ecosystem functioning are comparatively rare. Therefore, I also investigated functional consequences of species invasions (amphipods in the Rhine drainage system) on basic ecosystems processes. The results indicated that the replacement of

native species likely affects vital ecosystem services. Certain behavioral traits (like consistent individual differences in activity) may contribute to the invasiveness of some species. To investigate this possibility, I compared behavioral variation between native and invasive amphipod species and found that traits like activity are not necessarily higher in invasive compared to native species. However, the results indicate highly variable and unpredictable patterns of individual activity variation in the invasive species – a pattern that might indeed be advantageous when copying with new environmental conditions.

The second part of my thesis examined distribution patterns and phenotypic trait divergence in species that historically invaded new geographical areas. I investigated variation of abiotic and biotic selection factors along a stream gradient to study species' distributions and gradient evolution of morphological and life-history traits within species while focusing on two species of neotropical mosquitofishes (*Gambusia* spp.). This study exemplifies that even closely related, congeneric species can respond in unique ways to the same components of a river gradient. In another study, I investigated ecological and evolutionary consequences of species invasions to extreme habitats focusing on the colonization of two cave ecosystems by a neotropical fish. I found evidence for improved olfaction in one of two populations of cave fishes, suggesting independent evolutionary trajectories of both cave populations. Finally, I give first insights into a study that considers genetic and phenotypic trait divergence across both, the native (US American) and the invasive (European) distribution range of Eastern mosquitofish (*Gambusia holbrooki*). The results highlight the importance of simultaneously considering processes involved in natural invasions and during human-induced invasions to understand the success of invading species.

We often lack detailed information on the impacts of historical biological invasions. Also, we are currently lacking crucial knowledge about the time scales during which different mechanisms (behavioral flexibility, plastic phenotypic changes, and genetic adaptation) play a role during biological invasions and affect species exchange and establishment. Comparative analyses of historical, natural invasion and recent (man-made) invasions can provide insights into the relative importance of the processes governing adaptation to abiotic stressors and selection resulting from biotic interactions. Beyond their negative effects, the establishment of invasive species and the subsequent range expansion represent "natural experiments" to investigate fundamental questions in ecology and evolution. My comparison of natural and human-induced biological invasions revealed that in many cases preadaptation to altered abiotic conditions plays a key role during early stages of invasions and range expansions. Considering the evolutionary history of invasive species and the evolutionary history of the recipient native fauna might therefore help predict the consequences of biological invasions for the ecosystem and the future success of the invading species. This knowledge can also be implemented when formulating conservation strategies, including methods to mitigate and manage human-induced biological invasions.

ZUSAMMENFASSUNG

Invasive, nicht einheimische Arten sind ein wesentlicher Bestandteil des anthropogenen globalen Wandels und spielen eine bedeutende Rolle beim Verlust der Artenvielfalt, bei der Neuordnung von biotischen Interaktionen und der Veränderung von ökosystemaren Dienstleistungen. Vor allem limnische Ökosysteme sind von invasiven Arten bedroht, da sie räumlich begrenzte Lebensräume darstellen und viele Gewässer zudem bereits stark vom Menschen verändert worden sind. Gegenwärtige biologische Invasionen zeichnen sich durch eine außergewöhnlich schnelle und weite Ausbreitung der betroffenen Arten aus. Viele Arten waren in der Lage innerhalb kürzester Zeit weit über ihr natürliches Verbreitungsgebiet hinweg zu expandieren. Hierfür ist meist der Mensch verantwortlich, der zum Beispiel durch globale Handelswege oder den Bau von Kanälen geographisch getrennte Gebiete miteinander verbunden und so den Faunenaustausch ermöglicht hat. Wirft man allerdings einen Blick in die Geschichte, wird deutlich, dass biologische Invasionen ein sehr wesentlicher Bestandteil der Erdgeschichte sind. Biologische Invasionen beschreiben die Ankunft einer Art in einem Gebiet, in dem sie nicht ursprünglich evolviert ist. Auf natürlichem Weg ist dies z.B. durch veränderte klimatische Bedingungen, geotektonische Verschiebungen oder andere natürliche Ereignisse, wie Fluten oder Orkane, möglich. Die Vektoren gegenwärtiger biologischer Invasionen sind dagegen meist andere und stehen häufig unmittelbar mit dem Menschen in Verbindung. Man unterscheidet zwischen beabsichtigen und unbeabsichtigten Invasionen. Unbeabsichtigt breiten sich Arten zum Beispiel durch passive Verschleppung mit Handelsgütern oder im Ballastwasser von Schiffen aus. Viele Arten werden aber auch beabsichtigt über ihre natürlichen geographischen Grenzen hinweg verbreitet, wie z. B. Zier- und Nutzpflanzen oder Arten die fischereiwirtschaftlich genutzt werden. Die Konsequenzen sind zunächst einmal ähnlich, unabhängig davon, ob sie eine biologische Invasion auf natürliche Weise oder anthropogen hervorgerufen wurde. Die neu angekommene Art ist mit neuen abiotischen und biotischen Bedingungen und Interaktionen konfrontiert, woraus sich neue Selektionsdrücke ergeben. Gleichzeitig fungiert aber auch der Neuankömmling als Selektionsfaktor für die ansässige Fauna. An dieser Stelle knüpfen meine Untersuchungen an. Die vorliegende Arbeit umfasst sieben Studien, die verschiedene Aspekte biologischer Invasionen untersuchen. Meine Arbeit hat zum Ziel, den Einfluss abiotischer Faktoren und biotischer Interaktionen während der Etablierung bei verschiede-

nen invasiven Arten aufzuzeigen. Darüber hinaus untersuche ich Auswirkungen auf Ökosystemprozesse, die durch eine veränderte Artenzusammensetzung beeinträchtigt werden können und sich dadurch auf die Funktionalität des Ökosystems auswirken würden.

Der erste Teil meiner Arbeit untersucht anthropogen herbeigeführte biologische Invasionen. Die erste Studie dieses Teils widmet sich einem außergewöhnlichen Fließgewässer, das ganzjährig von Kühlwasser eines angrenzenden Kohlekraftwerks gespeist wird. Dadurch unterscheiden sich die abiotischen Bedingungen deutlich von denen umgebender Gewässer. Erste Berichte über eine ungewöhnliche Artenzusammensetzung stammen aus den 1970er Jahren und waren Anlass, den Bach etwa 40 Jahre später erneut zu untersuchen und die ansässigen Arten hinsichtlich ihrer Populationsdynamik sowie möglicher physiologischer Anpassungen genauer zu untersuchen. Die Artenzusammensetzung zeichnet sich durch eine ungewöhnliche Kombination einheimischer und invasiver Arten aus. Eine der invasiven Arten ist der Guppy (*Poecilia reticulata*), der wahrscheinlich durch Aquarianer dort eingebracht worden ist und seit 40 Jahren eine stabile, sich selbst erhaltende Population bildet. Die Untersuchung der unteren Thermotoleranzgrenze des Guppies zeigte allerdings, dass das Bestehen der Population nach wie vor vom Warmwasserzufluss des Kraftwerks abhängig ist und eine Ausbreitung in angrenzende Gewässer spätestens durch den Temperaturrückgang im Winter unterbunden wird. Meine zweite Studie untersucht Räuber-Beute Beziehungen, die durch das Einbringen invasiver Arten verändert werden. Dabei betrachtete ich die Auswirkung von chemischen Signalstoffen der Prädatoren (Kairomone) – in diesem Fall verschiedene einheimische und invasive Fischarten – auf die Larvalentwicklung der Gemeinen Stechmücke (*Culex pipiens*). Die Mückenlarven waren eindeutig in der Lage, den einheimischen, co-evolvierten Dreistachligen Stichling (*Gasterosteus aculeatus*) wahrzunehmen, was sich in einer spezifischen Reduktion der Körpergröße während der Larvalentwicklung äußerte. Der anschließende Fraßversuch bestätigte den Vorteil kleinerer Laven, da der Dreistachlige Stichling eine deutliche Fraßpräferenz für größere Larven zeigte. Bei invasiven Prädatoren zeigten die Larven hingegen keine spezifische Größenreduktion während ihrer Entwicklung, was die Gefahr verdeutlicht, dass viele Beuteorganismen nicht in der Lage sind neue Prädatoren wahrzunehmen und durch eine spezifische Reaktion die Prädationsgefahr zu mindern. Die dritte Studie widmet sich den Auswirkungen invasiver Arten auf Ökosystemprozesse. Der Energiekreislauf von Fließgewässern beruht im Wesentlichen auf dem Eintrag allochthonen organischen Materials, welches meist in der Form von Laub in die Gewässer eingetragen wird und als Grundlage des Nahrungsgefüges dient. Bachflohkrebse der Ordnung Amphipoda sind wesentlich am Abbau des organischen Materials beteiligt und werden daher als Schlüsselorganismen im Fließgewässer angesehen. In den letzten Jahrzehnten hat sich durch den Bau von Kanälen die Amphipodenfauna Mitteleuropas jedoch wesentlich verändert. So sind die Bundeswasserstraßen Deutschlands heutzutage ausschließlich von invasiven Amphipodenarten besiedelt, während die einheimischen Arten in die Zuflüsse zurückgedrängt wurden. Die Veränderung der Artenzusammensetzung wirft die Frage auf, inwiefern sich dadurch auch die Stoffwechselwege der Fließgewässer verändert haben. Dafür habe ich die Fraßraten der häufigsten einheimischen und invasiven mitteleuropäischen Amphipodenarten miteinander verglichen. Die Ergebnisse zeigen unverkennbar, dass die invasiven Arten wesentlich weniger Laub abbauen als ihre einheimischen Verwandten. Sofern andere Taxa diese Veränderung im Ökosystemprozess nicht auffangen können, ist also von einer Änderung des Energieflusses als Folge der neuen Artenzusammensetzung auszugehen, was das Risiko indirekter Konsequenzen biologischer Invasionen verdeutlicht. Die vierte Studie vergleicht anhand der gleichen Modellorganismen Verhaltenscharakteristika einheimischer und invasiver Amphipodenarten. Invasiven Arten werden häufig Eigenschaften zugeschrieben, die eine schnelle Ausbreitung im neuen Lebensraum begünstigen, zum Beispiel eine hohe Aktivität auf Verhaltensebene. Der untersuchte invasive Höckerflohkrebs (*Dikerogammarus villosus*) zeigte jedoch keine erhöhte Aktivität im Vergleich zu den einheimischen Amphipoden. Allerdings zeigte ausschließlich die invasive Art ein sehr variables, unvorhersehbares Verhaltensmuster individueller Aktivität, das die Ausbreitung in neue Areale begünstigen kann.

Der zweite Teil meiner Arbeit behandelt Konsequenzen natürlicher biologischer Invasionen. In der ersten Studie habe ich die Verbreitungsmuster von zwei nahverwandten Moskitofischarten der Gattung *Gambusia* entlang eines Flussgradienten untersucht. Die Arten gehören zur Familie Poeciliidae, die ihren evolutiven Ursprung in den Neotropen hat und in mehreren Ausbreitungsereignissen Zentralamerika besiedelt hat. Vertreter der Gattung *Gambusia* kommen natürlicherweise von Kolumbien bis in die Vereinigten Staaten von Amerika vor. Stellenweise besiedeln mehrere Arten der Gattung den gleichen Lebensraum. Im Río Grijalva Flusssystem im Süden Mexikos ist dies der Fall. Das Vorkommen von zwei nahverwandten Arten entlang von unterschiedlichen abiotischen und biotischen Bedingungen ermöglichte es den Einfluss verschiedener Selektionsfaktoren auf die Ausprägung phänotypischer Merkmale zu untersuchen. Die Ergebnisse der Studie belegen eindrucksvoll, dass die zwei nahverwandten Arten auf manche Umweltfaktoren gegensätzlich reagieren. Gründe hierfür liegen wahrscheinlich in der unterschiedlichen Evolutionsgeschichte der beider Arten. Während *G. sexradiata* und deren Schwesterarten ausschließlich auf dem Festland zu finden sind, ist *G. yucatana* mit ihren Schwesterarten stets küstennah und über die Großen Antillen verbreitet. Die Präferenz beider Arten hinsichtlich gewisser Umweltfaktoren wird jedoch nur in der phänotypischen Merkmalsausprägung deutlich; eine Zonierung im Vorkommen der beiden Arten entlang des Flussgradienten – wie sie anhand der generellen Verbreitungsmuster der Schwesterarten zu vermuten wäre – findet sich nicht. Dies erklärt sich vermutlich durch regelmäßige Flutereignisse, die in dem weitgehend unverbauten Flusssystem nach wie vor auftreten und die stochastischen Verbreitungsmuster beider Arten erklären. Innerhalb des Río Grijalva Flusssystems finden sich aber auch Habitate, die kaum fluktuierenden Umweltbedingungen ausgesetzt sind. Ein Beispiel dafür ist ein Höhlensystem, welches ich in zwei weiteren Studien untersucht habe. Die Cueva del Azufre ist eine Höhle die sich durch zwei gleichzeitig wirkende abiotische Stressoren auszeichnet: Dunkelheit und Schwefelwasserstoff. Trotz dieser extremen Bedingungen hat sich dort eine Population des Atlantikkärpflings (*Poecilia mexicana*) etabliert und an die lokalen Verhältnisse angepasst. Der in der Höhle zu findende Ökotyp des Atlantikkärpflings zeichnet sich durch verschiedene Anpassungen aus, die das Überleben im Extremhabitat gewährleisten. Obwohl dieses einzigartige Ökosystem bereits seit Jahrzehnten intensiv untersucht wird, fehlten lange Zeit Informationen zu grundlegenden Populationscharakteristika, wie zur Populationsgröße oder zur Mikrohabitatwahl. Diese Studie nun zeigte, dass der lokal angepasste Ökotyp in enormen Dichten mit über 200 Individuen pro m^2 vorkommt. Die darauffolgende Studie widmete sich Anpassungen zur nicht-visuellen Kommunikation der Höhlenfische und untersuchte die Fähigkeit zur Wahrnehmung von artgleichen Individuen anhand chemischer Signalstoffe. Dabei zeigte sich, dass die in der Cueva del Azufre beheimatete Population – im Gegensatz zu der nahegelegenen Population aus einer nicht-schwefelhaltigen Höhle – offensichtlich die Fähigkeit evolviert hat, artgleiche Individuen anhand chemischer Stoffe wahrzunehmen. Dieser Befund bekräftigt die Annahme, dass beide Höhlensysteme unabhängig voneinander besiedelt worden sind und es sich bei der Population in der Cueva del Azufre um die ältere der beiden

Höhlenformen handelt. Die größere Populationsgröße in der Cueva del Azufre begünstigt zudem vermutlich die Evolution von Merkmalen, die zum Beispiel die nicht-visuelle Kommunikation ermöglichen. Zuletzt gebe ich erste Einblicke in eine weitere Studie die sich mit der historischen und gegenwärtigen Ausbreitung des Östlichen Moskitofisches (*Gambusia holbrooki*) befasst. Die Art ist zusammen mit ihrer Schwesterart *G. affnis* der nördlichste Vertreter der Familie Poeciliidae und besiedelt küstennahe Gewässer von Florida bis New Jersey. Zusammen mit *G. affinis* besitzt die Art außerdem eine Invasionsgeschichte in vielen Teilen der Welt. Dort sind die beiden Arten in der kontrovers diskutierten Annahme ausgesetzt worden, sie würden Mückenlarven und damit Malaria effektiv bekämpfen. Heute zählen beide Arten zu den weltweit hundert bedrohlichsten invasiven Arten, da sie aufgrund ihrer räuberischen Lebensweise viele einheimische Arten gefährden. Der erste Teil dieser Untersuchungen bezieht sich auf das natürliche Verbreitungsgebiet von *G. holbrooki* und zeigt eine deutliche genetische sowie phänotypische Differenzierung von Süd nach Nord. Die in Europa angesiedelten Populationen entstammen dem nördlichen Verbreitungsgebiet der Art (North Carolina). Das könnte bedeutend sein, betrachtet man den heutigen Erfolg als invasive Art und die Evolutionsgeschichte in ihrem einheimischen Verbreitungsgebiet: Als nördlichster Vertreter der Poeciliiden war *G. holbrooki* wesentlich von der letzten Eiszeit betroffen und vor allem die nördlichen Populationen haben wahrscheinlich Mechanismen evolviert um ihr Verbreitungsgebiet rasch wieder ausweiten und auf variable abiotische Umweltbedingungen reagieren zu können. Diese Fähigkeit käme der Art auch bei ihrer derzeitigen vom Menschen verursachten Invasionsgeschichte zugute, indem sie durch ein hohes Maß an Flexibilität auf variable Umweltbedingungen reagieren können, was die rasche Ausbreitung in den invasiv besiedelten Gebieten erklären würde.

Im Gegensatz zu gegenwärtigen biologischen Invasionen fehlen uns bei historischen Invasionen Detailinformationen zum Faunenaustauch. Zudem fehlt uns entscheidendes Wissen über die Mechanismen, die während biologischer Invasionen von Bedeutung sind (Flexibilität im Verhalten, plastische phänotypische Veränderungen, oder genetische Anpassung). Vergleichende Analysen von (historischen) natürlichen Invasionen und (derzeitigen) anthropogenen hervorgerufenen Invasionen können entscheidend helfen um Anpassungsstrategien an abiotische Umweltfaktoren zu verstehen. Darüber hinaus zeigen sie, wie sich neue biotische Interaktionen auf die Selektion von invasiven und einheimischen Arten auswirken. Neben den nicht zu unterschätzenden negativen

Folgen gegenwärtiger biologischer Invasionen auf die einheimische Biodiversität bieten diese „natürliche Experimente“ Gegebenheiten, die es ermöglichen wesentliche Fragen der Ökologie und Evolutionsbiologie zu beantworten. Mein Vergleich zwischen natürlichen und anthropogen induzierten Invasionen zeigt, dass in vielen Fällen Präadaptation eine wesentliche Rolle bei der erfolgreichen Etablierung einer Art spielt. Zudem zeigt sich, dass der Rückblick auf die Evolutionsgeschichte entscheidend sein kann, um gegenwärtige Ausbreitungsmuster zu verstehen. Dieses Wissen kann in Naturschutzvorhaben berücksichtigt werden, wenn es darum geht zukünftige Ausbreitungen invasiver Arten zu prognostizieren und Regionen ausfindig zu machen, deren Biodiversität besonders von invasiven Arten bedroht werden könnte.

PREFACE

This PhD-thesis, entitled "*Role of abiotic factors and biotic interactions in biological invasions: a comparison of natural and human-induced invasions in freshwater ecosystems*", represents a compilation of experimental studies dealing with the evolutionary and ecological effects of human-induced (Chapter 2) and natural biological invasions (Chapter 3). Both phenomena differ in spatial and temporal scales, while basic principles are similar, as invading species have to deal with an array of novel abiotic and biotic selective pressures and simultaneously act as a novel selective agent for the native fauna.

My PhD thesis consists of seven articles, all of which have been submitted to peer-reviewed journals. Except for Jourdan *et al.* (*submitted*; Chapter 3.1) and Jourdan *et al.* (*major revision*; Chapter 3.3), all articles have already passed the peer reviewing process and have been accepted or are already published by the respective journal. I arranged them into subchapters, each of which comprises one publication. My contributions to each of the publications are highlighted at the beginning of each subchapter. During the time I worked on my thesis, I was partly also involved in other projects that are not part of this thesis (see my Curriculum Vitae for details). I analyzed, for instance, the genetic differentiation of extremophile lineages of *Gambusia* spp. and consequences of convergent colonizations of sulfidic springs (Riesch *et al.* in press). Furthermore, I conducted studies investigating social networks in *Poecilia mexicana* (Bierbach *et al*. 2014), and investigations of the invasive amphipod fauna in the river Lahn (Chen *et al.* 2012a).

In order to meet the prerequisites of a cumulative (publication-based) PhD thesis at the Goethe University of Frankfurt I introduce the reader to the general topic of biological invasions with a brief review, followed by a chapter overview, including an introduction and critical conclusive remarks for each study, followed by an outlook and critical conclusive remarks on my work as a whole. However, while all publications represent a cooperative achievement, combining the expertise of my own person and other participating scientists (as indicated by the author contributions provided for each publication), I use the 1st person singular in the first chapter to indicate that I was the sole author of this text.

1. GENERAL OVERVIEW

1.1 Introduction

1.1.1 General aspects of human-induced biological invasions

On a scale of millions of years, adaptation, speciation and extinction as well as geological processes and climate change have created unique assemblages of species around the globe (Futuyma 2005). Nowadays, the diversity of species is threatened by the exponential growth of the human population and associated effects like habitat destruction, land use change, human induced global warming (through CO_2 and other "greenhouse" gases), hunting and overfishing (Vitousek *et al.* 1996; Sala *et al.* 2000). Another major driver of biodiversity loss are biological invasions. Human activities rapidly erase the natural barriers that have historically isolated animal and plant species, leading to altered geographic distribution patterns of many species from local to continental scales (Vitousek *et al.* 1997; Mack *et al.* 2000; Sala *et al.* 2000; Sakai *et al.* 2001; Simberloff *et al.* 2013). As a consequence, the term 'invasive alien species' was established by the IUCN (2000) and defined as 'a species which becomes established in natural or semi-natural ecosystems or habitats, is an agent of change, and threatens native biological diversity' (IUCN 2000, p. 4). The concept of biological invasions has become increasingly popular and fostered the growth of a new research field, *invasion science* (Richardson 2011; Richardson & Ricciardi 2013; Simberloff *et al.* 2013). This new field aims to detect, understand, and mitigate the impact of biological invasions with broad ecological and conservation implications (Simberloff *et al.* 2013).

Species are able to enter new habitats either actively, e.g. by natural dispersal using newly constructed channels or passively, facilitated by human activities (Galil *et al.* 2007). Passive introduction can either happen intentionally (e.g., global distribution of domesticated animals) or unintentionally (e.g., through transcontinental transportation in ballast water or through travel activities; Vitousek *et al.* 1997; Gollasch 2007; Nentwig 2007a). However, biological invasions represent a multi-stage process, and a species has to pass successfully through all stages, including transport and introduction out of their native distribution range, population establishment and further spread (Lodge 1993; Jeschke & Strayer 2005; Chapple *et al.* 2012). At each stage, variation in physiological, morphological, life history, behavioral and other traits may hamper or promote invasion success (Kolar & Lodge 2001; Sakai *et al.* 2001). Once a species is established beyond its natural distribution range, various outcomes are possible: While the majority of introduced species have no obvious immediate negative effect (Nentwig 2007b), some ─ but not all ─ invasive taxa have ecological impacts on the invaded ecosystems (Vitousek *et al.* 1997; Chapin *et al.* 2000; Crooks 2002; Strayer *et al.* 2006; Nentwig 2007b). The most obvious, and therefore most commonly considered, negative impacts occur when invasive species prey on native species. One example are black rats (*Rattus rattus*), which were introduced to islands worldwide and have tremendous effects on seabird populations as they prey on eggs and nestlings. Especially the family Hydrobatidae and other small, burrow-nesting seabirds are heavily affected by introduced rats (Jones *et al.* 2008). Also the introduction of predatory fishes can have devastating effects on native populations of fishes and amphibians [e.g., Eastern Mosquitofish, *Gambusia holbrooki* (Pyke 2008); Nile perch, *Lates niloticus* (Witte *et al.* 1992); large-mouth bass, *Micropterus salmoides* (Gratwicke & Marshall 2001; Takamura 2007)]. Likewise, introduced herbivores caused substantial damage at both, local and landscape scales: The golden apple snail (*Pomacea canaliculata*) feeds on wetland crops and has caused enormous losses to rice crop production in Asia (Carlsson & Lacoursière 2005). European rabbits (*Oryctolagus cuniculus*) have been introduced worldwide and have degraded habitats by overgrazing (Lees & Bell 2008).

Introduced species can also compete with native species for resources like food. The fire ant (*Solenopsis invicta*), for example, invaded Central America northwards into the southeastern United States from South America and strongly decimated the indigenous ant fauna. Competitive replacement due to exploitation of food resources and aggressive interactions of the fire ant with other ant species appear to be the primary mechanisms behind the sharp decline of native ants (Porter & Savignano 1990; Holway *et al.* 2002). The gray squirrel (*Sciurus carolinensis*) was brought from North America to Great Britain and gradually replaced native red squirrels (*Sciurus vulgaris*), probably because it is a more efficient forager (Okubo *et al.* 1989; Gurnell *et al.* 2004).

Non-native species may also alter ecosystem processes in the invaded area. The European wild boar (*Sus scrofa*), for instance, caused enormous damage in many regions, turning up large patches of vegetation by rooting for food. The erosion caused by their foraging activity affects plant communities by disrupting species interactions and nutrient cycles, demonstrating their role as ecosystem engineers (Lowe *et al.* 2000; Cuevas *et al.* 2010; Barrios-Garcia & Ballari 2012). In addition to severe impacts on native biodiversity, some invasive species also cause major economic problems in agriculture and forestry with economic losses in excess of 137 billion US$ in the USA alone (Pimentel *et al.* 2000). Since 1970 the number of invasive species in Europe increased by 76% (Butchart *et al.* 2010), and the number of invasive species worldwide continues to increase (Hulme 2009).

1.1.2 Human-induced biological invasions in aquatic ecosystems

Freshwater ecosystems are especially susceptible to biological invasions, since they are spatially restricted environments and often already heavily altered by anthropogenic activities (such as flow modification, nutrient influx, and destruction or degradation of habitat) while simultaneously exhibiting a high degree of endemism (Dudgeon *et al.* 2006; Strayer 2006; Havel *et al.* 2015). Recent analyses suggest that the loss of biodiversity is far greater in freshwater compared to terrestrial ecosystems (Ricciardi & Rasmussen 1999; Sala *et al.* 2000). Dudgeon *et al.* (2006) classified the threats to global freshwater biodiversity in five interacting categories: overexploitation, water pollution, flow modification, destruction or degradation of habitat and invasion by non-native species. The latter adds to physical and chemical anthropogenic impacts, because invasive species are often better able to withstand the stressors than indigenous fauna and thus, readily colonize modified or degraded freshwater ecosystems (e.g., Bunn & Arthington 2002; Kennard *et al.* 2005).

The worldwide trend of increasing biological invasions in aquatic ecosystems is primarily related to human activities like aquaculture, global trade of certain species, construction of canals, ballast water of ships, aquarium releases and subsequent feralization, releases for sport fisheries, and recreational activities (Cohen & Carlton 1998; Padilla & Williams 2004; Galil *et al.* 2007; Roman & Darling 2007; Gozlan *et al.* 2010). One of the most popular examples of an aquatic invasion is the introduction of the predacious Nile perch (*Lates niloticus*) into Lake Victoria in the 1950s to support commercial fisheries. Lake Victoria originally harbored a unique species flock of endemic haplochromine cichlids, which presumably contained more than 500 species and whose members exhibited a wide range of trophic and other ecological specializations. The spread of the novel apex predator led to a dramatic decline of endemic species as it eliminated or seriously threatens more than 200 of the 500 species (Hughes 1986; Seehausen *et al.* 1997; Goldschmidt 1998; Pringle 2005). The lake's fisheries changed from multispecies fisheries, which subsisted the ecosystem's high diversity, to highly efficient commercial fisheries, where the majority of the catch consisted of only three species: the invasive Nile perch, the invasive Nile tilapia (*Oreochromis niloticus*), and the small native cyprinid *Rastrineobola argentea* (Balirwa *et al.* 2003; Pringle 2005). The substantial increase in biomass of the native zooplanktivorous cyprinid *R. argentea* can be explained by the disappearance of zooplanktivorous haplochromines (Wanink 1999) and illustrates that the loss of native species dramatically altered the ecology and functional diversity of Lake Victoria.

The introduction of Nile perch was accompanied by other forms of anthropogenic environmental change, such as overfishing and eutrophication, which further facilitated the extinction of the endemic cichlid species flock. The rapid eutrophication of Lake Victoria, for instance, has caused increased turbidity, which impaired mate recognition in cichlids (Seehausen *et al.* 1997; Hecky *et al.* 2010). Sympatric haplochromine cichlids have versatility colored males and mostly cryptically colored females (Seehausen & van Alphen 1998). Haplochromines lack strong intrinsic post-zygotic isolation, and reproductive isolation among sympatric species is maintained for the most part by assortative mate choice. The increased turbidity of Lake Victoria resulted in a reduced ability of cichlids to identify conspecifics, which led to hybridization and contributed to the loss of species diversity among cichlids (Seehausen *et al.* 1997). This illustrates that beside the immediate adverse effects caused by the introduction of Nile perch, other components of environmental change also play a vital role, although the Nile perch was certainly a major contributor to biodiversity loss.

The introduction of non-native species can have direct (e.g., predation) and indirect effects on native biodiversity. Indirect effects can be seen in the form of the simultaneous introduction of parasites or pathogens to which the invasive species is adapted while its native relatives are not (Prenter *et al.* 2004; Nentwig 2007a; Emde *et al.* 2015). A well-known example is the crayfish plague fungus, *Aphanomyces astaci* (Leptolegniaceae; Saprolegniales), a severe pathogen of freshwater crayfish that was introduced to

Europe by American crayfish species like *Pacifastacus leniusculus*, *Orconectes limosus* and *Procambarus clarkii* and which had devastating effects on European crayfish populations (Dièguez-Uribeondo & Söderhäll 1993; Vennerström *et al.* 1998; Westman 2002). While American crayfish are partly resistant to the fungus, they transfer the disease to susceptible native European crayfish species such as the noble crayfish, *Astacus astacus*, leading to significant declines in population numbers (estimates of decline rates in Sweden, Finland and Norway are as high as 78%, ~20% and 61%, respectively). The species is, therefore, now listed as *vulnerable* in the IUCN Red List of threatened species (Edsman *et al.* 2010). Those examples illustrate that the consequences of biological invasions are manifold and often result in a reduction and homogenization of biodiversity in the invaded habitat.

1.1.3 Natural biological invasions

Contemporary, human-induced biological invasions often involve long-distance dispersal, with many species having extended their original distribution range over large parts of the globe within a very short time frame. However, a long term view shows that biological invasions are basically a natural process, resulting from climatic changes, geotectonic activity or other natural events, like catastrophic floods or hurricanes. Hence, they have always been a substantial component of species interactions (Vermeij 1991; Lodge 1993). When major dispersal barriers break down ─ whether induced by anthropogenic activities or by forces of nature (e.g., by geological, climatic or other environmental changes) ─ species may expand their distribution ranges and invade to new geographical areas (Futuyma 2005).

Similar to human-induced invasions, naturally invading species face novel ecological conditions in their new environment, which can promote the evolution of novel traits, create novel species interactions, and alter existing ecosystem processes. In addition, invasions may initiate speciation (thus increasing local biodiversity) or cause species extinctions (Marshall 1988; Futuyma 2005; Webb 2006). A famous example of natural biological invasions is the so called Great Interchange between North- and South America. When the Isthmus of Panama closed in the late Pliocene, several families of plants and animals could bi-directionally expand their distribution ranges between South and North America (Rosen 1975; Marshall 1988; Webb 2006). The zoogeography of mammals, reptiles, amphibians, birds, and freshwater fish changed, as they were able to invade North America from the south and vice versa. This example of natural invasion provides unique insights into the mechanisms and processes at play when previously isolated biotas meet. However, temporal resolution of historical species range expansions is considerably lower compared to recent human-induced invasions (Swetnam *et al.* 1999). Therefore, it is impossible to determine whether the loss of biota (that took place subsequent to the formation of the Isthmus of Panama) happened due to competition with invasive taxa (Marshall *et al.* 1982; Marshall 1988). This possibility, however, is quite likely in several cases, as it is assumed that the initial migration was symmetrical (Marshall *et al.* 1982). However, northern taxa were apparently better able to compete for resources in their new habitats, may have caused species extinctions and underwent subsequent evolutionary radiation, while the southern, Neotropical species proved far less successful in invading northern areas (Marshall *et al.* 1982; Webb 2006). Marshall (1988) explained this phenomenon by the island character of South America during most of the Cenozoic. Due to its isolation, South American land mammals evolved in 'a world of their own' (Marshall 1988, p. 381), with several taxa being autochthonous and endemic to the continent. When the Isthmus of Panama formed and biotic interchange began, the land mammals of South America for the first time in their evolutionary history faced a major influx of potential new mammalian competitors and predators. On the other hand, South American taxa that dispersed to North America entered a fauna in Central America which had a long evolutionary history of invasions and subsequent changes in species interactions — not only in Central- and North America, but also prior to that while the North American continent was connected to Eurasia.

Differences in the evolutionary histories of North and South American faunas demonstrate that the directions and dimensions of biotic interchange will be different on each continent and for different taxonomic groups, partly depending on the biotic interactions biota experienced in the recent evolutionary history (Marshall 1988; Webb 2006). This view is congruent with current observations of human-induced biological invasions on islands. Geographical isolation and faunal compositions of Australia, for instance, strongly contribute to the serious impact of introduced predators on that continent, as seen in the dramatic decline of flightless birds after the introduction of eutherian mammals (Courchamp *et al.* 2003; Salo *et al.* 2007). Those species lacked behavioral or life-history adaptations to cope with the novel predators (Courchamp *et al.* 2003). By contrast, species in the Northern Hemisphere evolved in a much larger, more speciose and thus, more competitive area. Frequent historical range expansions between the connected continents of Eurasia, Africa and North America (e.g., during the middle to late Miocene) likely promoted the evolution of shared predator avoidance mechanisms, rendering those taxa pre-adapted to novel predator types (Courchamp *et al.* 2003; Webb 2006; but see 2.2).

There are also examples of more recent natural biological invasions, with species that having been able to expand their range rapidly and having crossed major barriers on their own. One example is the cattle egret (*Bubulcus ibis*), that has undergone a rapid and wide-reaching natural range expansion. Originally native to Southern Spain and Portugal, tropical and subtropical Africa as well as humid tropical and subtropical Asia, the cattle egret expanded its distribution range by the end of the 19th century and spread — unassisted by humans — into southern Africa, and further to warmer parts of South- and North America (Drury Jr *et al.* 1953; Crosby 1972; Arendt 1988; Massa *et al.* 2014).

1.1.4 Ecological and evolutionary consequences of biological invasions

In both, human-induced and natural biological invasions the invading species faces an array of novel abiotic and biotic ecological conditions in the invaded range to which they did not adapt, which may provide native species with a competitive advantage over invading taxa (Mooney & Cleland 2001; Sakai *et al.* 2001; Simberloff 2013). Interactions between invading taxa and native fauna as well as more recent invaders may indeed be vital factors determining whether the invasive species will become established (Platvoet *et al.* 2009; Hudina *et al.* 2011; Maazouzi *et al.* 2011). The available literature suggests that approximately 10% of human-induced species introductions will lead to the establishment of self-sustaining populations (Kolar & Lodge 2001). Common reasons for unsuccessful invasions include absence of preadaptation to altered climatic conditions, competition with or predation by native species, or susceptibility to diseases (Sakai *et al.* 2001). Moreover, populations of invasive species often have reduced genetic diversity as a result of founder effects and genetic bottlenecks (Tsutsui *et al.* 2000; Allendorf & Lundquist 2003). As reduced genetic diversity is expected to constrain the evolutionary potential of a given (invasive) population, it is even more surprising that some introduced species are able to establish self-sustaining populations and even become dominant in their new environment; this phenomenon has been referred to as the *paradox of invasion biology* (Allendorf & Lundquist 2003). However, the exact reasons for the failure or successful establishment of invasive species often remain unclear, as the ecological and evolutionary mechanisms allowing organisms to successfully colonize new environments and expand their range are far from being well understood. Studying and understanding the often complex interactions between invading species and the recipient biota, therefore, remains a fundamental challenge to ecologists and conservation managers (Kolar & Lodge 2001; Mooney & Cleland 2001; Hayes & Barry 2008).

Genetic variability is not required if the invading species is already preadapted to novel environmental conditions. Preadaptation can be brought about either because the introduced phenotype is already well adapted to the novel conditions due to similar selection pressures in the evolutionary history of the species or because the species possesses the appropriate phenotypic plasticity to produce the required phenotype(s) (Sakai *et al.* 2001; Suarez & Tsutsui 2008). Adaptive plasticity in particular might promote the establishment and persistence in a new environment, as plastic phenotypes can be expressed within a single generation (Ghalambor *et al.* 2007). An alternative explanation for successful establishment of an invasive species are adaptive evolutionary changes over short time-spans by means of selection on existing allelic variation, or selection on novel phenotypes arising from *de novo* mutations (i.e., contemporary evolution; Reznick & Ghalambor 2001).

1.2 Manuscript overview

In the following sections, I will outline the framework of the respective studies that form the backbone of the corresponding chapters. Furthermore, for each study I will provide a short discussion of some of the central findings. A broad discussion and outlook section of my work is given after the manuscript overview.

1.2.1 Human-induced biological invasions

Manuscript 1: Invasive species in an artificially heated stream

This subchapter consists of a research article in which I characterized a stream in Germany whose thermal conditions are altered by anthropogenic discharges of heated cooling waters from a power plant (Jourdan *et al.* 2014a). In subtropical and temperate regions thermally altered freshwater ecosystems have recently been documented to host reproducing populations of non-native species of tropical origin (e.g., Specziar 2004; Piazzini *et al.* 2010; Klotz *et al.* 2013; Emde *et al.* 2015). Such thermally influenced habitats with average temperatures much different from those in adjacent, non-heated waters and provide suitable conditions for invasive species that would not be able to establish populations in adjacent waters. The resulting species communities often comprise both, non-native and native species. The main pathways for the introduction of non-native species are ornamental pet trade and releases by aquarium hobbyists (Padilla & Williams 2004; Jourdan *et al.* 2014a). Although the aquarium trade has been increasingly recognized as a major vector of biological invasions that accounts for 21% of freshwater fish introductions worldwide (Gozlan *et al.* 2010; Strecker *et al.* 2011), it seems to play a minor role in temperate regions, since most of the ornamental fishes are of tropical origin and low winter temperatures prevent them from establishing self-sustaining populations in most parts of Europe.

In this article, I reported on species compositions in a system that constitutes a 'thermal refuge' for an exceptional species assembly, including a population of *Poecilia reticulata* that has established a self-sustaining population for at least three decades, as well as other tropical fishes (e.g., *Amatitlania nigrofasciata* and the mouthbrooding African cichlid *Oreochromis* sp.). Moreover, I determined the population size of guppies and investigated the lower thermal tolerance limit ($\mathrm{CT}_{\mathrm{min}}$) to see if the guppies already adapted to colder conditions. The source of the guppies is suspected to be ornamental animals. Nevertheless, I could not find evidence for adaptation to lower temperatures, as no difference in $\mathrm{C}_{\mathrm{Tmin}}$ between ornamental and Gillbach guppies was detected. Despite the limited invasion potential, the Gillbach population of introduced guppies with its presumed 'spread and diminish' characteristics (Jourdan *et al.* 2014a) may provide a valuable semi-natural experiment for future questions related to local adaptation of invasive populations and ecological interactions with indigenous faunal elements.

This study served as a starting point for subsequent studies: Emde *et al.* (2015) focused on the risk of the release and establishment of ornamental fish species for the introduction and spread of non-native metazoan parasites. This is of particular interest, since the thermal gradient of the Gillbach is directly connected to unaffected ecosystems and may serve as a source habitat for non-native parasites. Therefore, the authors investigated the population of *A. nigrofasciata* inhabiting the Gillbach and identified five parasite species in *A. nigrofasciata*, including one native (*Acanthocephalus anguillae*), three non-native species (*Anguillicoloides crassus*, *Camallanus cotti* and *Bothriocephalus acheilognathi*) and unspecified nematode cysts. Furthermore the authors found alarming evidence for the spread of nonnative parasites into adjacent fish communities, as *Camallanus cotti* was also found in native chub (*Squalius cephalus*) and gudgeon (*Gobio gobio*).

The well-characterized species composition in the Gillbach (Höfer & Staas 1998; Kempkes 2005; Kempkes 2010; Jourdan *et al.* 2014a) provides the possibility to study the role of preadaptation in predator-prey interactions. In a recent study, Sommer-Trembo *et al.* (2016) used the unique mixture of native and invasive species in the Gillbach to evaluate the relative importance of innate versus learned components of predator recognition in guppies. The authors compared avoidance reactions of both co-evolved piscine predators from their original distribution range and novel, presently co-occurring predator species. Wild-caught, predator-experienced as well as lab-reared, predator-naive individuals showed pronounced avoidance behavior towards all predators. While this finding supports earlier studies on visual predator avoidance in guppies (Kelley & Magurran 2003), the study showed that consistent individual differences in predator avoidance, as seen in predator-naive (laboratory-reared) fish, are homogenized by predator experience, as strikingly lower among individual variance in the behavioral responses to predators were seen in wild-caught fish.

In 2015 it was announced that the coal-fired power plant “Niederaußem” will be finally shut down by 2023 at the latest (e.g., Kölner-Stadt-Anzeiger 2015). The original watercourse of the Gillbach has been destroyed by brown coal surface mining and nowadays the Gillbach exclusively receives cooling water stemming from the power plant (Klotz *et al.* 2013; Jourdan *et al.* 2014a). As a consequence of the power plant decommissioning, the abiotic characteristics of the Gillbach will change immediately. Without warm water influx the architecture of the community will rapidly change, since overwinter survival of the tropical fish species inhabiting the Gillbach should not be possible in German river systems (for thermal tolerances of guppies see: Fujio *et al.* 1990; Jourdan *et al.* 2014a). The experimental conditions to investigate invasive species under semi-natural conditions will end at that time. However, the return to natural conditions will provide new, interesting conditions to study the gradual community recovery (i.e., the ability of the native communities to return to the pre-disturbance state following displacement). I recommend conducting a continuous monitoring of this unique system during its recovery with a special focus on changes in species composition.

Manuscript 2: Novel predator-prey interactions

Exposure to predation has a strong effect on virtually all aspects of a population's biology, as selection from predation is a powerful driver of phenotypic diversification including adaptive variation in prey species' life histories (Reznick & Endler 1982; Crowl & Covich 1990; Riesch *et al.* 2013), morphology (Krueger & Dodson 1981; Dodson 1989; Walker 1997; Langerhans *et al.* 2004; Hendry *et al.* 2006), and behavior (Dixon & Baker 1988; Lima & Dill 1990). There is ample evidence for co-evolutionary dynamics between predator and prey species, leading, e.g., to the evolution of various defense traits in morphology and adaptive life history shifts (for review see: Abrams 2000). One such co-evolutionary process should occur when larval stages encounter variation in predation regimes, which – if building defense traits bears costs – should select for phenotypically plastic expression of those traits (Hebert & Grewe 1985; Dodson 1989; Agrawal 2001; Benard 2004).

Invasive predators may have devastating effects on native species since naive prey may be unable to recognize the novel species as a predator or cannot show the appropriate anti-predator defense (Salo *et al.* 2007). Therefore, understanding how invasive predators affect their prey is a vital goal in conservation biology. Some studies picked up that question and found no behavioral anti-predator response of native species to an unknown predator (e.g., Kesavaraju & Juliano 2004; Kesavaraju *et al.* 2007), whereas others found morphological and behavioral adaptations to invasive predators (e.g., Flecker 1992; Pease & Wayne 2014). In my study (Jourdan *et al.* 2016a), I focused on predator-induced life-history shifts in native common house mosquitoes (*Culex pipiens*). Larvae of this species occur in both predator-free habitats (puddles) and under high predation in larger water bodies (Vinogradova 2000). The question I raised was whether mosquitoes respond with altered life-histories when exposed to native predators with which they had coevolved, but not with unknown, invasive predators. Previous studies reported on altered larval development in mosquitoes in response to chemical cues (kairomones) from native predators (Beketov & Liess 2007; van Uitregt *et al.* 2012). Therefore, I experimentally exposed mosquito larvae to kairomones of their native predators (sticklebacks, *Gasterosteus aculeatus*), invasive, not co-evolved eastern mosquitofish (*Gambusia holbrooki*) and algivorous, non-native suckermouth catfish (*Ancistrus* sp.). I found two major effects: First, mosquitoes were lighter in all kairomone treatments, likely reflecting reduced food uptake due to decreased activity in the presence of all fish species (co-evolved native and unfamiliar invasive). Second, I found reduced larval body size only in the stickleback treatment as a specific response to the co-evolved predator. A prey choice experiment confirmed the expected predilection of co-evolved sticklebacks for large-bodied larvae, providing an intriguing ultimate explanation for suppressed lengths growth under high stickleback predation.

Thus, this study demonstrates that the antipredator response can be finely nuanced and that only certain traits exhibit a predator-specific response, while others show similar responses across a range of potential (co-evolved native or unfamilar invasive) predators. The predator-specific response, however, was detected only when the co-evolved predator was involved, which is congruent with the conclusions reached by Salo *et al.* (2007): The authors compared several studies of predator-prey interactions and found that predator origin (native versus invasive) had a highly significant effect on prey responses, with invasive predators having a much higher impact than native predators. However, invasive predator species may also drive evolutionary divergence in native species (for review see Schlaepfer *et al.* 2005) and so it remains to be tested in future studies if mosquitoes will evolve predator recognition in regions where *Gambusia* is invasive.

Manuscript 3: The effect of invasive species on ecosystem services

This subchapter focuses on Central European stream ecosystems that naturally harbor diverse and unique macroinvertebrate communities. Stream communities typically consist of aquatic insects, annelids, plathelminthes, mollusks and crustaceans, whereby the macroinvertebrate diversity clearly exceeds that of fishes and macrophytes in streams (Allan & Flecker 1993). A growing body of literature focuses on the adverse effects of biological invasions, e.g., on the decline of indigenous biodiversity, while studies on the consequences of invasions on components of ecosystem functioning are comparatively rare. Benthic macroinvertebrates are essential contributors to the maintenance of ecosystem functions, including detritus processing, herbivory, interactions with microbes, and energy transfer to carnivores at higher trophic levels (Cummins & Klug 1979; Wallace & Webster 1996; Wallace *et al.* 1997; Graça 2001; Graça *et al.* 2015). Leaf litter produced by riparian woody vegetation is a major source of carbon for heterotrophic stream communities and the decomposition of leaf litter by macroinvertebrates represents a fundamentally important process in the headwaters of stream ecosystems (Vannote *et al.* 1980; Gessner *et al.* 1999). Some species are keystone species (sensu Mills *et al.* 1993) in their native ecosystems. Thus, their replacement by invasive species has more far-reaching consequences for ecosystem functioning than would be expected from individual numbers or biomass alone, leading, for example, to altered resource availability or energy flow dynamics (Power *et al.* 1996; Vitousek *et al.* 1996; Chapin *et al.* 2000).

For this study (Jourdan *et al.* 2016b) I focused on the river Rhine, a prime example of a dynamic and heavily invaded freshwater ecosystem due to the passive introduction of exotic species (e.g., in ballast water) as a consequence of intense industrial shipping, as well as active invasions along man-made canals connecting Central and Eastern European freshwater biomes (Bij de Vaate *et al.* 2002; Leuven *et al.* 2009; Panov *et al.* 2009). Since the early 20th century, the number of invasive species in the Rhine increased continuously, and nowadays invasive species constitute up to 90% of macroinvertebrates in terms of biomass and numerical abundance (Van Riel *et al.* 2006; Leuven *et al.* 2009). A central question emerging from those considerations is which of the indigenous and invasive faunal elements are significant to ecosystem functioning. Amphipods of the superfamily Gammaroidea are widespread and constitute an important functional element in European freshwater habitats (MacNeil *et al.* 1997; Väinölä *et al.* 2008). In terms of abundance, gammarid species often dominate littoral communities, particularly in rivers and streams (Marchant & Hynes 1981; Dangles *et al.* 2004), with densities of up to 8,500 individuals m^{-2} (Gee 1988).

Within two decades, the amphipod fauna of Europe have changed substantially following the introduction of non-native amphipod species that replaced many resident amphipod species, including previously successful invaders (Jażdżewski 1980; Bij de Vaate *et al.* 2002). The two formerly common native amphipod species *Gammarus fossa-*

rum and *G. pulex* are nowadays completely displaced by invasive species in the main channel of the Rhine and in its navigable tributaries (Leuven *et al.* 2009). However, while shifts in species compositions are well documented (e.g., Jażdżewski 1980; Bij de Vaate & Klink 1995; Dick & Platvoet 2000; Bij de Vaate *et al.* 2002; Josens *et al.* 2005; Leuven *et al.* 2009; Chen *et al.* 2012b; Chen *et al.* 2012a; Altermatt *et al.* 2016), consequences for ecosystem functions are still rarely considered (Piscart *et al.* 2011). Therefore, I conducted a comprehensive study that provides a broad overview on leaf litter consumption by the most common native and invasive amphipod species in Central Europe. I asked if invasive amphipods are able to maintain the ecosystem service of their native relatives which they replaced in the main channel of the river Rhine. The results showed that invasive amphipods are not able to maintain ecological functions of native amphipods (Jourdan *et al.* 2016b). Therefore, the species turnover observed in recent decades is likely accompanied by alterations in energy-flow dynamics and ecosystem functioning.

The results inspired me to conceive a follow-up study in cooperation with the Aquatic Ecotoxicology group at Goethe University of Frankfurt. The study (Jeetun 2016) investigated the impact of pollutants on leaf litter decomposition by native and invasive species to gain further insights into the factors that might affect leaf consumption rates and promote community structuring in Central European stream ecosystems. Treated sewage water is usually released into aquatic ecosystems and consists of a complex mixture of chemicals like pharmaceuticals and personal care products (Daughton & Ternes 1999). The river Rhine is heavily affected by human activities and suffers from a series of environmental modifications, including the discharge of treated sewage water (Leuven *et al.* 2009; Früh *et al.* 2012; Schneider *et al.* 2015). The study used treated sewage water from a sewage water treatment plant located within the Rhine drainage (for location details see Schneider *et al.* 2015) and the same experimental setup as used in Jourdan et al. (2016b). The negative effect of treated sewage water on leaf litter consumption is already reported for native *G. fossarum* (Bundschuh *et al.* 2011). However, comparable studies on invasive amphipods were so far missing. It was predicted that treated sewage water affects sensitive amphipods like *G. fossarum* most (Meijering 1991), followed by *G. pulex* (Meijering 1991), while tolerant, invasive *D. villosus* should be less affected (Grabowski *et al.* 2007; Maazouzi *et al.* 2011). This could mitigate the effect of reduced consumption rates of invasive species observed previously when I used artificial ADaM medium (Klüttgen *et al.* 1994) to maintain the test subjects (Jourdan *et al.* 2016b). The results, however, suggest that a short term exposure to treated sewage water did not affect the consumption rates of both, native and invasive amphipods, while the species-effect was robust with strongly reduced leaf consumption of invasive *D. villosus* (Jeetun 2016).

Altogether the results highlight the far-reaching consequences of biological invasions and the importance of studies focusing on functional consequences of biological invasions. Those studies are not only vital for basic ecological understanding, but also for biodiversity conservation, including restoration and maintenance of stream ecosystem services (Palmer *et al.* 1997; Walsh *et al.* 2016). In my experiments invasive amphipods failed to maintain the shredding activity of native amphipods. Within this context, the question arises if other taxonomic groups can compensate and maintain this function. Diverse communities tend to provide more stable ecosystem services than less diverse communities (Kremen 2005; Cardinale *et al.* 2012), and a compensatory community response may maintain ecosystem functions after the loss of certain taxonomic groups (for details see Discussion in Jourdan *et al.* 2016b). Long-term investigations on the trophic ecology (including stable isotope and gut content analyses, as well as feeding experiments) of a broader range of native and introduced aquatic taxa in the Rhine drainage would provide valuable insights into the temporal dynamics of possible compensatory mechanisms of leaf litter decomposition.

Manuscript 4: Behavioral differences between native and invasive species

Given the variability in the success of species introductions, a major objective of invasion biology is to identify the traits that accurately predict the success of an invader (e.g., Kolar & Lodge 2001). Successful invasive species might be those with traits that allow them to quickly reach high population densities (e.g., r-type life-histories; Sakai *et al.* 2001). Besides that, invaders are predicted to show certain behavioral types, like high dispersal tendencies (Chapple *et al.* 2012). Dispersers additionally tend to be, on average, more aggressive (Duckworth & Badyaev 2007) and less sociable than residents (Cote *et al.* 2010). A study on the famous example of invasive Argentine ant (*Linepithema humile*) shows another trend: Tsutsui *et al.* (2000) found reduced intraspecific aggression among spatially separate nests, which leads to the formation of massive 'supercolonies', while native populations exhibit pronounced intraspecific aggression. The authors interpreted altered intraspecific aggressiveness as a result of a genetic bottleneck (Tsutsui *et al.* 2000; Suarez & Tsutsui 2008). This exemplifies how behavior influences the success of species introductions. Previous studies often focused on species characteristics that might explain differences between invasive and non-invasive species (e.g., Rehage & Sih 2004), however, populations of invasive species likely represent a non-random sample of the initial native population, selected on the basis of interindividual variation in multiple traits (Cote *et al.* 2010; Canestrelli *et al.* 2016). A new approach considered this aspect by focusing on individual-level behavioral variation, which has become a key target of research on animal behavior in recent years (Sih *et al.* 2004; Réale *et al.* 2007; Cote *et al.* 2010). Therefore, investigating the role of individual behavioral traits might help to elucidate the mechanisms underlying the success of invaders.

The study presented in this subchapter (Bierbach *et al.* 2016) focused again on the amphipod community in the heavily invaded river Rhine (Van Riel *et al.* 2006; Leuven *et al.* 2009; 2.3). Individual swimming activity of individuals from four different amphipod species was repeatedly measured over the course of five days. The results indicated that invasive *D. villosus* exhibited no higher overall levels of activity compared to the three non-invasive species, which is in accordance with previous observations (Maazouzi *et al.* 2011). However, the non-invasive species, on average, changed their behavior predictably over the course of the experiment, while *D. villosus* did not exhibit any consistent change in activity. This individual activity variation in *D. villosus* might help this successful invader to cope with new and unpredictable environmental conditions encountered in the river Rhine system (Bierbach *et al.* 2016).

1.2.2 Natural biological invasions

Manuscript 5: Distribution and phenotypic trait divergence along environmental gradients

In this subchapter, I compared distribution patterns of two closely related, congeneric mosquitofishes (genus *Gambusia*) within the south Mexican Rìo Grijalva basin (Jourdan *et al.* submitted-a). The genus *Gambusia* belongs to the family of Poeciliidae (Rauchenberger 1989; Wischnath 1993), that was initially restricted to South America (Hrbek *et al.* 2007). Hrbek *et al.* (2007) predicted multiple colonization events of poeciliids to Middle America followed by a mix of vicariance and dispersal events. The main colonization of Middle and North America most likely occurred by a second invasion of Central and North America via the Aves land bridge thought the Greater Antilles. Whether species dispersed over connected land masses from the Antilles to Central America, or across salt water, is not known. The current distribution of species within the genus *Gambusia* might reflect more recent colonization events, including the hypothesized dispersal of some species into the Greater Antilles 13.49 Mya ago (95% CI 12.06– 15.19 Mya; Hrbek *et al.* 2007). However, the Caribbean basin is considered one of the most geologically complex regions in the world (Lydeard *et al.* 1995a). Understanding the historical dispersal of the genus *Gambusia* is therefore difficult to predict.

In the study presented here (Jourdan *et al.* submitted-a), I focused on the Río Grijalva basin in southern Mexico which is inhabited by two mosquitofishes, the teardrop mosquitofish (*Gambusia sexradiata*) and the Yucatan gambusia (*G. yucatana*). Previous studies on Mexican and Belizean ichthyofauna suggest that *G. yucatana* may occur more in coastal waters; however, the species is occasionally also found in inland waters (Greenfield *et al.* 1982; Miller *et al.* 2005), while the opposite pattern was reported for *G. sexradiata* (Carter 1981; Greenfield *et al.* 1982; Rauchenberger 1989). This distribution pattern is reflected by different salinity tolerances: *G. sexradiata* exhibits a lower tolerance to sea water compared to *G. yucatana* (Carter 1981), while *G. yucatana* is even known from some marine habitats (Carter 1981; Greenfield *et al.* 1982). *G. yucatana* is a member of the *Gambusia puncticulata*-species group, the members of which are distributed across the Greater Antilles (Rauchenberger 1989; Lydeard *et al.* 1995b; Lydeard *et al.* 1995a). Given its tolerance to sea water (Carter 1981), it is well conceivable that the species invaded Mexican watersheds through marine long-distance dispersal, e.g., during hurricanes (Gillespie *et al.* 2012). Such a close Caribbean–Central American relationship of freshwater biota also becomes evident for other groups of organisms, e.g., freshwater crabs (Rodríguez 1986). *Gambusia sexradiata* is part of the *Gambusia nobilis*-species group, all extant members of which occur on the North American mainland. *Gambusia sexradiata* is the only member with a wide distribution range, and it is the only member of the clade that is not entirely restricted to freshwater environments (Rauchenberger 1989; Lydeard *et al.* 1995a). Altogether, the evolutionary histories of both species suggest a competitive advantage of *G. yucatana* at coastal sites, while *G. sexradiata* should be better adapted to inland conditions.

I expected to find a zonation of *G. sexradiata* and *G. yucatana* along the investigated stream gradient, but instead, I found a patchy occurrence, with syntopic occurrences at only few sites. *Gambusia sexradiata* and *G. yucatana* are adapted to slow-flowing or stagnant conditions (Rauchenberger 1989; Miller *et al.* 2005) and are probably more affected by floods compared to co-occurring species like *Poecilia mexicana* and *Astyanax aeneus*, both of which are adapted to higher stream velocities (Miller *et al.* 2005; Plath *et al.* 2010). Contrary to other river systems of Mexico, damming projects were not as extensive in the Grijalva basin, and so the stream still experiences regular catastrophic flooding (Hudson *et al.* 2005). This may partly determine the seemingly stochastic distribution of both species along the river gradient, since massive dislocations during floods leave few individuals at a given site and subsequently allow either species to build up local populations again.

I focused on intraspecific trait divergence along the stream gradient with gradual variation of various selection factors. The results exemplified that even closely related, congeneric species can respond in unique ways to the same components of the river gradient, e.g., in traits like fat content or body shape. These responses in potentially fitness-related traits like fat content may still allude to site-specific ecological advantages of either species along the examined stretch of the Río Grijalva, which suggested *G. yucatana* might be better adapted to coastal regions relative to *G. sexradiata*. I conducted this study in a river drainage that has, until now, not been affected much by damming projects (Hudson *et al.* 2005), which have the potential to affect the evolutionary trajectories of populations (Franssen 2011; Franssen *et al.* 2013) and distribution patterns of species (Bunn & Arthington 2002). My study demonstrates complex patterns of microevolutionary phenotypic diversification that are threatened by the widespread practice to produce hydroelectric power from stream impoundments (Bunn & Arthington 2002).

Manuscript 6: The natural invasion of extreme habitats

Fluctuating environmental conditions might explain stochastic species distribution patterns in some parts of the Grijalva basin, however, the basin also offers habitats with stable, but extreme environmental conditions. Extreme habitats are characterized by adverse abiotic conditions, with chemical and/or physical stressors that are outside the range usually experienced by an organism (Rothschild & Mancinelli 2001; Tobler *et al.*

2015). Most often, extreme environments are inhabited by microbial taxa, whereby they have evolved a diversity of adaptations to cope with harsh conditions (Skirnisdottir *et al.* 2000; Rothschild & Mancinelli 2001). However, despite rough environmental conditions also some invertebrate and vertebrate species invaded those habitats (Rothschild & Mancinelli 2001). A unique example of an extreme habitat can be found in southern Mexico: the Cueva del Azufre cave system (Gordon & Rosen 1962; Hose & Pisarowicz 1999; Parzefall 2001; Tobler *et al.* 2006; Fig. 1). This cave ecosystem faces two abiotic stressors: darkness and hydrogen sulfide (H_2S). H_2S competes with oxygen in the respiratory chain and is highly toxic to most metazoans in micro-molar concentrations and further leads to extreme hypoxia in the water (Evans 1967; Bagarinao 1992). The Cueva del Azufre is the only known chemoautotrophic cave ecosystem which is inhabited by a vertebrate species (Plath & Tobler 2010) and provides a unique study system to investigate the adaptation to the simultaneous action of two strong selective forces (permanent darkness and H_2S).

In this subchapter I focused on the Atlantic molly (*Poecilia mexicana*; Fig. 1C, D), a teleost species that naturally invaded this cave ecosystem and adapted to vastly divergent ecological conditions inside the Cueva del Azufre (Gordon & Rosen 1962; Parzefall 2001; Jourdan *et al.* 2014b). In general, the reason for the colonization of caves is viewed as either accidental (entrapment or refugium) or as actively advantageous. These advantages include, for instance, environmental stability, protection from predation, or the exploitation of new niches (Romero & Green 2005). The colonization of this extreme habitat, probably happened no longer than few hundred thousand years ago (Pfenninger *et al.* 2014). Previous studies in the Cueva del Azufre have demonstrated that divergent natural selection due to darkness and H_2S drives evolutionary diversification in a number of traits. For example, the cave dwelling population shows energetically costly behavioral adaptations, like aquatic surface respiration (Plath *et al.* 2007a), while reducing energetically costly male sexual behavior and size-dependent alternative mating tactics (Plath *et al.* 2003; Plath 2008). Furthermore, physiological- (e.g., adaptations in the cytochrome-c oxidase complex; Pfenninger *et al.* 2014), morphological- (e.g., increased head size and number of taste buds; Parzefall 2001; Tobler *et al.* 2008a) and life-history adaptations (e.g., increased offspring size; Riesch *et al.* 2010) were described in this system. However, while various adaptations were known, basic ecological data on population characteristics was not yet available. Therefore, in this subchapter, I provide basic ecological data on the endemic cave molly population, including population densities, size class compositions and use of different microhabitats.

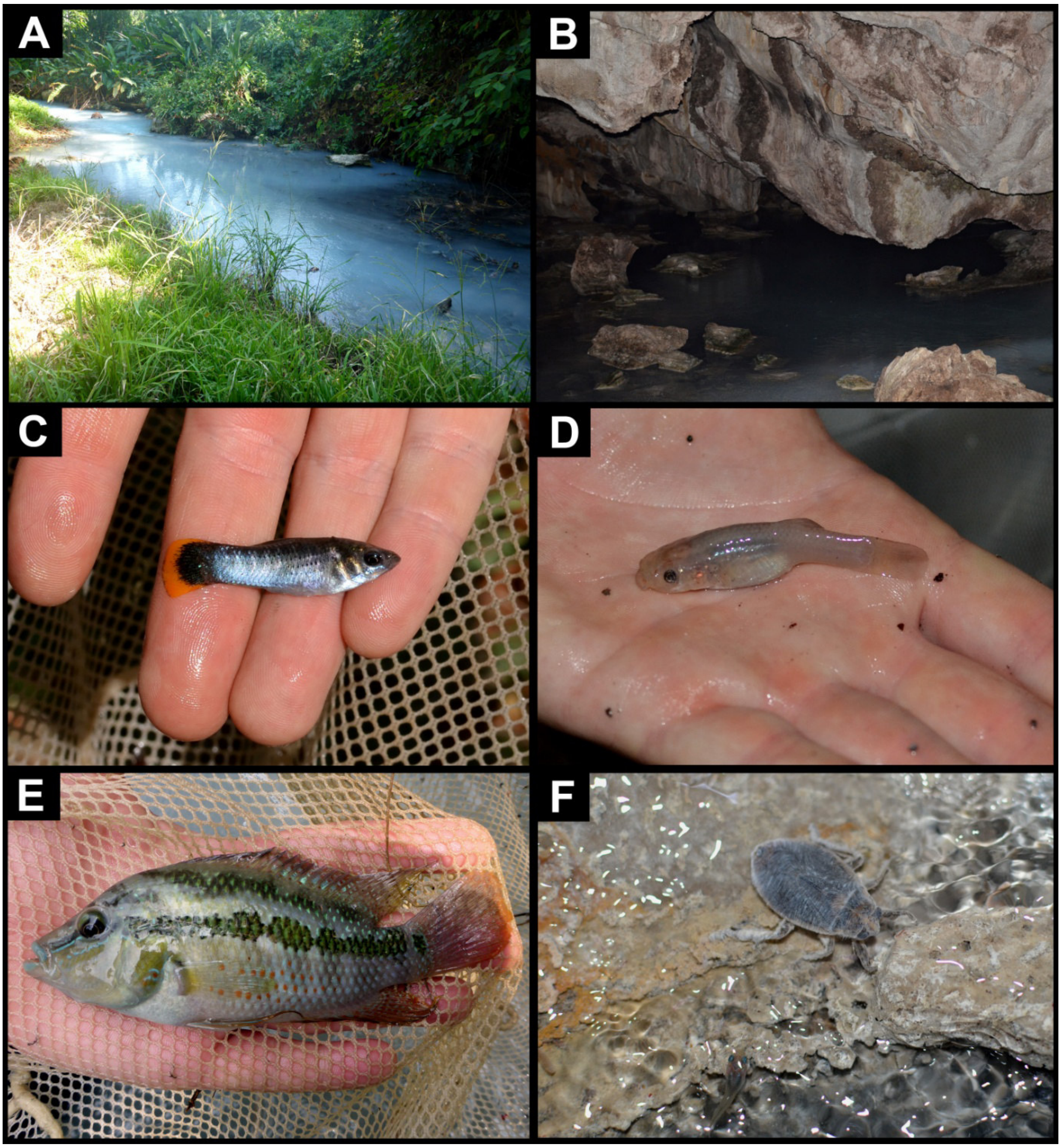

Figure 1: (**A**) The El Azufre is a sulfidic surface creek that forms the outflow of the (**B**) Cueva del Azufre, a nutrient-rich cave with high levels of H_2S and extreme hypoxia in the water. (**C**) A successful colonizer of the sulfidic El Azufre is the poeciliid fish *Poecilia mexicana*. Large, dominant males of the El Azufre population show orange margins of the dorsal and anal fins. (**D**) In contrast, the population of *P. mexicana* inhabiting the Cueva del Azufre is characterized by reduced pigmentation and eye size (shown is a female from chamber X). (**E**) While the El Azufre contains predatory fishes like '*Chiclasoma' salvini*, predatory fishes are lacking in the cave (Tobler *et al.* 2006). (**F**) Aquatic water bugs of the genus *Belostoma* act as one of the top predators in the subterranean food web, preying on the abundant *P. mexicana* (Tobler *et al.* 2007; Plath *et al.* 2011).

I found high overall densities in the cave and highest densities at the middle part of the cave with more than 200 individuals per square meter. These sites have lower H_2S concentrations compared to the inner parts where most large sulfide sources are located but they are annually exposed to a religious harvesting ceremony of local Zoque people called La Pesca (Hose & Pisarowicz 1999; Tobler *et al.* 2011). The indigenous Zoque people introduce barbasco, a fish toxicant, to harvest the endemic cave fish. Despite strong annual disturbance and harvesting overall fish densities were high, especially at sites annually exposed to the ritual. I found a marked shift in size/age compositions towards an overabundance of smaller, juvenile fish at those sites. I discuss these findings in relation to several environmental gradients within the cave (i.e., differences in toxicity and lighting conditions), but I also tentatively argue that the annual fish harvest during the La Pesca locally diminishes competition (and possibly, cannibalism by large adults), which might be followed by a phase of overcompensation of fish densities. This idea received support, for instance, from empirical studies on laboratory populations of the poeciliid fish *Heterandria formosa,* which showed that biomass of the juvenile size class increased in response to intermediate adult mortality rates (Schröder et al. 2009). Therefore, future studies should conduct a sampling before and after La Pesca in order to demonstrate the influence of the ritual. While human influences on highly endemic, locally adapted populations generally are to be evaluated as highly problematic, management plans for cave mollies ought to consider the important role La Pesca plays in the religion of the local human population.

Manuscript 7: Evolution of constructive traits in extreme habitats

The colonization of cave habitats is a classic example of regressive evolution (Jeffery 2001; Jeffery 2009). All cavefish species share some degree of eye loss and pigmentation reduction and usually evolved constructive changes, like enhanced feeding abilities (e.g., jaws, barbells or taste buds) and a mechanosensory system of cranial neuromasts (Jeffery 2001).

The Atlantic molly (*Poecilia mexicana*) colonized at least two cave systems, the Cueva del Azufre (see above) and the much smaller, non-sulfidic Cueva Luna Azufre (Tobler *et al.* 2008a; Tobler *et al.* 2008b). While 'regressive' evolutionary processes (eye and pigment reduction; Fig. 1D) are already well documented in both populations (e.g., Parzefall 2001; Plath *et al.* 2007b; Tobler *et al.* 2008a; Plath & Tobler 2010; Eifert *et al.* 2015), the evolution of 'constructive traits' (like improved non-visual senses of cave fishes) has received less attention in the scientific literature. I therefore focused on the ability to detect chemical cues from conspecifics in two surface- and two cave-dwelling populations of *P. mexicana* (Jourdan *et al.* submitted-b). The results of the study suggest that only the Cueva del Azufre cave population, but not the Luna Azufre population, has evolved improved chemoreception. This points towards independent evolutionary trajectories in both cave populations. The absence of an evolutionary response to selection imposed by perpetual darkness in the Luna Azufre population could be a consequence of lower evolvability due to a small population size (see Willi *et al.* 2006), or possibly due to a more recent invasion of the cave, compared to the Cueva del Azufre cave population. The results highlight the potential of cave-adapted *P. mexicana* for future investigations into the mechanisms involved in improved olfaction in cave fishes.

1.3 Further research prospects

Combined investigations across native and introduced distribution ranges

As outlined so far, natural and human-induced biological invasions have some basic mechanisms in common, e.g., by which invasive species evolve in response to their new biotic and abiotic environment. A constraint of many studies on invasive species is that they focus solely on the invasive range. However, when studying human-induced biological invasions, it is advisable to also consider similar investigations across the species' native range, in order to better understand their historical origin, and predict their current human-induced spread more accurately.

I started to investigate the evolutionary ecology of the ongoing invasion of eastern mosquitofish (*Gambusia holbrooki*) in Europe, with known native source populations. In an integrative approach, I focused on adaptive trait divergence in morphological and life-history traits, as well as on population genetic differentiation along south-north gradients in the species' natural range in the USA and in the invasive range in Europe, to uncover the relative contributions of preadaptation versus contemporary evolutionary change (experimental protocols followed those outlined in Jourdan *et al.* submitted-a).

The native range of the eastern mosquitofish ranges from southern Florida along the Atlantic coastal drainages to New Jersey (Rivas 1963; Rauchenberger 1989), where they colonize fresh- and brackish-water habitats (Nordlie & Mirandi 1996). Therefore, *G. holbrooki* is – together with its sister species *G. affinis* – the most northern occurring member of the family Poeciliidae.

Mosquitofishes (in fact the two closely related species *G. holbrooki* and *G. affinis*) have been intentionally introduced across the globe for malaria prophylaxis some 80 to 100 years ago, due to the common misconception that they are more efficient predators of mosquito larvae than native species (see Pyke 2008 for a review). Since the introduction of *G. holbrooki* to southern Spain in the 1920s (Krumholz 1948; Vidal et al. 2010) it has rapidly spread throughout most of southern Europe (Krumholz 1948; Garcia-Berthou *et al.* 2005; Grapputo *et al.* 2006; Beaudouin *et al.* 2008; Vidal *et al.* 2010; Sanz *et al.* 2013; personal observation; Fig. 2). Given its current presence in northern France, *G. holbrooki* might soon also invade Central Europe, since non-native species may fare better than native species in a warming climate (Dukes & Mooney 1999; Bellard *et al.* 2013).

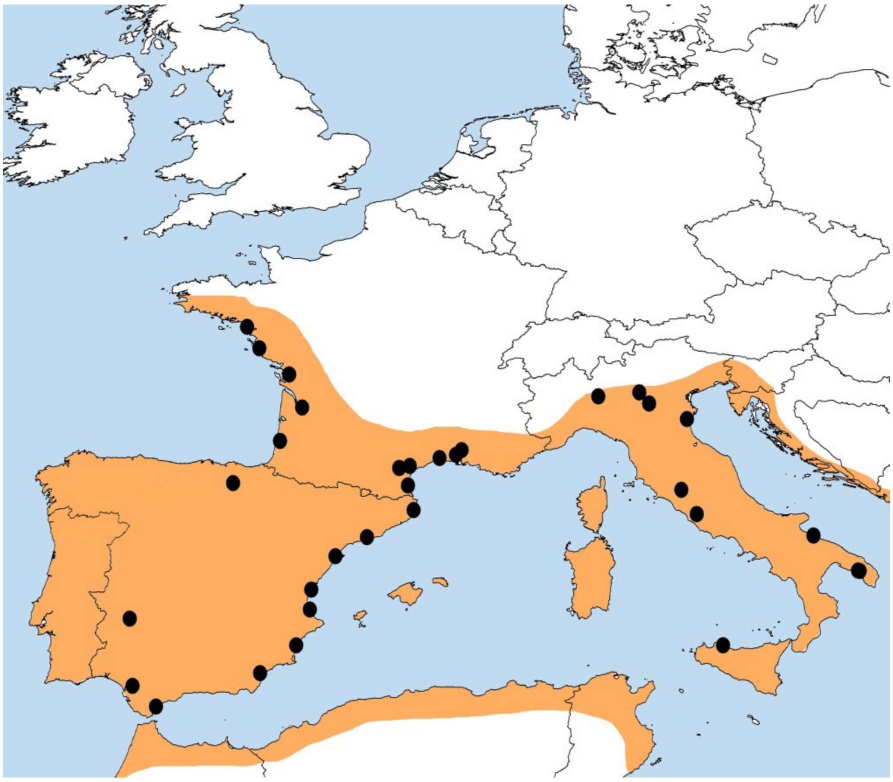

FIGURE 2: Current distribution range of *Gambusia holbrooki* in Europe (orange), according to Froese & Pauly (2016) and personal observations, as well as sampling sites at which *G. holbrooki* was collected for this study in 2013 (black circles).

Collectively, *G. holbrooki* and *G. affinis* are among the most invasive fish world-wide and are currently considered as one of the 100 most detrimental invasive species (Lowe *et al.* 2000). Their negative impact on local faunas stems partially from their carnivorous life style, and besides the desired effect on mosquito larvae, indigenous fish and amphibian larvae often decline after the introduction of mosquitofish (Barrier & Hicks 1994; Morgan & Buttemer 1996; Remon *et al.* 2016). In southern Europe, introduced *G. holbrooki* were thus considered to play a key role in freshwater ecosystems (e.g., Díez-del-Molino *et al.* 2016) by competing and displacing primarily endemic fishes like the Spanish toothcarp, *Aphanius iberus*, and the Valencia toothcarp, *Valencia hispanica* (Rincon *et al.* 2002; Caiola & Sostoa 2005; Alcaraz & García-Berthou 2007; Alcaraz *et al.* 2008; Carmona-Catot *et al.* 2013).

Beyond their negative effects on native fauna, however, invasive mosquitofish provide a unique opportunity to investigate climate-driven intra-specific diversification via local adaptation and phenotypic plasticity. I collected *G. holbrooki* from 32 sites across southern Europe, ranging from coastal, brackish lagoons to large alpine lakes in northern Italy (Fig. 2). The current, widespread distribution in Europe highlights the remarkable adaptability to different climatic conditions in its novel distribution range.

To explain the geographic patterns of *G. holbrooki's* invasion in Europe and to augment our understanding of the species' potential for further invasions and range expansions, I investigated genetic diversity and mechanisms of evolutionary change. First results confirmed previous assumptions as one region in the USA could be identified as the most likely origin of European mosquitofish populations. Furthermore, reduced genetic diversity was found in invasive European populations compared to native populations, which is, however, contrary to previous studies, as they did not found reduced genetic diversity of European populations (Díez-del-Molino *et al.* 2013; Sanz *et al.* 2013). Nevertheless, despite the detected genetic bottleneck, they were able to adapt to a wide range of habitats and climatic conditions in Europe.

Previous investigations across native populations of *G. holbooki* found higher genetic diversity in southern populations, with a gradual decrease towards northern populations (Sanz *et al.* 2013; Riesch *et al.* in prep.). These findings might reflect patterns of the species' natural history, indicating a postglacial colonization of northern distribution sites from source- or refuge populations in Florida. Interestingly, the European populations appear to have their origin in the more northern native distribution range (Krumholz 1948; Sanz *et al.* 2013), which might have been more affected by the last glacial retreat (or has even been colonized after the last glacial retreat). Therefore, the species probably acquired its potential to invade new territories already by evolutionary changes in these populations as a response to new environmental conditions during the post-glacial northward expansion (Díez-del-Molino *et al.* 2013). In addition, peripheral populations in general often display greater adaptation to abiotic stressors allowing persistence in unfavorable and unstable environments (Hardie & Hutchings 2010).

My first results on life-history divergence suggest that invasive populations show differentiations comparable to populations across their native distribution range. Common garden reared descendants of wild-caught invasive *G. holbrooki* will reveal if those adaptations are the results of contemporary evolutionary processes (i.e. selection on allelic variation in the founder population or on variation arising from *de novo* mutations) or phenotypic plasticity. The latter may be a key mechanism in biological invasions as it allows rapid adaptive phenotypic divergence (Ghalambor *et al.* 2007), to which the source populations might be selected for under fluctuating environmental conditions. This could explain the fast spread of mosquitofish across Europe. This study will therefore provide promising insights into both, past patterns of range expansions as well as the causes and consequences of current human-induced changes in species' ranges.

1.4 Concluding remarks

The current distribution of biota on a worldwide scale is the consequence of historical fluctuations in climate and tectonic relationships as well as current anthropogenic global change. In general, biological invasions are a fundamentally natural process resulting from climatic, geotectonic or other natural events and have always been a substantial component of species interactions (Vermeij 1991; Lodge 1993). However, compared to natural invasions, the rate of human-induced biological invasions today exceeds any rate ever seen in the past. This can be attributed for the most to increased international trade across continents facilitated by the construction of canals and highways, as well as frequent dispersal of species along large distances in planes or in ship ballast water. Invasions are further promoted by the interplay of other components of anthropogenic global change, like climate change and habitat deterioration (Vitousek *et al.* 1996; Dukes & Mooney 1999; Sala *et al.* 2000; Hulme 2009). Before the Age of Discoveries in the 15th century, dispersal of organisms across great biogeographic barriers was a rare event, while today it is highly common and geographically widespread (Mooney & Cleland 2001; Hulme 2009). We lack detailed information on the impacts of historical biological invasions, especially on time frames of less than millions of years, and analytical limitations in the temporal resolution of biotic interchange do not permit us to clearly understand the mechanisms of exchange and species establishment (Mooney & Cleland 2001).

Therefore, beside their severe negative effects, the establishment of invasive species and subsequent rapid range expansion provide unprecedented conditions (a 'natural experiment') to investigate basic principles in ecology and evolution, such as the relationship of adaptation, divergence, and speciation under natural conditions and in contemporary time scales (Kolar & Lodge 2001; Strayer *et al.* 2006). The investigation of invasive species provides us with new general insights into the question of how new species are integrated into existing ecosystems, what factors determine the geographical range of species, the relative roles of preadaptation versus rapid adaptive trait shifts during the early stages of range expansion, or how selective regimes are altered by the presence of congeners, and whether changes in species compositions affect ecosystem processes. On the other hand, we also need to consider the evolutionary history of a given taxon to understand and predict current, human-induced species invasions.

The case studies which form my thesis provide general insights into how species respond to novel abiotic and biotic environmental conditions. Overall, preadaptation ― including adaptive phenotypic plasticity (Gotthard & Nylin 1995; Ghalambor *et al.* 2007; see below) ― might be a key factor for a species to successfully persist when exposed to novel abiotic conditions and biotic interactions. For example, invasive guppies (2.1) have a tropical origin and are able to withstand new environmental conditions only under unusual climatic conditions. In this unique study system stable abiotic conditions are provided by the effluents of the power plant that ensure the establishment of a self-sustaining population of guppies and other tropical fishes. Additionally, introduced guppies were most probably descendants of ornamental fishes that were selected for withstanding temporarily harsh conditions during aquarium rearing and trading. Preadaptation is also a prerequisite for a prey organism to persist when facing a novel predator. As outlined in subchapter 2.2, the lack of a response of prey organisms might explain the local decrease of many species due to predation by invasive species. However, long term investigations will have to reveal if the prey organisms are able to adapt to the new biological interaction in response to the novel selection pressure. This could be due to rapid evolutionary responses or local extirpation followed by an invasion of other subpopulations that might be better (pre-)adapted to local predation regimes. Trophic preadaptation might also explain the success of invasive amphipods, like *Dikerogammarus villosus* and *Echinogammarus ischnus*, in the large European rivers, as they most probably originate from downstream regions and might be adapted to these conditions. This could also explain why we are currently observing population establishments only in downstream regions, whereas tributaries and upstream regions were mostly not invaded by invasive amphipods (e.g., Chen *et al.* 2012b; Altermatt *et al.* 2016). The results presented in subchapter 2.3 show that the progressive shift in basic food resources along the river continuum may be a delimiting factor for further range expansions, since native amphipods are better able to exploit the major energy source (leaf litter) in upstream regions, where they might out-compete invading taxa. Finally, increased activity is a behavioral trait that might be favorable for an invasive species when invading new territories. The results presented in subchapter 2.4 illustrate how an invasive species (the invasive amphipod *D. villosus*) can show pronounced differences in activity levels compared to native amphipods, with higher and largely unpredictable variation in activity levels found in the invasive but not in native amphipods, which might help this successful invader to cope with new environmental conditions.

Investigations of natural systems reveal insights into natural dispersal and adaptation to long-term exposure to altered ecological conditions, including abiotic stressors and biotic interactions among species with a co-evolutionary history. For example, the results of phenotypic trait divergence presented in subchapter 3.1 highlight the complex response of organisms to multiple selective agents along complex environmental gradients that drive adaptive diversification in two closely related species. This study exemplifies that even closely related, congeneric species can respond in unique ways to the same components of a river gradient, which might arise from different evolutionary histories. While one species (in this case *G. yucatana*) seems to be more competitive under stable environmental conditions at coastal sites, the other species (*G. sexradiata*) might be able to withstand more fluctuating environmental conditions. Furthermore, subchapters 3.2 and 3.3 deal with systems in which species are locally adapted to unique and stable (non-fluctuating) abiotic and biotic conditions. The investigated cave ecosystem provides a stable environment, where the loss of visual orientation favored the evolution of constructive traits that allow orientation and communication in absence of visual information, while phenotypic plasticity is predicted to be reduced or even lost under stable conditions (Gilbert & Epel 2009). However, even this example indicates that preadaptation might play an important role for the colonization of the cave, as livebearing fishes should be favored under harsh abiotic conditions due to parental protection of offspring during gestation (physiological homeostasis) and active microhabitat choice of pregnant females. Therefore, it is not surprising that many fishes which have invaded extreme habitats, such as sulfide-toxic freshwaters, are livebearers (Riesch *et al.* 2015). Finally, first insights into the natural and human-induced invasion history of *G. holbrooki* indicate the influence of historical selective agents on current invasion success. This highlights the potential of bridging knowledge about the natural invasion history of a given species with current human-induced invasion patterns and will give important insights into the mechanisms that result in the spread of invasive species.

In all cases, adaptive phenotypic plasticity (i.e. beneficial plasticity that is maintained by selection) might be an important component of preadaptation. Phenotypic plasticity describes the phenomenon that a given genotype can produce different phenotypes in response to fluctuation in the biotic or abiotic environment (Agrawal 2001). The continuous range of potential phenotypes produced by a genotype within a certain range of environmental conditions can be described as its reaction norm (Gilbert & Epel 2009). Phenotypic plasticity itself is a trait that can evolve in response to natural selection, resulting in a change of the reaction norm between the ancestral and derived populations or species (Gotthard & Nylin 1995). Empirical evidence comes from amphibians and fishes that show different reaction norm between ecotypes and species exposed to different selection pressures (e.g., Haugen & Vøllestad 2000; Morey & Reznick 2004; Gomez-Mestre & Buchholz 2006; Ledon-Rettig *et al.* 2008). However, until now, studies comparing reaction norms between invasive populations and their native sources are scarce. Most available studies considered plants, suggesting that invasive species have greater phenotypic plasticity than co-occurring non-invasive species (Davidson *et al.* 2011).

Future investigations on invasive animals promise to provide additional, important insights into the role of phenotypic plasticity as they might affect ecological success and evolutionary divergence in the new habitat. The eastern mosquitofish, *Gambusia holbrooki*, would be an ideal model organism for the comparison of reaction norms. Different populations could be used to study the phenotypic response to environmental conditions, for example, long term established populations from Florida that might have a reduced reaction norm, compared to populations at the range margin in New Jersey. Populations from New Jersey, however, should have similar reaction norms as seen in invasive populations in Europe. Moreover, within the last 100 years of invasion history reaction norms might already have changed, for example, because of drift or costs associated with maintaining plasticity when it is not favored by natural selection (e.g., Auld *et al.* 2009). As this could already be the case in European populations, different reaction norms might be observed at the invasion front in northern France compared to long-term established populations in southern Spain. To further expand this approach, relatives without a history of human-induced invasion like *G. sexradiata* or *G. yucatana* (see 3.1) could be included in the study, as they have a different evolutionary history and a smaller distribution range, which might result in the overall narrowest reaction norms. A target of such investigations could be the age and size at maturity as a response to different food supply or different temperature regimes. Population encountering a heterogeneous environment with respect to factors affecting body growth (i.e. populations from New Jersey or northern France) should evolve a broader reaction norm for the maturation age compared to species with smaller distribution ranges like *G. sexradiata*/ *G. yucatana* or populations of *G. holbrooki* from Florida.

In times during which humans profoundly alter the structure and functioning of natural ecosystems, understanding and predicting the consequences of biological invasions is urgently needed to develop appropriate conservation strategies, including methods to mitigate and manage human-induced biological invasions. The studies presented here provide basic information on how native and invasive species respond to abiotic and biotic factors and will contribute to a better understanding of human-induced species invasions. Furthermore my results suggest to consider the evolutionary history of an invasive species as well as the geographical history of a given ecosystem, as dimensions of species invasions will be different on each continent and for different taxonomic groups, partly depending on the biotic interactions which biota experienced in their recent evolutionary history (Marshall 1988; Courchamp *et al.* 2003; Webb 2006). This will help to predict consequences and the future success of the invader. However, human-induced biological invasions are irreversible in almost all cases and prevention of species invasions should be the main goal of all management strategies (Mack *et al.* 2000; Simberloff 2013; Simberloff *et al.* 2013). Therefore, conservation strategies should develop and implement integrated approaches to control human vectors of dispersal (Ricciardi 2006). This, however seems to be challenging, as pathways by which species were transferred are diverse and dynamic over time, influenced by trends in human trade and transport (Hulme 2009). An additional important factor is the prevention of habitat modification. Human activities are increasingly disturbing habitats and it is widely recognized that biological invasions are more likely to occur in anthropogenically altered environments (e.g., Lozon & MacIsaac 1997; Bunn & Arthington 2002; Kennard *et al.* 2005). This problem was formulated by Lee and Gelembiuk (2008), who emphasized that 'with increasing alterations to the environment, we are likely to create a world of invaders, adapted to ongoing disturbance' (Lee & Gelembiuk 2008, p. 441).

1.5 References

Abrams, P. A. (2000). The evolution of predator-prey interactions: theory and evidence. *Annual Review of Ecology and Systematics* **31**:79-105

Agrawal, A. A. (2001). Phenotypic plasticity in the interactions and evolution of species. *Science* **294**:321-326

Alcaraz, C. & García-Berthou, E. (2007). Food of an endangered cyprinodont (*Aphanius iberus*): ontogenetic diet shift and prey electivity. *Environmental Biology of Fishes* **78**:193-207

Alcaraz, C., Bisazza, A. & García-Berthou, E. (2008). Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish. *Oecologia* **155**:205-213

Allan, J. D. & Flecker, A. S. (1993). Biodiversity conservation in running waters. *Bioscience* **43**:32-43

Allendorf, F. W. & Lundquist, L. L. (2003). Introduction: Population biology, evolution, and control of invasive species. *Conservation Biology* **17**:24-30. doi: 10.1046/j.1523-1739.2003.02365.x

Altermatt, F., Alther, R. & Mächler, E. (2016). Spatial patterns of genetic diversity, community composition and occurrence of native and non-native amphipods in naturally replicated tributary streams. *BMC Ecology* **16**

Arendt, W. J. (1988). Range Expansion of the Cattle Egret (*Bubulcus ibis*) in the Greater Caribbean Basin. *Colonial Waterbirds* **11**:252-262. doi: 10.2307/1521007

Auld, J. R., Agrawal, A. A. & Relyea, R. A. (2009). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proceedings of the Royal Society of London B Biological Sciences* **277**:503-511

Bagarinao, T. (1992). Sulfide as an environmental factor and toxicant: tolerance and adaptations in aquatic organisms. *Aquatic Toxicology* **24**:21-62. doi: 10.1016/0166-445x(92)90015-f

Balirwa, J. S., Chapman, C. A., Chapman, L. J., Cowx, I. G., Geheb, K., Kaufman, L., Lowe-McConnell, R. H., Seehausen, O., Wanink, J. H. & Welcomme, R. L. (2003). Biodiversity and fishery sustainability in the Lake Victoria basin: an unexpected marriage? *Bioscience* **53**:703-715

Barrier, R. & Hicks, B. J. (1994). Behavioral interactions between black mudfish (*Neochanna diversus* Stokell, 1949: Galaxiidae) and mosquitofish (*Gambusia affinis* Baird & Girard, 1854). *Ecology of Freshwater Fish* **3**:93-99

Barrios-Garcia, M. N. & Ballari, S. A. (2012). Impact of wild boar (*Sus scrofa*) in its introduced and native range: a review. *Biological Invasions* **14**:2283-2300

Beaudouin, R., Ginot, V. & Monod, G. (2008). Growth characteristics of eastern mosquitofish *Gambusia holbrooki* in a northern habitat (Brittany, France). *Journal of Fish Biology* **73**:2468-2484. doi: 10.1111/j.1095-8649.2008.02101.x

Beketov, M. A. & Liess, M. (2007). Predation risk perception and food scarcity induce alterations of life-cycle traits of the mosquito *Culex pipiens*. *Ecological Entomology* **32**:405-410

Bellard, C., Thuiller, W., Leroy, B., Genovesi, P., Bakkenes, M. & Courchamp, F. (2013). Will climate change promote future invasions? *Global Change Biology* **19**:3740-3748

Benard, M. F. (2004). Predator-induced phenotypic plasticity in organisms with complex life histories. *Annual Review of Ecology and Systematics* **35**:651-673

Bierbach, D., Oster, S., Jourdan, J., Arias-Rodriguez, L., Krause, J., Wilson, A. M. & Plath, M. (2014). Social network analysis resolves temporal dynamics of male dominance relationships. *Behavioral Ecology and Sociobiology* **68**:935-345. doi: 10.1007/s00265-014-1706-y

Bierbach, D., Laskowski, K. L., Brandt, A.-L., Chen, W., Jourdan, J., Streit, B. & Plath, M. (2016). Highly variable, unpredictable activity patterns in invasive, but not native amphipod species. *Aquatic Ecology* **50**:261-271

Bij de Vaate, A. & Klink, A. G. (1995). *Dikerogammarus villosus* Sowinsky (Crustacea: Gammaridae) a new immigrant in the Dutch part of the Lower Rhine. *Lauterbornia* **20**:51-54

Bij de Vaate, A., Jazdzewski, K., Ketelaars, H. A., 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 Sciences* **59**:1159-1174

Bundschuh, M., Zubrod, J. P. & Schulz, R. (2011). The functional and physiological status of *Gammarus fossarum* (Crustacea; Amphipoda) exposed to secondary treated wastewater. *Environmental Pollution* **159**:244-249

Bunn, S. E. & Arthington, A. H. (2002). Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental Management* **30**:492-507

Butchart, S. H., Walpole, M., Collen, B., Van Strien, A., Scharlemann, J. P., Almond, R. E., Baillie, J. E., Bomhard, B., Brown, C. & Bruno, J. (2010). Global biodiversity: indicators of recent declines. *Science* **328**:1164-1168

Caiola, N. & Sostoa, A. d. (2005). Possible reasons for the decline of two native toothcarps in the Iberian Peninsula: evidence of competition with the introduced Eastern mosquitofish. *Journal of Applied Ichthyology* **21**:358-363

Canestrelli, D., Bisconti, R. & Carere, C. (2016). Bolder Takes All? The Behavioral Dimension of Biogeography. *Trends in Ecology & Evolution* **31**:35-43

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., Narwani, A., Mace, G. M., Tilman, D. & Wardle, D. A. (2012). Biodiversity loss and its impact on humanity. *Nature* **486**:59-67

Carlsson, N. O. & Lacoursière, J. O. (2005). Herbivory on aquatic vascular plants by the introduced golden apple snail (*Pomacea canaliculata*) in Lao PDR. *Biological Invasions* **7**:233-241

Carmona-Catot, G., Magellan, K. & García-Berthou, E. (2013). Temperature-specific competition between invasive mosquitofish and an endangered cyprinodontid fish. *PLoS One* **8**:e54734

Carter, H. J. (1981). Aspects of the physiological ecology of species of *Gambusia* from Belize, Central America. *Copeia* **1981**:694-700

Chapin, F. S. I., Zavaleta, E. S., Eviner, V. T., Naylor, R. L., Vitousek, P. M., Reynolds, H. L., Hooper, D. U., Lavorel, S., Sala, O. E. & Hobbie, S. E. (2000). Consequences of changing biodiversity. *Nature* **405**:234-242

Chapple, D. G., Simmonds, S. M. & Wong, B. B. (2012). Can behavioral and personality traits influence the success of unintentional species introductions? *Trends in Ecology & Evolution* **27**:57-64

Chen, W., Lipkowski, K., Jourdan, J., Plath, M., Streit, B. & Klaus, S. (2012a). *Dikerogammarus haemobaphes* in the River Lahn, Germany, with notes on the dynamics of Amphipoda assemblages. *Lauterbornia* **75**:87-93

Chen, W., Bierbach, D., Plath, M., Streit, B. & Klaus, S. (2012b). Distribution of amphipod communities in the Middle to Upper Rhine and five of its tributaries. *BioInvasions Records* **1**:263-271

Cohen, A. N. & Carlton, J. T. (1998). Accelerating invasion rate in a highly invaded estuary. *Science* **279**:555-558

Cote, J., Fogarty, S., Weinersmith, K., Brodin, T. & Sih, A. (2010). Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). *Proceedings of the Royal Society of London B: Biological Sciences* **277**:1574-1579

Courchamp, F., Chapuis, J.-L. & Pascal, M. (2003). Mammal invaders on islands: impact, control and control impact. *Biological reviews* **78**:347-383. doi: 10.1017/s1464793102006061

Crooks, J. A. (2002). Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos* **97**:153-166

Crosby, G. T. (1972). Spread of the cattle egret in the Western Hemisphere. *Bird-Banding* **43**:205-212

Crowl, T. A. & Covich, A. P. (1990). Predator-induced life-history shifts in a freshwater snail. *Science* **247**:949-951

Cuevas, M. F., Novillo, A., Campos, C., Dacar, M. A. & Ojeda, R. A. (2010). Food habits and impact of rooting behaviour of the invasive wild boar, *Sus scrofa*, in a protected area of the Monte Desert, Argentina. *Journal of Arid Environments* **74**:1582-1585

Cummins, K. W. & Klug, M. J. (1979). Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics* **10**:147-172

Dangles, O., Gessner, M. O., Guerold, F. & Chauvet, E. (2004). Impacts of stream acidification on litter breakdown: implications for assessing ecosystem functioning. *Journal of Applied Ecology* **41**:365-378

Daughton, C. G. & Ternes, T. A. (1999). Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environmental Health Perspectives* **107**:907-938

Davidson, A. M., Jennions, M. & Nicotra, A. B. (2011). Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters* **14**:419-431

Dick, J. T. & Platvoet, D. (2000). Invading predatory crustacean *Dikerogammarus villosus* eliminates both native and exotic species. *Proceedings of the Royal Society of London B Biological Sciences* **267**:977-983

Dièguez-Uribeondo, J. & Söderhäll, K. (1993). *Procambarus clarkii* Girard as a vector for the crayfish plague fungus, *Aphanomyces astaci* Schikora. *Aquaculture Research* **24**:761-765

Díez-del-Molino, D., Carmona-Catot, G., Araguas, R.-M., Vidal, O., Sanz, N., García-Berthou, E. & García-Marín, J.-L. (2013). Gene flow and maintenance of genetic diversity in invasive mosquitofish (*Gambusia holbrooki*). *PLoS One* **8**:e82501

Díez-del-Molino, D., Araguas, R.-M., Vera, M., Vidal, O., Sanz, N. & García-Marín, J.-L. (2016). Temporal genetic dynamics among mosquitofish (*Gambusia holbrooki*) populations in invaded watersheds. *Biological Invasions* **18**:841-855

Dixon, S. & Baker, R. (1988). Effects of size on predation risk, behavioural response to fish, and cost of reduced feeding in larval *Ischnura verticalis* (Coenagrionidae: Odonata). *Oecologia* **76**:200-205

Dodson, S. (1989). Predator-induced reaction norms. *Bioscience* **39**:447-452

Drury Jr, W. H., Morgan, A. H. & Stackpole, R. (1953). Occurrence of an African Cattle Egret (*Ardeola ibis ibis*) in Massachusetts. *The Auk* **70**:364-365

Duckworth, R. A. & Badyaev, A. V. (2007). Coupling of dispersal and aggression facilitates the rapid range expansion of a passerine bird. *Proceedings of the National Academy of Sciences* **104**:15017-15022. doi: 10.1073/pnas.0706174104

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., Naiman, R. J., Prieur-Richard, A.-H., Soto, D. & Stiassny, M. L. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological reviews* **81**:163-182

Dukes, J. S. & Mooney, H. A. (1999). Does global change increase the success of biological invaders? *Trends in Ecology & Evolution* **14**:135-139

Edsman, L., Füreder, L., Gherardi, F. & Souty-Grosset, C. (2010). *Astacus astacus*. The IUCN Red List of Threatened Species 2010: e.T2191A9338388. http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T2191A9338388.en. Accessed: 01-05-2016.

Eifert, C., Farnworth, M., Schulz-Mirbach, T., Riesch, R., Bierbach, D., Klaus, S., Wurster, A., Tobler, M., Streit, B. & Indy, J. (2015). Brain size variation in extremophile fish: local adaptation versus phenotypic plasticity. *Journal of Zoology* **295**:143-153

Emde, S., Kochmann, J., Kuhn, T., Dörge, D. D., Plath, M., Miesen, F. W. & Klimpel, S. (2015). Cooling water of power plant creates "hot spots" for tropical fishes and parasites. *Parasitology Research* **115**:85-98. doi: 10.1007/s00436-015-4724-4

Evans, C. L. (1967). The toxicity of hydrogen sulphide and other sulphides. *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences* **52**:231-248

Flecker, A. S. (1992). Fish predation and the evolution of invertebrate drift periodicity: evidence from neotropical streams. *Ecology* **73**:438-448

Franssen, N. R. (2011). Anthropogenic habitat alteration induces rapid morphological divergence in a native stream fish. *Evolutionary Applications* **4**:791-804

Franssen, N. R., Harris, J., Clark, S. R., Schaefer, J. F. & Stewart, L. K. (2013). Shared and unique morphological responses of stream fishes to anthropogenic habitat alteration. *Proceedings of the Royal Society B-Biological Sciences* **280**. doi: 10.1098/rspb.2012.2715

Froese, R. & Pauly, D. (2016). FishBase. World Wide Web electronic publication. www.fishbase.org, version (01/2016).

Früh, D., Stoll, S. & Haase, P. (2012). Physicochemical and morphological degradation of stream and river habitats increases invasion risk. *Biological Invasions* **14**:2243-2253

Fujio, Y., Nakajima, M. & Nagahama, Y. (1990). Detection of a low temperature-resistant gene in the guppy (*Poecilia reticulata*), with reference to sex-linked inheritance. *Japanese Journal of Genetics* **65**:201-207

Futuyma, D. J. (2005). *Evolution*. Sinauer Associates, Inc., Sunderland, USA

Galil, B. S., Nehring, S. & Panov, V. (2007) Waterways as invasion highways–Impact of climate change and globalization. In: Nentwig, W. (ed) *Biological Invasions*. Springer, Berlin, pp 59-74

Garcia-Berthou, E., Alcaraz, C., Pou-Rovira, Q., Zamora, L., Coenders, G. & Feo, C. (2005). Introduction pathways and establishment rates of invasive aquatic species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences* **62**:453-463. doi: 10.1139/f05-017

Gee, J. H. (1988). Population dynamics and morphometries of *Gammarus pulex* L.: evidence of seasonal food limitation in a freshwater detritivore. *Freshwater Biology* **19**:333-343

Gessner, M. O., Chauvet, E. & Dobson, M. (1999). A perspective on leaf litter breakdown in streams. *Oikos* **85**:377-384

Ghalambor, C. K., McKay, J. K., Carroll, S. P. & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* **21**:394-407

Gilbert, S. F. & Epel, D. (2009). *Ecological Developmental Biology: Integrating Epigenetics, Medicine, and Evolution*. Sinauer Associates, Sunderland, MA, USA.

Gillespie, R. G., Baldwin, B. G., Waters, J. M., Fraser, C. I., Nikula, R. & Roderick, G. K. (2012). Long-distance dispersal: a framework for hypothesis testing. *Trends in Ecology & Evolution* **27**:47-56

Goldschmidt, T. (1998). *Darwin's dreampond: Drama in Lake Victoria*. Massachusetts Institute of Technology Press, Cambridge, Massachusetts, USA.

Gollasch, S. (2007) Is ballast water a major dispersal mechanism for marine organisms? In: Nentwing, W. (ed) *Biological Invasions*. Springer, Berlin, pp 49-57

Gomez-Mestre, I. & Buchholz, D. R. (2006). Developmental plasticity mirrors differences among taxa in spadefoot toads linking plasticity and diversity. *Proceedings of the National Academy of Sciences* **103**:19021-19026

Gordon, M. S. & Rosen, D. E. (1962). A cavernicolous form of the poeciliid fish *Poecilia sphenop*s from Tabasco, Mexico. *Copeia* **2**:360-368

Gotthard, K. & Nylin, S. (1995). Adaptive plasticity and plasticity as an adaptation: a selective review of plasticity in animal morphology and life history. *Oikos* **74**:3-17

Gozlan, R., Britton, J., Cowx, I. & Copp, G. (2010). Current knowledge on non-native freshwater fish introductions. *Journal of Fish Biology* **76**:751-786

Grabowski, M., Bacela, K. & Konopacka, A. (2007). How to be an invasive gammarid (Amphipoda: Gammaroidea)–comparison of life history traits. *Hydrobiologia* **590**:75-84

Graça, M. A. S. (2001). The role of invertebrates on leaf litter decomposition in streams–a review. *International Review of Hydrobiology* **86**:383-393

Graça, M. A. S., Ferreira, V., Canhoto, C., Encalada, A. C., Guerrero-Bolaño, F., Wantzen, K. M. & Boyero, L. (2015). A conceptual model of litter breakdown in low order streams. *International Review of Hydrobiology* **100**:1-12. doi: 10.1002/iroh.201401757

Grapputo, A., Bisazza, A. & Pilastro, A. (2006). Invasion success despite reduction of genetic diversity in the European populations of eastern mosquitofish (*Gambusia holbrooki*). *Italian Journal of Zoology* **73**:67-73. doi: 10.1080/11250000500502111

Gratwicke, B. & Marshall, B. (2001). The relationship between the exotic predators *Micropterus salmoides* and *Serranochromis robustus* and native stream fishes in Zimbabwe. *Journal of Fish Biology* **58**:68-75

Greenfield, D. W., Greenfield, T. A. & Wildrick, D. M. (1982). The taxonomy and distribution of the species of *Gambusia* (Pisces: Poeciliidae) in Belize, Central America. *Copeia* **1982**:128-147

Gurnell, J., Wauters, L. A., Lurz, P. W. & 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

Hardie, D. C. & Hutchings, J. A. (2010). Evolutionary ecology at the extremes of species' ranges. *Environmental Reviews* **18**:1-20

Haugen, T. & Vøllestad, L. (2000). Population differences in early life-history traits in grayling. *Journal of Evolutionary Biology* **13**:897-905

Havel, J. E., Kovalenko, K. E., Thomaz, S. M., Amalfitano, S. & Kats, L. B. (2015). Aquatic invasive species: challenges for the future. *Hydrobiologia* **750**:147-170

Hayes, K. R. & Barry, S. C. (2008). Are there any consistent predictors of invasion success? *Biological Invasions* **10**:483-506

Hebert, P. D. & Grewe, P. M. (1985). Chaoborus-induced shifts in the morphology of *Daphnia ambigua*. *Limnol. Oceanogr* **30**:1291-1297

Hecky, R., Mugidde, R., Ramlal, P., Talbot, M. & Kling, G. (2010). Multiple stressors cause rapid ecosystem change in Lake Victoria. *Freshwater Biology* **55**:19-42

Hendry, A., Kelly, M., Kinnison, M. & Reznick, D. (2006). Parallel evolution of the sexes? Effects of predation and habitat features on the size and shape of wild guppies. *Journal of Evolutionary Biology* **19**:741-754

Höfer, S. & Staas, S. (1998) Bericht zur fischereibiologischen Untersuchug des Gillbaches im Bereich Bergheim-Auenheim (Okt/Nov. 1998). Zoologisches Institut der Universität zu Köln, Abt. Allgemeine Ökologie und Limnologie, Köln

Holway, D. A., Lach, L., Suarez, A. V., Tsutsui, N. D. & Case, T. J. (2002). The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics* **33**:181-233

Hose, L. D. & Pisarowicz, J. A. (1999). Cueva de Villa Luz, Tabasco, Mexico: Reconnaissance study of an active sulfur spring cave and ecosystem. *Journal of Cave and Karst Studies* **61**:13-21

Hrbek, T., Seckinger, J. & Meyer, A. (2007). A phylogenetic and biogeographic perspective on the evolution of poeciliid fishes. *Molecular Phylogenetics and Evolution* **43**:986-998

Hudina, S., Galić, N., Roessink, I. & Hock, K. (2011). Competitive interactions between co-occurring invaders: identifying asymmetries between two invasive crayfish species. *Biological Invasions* **13**:1791-1803

Hudson, P. H., Hendrickson, D. A., Benke, A. C., Varela-Romero, A., Rodiles-Hernández, R. & Minckley, W. L. (2005) Rivers of Mexico. In: Benke, A. C. & Cushing, C. E. (eds) *Rivers of North America*. Elsevier, Massachusetts, pp 1031–1085

Hughes, N. (1986). Changes in the feeding biology of the Nile perch, *Lates niloticus* (L.)(Pisces: Centropomidae), in Lake Victoria, East Africa since its introduction in 1960, and its impact on the native fish community of the Nyanza Gulf. *Journal of Fish Biology* **29**:541-548

Hulme, P. E. (2009). Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology* **46**:10-18

IUCN (2000). IUCN guidelines for the prevention of biodiversity loss caused by alien invasive species. *Gland: SSC Invasive Species Specialist Group, IUCN*

Jażdżewski, K. (1980). Range extensions of some gammaridean species in European inland waters caused by human activity. *Crustaceana. Supplement* **6**:84-107

Jeetun, N. (2016) Einfluss von Klärwasser auf funktionelle, physiologische und morphologische Merkmale einheimischer und invasiver Flohkrebse (Amphipoda: Gammaridae) M.Sc.-Thesis, Johann Wolfgang Goethe-Universität, Frankfurt am Main angefertigt am Institut für Ökologie und Evolution des Fachbereichs 15

Jeffery, W. R. (2001). Cavefish as a model system in evolutionary developmental biology. *Developmental Biology* **231**:1-12. doi: 10.1006/dbio.2000.0121

Jeffery, W. R. (2009). Regressive evolution in *Astyanax* cavefish. *Annual Review of Genetics* **43**:25

Jeschke, J. M. & Strayer, D. L. (2005). Invasion success of vertebrates in Europe and North America. *Proceedings of the National Academy of Sciences of the United States of America* **102**:7198-7202

Jones, H. P., Tershy, B. R., Zavaleta, E. S., Croll, D. A., Keitt, B. S., Finkelstein, M. E. & Howald, G. R. (2008). Severity of the effects of invasive rats on seabirds: a global review. *Conservation Biology* **22**:16-26

Josens, G., de Bij Vaate, A., Usseglio-Polatera, P., Cammaerts, R., Cherot, F., Grisez, F., Verboonen, P. & Bossche, J.-P. V. (2005). Native and exotic Amphipoda and other Peracarida in the River Meuse: new assemblages emerge from a fast changing fauna. *Hydrobiologia* **542**:203-220

Jourdan, J., Miesen, F. W., Zimmer, C., Gasch, K., Herder, F., Schleucher, E., Plath, M. & Bierbach, D. (2014a). On the natural history of an introduced population of guppies (*Poecilia reticulata* Peters, 1859) in Germany. *BioInvasions Records* **3**:175–184. doi: 10.3391/bir.2014.3.3.07

Jourdan, J., Bierbach, D., Riesch, R., Schießl, A., Wigh, A., Arias-Rodriguez, L., Indy, J. R., Klaus, S., Zimmer, C. & Plath, M. (2014b). Microhabitat use, population densities, and size distributions of sulfur cave-dwelling *Poecilia mexicana*. *PeerJ* **2**:e490. doi: 10.7717/peerj.490

Jourdan, J., Baier, J., Riesch, R., Klimpel, S., Streit, B., Müller, R. & Plath, M. (2016a). Adaptive growth reduction in response to fish kairomones allows mosquito larvae (*Culex pipiens*) to reduce predation risk. *Aquatic Sciences* **78**:303-314

Jourdan, J., Westerwald, B., Kiechle, A., Chen, W., Streit, B., Klaus, S., Oetken, M. & Plath, M. (2016b). Pronounced species turnover, but no functional equivalence in leaf consumption of invasive amphipods in the river Rhine. *Biological Invasions* **18**:763-774. doi: 10.1007/s10530-015-1046-5

Jourdan, J., Krause, S., Lazar, V. M., Zimmer, C., Sommer-Trembo, C., Arias-Rodriguez, L., Klaus, S., Riesch, R. & Plath, M. (submitted-a). Shared and unique patterns of phenotypic diversification along a stream gradient in two congeneric species.

Jourdan, J., Jordan, M., Zimmer, C., Eifert, C., Arias-Rodriguez, L. & Plath, M. (submitted-b). Response to conspecific chemical cues in surface- and cave-dwelling populations of Atlantic mollies, *Poecilia mexicana*.

Kelley, J. L. & Magurran, A. E. (2003). Effects of relaxed predation pressure on visual predator recognition in the guppy. *Behavioral Ecology and Sociobiology* **54**:225-232. doi: 10.1007/s00265-003-0621-4

Kempkes, M. (2005). Neues von den "rheinischen". *Die Aquarien- and Terrarienzeitschrift, DATZ* **09/2005**:64-68

Kempkes, M. (2010). *Die Guppys Band 1*. Westarp Wissenschaften-Verlagsgesellschaft mbH, Hohenwarsleben

Kennard, M. J., Arthington, A., Pusey, B. J. & Harch, B. (2005). Are alien fish a reliable indicator of river health? *Freshwater Biology* **50**:174-193

Kesavaraju, B. & Juliano, S. (2004). Differential behavioral responses to water-borne cues to predation in two container-dwelling mosquitoes. *Annals of the Entomological Society of America* **97**:194

Kesavaraju, B., Alto, B. W., Lounibos, L. P. & Juliano, S. A. (2007). Behavioural responses of larval container mosquitoes to a size-selective predator. *Ecological Entomology* **32**:262-272

Klotz, W., Miesen, F. W., Hüllen, S. & Herder, F. (2013). Two Asian fresh water shrimp species found in a thermally polluted stream system in North Rhine-Westphalia, Germany. *Aquatic Invasions* **8**:333-339

Klüttgen, B., Dülmer, U., Engels, M. & Ratte, H. (1994). ADaM, an artificial freshwater for the culture of zooplankton. *Water Research* **28**:743-746

Kolar, C. S. & Lodge, D. M. (2001). Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution* **16**:199-204

Kölner-Stadt-Anzeiger (2015) http://www.ksta.de/region/rhein-erft/bergheim/--sote-bergheim-stilllegung-kraftwerksbloecke-22980674, Accessed: 25-05-2016

Kremen, C. (2005). Managing ecosystem services: what do we need to know about their ecology? *Ecology Letters* **8**:468-479

Krueger, D. A. & Dodson, S. I. (1981). Embryological induction and predation ecology in *Daphnia pulex*. *Limnol. Oceanogr* **26**:219-223

Krumholz, L. A. (1948). Reproduction in the Western Mosquitofish, *Gambusia affinis affinis* (Baird & Girard) and its use in mosquito control. *Ecological Monographs* **18**:1-43. doi: 10.2307/1948627

Langerhans, R. B., Layman, C. A., Shokrollahi, A. & DeWitt, T. J. (2004). Predator-driven phenotypic diversification in *Gambusia affinis*. *Evolution* **58**:2305-2318

Ledon-Rettig, C. C., Pfennig, D. W. & Nascone-Yoder, N. (2008). Ancestral variation and the potential for genetic accommodation in larval amphibians: implications for the evolution of novel feeding strategies. *Evolution & development* **10**:316-325

Lee, C. E. & Gelembiuk, G. W. (2008). Evolutionary origins of invasive populations. *Evolutionary Applications* **1**:427-448

Lees, A. C. & Bell, D. J. (2008). A conservation paradox for the 21st century: the European wild rabbit *Oryctolagus cuniculus*, an invasive alien and an endangered native species. *Mammal Review* **38**:304-320

Leuven, R. S., van der Velde, G., Baijens, I., Snijders, J., van der Zwart, C., Lenders, H. R. & bij de Vaate, A. (2009). The river Rhine: a global highway for dispersal of aquatic invasive species. *Biological Invasions* **11**:1989-2008

Lima, S. L. & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* **68**:619-640

Lodge, D. M. (1993). Biological invasions: lessons for ecology. *Trends in Ecology & Evolution* **8**:133-137

Lowe, S., Browne, M. & Boudjelas, S. (2000). *100 of the World's Worst Invasive Alien Species: a Selection from the Global Invasive Species Database*. Invasive Species Specialist Group Auckland, New Zealand

Lozon, J. D. & MacIsaac, H. J. (1997). Biological invasions: are they dependent on disturbance? *Environmental Reviews* **5**:131-144

Lydeard, C., Wooten, M. C. & Meyer, A. (1995a). Molecules, morphology, and area cladograms: a cladistic and biogeographic analysis of *Gambusia* (Teleostei: Poeciliidae). *Systematic Biology* **44**:221-236

Lydeard, C., Wooten, M. C. & Meyer, A. (1995b). Cytochrome b sequence variation and a molecular phylogeny of the live-bearing fish genus *Gambusia* (Cyprinodontiformes: Poeciliidae). *Canadian Journal of Zoology* **73**:213-227

Maazouzi, C., Piscart, C., Legier, F. & Hervant, F. (2011). Ecophysiological responses to temperature of the “killer shrimp” *Dikerogammarus villosus*: is the invader really stronger than the native *Gammarus pulex*? *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **159**:268-274

Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M. & Bazzaz, F. A. (2000). Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* **10**:689-710. doi: 10.2307/2641039

MacNeil, C., Dick, J. T. & Elwood, R. W. (1997). The trophic ecology of freshwater *Gammarus* spp.(Crustacea: Amphipoda): problems and perspectives concerning the functional feeding group concept. *Biological Reviews of the Cambridge Philosophical Society* **72**:349-364

Marchant, R. & Hynes, H. (1981). The distribution and production of *Gammarus pseudolimnaeus* (Crustacea: Amphipoda) along a reach of the Credit River, Ontario. *Freshwater Biology* **11**:169-182

Marshall, L. G., Webb, S. D., Sepkoski, J. J. & Raup, D. M. (1982). Mammalian evolution and the great American interchange. *Science* **215**:1351-1357

Marshall, L. G. (1988). Land mammals and the great American interchange. *American Scientist* **76**:380-388

Massa, C., Doyle, M. & Fortunato, R. C. (2014). On how Cattle Egret (*Bubulcus ibis*) spread to the Americas: meteorological tools to assess probable colonization trajectories. *International Journal of Biometeorology* **58**:1879-1891

Meijering, M. P. (1991). Lack of oxygen and low pH as limiting factors for *Gammarus* in Hessian brooks and rivers. *Hydrobiologia* **223**:159-169

Miller, R., Minckley, W. & Norris, S. (2005). Freshwater fishes of Mexico. *University of Chicago, Chicago*

Mills, L. S., Soulé, M. E. & Doak, D. F. (1993). The keystone-species concept in ecology and conservation. *Bioscience* **43**:219-224

Mooney, H. A. & Cleland, E. E. (2001). The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences* **98**:5446-5451

Morey, S. R. & Reznick, D. N. (2004). The relationship between habitat permanence and larval development in California spadefoot toads: field and laboratory comparisons of developmental plasticity. *Oikos* **104**:172-190

Morgan, L. & Buttemer, W. (1996). Predation by the non-native fish *Gambusia holbrooki* on small *Litoria aurea* and *L. dentata* tadpoles. *Australian Zoologist* **30**:143-149

Nentwig, W. (2007a) Pathways in animal invasions. In: Nentwig, W. (ed) *Biological Invasions*. Springer, Berlin, pp 11-27

Nentwig, W. (2007b) Biological Invasions: why it matters. In: Nentwig, W. (ed) *Biological Invasions*. Springer, Berlin, pp 1-6

Nordlie, F. & Mirandi, A. (1996). Salinity relationships in a freshwater population of eastern mosquitofish. *Journal of Fish Biology* **49**:1226-1232

Okubo, A., Maini, P., Williamson, M. & Murray, J. (1989). On the spatial spread of the grey squirrel in Britain. *Proceedings of the Royal Society of London B: Biological Sciences* **238**:113-125

Padilla, D. K. & Williams, S. L. (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

Palmer, M. A., Ambrose, R. F. & Poff, N. L. (1997). Ecological theory and community restoration ecology. *Restoration Ecology* **5**:291-300

Panov, V. E., Alexandrov, B., Arbačiauskas, K., Binimelis, R., Copp, G. H., Grabowski, M., Lucy, F., Leuven, R. S., Nehring, S. & Paunović, M. (2009). Assessing the risks of aquatic species invasions via European inland waterways: from concepts to environmental indicators. *Integrated Environmental Assessment and Management* **5**:110-126

Parzefall, J. (2001). A review of morphological and behavioural changes in the cave molly, *Poecilia mexicana*, from Tabasco, Mexico. *Environmental Biology of Fishes* **62**:263-275. doi: 10.1023/a:1011899817764

Pease, K. M. & Wayne, R. K. (2014). Divergent responses of exposed and naive Pacific tree frog tadpoles to invasive predatory crayfish. *Oecologia* **174**:241-252. doi: 10.1007/s00442-013-2745-1

Pfenninger, M., Lerp, H., Tobler, M., Passow, C., Kelley, J. L., Funke, E., Greshake, B., Erkoc, U. K., Berberich, T. & Plath, M. (2014). Parallel evolution of *cox* genes in H_2S-tolerant fish as key adaptation to a toxic environment. *Nature Communications* **5**:3873

Piazzini, S., Lori, E., Favilli, L., Cianfanelli, S., Vanni, S. & Manganelli, G. (2010). A tropical fish community in thermal waters of southern Tuscany. *Biological Invasions* **12**:2959-2965

Pimentel, D., Lach, L., Zuniga, R. & Morrison, D. (2000). Environmental and economic costs of nonindigenous species in the United States. *Bioscience* **50**:53-65

Piscart, C., Mermillod-Blondin, F., Maazouzi, C., Merigoux, S. & Marmonier, P. (2011). Potential impact of invasive amphipods on leaf litter recycling in aquatic ecosystems. *Biological Invasions* **13**:2861-2868

Plath, M., Parzefall, J. & Schlupp, I. (2003). The role of sexual harassment in cave and surface dwelling populations of the Atlantic molly, *Poecilia mexicana* (Poeciliidae, Teleostei). *Behavioral Ecology and Sociobiology* **54**:303-309

Plath, M., Tobler, M., Riesch, R., de León, F. J. G., Giere, O. & Schlupp, I. (2007a). Survival in an extreme habitat: the roles of behaviour and energy limitation. *Naturwissenschaften* **94**:991-996

Plath, M., Hauswaldt, J. S., Moll, K., Tobler, M., De Leon, F. J. G., Schlupp, I. & Tiedemann, R. (2007b). Local adaptation and pronounced genetic differentiation in an extremophile fish, *Poecilia mexicana*, inhabiting a Mexican cave with toxic hydrogen sulphide. *Molecular Ecology* **16**:967-976. doi: 10.1111/j.1365-294X.2006.03212.x

Plath, M. (2008). Male mating behavior and costs of sexual harassment for females in cavernicolous and extremophile populations of Atlantic mollies (*Poecilia mexicana*). *Behaviour* **145**:73-98

Plath, M. & Tobler, M. (2010) Subterranean fishes of Mexico (*Poecilia mexicana*, Poeciliidae). In: Trajano, E., Bichuette, M. E. & Kapoor, B. G. (eds) *The Biology of Subterranean Fishes*. Science Publishers, Enfield, New Hampshire, USA, pp 283-332

Plath, M., Hermann, B., Schroder, C., Riesch, R., Tobler, M., de Leon, F. J. G., Schlupp, I. & Tiedemann, R. (2010). Locally adapted fish populations maintain small-scale genetic differentiation despite perturbation by a catastrophic flood event. *BMC Evolutionary Biology* **10**:256. doi: 25610.1186/1471-2148-10-256

Plath, M., Riesch, R., Culumber, Z., Streit, B. & Tobler, M. (2011). Giant water bug (*Belostoma* sp.) predation on a cave fish (*Poecilia mexicana*): effects of female body size and gestational state. *Evolutionary Ecology Research* **13**:133-144

Platvoet, D., Dick, J. T., MacNeil, C., van Riel, M. C. & van der Velde, G. (2009). Invader–invader interactions in relation to environmental heterogeneity leads to zonation of two invasive amphipods, *Dikerogammarus villosus* (Sowinsky) and *Gammarus tigrinus* Sexton: amphipod pilot species project (AMPIS) report 6. *Biological Invasions* **11**:2085-2093

Porter, S. D. & Savignano, D. A. (1990). Invasion of polygyne fire ants decimates native ants and disrupts arthropod community. *Ecology* **71**:2095-2106

Power, M. E., Tilman, D., Estes, J. A., Menge, B. A., Bond, W. J., Mills, L. S., Daily, G., Castilla, J. C., Lubchenco, J. & Paine, R. T. (1996). Challenges in the quest for keystones. *Bioscience* **46**:609-620

Prenter, J., MacNeil, C., Dick, J. T. & Dunn, A. M. (2004). Roles of parasites in animal invasions. *Trends in Ecology & Evolution* **19**:385-390

Pringle, R. M. (2005). The origins of the Nile perch in Lake Victoria. *Bioscience* **55**:780-787

Pyke, G. H. (2008). Plague minnow or mosquito fish? A review of the biology and impacts of introduced *Gambusia* species. *Annual Review of Ecology Evolution and Systematics* **39**:171-191. doi: 10.1146/annurev.ecolsys.39.110707.173451

Rauchenberger, M. (1989). *Systematics and biogeography of the genus Gambusia (Cyprinodontiformes, Poecilidae)*. American Museum of Natural History

Réale, D., Reader, S. M., Sol, D., McDougall, P. T. & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological reviews* **82**:291-318

Rehage, J. S. & Sih, A. (2004). Dispersal behavior, boldness, and the link to invasiveness: a comparison of four *Gambusia* species. *Biological Invasions* **6**:379-391

Remon, J., Bower, D. S., Gaston, T. F., Clulow, J. & Mahony, M. J. (2016). Stable isotope analyses reveal predation on amphibians by a globally invasive fish (*Gambusia holbrooki*). *Aquatic Conservation: Marine and Freshwater Ecosystems*

Reznick, D. & Endler, J. A. (1982). The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* **36**:160-177. doi: 10.2307/2407978

Reznick, D. N. & Ghalambor, C. K. (2001). The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica* **112**:183-198

Ricciardi, A. & Rasmussen, J. B. (1999). Extinction rates of North American freshwater fauna. *Conservation Biology* **13**:1220-1222

Ricciardi, A. (2006). Patterns of invasion in the Laurentian Great Lakes in relation to changes in vector activity. *Diversity and Distributions* **12**:425-433

Richardson, D. M. (2011) Invasion science: the roads travelled and the roads ahead. In: Richardson, D. M. (ed) *Fifty years of invasion ecology: The legacy of Charles Elton*. Blackwell Publishing, pp 397-401

Richardson, D. M. & Ricciardi, A. (2013). Misleading criticisms of invasion science: a field guide. *Diversity and Distributions* **19**:1461-1467

Riesch, R., Plath, M. & Schlupp, I. (2010). Toxic hydrogen sulfide and dark caves: life-history adaptations in a livebearing fish (*Poecilia mexicana*, Poeciliidae). *Ecology* **91**:1494-1505. doi: 10.1890/09-1008.1

Riesch, R., Martin, R. A. & Langerhans, R. B. (2013). Predation's role in life-history evolution of a livebearing fish and a test of the Trexler-DeAngelis model of maternal provisioning. *American Naturalist* **181**:78-93

Riesch, R., Tobler, M. & Plath, M. (2015) Hydrogen Sulfide-Toxic Habitats. In: Riesch, R., Tobler, M. & Plath, M. (eds) *Extremophile Fishes*. Springer, pp 137-159

Riesch, R., Martin, R. A., Diamond, S. E., Jourdan, J., Plath, M. & Langerhans, R. B. (in prep.). Thermal regime drives a latitudinal gradient in morphology and life-history in a livebearing fish.

Riesch, R., Tobler, M., Lerp, H., Jourdan, J., Doumas, T., Nosil, P., Langerhans, R. B. & Plath, M. (in press). Extremophile Poeciliidae: multivariate insights into the complexity of speciation along replicated ecological gradients. *BMC Evolutionary Biology*

Rincon, P. A., Correas, A. M., Morcillo, F., Risueno, P. & Lobon-Cervia, J. (2002). Interaction between the introduced eastern mosquitofish and two autochthonous Spanish toothcarps. *Journal of Fish Biology* **61**:1560-1585. doi: 10.1006/jfbi.2002.2175

Rivas, L. R. (1963). Subgenera and species groups in the poeciliid fish genus *Gambusia* Poey. *Copeia* **1963**:331-347

Rodríguez, G. (1986). Centers of radiation of freshwater crabs in the Neotropics. *Crustacean Issues* **4**:51-67

Roman, J. & Darling, J. A. (2007). Paradox lost: genetic diversity and the success of aquatic invasions. *Trends in Ecology & Evolution* **22**:454-464

Romero, A. & Green, S. (2005). The end of regressive evolution: examining and interpreting the evidence from cave fishes. *Journal of Fish Biology* **67**:3-32

Rosen, D. E. (1975). A vicariance model of Caribbean biogeography. *Systematic Biology* **24**:431-464

Rothschild, L. J. & Mancinelli, R. L. (2001). Life in extreme environments. *Nature* **409**:1092-1101

Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., Baughman, S., Cabin, R. J., Cohen, J. E., Ellstrand, N. C., McCauley, D. E., O'Neil, P., Parker, I. M., Thompson, J. N. & Weller, S. G. (2001). The population biology of invasive species. *Annual Review of Ecology and Systematics* **32**:305-332. doi: 10.1146/annurev.ecolsys.32.081501.114037

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L. F., Jackson, R. B., Kinzig, A., Leemans, R., Lodge, D. M., Mooney, H. A., Oesterheld, M., Poff, N. L., Sykes, M. T., Walker, B. H., Walker, M. & Wall, D. H. (2000). Biodiversity - Global biodiversity scenarios for the year 2100. *Science* **287**:1770-1774. doi: 10.1126/science.287.5459.1770

Salo, P., Korpimäki, E., Banks, P. B., Nordström, M. & Dickman, C. R. (2007). Alien predators are more dangerous than native predators to prey populations. *Proceedings of the Royal Society of London B Biological Sciences* **274**:1237-1243

Sanz, N., Araguas, R. M., Vidal, O., Diez-del-Molino, D., Fernández-Cebrián, R. & García-Marín, J. L. (2013). Genetic characterization of the invasive mosquitofish (*Gambusia* spp.) introduced to Europe: population structure and colonization routes. *Biological Invasions* **15**:2333-2346. doi: 10.1007/s10530-013-0456-5

Schlaepfer, M. A., Sherman, P. W., Blossey, B. & Runge, M. C. (2005). Introduced species as evolutionary traps. *Ecology Letters* **8**:241-246

Schneider, I., Oehlmann, J. & Oetken, M. (2015). Impact of an estrogenic sewage treatment plant effluent on life-history traits of the freshwater amphipod *Gammarus pulex*. *Journal of Environmental Science and Health, Part A* **50**:272-281

Schröder, A., Persson, L. & de Roos, A. M. (2009). Culling experiments demonstrate size-class specific biomass increases with mortality. *Proceedings of the National Academy of Sciences of the United States of America* **106**:2671-2676. doi: 10.1073/pnas.0808279106

Seehausen, O., Witte, F., Katunzi, E. F., Smits, J. & Bouton, N. (1997). Patterns of the remnant cichlid fauna in southern Lake Victoria. *Conservation Biology* **11**:890-904

Seehausen, O. & van Alphen, J. J. (1998). The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex). *Behavioral Ecology and Sociobiology* **42**:1-8

Sih, A., Bell, A. & Johnson, J. C. (2004). Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution* **19**:372-378

Simberloff, D. (2013). *Invasive species: what everyone needs to know*. Oxford University Press

Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E. & Pascal, M. (2013). Impacts of biological invasions: what's what and the way forward. *Trends in Ecology & Evolution* **28**:58-66

Skirnisdottir, S., Hreggvidsson, G. O., Hjörleifsdottir, S., Marteinsson, V. T., Petursdottir, S. K., Holst, O. & Kristjansson, J. K. (2000). Influence of sulfide and temperature on species composition and community structure of hot spring microbial mats. *Applied and Environmental Microbiology* **66**:2835-2841

Sommer-Trembo, C., Zimmer, C., Jourdan, J., Bierbach, D. & Plath, M. (2016). Predator experience homogenizes consistent individual differences in predator avoidance. *Journal of Ethology* **34**:155-165

Specziar, A. (2004). Life history pattern and feeding ecology of the introduced eastern mosquitofish, *Gambusia holbrooki*, in a thermal spa under temperate climate, of

Lake Heviz, Hungary. *Hydrobiologia* **522**:249-260. doi: 10.1023/B:HYDR.0000029978.46013.d1

Strayer, D. L. (2006). Challenges for freshwater invertebrate conservation. *Journal of the North American Benthological Society* **25**:271-287

Strayer, D. L., Eviner, V. T., Jeschke, J. M. & Pace, M. L. (2006). Understanding the long-term effects of species invasions. *Trends in Ecology & Evolution* **21**:645-651

Strecker, A. L., Campbell, P. M. & Olden, J. D. (2011). The aquarium trade as an invasion pathway in the Pacific Northwest. *Fisheries* **36**:74-85

Suarez, A. V. & Tsutsui, N. D. (2008). The evolutionary consequences of biological invasions. *Molecular Ecology* **17**:351-360. doi: 10.1111/j.1365-294X.2007.03456.x

Swetnam, T. W., Allen, C. D. & Betancourt, J. L. (1999). Applied historical ecology: using the past to manage for the future. *Ecological Applications* **9**:1189-1206

Takamura, K. (2007). Performance as a fish predator of largemouth bass [*Micropterus salmoides* (Lacepede)] invading Japanese freshwaters: a review. *Ecological Research* **22**:940-946

Tobler, M., Schlupp, I., Heubel, K. U., Riesch, R., de Leon, F. J. G., Giere, O. & Plath, M. (2006). Life on the edge: hydrogen sulfide and the fish communities of a Mexican cave and surrounding waters. *Extremophiles* **10**:577-585. doi: 10.1007/s00792-006-0531-2

Tobler, M., Schlupp, I. & Plath, M. (2007). Predation of a cave fish (*Poecilia mexicana*, Poeciliidae) by a giant water-bug (*Belostoma*, Belostomatidae) in a Mexican sulphur cave. *Ecological Entomology* **32**:492-495. doi: 10.1111/j.1365-2311.2007.00892.x

Tobler, M., DeWitt, T. J., Schlupp, I., García de León, F. J., Herrmann, R., Feulner, P. G., Tiedemann, R. & Plath, M. (2008a). Toxic hydrogen sulfide and dark caves: phenotypic and genetic divergence across two abiotic environmental gradients in *Poecilia mexicana*. *Evolution* **62**:2643-2659. doi: 10.1111/j.1558-5646.2008.00466.x

Tobler, M., Riesch, R., de Leon, F. J. G., Schupp, I. & Plath, M. (2008b). A new and morphologically distinct population of cavernicolous *Poecilia mexicana* (Poeciliidae: Teleostei). *Environmental Biology of Fishes* **82**:101-108. doi: 10.1007/s10641-007-9258-x

Tobler, M., Culumber, Z., Plath, M., Winemiller, K. & Rosenthal, G. (2011). An indigenous religious ritual selects for resistance to a toxicant in a livebearing fish. *Biology Letters* **7**:229-232

Tobler, M., Riesch, R. & Plath, M. (2015) Extremophile Fishes: An Integrative Synthesis. In: Riesch, R., Tobler, M. & Plath, M. (eds) *Extremophile Fishes*. Springer, pp 279-296

Tsutsui, N. D., Suarez, A. V., Holway, D. A. & Case, T. J. (2000). Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences* **97**:5948-5953

Väinölä, R., Witt, J., Grabowski, M., Bradbury, J. H., Jazdzewski, K. & Sket, B. (2008). Global diversity of amphipods (Amphipoda; Crustacea) in freshwater. *Hydrobiologia* **595**:241-255

Van Riel, M., Van der Velde, G., Rajagopal, S., Marguillier, S., Dehairs, F. & Bij de Vaate, A. (2006). Trophic relationships in the Rhine food web during invasion and after establishment of the Ponto-Caspian invader *Dikerogammarus villosus*. *Hydrobiologia* **565**:39-58

van Uitregt, V. O., Hurst, T. P. & Wilson, R. S. (2012). Reduced size and starvation resistance in adult mosquitoes, *Aedes notoscriptus*, exposed to predation cues as larvae. *Journal of Animal Ecology* **81**:108-115. doi: 10.1111/j.1365-2656.2011.01880.x

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R. & Cushing, C. E. (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**:130-137

Vennerström, P., Söderhäll, K. & Cerenius, L. (1998). The origin of two crayfish plague (*Aphanomyces astaci*) epizootics in Finland on noble crayfish, *Astacus astacus*. *Annales Zoologici Fennici* **35**:43-46

Vermeij, G. J. (1991). Whem Biotas Meet: Understanding Biotic Interchange. *Science* **253**:1099-1104

Vidal, O., Garcia-Berthou, E., Tedesco, P. A. & Garcia-Marin, J.-L. (2010). Origin and genetic diversity of mosquitofish (*Gambusia holbrooki*) introduced to Europe. *Biological Invasions* **12**:841-851. doi: 10.1007/s10530-009-9505-5

Vinogradova, E. B. (2000). *Culex pipiens pipiens mosquitoes: taxonomy, distribution, ecology, physiology, genetic, applied importance and control*. Pensoft Publishers, Sofia, Bulgaria

Vitousek, P. M., D'Antonio, C. M., Loope, L. L. & Westbrooks, R. (1996). Biological invasions as global environmental change. *American Scientist* **84**:468-478

Vitousek, P. M., D'Antonio, C. M., Loope, L. L., Rejmanek, M. & Westbrooks, R. (1997). Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology* **21**:1-16

Walker, J. A. (1997). Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L.(Gasterosteidae) body shape. *Biological Journal of the Linnean Society* **61**:3-50

Wallace, J. B. & Webster, J. R. (1996). The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology* **41**:115-139

Wallace, J. B., Eggert, S. L., Meyer, J. L. & Webster, J. R. (1997). Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* **277**:102-104

Walsh, J. R., Carpenter, S. R. & Vander Zanden, M. J. (2016). Invasive species triggers a massive loss of ecosystem services through a trophic cascade. *Proceedings of the National Academy of Sciences* **113**:4081-4085

Wanink, J. H. (1999). Prospects for the fishery on the small pelagic *Rastrineobola argentea* in Lake Victoria. *Hydrobiologia* **407**:183-189

Webb, S. D. (2006). The Great American Biotic Interchange: patterns and processes. *Annals of the Missouri Botanical Garden* **93**:245-257

Westman, K. (2002) Alien crayfish in Europe: negative and positive impacts and interactions with native crayfish. In: Leppäkoski, E., Gollasch, S. & Olenin, S. (eds) *Invasive aquatic species of Europe. Distribution, impacts and management*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 76-95

Willi, Y., Van Buskirk, J. & Hoffmann, A. A. (2006). Limits to the adaptive potential of small populations. *Annual Review of Ecology, Evolution, and Systematics* **37**:433-458. doi: 10.1146/annurev.ecolsys.37.091305.110145

Wischnath, L. (1993). *Atlas of livebearers of the world*. TFH Publications; National Book Network

Witte, F., Goldschmidt, T., Wanink, J., van Oijen, M., Goudswaard, K., Witte-Maas, E. & Bouton, N. (1992). The destruction of an endemic species flock: quantitative data on the decline of the haplochromine cichlids of Lake Victoria. *Environmental Biology of Fishes* **34**:1-28

2. HUMAN-INDUCED BIOLOGICAL INVASIONS

2.1 Invasive species in an artificially heated stream

Erklärung zu den Autorenanteilen

an der Publikation: **On the natural history of an introduced population of guppies (*Poecilia reticulata* Peters, 1859) in Germany**

Status (*submitted, under review, in revision, accepted*): published
Ab *accepted* Name der Zeitschrift*[1]: ***BioInvasions Records***
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- Jourdan J (JJ)
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- Gasch K (KG)
- Herder F (FH)
- Schleucher E (ES)
- Plath M (MP)
- Bierbach D (DB)

Was hat der Promovierende bzw. was haben die Koautoren beigetragen?*[3]
(1) zu Entwicklung und Planung
Promovierender: 50%
Co-Autor MP: 30%
Co-Autor DB: 20%
(2) zur Durchführung der einzelnen Untersuchungen und Experimente
Promovierender: 30% initiale Durchführung und Etablierung der Versuchsprotokolle zur Thermotoleranz, Durchführung der Fang-Wiederfangversuche
Co-Autor KG: 40% Durchführung der Thermotoleranzversuche
Co-Autor FWM: 20% Durchführung der Elektrobefischung
Co-Autor DB: 10% Durchführung der Fang-Wiederfangversuche
(3) zur Erstellung der Datensammlung und Abbildungen
Promovierender: 100% Zusammentragen der Daten und Erstellung der Abbildungen
(4) zur Analyse und Interpretation der Daten
Promovierender: 70% Initiale Analyse und Interpretation der Daten
Co-Autor MP: 15% Diskussion der analysierten Daten
Co-Autor DB: 15% Diskussion der analysierten Daten
(5) zum Verfassen des Manuskripts
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Research Article

On the natural history of an introduced population of guppies (*Poecilia reticulata* Peters, 1859) in Germany

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Abstract

Artificially heated water bodies represent unusual habitats in temperate regions and form a refuge for exceptional fish communities. The Gillbach, a tributary of the river Erft in Germany, receives thermally polluted cooling water from a power plant. Here, we present data on the composition of the fish community in the Gillbach and found a high abundance of invasive species from all over the world, mostly introduced by releases from home aquaria. We found a species composition that is dominated by invasive species containing the same species as 15 years ago. We focused on guppies (*Poecilia reticulata*) and determined population size using the mark-recapture method. Furthermore, we investigated the lower thermal tolerance limit (C_{Tmin}) to determine if Gillbach guppies have already adapted to colder conditions compared to ornamental and Venezuelan wild type fish. We caught guppies of all sizes, and densities of 3.6 adult guppies per square meter were comparable to densities found in their natural distribution area, pointing toward a self-sustaining population in the Gillbach. The C_{Tmin} varied between populations and was significantly lower in ornamental and Gillbach guppies compared to guppies from Venezuela. Despite differences in C_{Tmin} and their well-known potential to adapt to new environments, guppies originally stem from the tropics, and a further spread will likely be restricted by low winter temperatures. Thus, *P. reticulata* in the Gillbach might not represent a threat for local fauna in Central Europe, but provide a unique semi-natural experiment for various questions related to local adaptation of invasive species, as well as ecological interactions with indigenous species.

Key words: *Amatitlania nigrofasciata*, Europe, exotic species, invasive, Rhine drainage, thermal pollution, thermal tolerances

Introduction

Alien species are among the major drivers of species extinctions and, thus, loss of biodiversity (Millennium Ecosystem Assessment 2005), especially in freshwater ecosystems (Mack et al. 2000). In the European Union alone, 12,122 non-native species have been reported so far (DAISIE European Invasive Alien Species Gateway 2013). However, not all of these are predicted to reproduce and expand their current distribution ranges ('invasive alien species', IAS; Williamson and Fitter 1996; Sakai et al. 2001). Nevertheless, even species that are not currently assumed to successfully reproduce in their new environments, or have a very localized occurrence, may occasionally overcome reproductive constraints – and thus reach IAS status – due to global warming, niche shifts, or local adaptation to altered environmental conditions (Whitney and Gabler 2008).

While most species introductions are accidental (Mack et al. 2000), several active introductions have also been documented. An example is the introduction of Nile perch (*Lates niloticus* Linnaeus, 1758) into Lake Victoria, with its disastrous consequences for the endemic fish fauna (Ogutu-Ohwayo 1990; Seehausen et al. 1997; Goldschmidt 1998). Live-bearing fishes of the family Poeciliidae have been widely introduced to tropical and subtropical countries for malaria prophylaxis, i.e., to help control vector (mosquito) populations (Stockwell and Henkanaththegedara 2011). In addition, some poeciliids like guppies (*Poecilia* (*Acanthophacelus*) *reticulata* Peters, 1859), swordtails and platyfish (*Xiphophorus* spp.), and mollies (*Poecilia* (*Mollienesia*) spp.) are among the most popular ornamental fishes, and many introductions may have been the result of occasional releases from home aquaria (Padilla and Williams 2004; Gozlan et al. 2010a; Stockwell and Henkanaththegedara 2011; Strecker et al. 2011). The ability to store sperm from multiple mates for several months secures several consecutive broods and allows a single gravid poeciliid female to found an entire new population (Zane et al. 1999; Evans and Magurran 2000).

Guppies are native to northern South America between Venezuela and northern Brazil, and to several nearby islands like Trinidad and Tobago (Rosen and Bailey 1963; Magurran 2005). Studies on natural populations of Trinidadian guppies reported on geographical variation in morphological, behavioral and life history characteristics, primarily explained by variation in predator regimes (Magurran et al. 1995; Magurran 2005). Within few generations after the exposure to an experimentally altered predator regime, guppy populations responded with an earlier onset of sexual maturity coupled with smaller offspring size at birth (high predation), or delayed onset of sexual maturity and increased offspring size at birth (low predation; Reznick et al. 2008). This ability to rapidly respond to altered selective regimes highlights the invasive potential of guppies (Magurran 2005; Deacon et al. 2011). Guppies have broad environmental tolerances and can withstand – at least for short periods of time – marine salinity (Chervinski 1984), as well as temperatures dropping to 12°C (Fujio et al. 1990) or rising to over 40°C (Chung 2001). This renders a wide range of habitats suitable for guppies and non-native guppy populations are currently reported from at least 69 countries in North and South America, Europe, Asia, Australasia, and Africa (Deacon et al. 2011). However, in contrast to Eastern mosquitofish (*Gambusia holbrooki* Girard, 1859) that were actively released in southern Europe during the 20th century for mosquito prophylaxis, and are nowadays present in virtually any southern European freshwater system (Vidal et al. 2010; pers. obs. for Italian, Spanish and southern French streams), guppies are not widely established in Europe. Exceptions are some isolated populations in a few southern European rivers probably established in recent years (Elvira and Almodovar 2001). Due to their native distribution in the tropics, low winter temperatures prevent self-sustaining populations in large parts of Europe. Nonetheless, there are occasional reports of small populations in Canada, Russia and parts of northern Europe, but these inhabit either geothermal springs or water bodies with artificially increased water temperatures due to thermal pollution arising through influx of cooling water from power plants or surface mining (Arnold 1990; Deacon et al. 2011). Such permanently warm refuges, however, might serve as source populations from where individuals might start spreading into hitherto uninhabited areas following adaptation to cooler conditions (Klotz et al. 2013) or elevated temperatures as a result of climate change (Rahel and Olden 2008; Walther et al. 2009; Wiesner et al. 2010; Bellard et al. 2013).

The present study reports on an artificially heated ecosystem, the upper Gillbach, that is verifiably inhabited by a guppy population since the mid-1970s (Kempkes 2010) and receives cooling water from a power plant. Although other non-native fish are regularly found in the Gillbach (Höfer and Staas 1998), we focused on the guppy due their status as a model species in evolutionary ecology (Magurran 2005; Evans et al. 2011), their well-known success as an invasive species in other parts of the world (Deacon et al. 2011) and the present lack of information regarding their invasiveness for central Europe (Nehring et al. 2010). Our major aims were (1) to provide an overview of the fish community found in the Gillbach 15 years after the last survey (Höfer and Staas 1998), (2) to estimate the population size of guppies using mark-recapture analysis, and (3) to evaluate whether guppies from the Gillbach have already been adapted to colder conditions and tolerate a lower critical minimum temperature (C_{Tmin}) compared to guppies from Venezuela and domesticated ornamental guppies. Our study – even though largely descriptive – is intended as a primer to future projects assessing the status (and invasion potential) of guppies in Central Europe.

Materials and methods

The Gillbach is a stream located west of Cologne in North Rhine-Westphalia (Germany), and meanders for approximately 28 km before it drains into the river Erft, a Rhine tributary (Figure 1). It receives thermally polluted water from the coal-fired power plant “Niederaußem” (50°59'46.82", 6°39'50.56", RWE Power Inc.). The Gillbach is approximately three meters wide and 30–80 cm deep. We measured abiotic water parameters (pH, conductivity, dissolved oxygen and temperature) with a Multi-Parameter Meter (HQ40d Portable Meter, HACH, Loveland, USA) approximately 100 m downstream of the influx pipe at least once a month between August 2011 and April 2012.

To characterize the fish community in the upper Gillbach, we performed electrofishing during 4 days in June 2013 along the first 400 meters downstream of the influx pipe (section I; Figure 1), the next 400 m downstream (section II), and for several hundred meters further downstream using a portable electro fishing device (Hans Grassl IG200-1).

First reports of introduced guppies date back to the 1970s, when first individuals were initially released to the Gillbach by hobbyist fish breeders (Kempkes 2010). Although we assume this feral population has been derived from a mixture of various domesticated ornamental strains, guppies nowadays caught in the Gillbach show an amazing male color polymorphism typically found in natural guppy populations (Figure 2; Haskins and Haskins 1951; Endler 1983; Houde 1997; Brooks 2002). Electrofishing is not an ideal method for catching guppies, mainly due to their small size. To estimate the population size of guppies, we, thus, used a standard mark-recapture approach (Reznick et al. 2001). Guppies were collected in June using a seine (2 mm mesh width) and dip nets along sections I and II. Only adult fish were considered in this approach. Females were included if their body size exceeded 10 mm standard length (SL), they appear to be gravid and they had a clearly visible dark gravid spot above the anal fin, while maturity in males was evaluated by inspecting their gonopodium (the transformed anal fin that develops into a copulatory organ at maturation) and checking for color ornaments on the body. Fish were transferred into well-aerated coolers and subsequently anesthetized with clove oil. Anesthetized fish were marked individually with color polymer tags and transferred to a tank with aerated water for recovery for at least half an hour. No mortality was detected. Recapture took place one week later at the same sites and with comparable sampling effort. At both capture events, body size of adult fish was measured to the nearest millimetre using plastic rulers.

We initially captured 93 adult males and 145 adult females, all of which were marked and released on the same day. During the second capture event, a total of 131 males and 235 females were captured, of which 7 males and 13 females were recaptured individuals. For the estimation of population size (with 95% CI), we used the R package Rcapture (Baillargeon and Rivest 2007; R_Core_Team 2013) assuming a closed population (Mt model). For the Gillbach, this assumption seems reasonable, at least over short periods of time, as the sampling area starts at the influx pipe of the power plant and is confined downstream by a railroad tunnel (Figure 1).

For the measurement of lower thermal tolerance limits (C_{Tmin}), guppies were collected from the Gillbach, and carefully transported to the laboratory of the University of Frankfurt/Main. Fish were acclimated to the laboratory conditions for three months before the measurements started. We further included a color polymorphic stock of guppies derived from various ornamental strains and a stock of guppies descended from animals imported from Venezuela by *Aquarium Dietzenbach*. All fish were maintained in mixed-sex stock tanks (80 to 180-l) at a constant temperature of 28°C under a 12:12 h illumination cycle. Tanks were equipped with natural gravel, internal filters, as well as stones and artificial plants for shelter. Fish were fed twice daily with commercial flake food. For the investigation of C_{Tmin}, we concentrated on females and followed the protocol provided by Bierbach et al. (2010). Test fish were acclimated to 25°C prior to the experiments in 60-l tanks for at least two weeks. The test apparatus consisted of a 10-l test tank connected to a circulating pump with an internal cooling aggregate. An air-pump ensured saturated oxygen concentrations throughout the tests. We gently introduced a test fish and started to decrease water temperature at a constant rate of $0.780\pm0.007°C\ min^{-1}$ once the fish was swimming calmly. Down-regulation of the water temperature was aided by the addition of ice cubes every two minutes. We noted at which temperature the test fish lost motion control as a proxy for the test subjects’ absolute physiological tolerance. Directly after the trials, fish were transferred into an aerated 10-l tank in which temperatures were gradually increased again to 25°C. All test fish

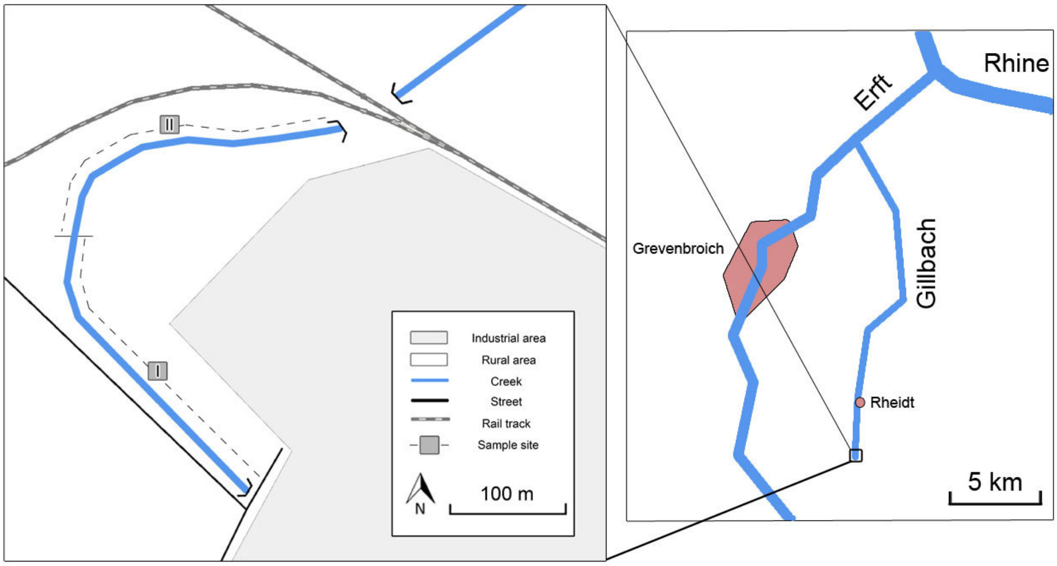

Figure 1. Overview of the study area. Schematic view of the watercourses (blue) and electrofishing sites (I and II) in the Gillbach (North Rhine-Westphalia, Germany).

Figure 2. Variation in body coloration in a sample of males caught from the Gillbach. Photographs by the authors.

regained motion control within few minutes, and no mortality was associated with this experiment. After completion of a trial, test fish were weighed to the nearest 0.1 g using a Sartorius PT 600 scale (accuracy ± 0.1%). In order to compare C_{Tmin} among populations, we used a linear mixed model with population as a fixed factor and log-transformed body mass as a covariate. The interaction term 'population by body mass' was not significant ($F_{2,22}$=2.77, P=0.085), but since the Akaike's Information Criterion (AIC) increased by 21.6% in the simplified model, we used the more complex model retaining the interaction term. To identify significant differences between populations, we used LSD tests for pairwise *post hoc* comparisons of the estimated marginal means derived from our model where body mass was adjusted to -0.29 log(g). The analysis was conducted using SPSS 22 (SPSS Inc. 2013).

Results

Fish community

Electrofishing revealed 11 different fish species, seven of which were non-native (Table 1). The most abundant native species were chub (*Squalius cephalus* Linnaeus, 1758) and barbel (*Barbus barbus* Linnaeus, 1758). Guppies were the most abundant non-native species, but – given the high capture success during the mark recapture approach (see below) – were clearly under-represented during electrofishing. Beside guppies, six other non-native species were recorded (Table 1), of which the Central American convict cichlid (*Amatitlania nigrofasciata* Günther, 1867) was the most abundant.

Sex ratio, population size and body size distribution of guppies

The sex ratio, combined from both capturing events, was female-biased (#males/#females = 0.58). We estimated a total population size of 4,305 (95% CI: 2,963–6,726) adult guppies, translating into an approximated average density of 3.6 adults per square meter. Males showed a very narrow body size range with a mean SL of 16.2 mm (95% CI: 16.0–16.4 mm) while female body size was much more variable, with a mean SL of 19.7 mm (95% CI: 19.2–20.1 mm; Figure 3).

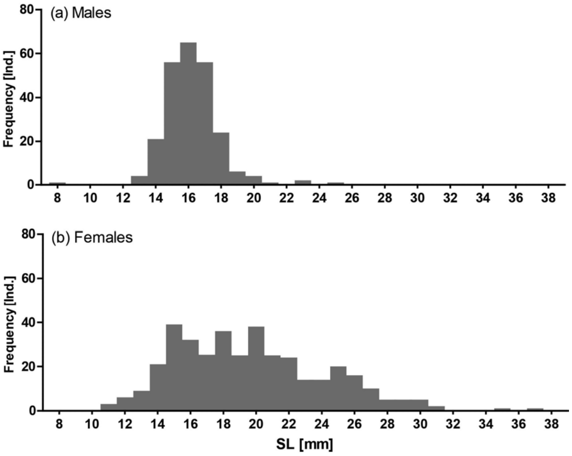

Figure 3. Body size distribution of (a) male and (b) female guppies collected in the Gillbach.

Abiotic habitat characteristics

As a consequence of a constant warm water discharge from the power plant, abiotic water parameters (temperature, pH, specific conductivity and dissolved oxygen) remained stable during the winter months (Table 2) and water temperatures

Table 1. Fish communities in the Gillbach inferred by electrofishing in June 2013.

Species	Origin	I	II	Further down-stream	Total
Cichlidae					
Amatitlania nigrofasciata (Günther, 1867)	Central America	11	6	3	20
Oreochromis sp.	Africa	6	0	1	7
Loricariidae					
Ancistrus sp.	South America	1	2	10	13
Cyprinidae					
Barbus barbus (Linnaeus, 1758)	native	2	1	37	40
Carassius auratus (Linnaeus, 1758)	Ornamental fish	0	5	0	5
Chondrostoma nasus (Linnaeus, 1758)	native	0	0	1	1
Cyprinus carpio (Linnaeus, 1758)	Asia	0	0	2	2
Gobio gobio (Linnaeus, 1758)	native	0	0	22	22
Pseudorasbora parva (Temminck & Schlegel, 1846)	Asia	1	0	0	1
Squalius cephalus (Linnaeus, 1758)	native	6	12	70	88
Poeciliidae					
Poecilia reticulata (Peters, 1859)	South America	10	20	11	32
Total		37	46	148	224

Table 2. Fluctuation in temperature and water chemistry of the Gillbach in 2011 and 2012.

Date	Daytime	t [°C]	pH	Specific conductivity [µS/cm]	DO [mg/L]	DO saturation [%]
Sept. 2011	11:30	23.2	8.10	1815	8.96	109.06
Oct. 2011	11:30	21.3	8.06	1877	8.46	99.16
Nov. 2011	12:00	19.4	8.34	1741	9.21	104.06
Dec. 2012	11:30	20.8	8.45	1778	9.14	98.13
Jan. 2012	16:00	19.0	8.28	1654	9.46	106.04
Feb. 2012	16:00	22.0	8.43	1915	8.73	103.72
Mar. 2012	14:30	23.1	8.46	1906	8.67	105.14
Apr. 2012	16:00	23.0	8.36	2026	8.30	100.50

never dropped below 19°C at the core area around the water influx. However, additional measurements approximately 2 km downstream in Rheidt (+51°00'50.88", +6°41'03.3") revealed a decline to 13.7°C in February 2012.

Thermal tolerances

Our linear mixed model detected a significant difference in C_{Tmin} between populations ($F_{2,22}$=5.484, P=0.012). Venezuelan guppies had a C_{Tmin} of 14.6 ± 0.6°C (mean ± SE), while it was significantly lower in fish from the Gillbach (12.4 ± 0.4°C; *Post-hoc* LSD test: P=0.007) and the ornamental population (12.5 ± 0.2°C; *Post-hoc* LSD test: P=0.004; Figure 4). The Gillbach and ornamental populations did not differ significantly in their thermal tolerance (*Post-hoc* LSD test: P=0.842).

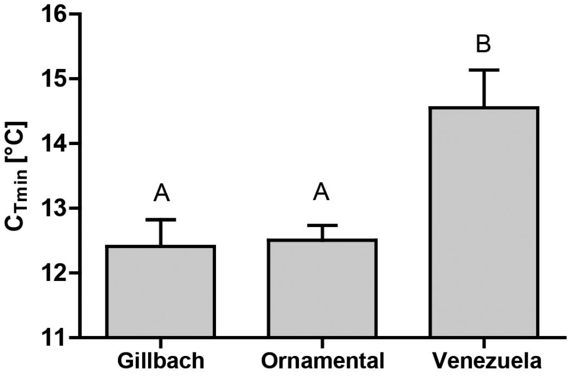

Figure 4. Lower critical minimum temperature (C_{Tmin}) of guppies from the Gillbach, an ornamental strain and descendants of wild-caught fish from Venezuela. Shown are estimated marginal means (EMM) from a linear model with (log-transformed) body mass as covariate. Different letters indicate significant differences in *post-hoc* LSD tests.

Discussion

Due to a constant warm water influx from the coal power plant 'Niederaußem', the Gillbach serves as a refuge for many tropical fish species and seven out of eleven species found in our survey are non-natives. Although most introduced species fail to establish self-sustaining populations (Williamson and Fitter 1996), high local densities and the presence of juveniles are indicators of a well-established population of guppies (*P. reticulata*) in the Gillbach. This assumption is further underpinned by consistent reports on guppies that date back to the mid-1970s (Kempkes 2010) and their wild-type-like morphology. Likewise, convict cichlids (*A. nigrofasciata*) were reported already 15 years ago (Höfer and Staas 1998) and different age-classes have been found in the current study. Beside guppies and convict cichlids, the survey from 1998 reported on other tropical species like Lake Malawi cichlids (*Pseudotropheus* sp.), as well as two additional undetermined representatives of the family Poeciliidae inhabiting the Gillbach. Native fishes recorded in 1998 were chub (*Squalius cephalus*), gudgeon (*Gobio gobio* Linnaeus, 1758), barb (*Barbus barbus*) and European eel (*Anguilla anguilla* Linnaeus, 1758). In our present study, we did not detect European eel or Lake Malawi cichlids, and the guppy was the only representative of poeciliid fishes. The undetermined poeciliids found in 1998 were presumably released by private pet fish keepers but, unlike guppies, failed to establish. Nevertheless, in addition to the previous report, we found a single specimen of common nase (*Chondrostoma nasus* Linnaeus, 1758), and two specimens of common carp (*Cyprinus carpio* Linnaeus, 1758). Like in 1998, we also caught one specimen of the Asian cyprinid *Pseudorasbora parva* Temminck & Schlegel, 1846, which has been accidently introduced in the 1960s with translocations of cyprinids for aquaculture, and is nowadays widely established in Europe (Kottelat and Freyhof 2007; Gozlan et al. 2010b). Interestingly, our survey found several adult individuals, breeding females as well as juveniles, of the mouthbrooding African cichlid *Oreochromis* sp., well known as 'tilapia' in aquaculture. This species was deliberately introduced throughout the world to facilitate aquaculture development, and is nowadays invasive in many tropical countries, but has failed to establish in Europe (Canonico et al. 2005; Garcia-Berthou et al. 2005). In the Gillbach, the occurrence of breeding adults and juveniles suggests a stable population; founder individuals most likely stemmed from a recently closed aquaculture facility that used the power plant's cooling water. Beside the variety of invasive fish species, also the invertebrate fauna of the Gillbach is affected by exotic species. Recently, Klotz et al. (2013) reported on two invasive species of freshwater shrimps (*Neocaridina davidi* Bouvier, 1904 and *Macrobrachium dayanum* Henderson, 1893) from Asia in this stream.

Our density estimates of 3.6 adult guppies per m^2 are similar to those reported on Trinidadian populations. Reznick and Endler (1982) found slightly lower densities (approximately 2 guppies per m^2) in high predation sites ("Crenicichla-sites") and higher densities (app. 9 guppies per m^2) in low predation sites ("Rivulus-sites"), while a subsequent study reported densities of approximately 4 individuals per m^2 at both high and low predation sites (Reznick et al. 2001). Similar to the Gillbach population, adult sex ratios in Trinidadian guppy populations are often female-biased, since males likely face higher predation rates (Magurran 2005; Arendt et al. 2014).

Our data on adult body size distributions reflect the findings from previous studies (Endler 1995). Like in many other poeciliids (Hughes 1985; Plath et al. 2003), male Trinidadian guppies are smaller (SL: 13–19 mm) than females (18–24 mm; Magurran 2005) and wild-type guppies are usually smaller than fish from domesticated strains (males: 21.5–27.5 mm, females: 23.8–35.5 mm; Zimmer et al. 2014). Predation has been identified as a major selective force for body size evolution, whereby high predation rates select for smaller body size in Trinidadian guppies. For example, Reznick and Endler (1982) reported on body lengths of 14.88 ± 0.10 mm (SL ± SE) for males caught at 'high predation' sites with abundant cichlid predators and 16.42 ± 0.14 mm for 'low predation' sites without predatory cichlids present (Reznick and Endler 1982).

Exposure to predation has a strong effect on virtually all aspects of a population's biology, as selection from predation is a powerful driver of behavioral, morphological and life-history trait evolution (Endler 1995). Predation also influences male ornamentation (Endler 1980), and guppy males from 'high predation' sites are often less conspicuous and bright (Rodd and Reznick 1997; Magurran 2005). Even though body coloration was not quantified in the present study, we found color polymorphic male phenotypes qualitatively resembling those found in natural guppy populations. Investigations of Gillbach guppies in the 1970s reported males carrying traits typical for ornamental fish, like elongated fins and single-color morphs (Kempkes 2010), and further releases of ornamental breeds over the last years cannot be ruled out. However, these forms seem to have disappeared, leaving polymorphic traits similar to native guppies from Trinidad (Endler 1983) or feral guppies from Japan (Karino and Haijima 2001) with a high variation of dorsal and caudal fin lengths and color spot patterns. As a logical extension of this interpretation, we argue that the Gillbach population likely faces predation pressures comparable to natural Trinidadian and South American 'high predation' populations (Reznick and Endler 1982; Figure 2) – most likely by piscivorous species like the native *S. cephalus* and *B. barbus*, as well as the invasive *A. nigrofasciata*. Furthermore, bird predation (e.g. by *Alcedo atthis* Linnaeus, 1758 or *Ardea cinerea* Linnaeus, 1758) is expected to occur. An alternative explanation is that the ornamental guppy strains are not hardy enough (i.e. insufficient thermal tolerance, handicapped due to elongated fins), so that only wild-type strains survived.

With a minimum water temperature of 19.2°C in January 2012, and a total annual temperature range of only 4.2°C, the Gillbach provides suitable temperature conditions for guppies along its first few kilometers. The constant outflow of cooling water provides even more stable conditions than in some natural Trinidadian habitats, where water temperatures can be highly variable (up to 7°C per day, Reeve et al. 2014). Nevertheless, water temperatures dropped to 13.7°C within the first 2 km in February 2012 and even though Gillbach guppies (and ornamental breeds) tolerated a lower critical minimum temperature (C_{Tmin}) than fish from a wild-type Venezuelan stock, it is unlikely that guppies will survive outside the core area close to the warm water influx during winter.

While increasing air temperatures are expected for the Rhine basin as an effect of climate change (up to 2.3°C in the lowland area during winter, projected to the year 2050; Middelkoop et al. 2001), water temperatures sometimes decrease to less than +1°C in the river Rhine (e.g. February 2012; measuring station Mainz-Wiesbaden, HLUG 2013). Even if climate change alters pathways of invasive species and modify ecological impacts (Rahel and Olden 2008; Walther et al. 2009), the overwinter survival of guppies should not be possible in German river areas without warm water influx. Poeciliids exhibit a great potential to adapt to new environments (Meffe and Snelson 1989; Stockwell and Henkanaththegedara 2011), and the C_{Tmin} of the Gillbach population recorded here is congruent with another study reporting on some domesticated guppy strains that tolerate temperatures of 12°C for at least 24 h (Fujio et al. 1990). However, guppies stem from the tropics and a further spread in Central Europe will probably be restricted by low winter temperatures. Similarly, the survival of the other non-native tropical fishes found in the Gillbach can be assumed to fully rely on the power plant's cooling water discharge.

In summary, the Gillbach is characterized by an unusual species composition, dominated by invasive species that established stable populations in the artificially heated creek. The guppy population consists of more than 4000 individuals in the core area around the water influx. The source of the guppies is suspected to be ornamental animals; nevertheless, we could not find evidence for adaptation to lower temperatures, as no difference in C_{Tmin} between ornamental- and Gillbach guppies was detected. The establishment of introduced species is influenced by many factors. Understanding the complex interactions between the invading species and the recipient environment is a fundamental challenge to ecologists and conservation managers (Mooney and Cleland 2001; Hayes and Barry 2008). Beside the limited invasive potential, the Gillbach population of introduced guppies with its assumed 'spread and diminish' characteristic may provide a fruitful semi-natural experiment for questions related to local adaptation of invasive populations and ecological interactions with indigenous ones. Even though the risk of a further spread of guppies in Central Europe may seem unlikely, we recommend the continuous monitoring of this system with a special focus on changes in the invasive status of the species inhabiting the Gillbach, since the thermal gradient is connected directly to native ecosystems and may serve as a source habitat for species invasions.

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References

Arendt JD, Reznick DN, López-Sepulcre A (2014) Replicated origin of female-biased adult sex ratio in introduced populations of the Trinidadian guppy (*Poecilia reticulata*). *Evolution* 68(8): 2343–2356, http://dx.doi.org/10.1111/evo.12445

Arnold A (1990) Eingebürgerte Fischarten: Zur Biologie und Verbreitung allochthoner Wildfische in Europa. Die Neue Brehm Bücherei, A. Ziemsen Verlag, Wittenberg, Lutherstadt

Baillargeon S, Rivest L-P (2007) Rcapture: Loglinear Models for Capture-Recapture. *Journal of Statistical Software* 19(5)

Bellard C, Thuiller W, Leroy B, Genovesi P, Bakkenes M, Courchamp F (2013) Will climate change promote future invasions? *Global Change Biology* 19(12): 3740–3748, http://dx.doi.org/10.1111/gcb.12344

Bierbach D, Schleucher E, Hildenbrand P, Köhler A, Arias-Rodriguez L, Riesch R, Plath M (2010) Thermal tolerances in mollies (*Poecilia* spp.): reduced physiological flexibility in stable environments? *Bulletin of Fish Biology* 12: 83–89

Brooks R (2002) Variation in female mate choice within guppy populations: population divergence, multiple ornaments and the maintenance of polymorphism. *Genetica* 116(2–3): 343–358, http://dx.doi.org/10.1023/A:1021228308636

Canonico GC, Arthington A, McCrary JK, Thieme ML (2005) The effects of introduced tilapias on native biodiversity. Aquatic Conservation: *Marine and Freshwater Ecosystems* 15(5): 463–483, http://dx.doi.org/10.1002/aqc.699

Chervinski J (1984) Salinity tolerance of the guppy, *Poecilia reticulata* Peters. *Journal of Fish Biology* 24(4): 449–452, http://dx.doi.org/10.1111/j.1095-8649.1984.tb04815.x

Chung K (2001) Critical thermal maxima and acclimation rate of the tropical guppy *Poecilla reticulata*. *Hydrobiologia* 462(1–3): 253–257, http://dx.doi.org/10.1023/A:1013158904036

DAISIE European Invasive Alien Species Gateway (2013) http://www.europe-aliens.org/ (Accessed 18th October 2013)

Deacon AE, Ramnarine IW, Magurran AE (2011) How Reproductive Ecology Contributes to the Spread of a Globally Invasive Fish. *Plos ONE* 6(9): e24416, http://dx.doi.org/10.1371/journal.pone.0024416

Elvira B, Almodovar A (2001) Freshwater fish introductions in Spain: facts and figures at the beginning of the 21st century. *Journal of Fish Biology* 59: 323–331, http://dx.doi.org/10.1111/j.1095-8649.2001.tb01393.x

Endler JA (1980) Natural selection on color patterns in *Poecilia reticulata*. *Evolution* 34: 76–91, http://dx.doi.org/10.2307/2408316

Endler JA (1983) Natural and sexual selection on color patterns in poeciliid fishes. *Environmental Biology of Fishes* 9(2): 173–190, http://dx.doi.org/10.1007/BF00690861

Endler JA (1995) Multiple-trait coevolution and environmental gradients in guppies. *Trends in Ecology & Evolution* 10(1): 22–29, http://dx.doi.org/10.1016/S0169-5347(00)88956-9

Evans JP, Magurran AE (2000) Multiple benefits of multiple mating in guppies. *Proceedings of the National Academy of Sciences* 97(18): 10074–10076, http://dx.doi.org/10.1073/pnas.180207297

Evans JP, Pilastro A, Schlupp I (2011) Ecology and evolution of poeciliid fishes. University of Chicago Press Chicago, IL, http://dx.doi.org/10.7208/chicago/9780226222769.001.0001

Fujio Y, Nakajima M, Nagahama Y (1990) Detection of a low temperature-resistant gene in the guppy (*Poecilia reticulata*), with reference to sex-linked inheritance. *Japanese Journal of Genetics* 65(4): 201–207, http://dx.doi.org/10.1266/jjg.65.201

Garcia-Berthou E, Alcaraz C, Pou-Rovira Q, Zamora L, Coenders G, Feo C (2005) Introduction pathways and establishment rates of invasive aquatic species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences* 62(2): 453–463, http://dx.doi.org/10.1139/f05-017

Goldschmidt T (1998) Darwin's dreampond: Drama in Lake Victoria. Massachusetts Institute of Technology Press, Cambridge, Massachusetts, USA

Gozlan R, Britton J, Cowx I, Copp G (2010a) Current knowledge on non-native freshwater fish introductions. *Journal of Fish Biology* 76(4): 751–786, http://dx.doi.org/10.1111/j.1095-8649.2010.02566.x

Gozlan RE, Andreou D, Asaeda T, Beyer K, Bouhadad R, Burnard D, Caiola N, Cakic P, Djikanovic V, Esmaeili HR (2010b) Pan-continental invasion of *Pseudorasbora parva*: towards a better understanding of freshwater fish invasions. *Fish and Fisheries* 11(4): 315–340, http://dx.doi.org/10.1111/j.1467-2979.2010.00361.x

Haskins CP, Haskins EF (1951) The inheritance of certain color patterns in wild populations of *Lebistes reticulatus* in Trinidad. *Evolution* 5: 216–225, http://dx.doi.org/10.2307/2405461

Hayes KR, Barry SC (2008) Are there any consistent predictors of invasion success? *Biological Invasions* 10(4): 483–506, http://dx.doi.org/10.1007/s10530-007-9146-5

HLUG (2013) Rheinwasser-Untersuchungsstation Mainz-Wiesbaden; Database accessible at: http://www.hlug.de/?id=7124&view=messwerte&detail=tabelle&station=10001 (Accessed 20th October 2013)

Höfer S, Staas S (1998) Bericht zur fischereibiologischen Untersuchug des Gillbaches im Bereich Bergheim-Auenheim (Okt/Nov. 1998). Zoologisches Institut der Universität zu Köln, Abt. Allgemeine Ökologie und Limnologie, Köln

Houde AE (1997) Sex, color, and mate choice in guppies. Princeton University Press, Princeton, NJ

Hughes AL (1985) Male size, mating success, and mating strategy in the mosquitofish *Gambusia affinis* (Poeciliidae). *Behavioral Ecology and Sociobiology* 17(3): 271–278, http://dx.doi.org/10.1007/BF00300146

Karino K, Haijima Y (2001) Heritability of male secondary sexual traits in feral guppies in Japan. *Journal of Ethology* 19(1): 33–37, http://dx.doi.org/10.1007/s101640170015

Kempkes M (2010) Die Guppys Band 1. Westarp Wissenschaften-Verlagsgesellschaft mbH, Hohenwarsleben

Klotz W, Miesen FW, Hüllen S, Herder F (2013) Two Asian fresh water shrimp species found in a thermally polluted stream system in North Rhine-Westphalia, Germany. *Aquatic Invasions* 8(3): 333–339, http://dx.doi.org/10.3391/ai.2013.8.3.09

Kottelat M, Freyhof J (2007) Handbook of European freshwater fishes, vol 13. Publications Kottelat, Cornol, Switzerland

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* 10(3): 689–710, http://dx.doi.org/10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2

Magurran A, Seghers B, Shaw P, Carvalho G (1995) The Behavioral Diversity and Evolution of Guppy, *Poecilia reticulata*, Populations in Trinidad. *Advances in the Study of Behavior* 24: 155–202, http://dx.doi.org/10.1016/S0065-3454(08)60394-0

Magurran AE (2005) Evolutionary ecology: the Trinidadian guppy. Oxford University Press, Oxford, http://dx.doi.org/10.1093/acprof:oso/9780198527855.001.0001

Meffe GK, Snelson F (1989) An ecological overview of poeciliid fishes. In: Meffe GK, Snelson FFJ (eds), Ecology and evolution of livebearing fishes (Poeciliidae). Englewood Cliffs, NJ: Prentice Hall, pp 13–31

Middelkoop H, Daamen K, Gellens D, Grabs W, Kwadijk JCJ, Lang H, Parmet B, Schadler B, Schulla J, Wilke K (2001) Impact of climate change on hydrological regimes and water resources management in the Rhine basin. *Climatic Change* 49(1–2): 105–128, http://dx.doi.org/10.1023/A:1010784727448

Millennium Ecosystem Assessment (2005) Ecosystems and Human Well-Being: Synthesis. Island Press, Washington, DC

Mooney HA, Cleland EE (2001) The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences* 98(10): 5446–5451, http://dx.doi.org/10.1073/pnas.091093398

Nehring S, Essl F, Klingenstein F, Nowack C, Stöhr O, Rabitsch W (2010) Schwarze Liste invasiver Arten: Kriteriensystem und Schwarze Listen invasiver Fische für Deutschland und für Österreich. BfN-Skripten 285. Bonn (Germany): Bundesamt für Naturschutz

Ogutu-Ohwayo R (1990) The decline of the native fishes of lakes Victoria and Kyoga (East Africa) and the impact of introduced species, especially the Nile perch, *Lates niloticus*, and the Nile tilapia, *Oreochromis niloticus*. *Environmental Biology of Fishes* 27(2): 81–96, http://dx.doi.org/10.1007/BF00001938

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(3): 131–138, http://dx.doi.org/10.1890/1540-9295(2004)002[0131:BBWAAO]2.0.CO;2

Plath M, Parzefall J, Schlupp I (2003) The role of sexual harassment in cave and surface dwelling populations of the Atlantic molly, *Poecilia mexicana* (Poeciliidae, Teleostei). *Behavioral Ecology and Sociobiology* 54(3): 303–309, http://dx.doi.org/10.1007/s00265-003-0625-0

R_Core_Team (2013) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. http://www.R-project.org/

Rahel FJ, Olden JD (2008) Assessing the effects of climate change on aquatic invasive species. *Conservation Biology* 22 (3): 521–533, http://dx.doi.org/10.1111/j.1523-1739.2008.00950.x

Reeve AJ, Ojanguren AF, Deacon AE, Shimadzu H, Ramnarine IW, Magurran AE (2014) Interplay of temperature and light influences wild guppy (*Poecilia reticulata*) daily reproductive activity. *Biological Journal of the Linnean Society* 111(3): 511–520, http://dx.doi.org/10.1111/bij.12217

Reznick D, Endler JA (1982) The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* 36(1): 160–177, http://dx.doi.org/10.2307/2407978

Reznick D, Iv MJB, Rodd H (2001) Life-History Evolution in Guppies. VII. The Comparative Ecology of High- and Low-Predation Environments. *The American Naturalist* 157(2): 126–140, http://dx.doi.org/10.1086/318627

Reznick DN, Ghalambor CK, Crooks K (2008) Experimental studies of evolution in guppies: a model for understanding the evolutionary consequences of predator removal in natural communities. *Molecular Ecology* 17: 97–107, http://dx.doi.org/10.1111/j.1365-294X.2007.03474.x

Rodd FH, Reznick DN (1997) Variation in the demography of guppy populations: the importance of predation and life histories. *Ecology* 78(2): 405–418

Rosen DE, Bailey RM (1963) The poeciliid fishes (Cyprinodontiformes), their structure, zoogeography, and systematics. *Bulletin of the American Museum of Natural History* 126: 1–176

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics* 32(1): 305–332, http://dx.doi.org/10.1146/annurev.ecolsys.32.081501.114037

Seehausen O, Witte F, Katunzi EF, Smits J, Bouton N (1997) Patterns of the remnant cichlid fauna in southern Lake Victoria. *Conservation Biology* 11: 890–904, http://dx.doi.org/10.1046/j.1523-1739.1997.95346.x

Stockwell CA, Henkanaththegedara SM (2011) Evolutionary conservation biology. In: Evans J, Pilastro A, Schlupp I (eds), Ecology and Evolution of Poeciliid Fishes. University of Chicago Press, Chicago, pp 128–141

Strecker AL, Campbell PM, Olden JD (2011) The aquarium trade as an invasion pathway in the Pacific Northwest. *Fisheries* 36(2): 74–85, http://dx.doi.org/10.1577/03632415.2011.10389070

Vidal O, Garcia-Berthou E, Tedesco PA, Garcia-Marin J-L (2010) Origin and genetic diversity of mosquitofish (*Gambusia holbrooki*) introduced to Europe. *Biological Invasions* 12(4): 841–851, http://dx.doi.org/10.1007/s10530-009-9505-5

Walther G-R, Roques A, Hulme PE, Sykes MT, Pyšek P, Kühn I, Zobel M, Bacher S, Botta-Dukát Z, Bugmann H (2009) Alien species in a warmer world: risks and opportunities. *Trends in Ecology & Evolution* 24(12): 686–693, http://dx.doi.org/10.1016/j.tree.2009.06.008

Whitney KD, Gabler CA (2008) Rapid evolution in introduced species, 'invasive traits' and recipient communities: challenges for predicting invasive potential. *Diversity and Distributions* 14(4): 569–580, http://dx.doi.org/10.1111/j.1472-4642.2008.00473.x

Wiesner C, Wolter C, Rabitsch W, Nehring S (2010) Gebietsfremde Fische in Deutschland und Österreich und mögliche Auswirkungen des Klimawandels. Bonn (Germany): Bundesamt für Naturschutz

Williamson MH, Fitter A (1996) The characters of successful invaders. *Biological Conservation* 78(1): 163–170, http://dx.doi.org/10.1016/0006-3207(96)00025-0

Zane L, Nelson WS, Jones AG, Avise JC (1999) Microsatellite assessment of multiple paternity in natural populations of a live bearing fish, *Gambusia holbrooki*. *Journal of Evolutionary Biology* 12(1): 61–69, http://dx.doi.org/10.1046/j.1420-9101.1999.00006.x

Zimmer C, Gavalas AS, Kunkel B, Hanisch J, Martin S, Bischoff S, Plath M, Bierbach D (2014) Mate choice copying in both sexes of the guppy: The role of sperm competition risk and sexual harassment. In: Geldani RM, Davin MA (eds), Sexual Selection: Evolutionary perspectives, mating strategies and long-term effects on genetic variation. Nova Science Publishers, Hauppauge, NY, pp 69–92

2.2 Novel predator-prey interactions

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Aquatic Sciences

RESEARCH ARTICLE

Adaptive growth reduction in response to fish kairomones allows mosquito larvae (*Culex pipiens*) to reduce predation risk

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Abstract Phenotypic plasticity is predicted to evolve when subsequent generations are likely to experience alternating selection pressures; e.g., piscine predation on mosquitoes (*Culex pipiens*) varies strongly depending on habitat type. A prey-choice experiment (exp. 1) detected a predilection of common mosquito predators (sticklebacks, *Gasterosteus aculeatus*) for large-bodied mosquito larvae, suggesting that larvae could benefit from suppressing growth under predation risk, and experiment 2 confirmed reduced pupa size and weight when we exposed larvae to stickleback kairomones. In experiment 3, we measured adult (imago) size instead to test if altered larval growth-patterns affect adult life-history traits. We further asked how specific life-history responses are, and thus, also used kairomones from introduced Eastern mosquitofish (*Gambusia holbrooki*), and from algivorous, non-native catfish (*Ancistrus* sp.). Adult body mass was equally reduced in all three kairomone treatments, suggesting that a non-specific anti-predator response (e.g., reduced activity) results in reduced food uptake. However, imagines were distinctly smaller only in the stickleback treatment, pointing towards a specific, adaptive life-history shift in response to the presence of a coevolved predator: mosquito larvae appear to suppress growth when exposed to their native predator, which presumably reduces predation risk, but also affects body size after pupation. Our study suggests that (1) not all antipredator responses are necessarily predator-specific, and (2) fluctuation in the cost-benefit ratio of suppressing larval growth has selected for phenotypic plasticity in *C. pipiens* larval life histories. This implies costs associated with suppressed growth, for example, in the form of lower lifetime reproductive success.

Keywords Chemical cues · Inducible defense trait · Invasive species · Phenotypic plasticity · Predator–prey interaction · Predator avoidance

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Introduction

Predation is a major selective force driving phenotypic diversification including adaptive variation in prey species' life histories (Reznick and Endler 1982; Crowl and Covich 1990; Riesch et al. 2013), morphology (Walker 1997; Langerhans et al. 2004), and behavior (Dixon and Baker 1988; Lima and Dill 1990). Characters that provide protection from predation often show heritable (evolved) differences in mean trait expression between species or populations that are exposed to different predation regimes (Reznick 1982; Langerhans et al. 2004). However, building defensive traits can be associated with considerable costs (e.g., Stevens et al. 1999), and so inducible trait expression (i.e., phenotypic plasticity) ought to be favored under certain conditions (Hebert and Grewe 1985; Dodson 1989; Agrawal 2001; Benard 2004); but see, e.g., DeWitt et al. (1998) and Auld et al. (2009) for costs of phenotypic plasticity. Classic examples of inducible morphological responses to predation risk come from studies on water flees (genus *Daphnia*), where juveniles produce neck-teeth, helmet-like structures, or elongated spines on the dorsal surface of the carapace in response to predator presence (Krueger and Dodson 1981; Hebert and Grewe 1985; Dodson 1989). One condition that favors the evolution of inducible predator-defense traits is provided when individuals can move between habitats that differ in predation risk across generations. For example, insects with complex life cycles can experience starkly divergent predatory regimes during their (aquatic) larval stages (Wilbur 1980; Palmer and Poff 1997), but since imagines can move freely between water bodies for oviposition, successive generations often experience different predatory regimes, and so plastic responses to predators in larval life histories, morphology and behavior are to be expected (reviewed in Benard 2004).

Predation is often size-selective, and predators tend to consume large individuals to optimize their net energy uptake (Brooks and Dodson 1965; Reznick 1982; Wellborn 1994; Plath et al. 2003) unless gape limitation or problems of handling large prey items lead to different patterns (Werner 1974; Nilsson and Brönmark 2000). In holometabolous insects, life history responses to predation on larval stages ought to be governed by early emergence to evade predation, which can result in reduced body size at metamorphosis (Peckarsky et al. 2001; Benard 2004; Beketov and Liess 2007), as somatic growth is largely restricted to the larval stage (Nijhout and Wheeler 1996). However, even if accelerating larval development is impossible, larvae could still suppress growth to evade size-specific predation. On the other hand, previous studies on culicid larvae reported on delayed developmental times when exposed to kairomones, probably because larvae reduced activity and fed less, resulting in delayed development (Beketov and Liess 2007; van Uitregt et al. 2012). Some studies, however, found no developmental response to predation risk at all (e.g., Caudill and Peckarsky 2003 for larvae of the mayfly *Callibaetis ferrugineus hageni*), and so holometabolous insects seem to react to predator cues along a continuum of reaction norms.

Accelerated emergence times and/or suppressed larval growth ought to be balanced by trade-offs (sensu Wilbur 1980; Stearns 1989) involving the competitiveness of larvae and ultimately, adults' realized reproductive potential (Bradshaw and Holzapfel 1992). Such a trade-off was demonstrated for the mosquito *Aedes notoscriptus*: when larvae of this Australian species were exposed to chemical cues of the native piscine predator *Hypseleotris galii* they reduced activity and, therefore, were better at avoiding predation than larvae that were not previously exposed to predator cues. However, predator-exposed larvae had reduced larval growth and development, were smaller at metamorphosis and less resistant to starvation than non-exposed ones (van Uitregt et al. 2012). Larvae of our study species, the common house mosquito *Culex pipiens molestus* (Forskal), inhabit stagnant or slow-flowing waters, ranging from small temporary puddles to large oxbow lakes (Vinogradova 2000). While transient water bodies are mostly predator-free environments, larvae in permanent (and mostly larger) water bodies are heavily preyed upon by multiple predators, including fishes, notonectid backswimmers, dytiscid beetles, dragonfly larvae, and others (Vinogradova 2000). Like several other aquatic invertebrates, *Culex* larvae detect predators through kairomones—natural chemicals released by their potential predators (Dodson et al. 1994; Ferrari et al. 2010). Little is known about the chemical structure of kairomones; an ongoing debate considers the question of whether (and to what extent) the predator's diet (Huryn and Chivers 1999; Beketov and Liess 2007), its cutaneous mucus (Forward and Rittschof 2000; Alvarez et al. 2014) or its bacterial flora (Beklioglu et al. 2006) affect the chemical composition of kairomones. However, there is evidence that kairomones differ widely even among similar taxa (Lass and Spaak 2003; Relyea 2003; Ferrari et al. 2010). When exposed to kairomones originating from aquatic heteropterans or beetles, mosquito larvae (*Culex* spp. and *Aedes* spp.) reduce their activity (Sih 1986; Ohba et al. 2012), and imagines appear to use kairomones of larval predators as a cue to avoid high-predation environments when searching for oviposition sites (Spencer et al. 2002; Ohba et al. 2012; Afify and Galizia 2015).

A number of studies focused on predator–prey interactions between culicid mosquito larvae (including the genus

Culex) and non-indigenous predators that have received attention as potential biological control agents against these vectors of human diseases (e.g., Krumholz 1948; Rosenheim et al. 1995; Kumar and Hwang 2006). For example, Offill and Walton (1999) compared a common native predator of *Culex* spp., the three-spined stickleback (*Gasterosteus aculeatus* Linnaeus) and *Gambusia affinis* (Baird and Girard), an introduced predator that is often used in mosquito control programs (Krumholz 1948), in terms of their efficiency of larval predation and found higher predation rates in *G. affinis*. However, predator–prey interactions between the widely distributed common house mosquito and its common native predator in Europe, *G. aculeatus* (see Medlock and Snow 2008), have received little attention in empirical research. Therefore, our first question was whether stickleback predation on *C. pipiens* is size-selective, and we conducted a prey choice experiment in which individual sticklebacks were offered two different size classes of mosquito larvae to answer this question. Our first experiment corroborated an increased predation risk of large-bodied larvae (see “Results”), and so we conducted another experiment to investigate potential predator-induced larval life history responses by raising larvae in water containing kairomones from sticklebacks, or water without kairomones in the control treatment. Specifically, we asked whether (size-specific) predation risk results in an accelerated developmental time and/or reduced larval body size, as measured by the size at pupation. Because we detected several life history responses when larvae were exposed to stickleback kairomones, we expanded our study and asked if mosquito larvae respond specifically to the presence of naturally co-occurring insectivorous fishes, or if any fish species elicits those responses. This question is of particular interest because of the increasing impact of invasive species—including non-native teleost fishes—in freshwaters worldwide (Mack et al. 2000; Sakai et al. 2001). Native, predator-naive prey species may be more vulnerable to predation by introduced, unfamiliar predators due to their inability to recognize novel predators and to show adaptive antipredator responses (Salo et al. 2007). In our third experiment we therefore compared life histories of native mosquito larvae exposed to no kairomones (control), kairomones from a non-native algivorous fish (*Ancistrus* sp.), sticklebacks, and from Eastern mosquitofish (*Gambusia holbrooki* Girard). The latter species is a severe predator of mosquitoes that has been introduced to southern Europe from the USA for malaria prophylaxis in the 1920s (Vidal et al. 2010), but does not presently co-occur with mosquitoes in Germany, where our study population of *C. pipiens* originated. We predicted the strongest shift in life histories to occur in response to chemical cues of sticklebacks, but a weaker or no response at all when exposed to kairomones from *Ancistrus* and mosquitofish. In this experiment, we focused on size and weight measurements of imagines, which allowed determination of whether and how larval life history shifts (as seen in our second experiment) translate into an altered adult body size. Taken together, our present study is the first of its kind to not only demonstrate altered larval development in response to predator cues, but to demonstrate how developmental plasticity relates to size-specific predation risk, and to what extent co-evolved and invasive alien (i.e., not co-evolved) predators elicit the same or different responses in essential *Culex* life-history traits.

Materials and methods

Study organisms and their maintenance

A randomly outbred laboratory strain of *Culex pipiens molestus* (Culicidae), founded from wild-caught animals collected near Regensburg, Germany, was obtained from Biogents AG (Regensburg). The subspecies *C. p. molestus* is autogenous, stenogamous, remains active throughout the year, and mainly feeds on mammalian, especially human blood (Harbach et al. 1984). Females produce at least one egg-raft after emergence, for which they do not obligatorily require a blood meal (Twohy and Rozeboom 1957). Mosquitoes were maintained as randomly outbred stocks consisting of several hundred imagines at 22 °C in two cages (60 cm × 60 cm × 60 cm gauze-covered frames), which were equipped with a water-filled Petri dish. Adult females were fed on saturated grape-sugar solution, which we offered ad libitum in the form of sugar-water soaked paper towels. Egg-rafts were removed from the culture and transferred to 10-L aquaria with equal amounts of deionized and tap water for hatching. Larvae were fed weekly on commercially available fine-ground fish food (Tetra Min$^{®}$).

All fishes used to produce kairomones for the tests were maintained in aerated and filtered $\geq$80-L tanks at 21 °C and fed ad libitum with flake food twice a day. Three-spined stickleback (*Gasterosteus aculeatus*; Gasterosteidae) are widely distributed throughout Europe; they feed mainly on crustaceans and aquatic insects (Hynes 1950). Sticklebacks were collected in a small creek in Niederursel, Germany, and were kept in the laboratory for 2–3 weeks before experimental use. In our third experiment we also included a laboratory strain of Eastern mosquitofish (*Gambusia holbrooki*; Poeciliidae), presumably of Floridian origin. Mosquitofish were actively released in southern Europe during the 20th century for mosquito prophylaxis, and are nowadays present in nearly all southern European water bodies (Vidal et al. 2010; pers. obs. for Italian, Spanish and southern French streams), but are currently not known to have established permanent populations in

Germany. Our third experiment included a domestic form of the algivorous South American armored catfish (*Ancistrus* sp. Loricariidae), which we obtained from a commercial aquarium breeder.

To prepare water containing specific fish kairomones, groups of fish were transferred into aerated and filtered 10-L aquaria that were maintained at 21 °C. In an attempt to standardize kairomone concentrations, we combined small groups of stimulus fish such that their cumulative body size (standard length, SL) would equal about 160 mm [4 individuals per aquarium for *Ancistrus* (mean ± SE, SL = 39.3 ± 1.0 mm), 4 sticklebacks (39.6 ± 2.4 mm) and 5–6 mosquitofish (26.8 ± 1.5 mm)]. Fishes were daily fed ad libitum with Tetra Min® fish food. Water was refilled every day after water had been removed for the experiments described below (Experiments 2 and 3).

Experiment 1: prey choice of three-spined sticklebacks

Prey choice tests with sticklebacks were performed in 12-L aquaria. Test tanks were aerated between trials, but the airstone was removed before mosquito larvae were introduced. Before each trial, we collected 14 larvae from our stock culture, i.e., seven from each of two visibly different size classes. Two individuals per size class were randomly taken from this sample and fixed in 70 % ethanol for subsequent size determination [large size class, mean (±SE) length from the cephalothorax to the tip of abdominal segment VIII: 5.2 ± 0.1 mm; small size class: 2.8 ± 0.1 mm). Since our aim was to detect general patterns of size-selective predation in sticklebacks, we chose mosquito larvae that clearly differed in size, which allowed visual differentiation of size classes by the observer. To initiate a trial, an individual stickleback (SL: 39.2 ± 1.4 mm) was introduced into the test tank and allowed to acclimate for 10 min. Afterwards, the remaining 10 larvae were gently introduced into the test tank. We observed the behavior of the focal fish from approximately 1 m distance and terminated a trial (i.e., removed the focal fish from the test tank) after five larvae had been eaten. All surplus larvae were retrieved from the test tank and their size class noted. In total, we conducted $n = 31$ independent trials.

Experiment 2: life history responses to stickleback kairomones (pupae size)

To determine larval responses to stickleback kairomones, larvae were reared in 100 mL vessels filled with 60 mL water (equal amounts of deionized and tap water). Each vessel was covered with fine nylon gauze. We introduced eight newly hatched (L_1) larvae per vessel and thus tested 10 replicates (80 L_1 larvae) in both treatments (control and stickleback kairomones; i.e., $n = 160$ larvae altogether). Larvae were fed on ground fish food until pupation. The consumption of food per larvae increased stepwise as follows: 0.5 mg (hatching day), 0.5 mg (day 2), 0.5 mg (day 4), 0.5 mg (day 5), and 1 mg per day from day 7 onwards. All experiments were conducted in a climate chamber at 21 °C, with 60 % humidity and a 12:12 h LD photoperiod. We exchanged 50 % of the water in the test vessels every day and replaced it with kairomone-water from the respective fish tanks or untreated water (according to the treatment). We used water from two replicate stimulus tanks containing sticklebacks (cumulative SL = 166 ± 3 mm) and two equal-sized and similarly equipped tanks without fish (control treatment).

Twice a day all mosquito test vessels were checked for mortality, and all dead larvae were immediately removed. Simultaneously, we checked for pupae, which were fixed in 70 % ethanol. We determined the sex of pupae based on the gonocoxopodites, which are large and partially bilobed in males but small and spiculate in females (cp. Harbach et al. 1984; personal observation). The same parameters as described in Müller et al. (2013) were measured under a stereo microscope: abdominal length (AL) from the third to the eighth segment, abdominal width (AW) at the fifth segment, and the area of the cephalothorax (CT) in lateral view.

We asked if any observable life history responses of the mosquito larvae can be ascribed to the presence of fish kairomones or whether it represents a response to metabolic waste products of fish and their related degradation products. We therefore analyzed nitrate and phosphate concentrations in randomly selected experimental vessels, the fish tanks and in the tanks containing control water using colorimetric tests (Merck KGaA, Darmstadt, Germany). Two nitrate and phosphate measurements were conducted per treatment. Sensitivity of the colorimetric tests is low, however, all measurements for nitrate and phosphate were uniformly high in the experimental mosquito vessels (~ 10 mg l^{-1} NO_3^-; >0.43 mg l^{-1} H_3PO_4) and considerably lower in the fish and control tanks (~ 5 mg l^{-1} NO_3^-; ~ 0.3 mg l^{-1} H_3PO_4). Therefore, waste products of the larvae themselves seem to have affected nitrate and phosphate concentrations in the test vessels, but the addition of fish (or control) water had no obvious effect on this.

Experiment 3: life history responses to different predator types (imago size)

In our third experiment, we used a similar approach as described before, but collected imagines directly after hatching. We set up three replicate stimulus tanks for each of the following fish species and three (empty) control tanks (i.e., 12 stimulus tanks in total): *Ancistrus* sp. (cumulative SL = 157 ± 4 mm), *G. aculeatus* (158 ± 3 mm) and *G. holbrooki* (152 ± 1 mm). We conducted 15 replicates (using 120 larvae) per treatment, amounting to a total sample size of $n = 480$ larvae.

Emerged imagines were frozen in individual 1.5 mL Eppendorf tubes at −80 °C until further processing. Mosquitoes were then dried at 60 °C for a minimum of 24 h, after which their dry weight was recorded using a Sartorius 4503 microbalance (accuracy: 1 µg). Wing length (as a proxy for body size) was determined to the nearest 0.1 mm as the distance from the axial incision to the R1 vein (Kreß et al. 2014) using a dissecting microscope (Nikon AZ100 Multizoom, Nikon Instruments Europe, Amsterdam, Netherlands) connected to a digital camera (Nikon DS-Fi1) with an image-analyzing system (NIS Elements BR, version 3.22.11, Laboratory Imaging). To determine fat content mosquitos were rinsed four times for at least 1.5 h with petroleum ether to extract nonpolar, non-structural lipids, then dried again and reweighed (Heulett et al. 1995; Riesch et al. 2010).

Statistical analysis

All statistical analyses were conducted using SPSS 22 (SPSS Inc., Chicago, IL). Numbers of large and small larvae consumed in experiment 1 were compared using a Wilcoxon signed rank test. We used multivariate General Linear Models (GLM) to compare life history traits between treatments in experiments 2 and 3; dependent data were *z*-transformed to standardize units. In experiment 2, ‘developmental time until pupation’ (days), ‘dry weight’ (mg), ‘cephalotorax area’ (mm^2) ‘abdominal width’ (mm) and ‘abdominal length’ (mm) were treated as the dependent variables, and in experiment 3 ‘developmental time until emergence’ (days), ‘dry weight’ (mg), ‘wing length’ (mm) and arcsine(square root)-transformed ‘fat-content’ (%). ‘Sex’, ‘treatment’ and their interaction were used as fixed factors. Since the interaction terms had no significant effects (experiment 2: $F_{5,127} = 1.37$, $P = 0.24$; experiment 3: $F_{12,784} = 0.99$, $P = 0.46$), they were removed from the final models. To identify the source of variation in case of significant treatment effects we used univariate GLMs on all four variables separately (using non-transformed data) and employed LSD tests for post hoc pairwise comparisons between treatments. We illustrated significant effects using estimated marginal means from the respective analytical models. Mortality data were analyzed using survival analysis: a log-rank test was performed to check if larval mortality rates varied among treatments.

Results

Experiment 1: prey choice of three-spined sticklebacks

Sticklebacks consumed more large than small larvae in 29 of 31 trials, and a Wilcoxon signed rank test confirmed that overall more large larvae were consumed (i.e., 72.9 ± 3.2 % of consumed larvae; $z = -4.63$, $P < 0.01$, $n = 31$; Fig. 1).

Experiment 2: life history responses to stickleback kairomones (pupae size)

A multivariate GLM uncovered significant treatment ($F_{5,128} = 7.15$, $P < 0.001$) and sex effects ($F_{5,128} = 179.25$, $P < 0.001$) on the four investigated life history traits. Univariate GLMs—as a touch-down approach to uncover the source of variation—revealed that developmental time was not affected by exposure to stickleback kairomones, while cephalothorax area, abdominal width, abdominal length, and dry weight were significantly reduced in the stickleback treatment (Table 1; Fig. 2). Differences between sexes are reported in supplementary Table S1.

Survival analysis revealed that the mortality of larvae did not differ between treatments (log-rank test, $\chi^2 = 3.59$, $P = 0.06$). While the marginally non-significant effect suggests a treatment effect, larvae exposed to kairomones actually had slightly increased (not decreased) survival (Fig. 3a). Mortality in the control treatment was 20.7 %, which meets the criteria of acceptable experimental baseline mortality for ecotoxicological tests in the non-biting midge *Chironomus riparius* (Chironomidae) provided in OECD guideline no. 219 (i.e., 30 %; OECD 2004).

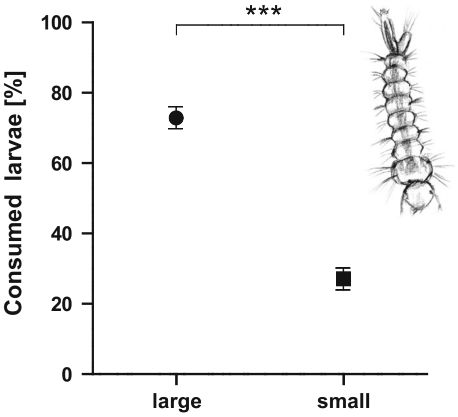


Fig. 1 Prey-choice preference of sticklebacks (*Gasterosteus aculeatus*) between large- (body length: 5.2 ± 0.1 mm) and small-bodied (2.8 ± 0.1 mm) mosquito larvae

Table 1 Results from ANCOVAs of the experiment 2 using 'kairomone treatment' and 'sex' as fixed factors

Source	Dependent variable	*F*	*df*	*P*
Treatment	Time until pupation	0.194	1	0.661
	Cephalothorax area	26.023	1	<0.001
	Abdominal width	6.628	1	0.011
	Abdominal length	13.031	1	<0.001
	Dry weight	25.801	1	<0.001
Sex	Time until pupation	131.191	1	<0.001
	Cephalothorax area	305.332	1	<0.001
	Abdominal width	189.537	1	<0.001
	Abdominal length	222.283	1	<0.001
	Dry weight	318.327	1	<0.001

The interaction term was not significant ($F_{5,127} = 1.372$, $P = 0.24$) such that only the main effects were analyzed

Experiment 3: life history responses to different predator types (imago size)

A multivariate GLM detected significant treatment ($F_{12,791} = 2.86$, $P = 0.001$) and sex effects ($F_{4,299} = 342.61$, $P < 0.001$) on the four investigated life history traits. Univariate GLMs found significant differences in dry weight (Fig. 4b) and body size (Fig. 4c), while developmental duration (Fig. 4a) and fat content (Fig. 4d) did not differ between treatments (Table 2). *Post-hoc* tests revealed that average dry weight was significantly lower in larvae exposed to any of the three fish treatments compared to the control treatment (LSD tests: $P < 0.01$ in all cases; Fig. 4b). Body size (wing length) was significantly smaller in the stickleback treatment than in the control ($P = 0.011$) and *Ancistrus* treatments ($P = 0.004$; Fig. 4c). Body size of mosquitoes from the *G. holbrooki* treatment was also slightly reduced (1.03 %), but was not significantly different from the other treatments ($P > 0.05$ in all cases). In addition, we found pronounced differences between sexes, with males emerging faster than females, having a lower dry weight, smaller wing length and a higher fat content than females (Table 2; for descriptive statistics see supplementary Table 2).

The survival of *C. pipiens* larvae did not significantly differ between treatments (log-rank test, $\chi^2 = 3.50$, $P = 0.32$); mortality in control treatment was 16.6 % (Fig. 3b).

Discussion

We found sticklebacks to exert size-selective predation upon *C. pipiens* larvae ("Experiment 1"). In case of newly hatched (L_1) larvae exposed to stickleback kairomones, pupae became significantly smaller and lighter compared to the control treatment without kairomones ("Experiment 2"). These observations were corroborated by investigating imagines, where we found a strong reduction in body size after exposure to stickleback kairomones ("Experiment 3"); however, body weight was reduced in all fish kairomone treatments irrespective of fish identity.

Size selective predation on aquatic arthropods by piscine predators is well documented for other teleost fishes; e.g., bluegill sunfish (*Lepomis macrochirus*) prefer large amphipod prey (Wellborn 1994), pumpkinseed sunfish (*L. gibbosus*) prefer large dragonfly larvae (Dixon and Baker 1988), and brook trout (*Salvelinus fontinalis*) exhibit a preference for large mayfly larvae (Allan 1978). We argue that lower predation risk of small-sized *C. pipiens* larvae by their common piscine predator in European freshwaters, the three-spined stickleback (*G. aculeatus*), provides them with a relative advantage over larger larvae in sites with high levels of predation, and so we asked if mosquitoes respond to predation risk with altered larval life histories, especially with suppressed growth, which was confirmed in experiment 2.

We further asked how specific this response is, and whether mosquito larvae differ in their responses to a co-evolved piscine predator, a not co-evolved (invasive) predator, and a non-insectivorous fish. Investigating the response of native prey organisms to novel (invasive) predators is of particular interest in light of the steady increase of biological invasions worldwide (Mack et al. 2000; Sakai et al. 2001). Different predators usually release predator-specific chemical profiles (Relyea 2001; Iyengar and Harvell 2002; Relyea 2003), but it remains to be investigated if prey species can respond to novel predator types with which they have not coevolved. Moreover, our second experiment as well as several previous studies reporting suppressed growth of mosquito larvae exposed to predator kairomones (e.g., van Uitregt et al. 2012 for *A. notoscriptus*; Ohba et al. 2012 for *C. tritaeniorhynchus*) did not answer the question of whether suppressed larval body growth is merely an *indirect* consequence of reduced activity (and thus, reduced feeding), or if mosquito larvae *actively* alter larval growth patterns as an adaptive life history response to evade predation—experiment 3 in our present study provides answers to both questions.

Our prediction for an adaptive response to predation risk was that larvae might accelerate larval development to evade predation (see "Introduction"), but we found no support for such a pattern. Neither did we find delayed developmental times in our present study (an effect found in previous studies, e.g., Beketov and Liess 2007; van Uitregt et al. 2012), and so it seems that developmental times show rather narrow reaction norms in our study population. Still, the results of experiment 3 suggest that

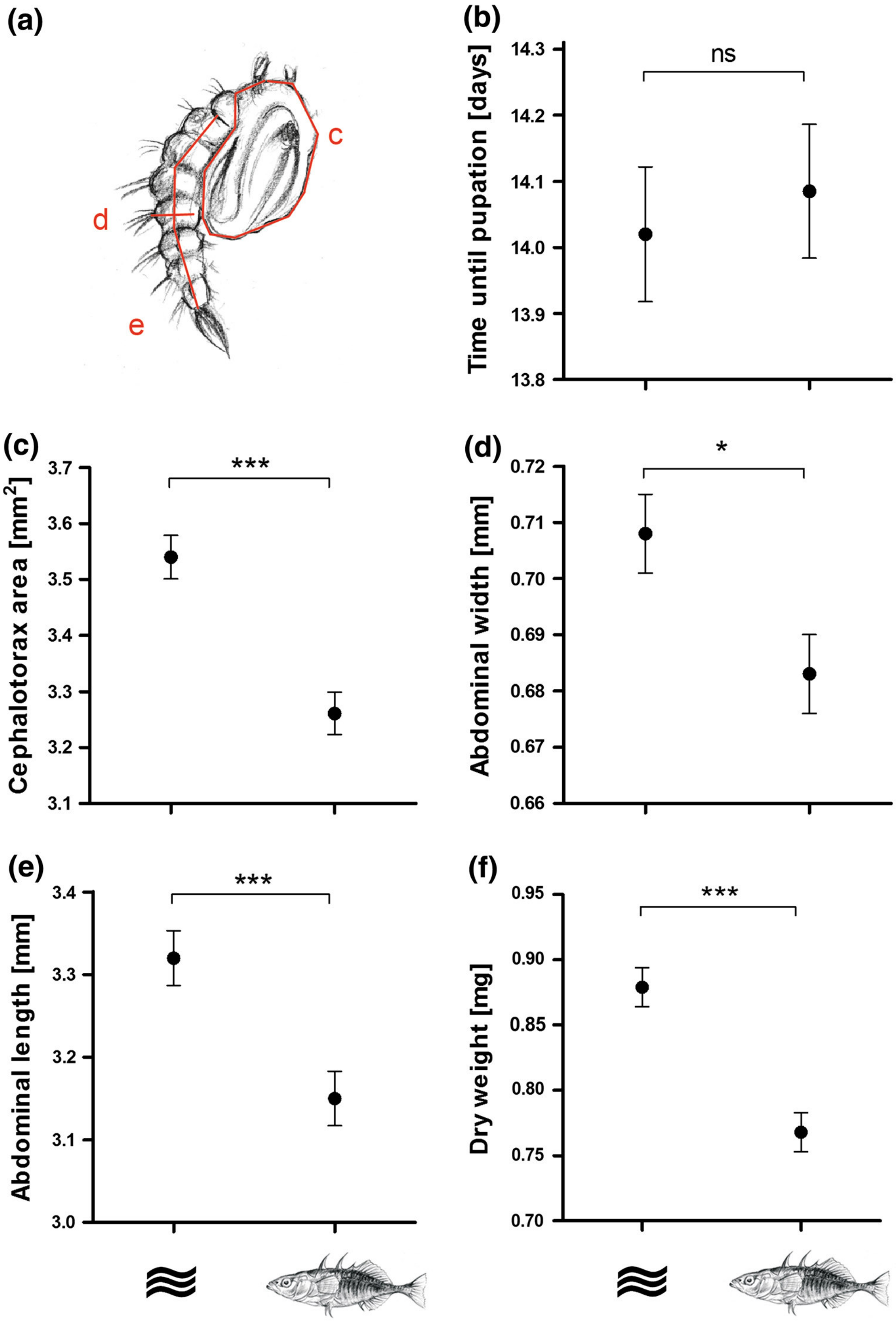


Fig. 2 Effects of kairomones on life history traits of *Culex pipiens* pupae reared as larvae in absence (*wave symbol*, *left*) or presence (*stickleback drawing*, *right*) of chemical cues from *Gasterosteus aculeatus*. **a** Pupae of *C. pipiens* with additional illustration of the measured distances: *c* cephalothorax area, *d* abdominal width and *e* abdominal length. **b**–**f** Dependent variables assessed in experiment 2 are depicted as back-transformed estimated marginal means (±SE) from a GLM. Shown are **b** the time until pupation, **c** cephalothorax area, **d** abdominal width, **e** abdominal length and **f** dry weight of pupae

mosquito larvae exhibit an unspecific stress response to kairomones from different fish species, as it seems plausible to explain reduced body weight as a consequence of reduced activity and thus, reduced food uptake (compare Beketov and Liess 2007; van Uitregt et al. 2012). Generally, time spent foraging correlates positively with the likelihood of being detected by visual predators or encountering ambush predators, and so individuals typically decrease foraging under predation risk (Lima and Dill 1990; Benard 2004; Stoks et al. 2005). Also *C. pipiens* larvae were found to move less when exposed to kairomones from a heteropteran predator, the backswimmer *Notonecta undulata* (Sih 1986), and reduced foraging under the influence of predator kairomones was also reported from other culicid larvae (Kesavaraju and Juliano 2004; Ohba et al. 2012).

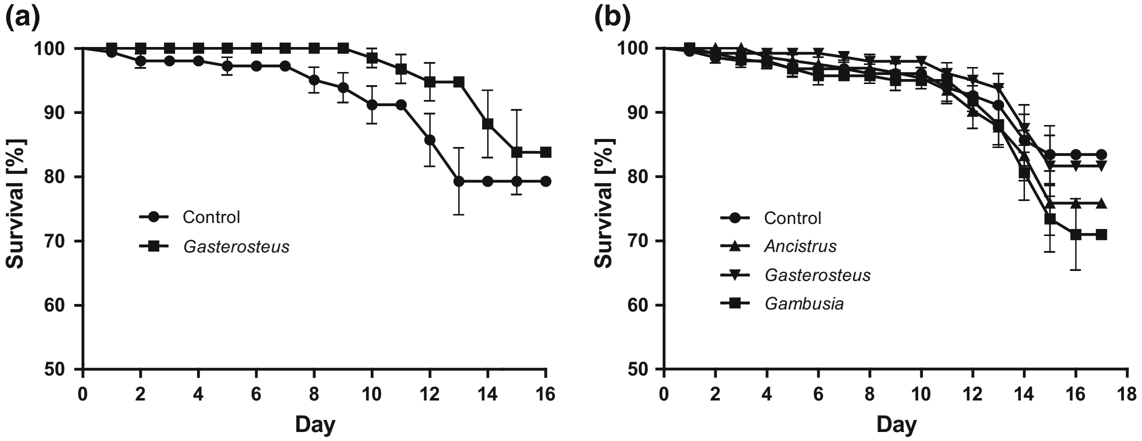
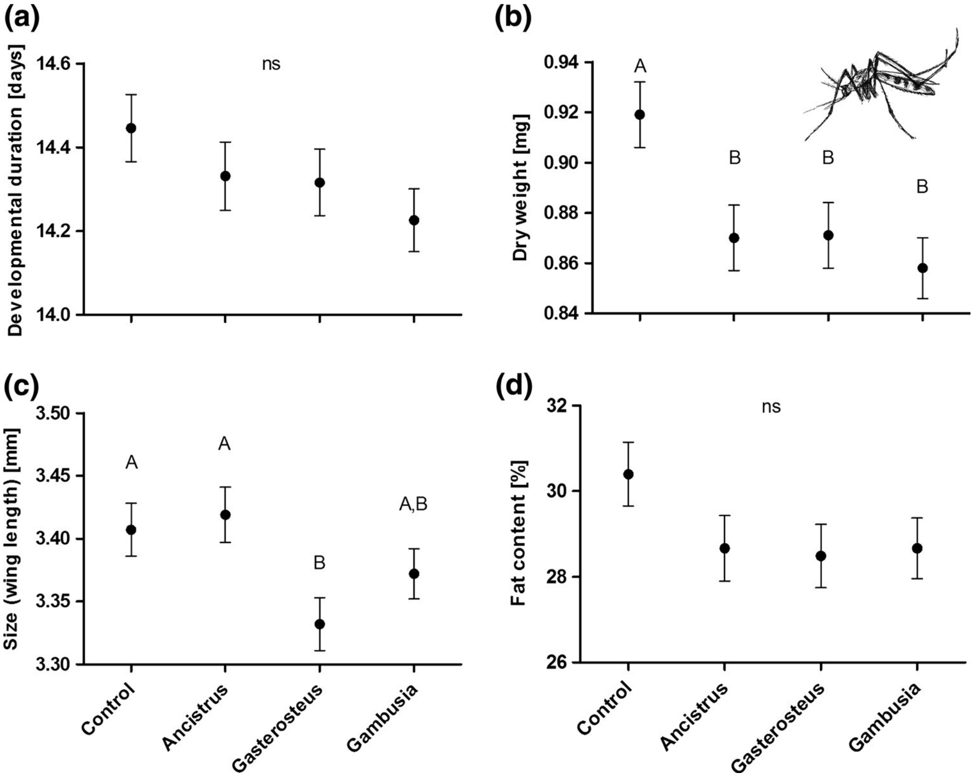


Fig. 3 Survival curve of *Culex pipiens* larvae reared **a** in experiment 2, and **b** in experiment 3

Fig. 4 Effects of kairomones on life history traits of *Culex pipiens* imagines when larvae were reared in absence (control) or presence of chemical cues from non-predatory, algivorous *Ancistrus* sp., the native predator *Gasterosteus aculeatus* and the non-native predator *Gambusia holbrooki*. Shown are back-transformed estimated marginal means (±SE) from a GLM, for **a** developmental time until emergence, **b** dry weight, **c** body size and **d** fat content of emerged imagines. *Letters* above the *bars* represent the results of LSD tests for pairwise post hoc comparisons, where *different letters* indicate significant differences between treatments ($P < 0.05$)



Moreover, we detected another effect—reduced body size—that was strongest in the treatment with stickleback kairomones, considerably weaker when kairomones of insectivorous, but not co-evolved, *Gambusia* were present, while no effect at all was seen in the *Ancistrus* treatment. Since we did not measure activity in our study, a link between reduced foraging and reduced body size cannot be excluded. However, if this explanation was true, and if our interpretation is correct that equally reduced body weight in all three fish kairomone treatments is the result of reduced activity (see above), then we would have expected at least slightly reduced body size also in the treatment using *Ancistrus* kairomones, but no such pattern was uncovered (Fig. 4c). We calculated the ratio between body weight and body size. The non-specific body weight reduction in all kairomone treatments was reflected by a high body mass-to-body size ratio in the control treatment (0.270; calculated from EMMs), but the highest ratio among the fish kairomone treatments was detected when *Gasterosteus* kairomones were presented (*Ancistrus*: 0.254,

Table 2 Results from ANCOVAs on four variables assessed in experiment 3 using 'kairomone treatment' and 'sex' as fixed factors, and 'developmental duration', 'dry weight', 'wing length' and 'fat content' as the dependent variables

Source	Dependent variable	*F*	*df*	*P*
Treatment	Developmental duration	1.357	3	0.256
	Dry weight	4.498	3	0.004
	Wing length	3.490	3	0.016
	Fat content	1.511	3	0.212
Sex	Developmental duration	155.504	1	<0.001
	Dry weight	1,097.906	1	<0.001
	Wing length	677.562	1	<0.001
	Fat content	38.940	1	<0.001

The interaction term was not significant ($F_{12,784} = 0.99$, $P = 0.46$) such that only the main effects were analyzed

Gasterosteus: 0.261, *Gambusia*: 0.254), and so it appears as if larvae are indeed actively suppressing growth and thus, become 'denser' (i.e., have a higher weight-to-body size ratio compared to the *Ancistrus* and *Gambusia* treatments) to reduce predation risk in the presence of their common predator. This suggests that, at least in *C. pipiens*, the evolutionary history of this predator–prey interaction seems to be more important than the relative risk of predation posed by the predator, and some component of sticklebacks' chemical cues may allow a specific recognition of the coevolved predator. The significance of evolutionary history in predator–prey recognition was also observed by Alvarez et al. (2014), who investigated predator avoidance behavior of mayfly larvae (*Baetis* spp.) to chemical cues (cutaneous mucus). The authors showed that larvae exhibited no behavioral response to novel predator species, while larvae did respond to five co-occurring freshwater fishes. However, the response to co-occurring fishes was not predator-specific even though fish species differed in their strength of predation on mayfly larvae. Given that all fish species in our study obtained the same diet, species-specific kairomones likely stemmed from a combination of cutaneous mucus and mucosa-associated bacteria (Beklioglu et al. 2006; Alvarez et al. 2014).

With regards to reduced larval body size, our results suggest that size reduction confers benefits under predation risk. However, since body size of female mosquitoes correlates positively with fecundity (Briegel 1990; Lyimo and Takken 1993; McCann et al. 2009), this reduction may come at a cost at the adult stage, essentially leading to a life-history trade-off. Furthermore, van Uitregt et al. (2012) found smaller mosquito imagines to be less resistant to starvation, while larger imagines can have longer reproductive life-spans (Neems et al. 1990), have an increased ability to disperse (Kaufmann et al. 2013), and tend to be superior in mate competition (Wellborn and Bartholf 2005). This trade-off between costs and benefits of suppressed larval growth likely governs the evolution of the remarkable plasticity of larval life histories in *C. pipiens* we describe here, as subsequent larval generations can experience starkly different piscine predation pressures.

Several studies have shown how alternative phenotypes can be induced through alterations of hormone release and enzymatic activity, as well as altered gene expression mediated by DNA methylation and transcription factor activation (Gilbert and Epel 2009; Miyakawa et al. 2010; Snell-Rood et al. 2010; Beldade et al. 2011; Sommer and Ogawa 2011; Schneider et al. 2014). These mechanisms are highly interactive: environmental cues can affect the dynamics of hormone production and thus, trigger changes in hormone titers, while hormones can affect gene expression (Gilbert and Epel 2009; Beldade et al. 2011). Future studies will need to address the molecular mechanisms underlying adaptive life-history shifts of mosquito larvae exposed to predator kairomones. This may also answer the question of how specific kairomones of the investigated fish species are and how these affect the entailing cascades. The reduced body weight in all fish treatments (Fig. 4b) implies that this particular response is triggered by chemical cues shared by a broad array of teleost species, whereas the reduction in body size was much more specific: the co-evolved predator triggered the strongest response, but a weak (albeit not significant) response was also triggered by *Gambusia*. This may be explained by the greater phylogenetic proximity between sticklebacks and *Gambusia* (Betancur-R et al. 2013), resulting in somewhat similar chemical profiles. Furthermore, it remains to be tested if mosquitoes will evolve predator recognition in regions where *Gambusia* is invasive.

Finally, the picture becomes even more complex when other studies on adaptive responses to invasive alien species are evaluated. Some studies found no behavioral antipredator response of native species to an unknown predator (e.g., Kesavaraju and Juliano 2004; Kesavaraju et al. 2007), whereas others found morphological and behavioral adaptations to invasive predators (e.g., Flecker 1992; Pease and Wayne 2014). Thus, our study demonstrates that—with regards to invertebrate prey presented with chemical cues of potential predators—the lack of a specific response does not always equate to an inability of the prey species to discriminate. Rather, the antipredator response can be finely nuanced so that only certain traits exhibit a predator-specific response, while other traits show similar responses across a range of potential (co-evolved native or invasive alien) predators.

Author contribution statement JJ, RM and MP conceived and designed the study. JJ and JB conducted the experiments. JJ, RR, RM and MP analyzed data. RM and MP provided laboratory, rearing and infrastructural possibilities. All authors wrote, read and approved the manuscript.

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References

Afify A, Galizia CG (2015) Chemosensory cues for mosquito oviposition site selection. J Med Entomol. doi:10.1093/jme/tju024

Agrawal AA (2001) Phenotypic plasticity in the interactions and evolution of species. Science 294:321–326

Allan JD (1978) Trout predation and the size composition of stream drift. Limnol Oceanogr 23:1231–1237

Alvarez M, Landeira-Dabarca A, Peckarsky B (2014) Origin and specificity of predatory fish cues detected by *Baetis* larvae (Ephemeroptera; Insecta). Anim Behav 96:141–149

Auld JR, Agrawal AA, Relyea RA (2009) Re-evaluating the costs and limits of adaptive phenotypic plasticity. Proc R Soc Lond B Biol Sci 277:503–511

Beketov MA, Liess M (2007) Predation risk perception and food scarcity induce alterations of life-cycle traits of the mosquito *Culex pipiens*. Ecol Entomol 32:405–410

Beklioglu M, Telli M, Gozen AG (2006) Fish and mucus-dwelling bacteria interact to produce a kairomone that induces diel vertical migration in *Daphnia*. Freshw Biol 51:2200–2206

Beldade P, Mateus ARA, Keller RA (2011) Evolution and molecular mechanisms of adaptive developmental plasticity. Mol Ecol 20:1347–1363

Benard MF (2004) Predator-induced phenotypic plasticity in organisms with complex life histories. Annu Rev Ecol Syst 35:651–673

Betancur-R R et al (2013) The tree of life and a new classification of bony fishes. PLoS Curr 2013:5. doi:10.1371/currents.tol.53ba26640df0ccaee75bb165c8c26288

Bradshaw WE, Holzapfel CM (1992) Reproductive consequences of density-dependent size variation in the pitcherplant mosquito, *Wyeomyia smithii* (Diptera: Culicidae). Ann Entomol Soc Am 85:274–281

Briegel H (1990) Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti*. J Insect Physiol 36:165–172

Brooks JL, Dodson SI (1965) Predation, body size, and composition of plankton. Science 150:28–35

Caudill CC, Peckarsky BL (2003) Lack of appropriate behavioral or developmental responses by mayfly larvae to trout predators. Ecology 84:2133–2144

Crowl TA, Covich AP (1990) Predator-induced life-history shifts in a freshwater snail. Science 247:949–951

DeWitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. Trends Ecol Evol 13:77–81

Dixon S, Baker R (1988) Effects of size on predation risk, behavioural response to fish, and cost of reduced feeding in larval *Ischnura verticalis* (Coenagrionidae: Odonata). Oecologia 76:200–205

Dodson S (1989) Predator-induced reaction norms. Bioscience 39:447–452

Dodson SI, Crowl TA, Peckarsky BL, Kats LB, Covich AP, Culp JM (1994) Non-visual communication in freshwater benthos: an overview. J North Am Benthol Soc 13:268–282. doi:10.2307/1467245

Ferrari MC, Wisenden BD, Chivers DP (2010) Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. Can J Zool 88:698–724

Flecker AS (1992) Fish predation and the evolution of invertebrate drift periodicity: evidence from neotropical streams. Ecology 73:438–448

Forward RB, Rittschof D (2000) Alteration of photoresponses involved in diel vertical migration of a crab larva by fish mucus and degradation products of mucopolysaccharides. J Exp Mar Biol Ecol 245:277–292

Gilbert SF, Epel D (2009) Ecological developmental biology: integrating epigenetics, medicine, and evolution. Sinauer Associates, Sunderland

Harbach RE, Harrison BA, Gad AM (1984) *Culex (culex) molestus* Forskal (Diptera: Culicidae): neotype designation, description, variation, and taxonomic status. Proc Entomol Soc Wash 86:521–542

Hebert PD, Grewe PM (1985) Chaoborus-induced shifts in the morphology of *Daphnia ambigua*. Limnol Oceanogr 30:1291–1297

Heulett ST, Weeks SC, Meffe GK (1995) Lipid dynamics and growth relative to resource level in juvenile eastern mosquitofish (*Gambusia holbrooki*: Poeciliidae). Copeia 1995:97–104

Huryn AD, Chivers DP (1999) Contrasting behavioral responses by detritivorous and predatory mayflies to chemicals released by injured conspecifics and their predators. J Chem Ecol 25:2729–2740

Hynes H (1950) The food of fresh-water sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*), with a review of methods used in studies of the food of fishes. J Anim Ecol 19:36–58

Iyengar EV, Harvell CD (2002) Specificity of cues inducing defensive spines in the bryozoan *Membranipora membranacea*. Mar Ecol Prog Ser 225:205–218

Kaufmann C, Reim C, Blanckenhorn WU (2013) Size-dependent insect flight energetics at different sugar supplies. Biol J Linn Soc 108:565–578. doi:10.1111/j.1095-8312.2012.02042.x

Kesavaraju B, Juliano S (2004) Differential behavioral responses to water-borne cues to predation in two container-dwelling mosquitoes. Ann Entomol Soc Am 97:194

Kesavaraju B, Alto BW, Lounibos LP, Juliano SA (2007) Behavioural responses of larval container mosquitoes to a size-selective predator. Ecol Entomol 32:262–272

Kreß A, Oehlmann J, Kuch U, Müller R (2014) Impact of temperature and nutrition on the toxicity of the insecticide λ-cyhalothrin in fulllifecycle tests with the target mosquito species *Aedes albopictus* and *Culex pipiens*. J Pest Sci 87:739–750. doi:10.1007/s10340-014-0620-4

Krueger DA, Dodson SI (1981) Embryological induction and predation ecology in *Daphnia pulex*. Limnol Oceanogr 26:219–223

Krumholz LA (1948) Reproduction in the Western Mosquitofish, *Gambusia affinis affinis* (Baird & Girard) and its use in mosquito control. Ecol Monogr 18:1–43. doi:10.2307/1948627

Kumar R, Hwang JS (2006) Larvicidal efficiency of aquatic predators: a perspective for mosquito biocontrol. Zool Stud 45:447–466

Langerhans RB, Layman CA, Shokrollahi A, DeWitt TJ (2004) Predator-driven phenotypic diversification in *Gambusia affinis*. Evolution 58:2305–2318

Lass S, Spaak P (2003) Chemically induced anti-predator defences in plankton: a review. Hydrobiologia 491:221–239

Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. Can J Zool 68:619–640

Lyimo EO, Takken W (1993) Effects of adult body size on fecundity and the pre-gravid rate of *Anopheles gambiae* females in Tanzania. Med Vet Entomol 7:328–332

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecol Appl 10:689–710. doi:10.2307/2641039

McCann S, Day JF, Allan S, Lord CC (2009) Age modifies the effect of body size on fecundity in *Culex quinquefasciatus* Say (Diptera: Culicidae). J Vector Ecol 34:174–181

Medlock J, Snow K (2008) Natural predators and parasites of British mosquitoes—a review. European Mosquito Bulletin 25:1–11

Miyakawa H et al (2010) Gene up-regulation in response to predator kairomones in the water flea, *Daphnia pulex*. BMC Dev Biol 10:45

Müller R, Knautz T, Völker J, Kreß A, Kuch U, Oehlmann J (2013) Appropriate larval food quality and quantity for *Aedes albopictus* (Diptera: Culicidae). J Med Entomol 50:668–673

Neems R, McLachlan A, Chambers R (1990) Body size and lifetime mating success of male midges (Diptera: Chironomidae). Anim Behav 40:648–652

Nijhout H, Wheeler D (1996) Growth models of complex allometries in holometabolous insects. Am Nat 148:40–56

Nilsson PA, Brönmark C (2000) Prey vulnerability to a gape-size limited predator: behavioural and morphological impacts on northern pike piscivory. Oikos 88:539–546

OECD (2004) OECD guideline for testing chemicals: Test No. 219: Sediment-water chironomid toxicity using 474 spiked water. Organization for Economic Cooperation and Development, Paris

Offill Y, Walton W (1999) Comparative efficacy of the threespine stickleback (*Gasterosteus aculeatus*) and the mosquitofish (*Gambusia affinis*) for mosquito control. J Am Mosq Control Assoc 15:380–390

Ohba S-Y, Ohtsuka M, Sunahara T, Sonoda Y, Kawashima E, Takagi M (2012) Differential responses to predator cues between two mosquito species breeding in different habitats. Ecol Entomol 37:410–418

Palmer MA, Poff NL (1997) The influence of environmental heterogeneity on patterns and processes in streams. J North Am Benthol Soc 16:169–173

Pease KM, Wayne RK (2014) Divergent responses of exposed and naive Pacific tree frog tadpoles to invasive predatory crayfish. Oecologia 174:241–252. doi:10.1007/s00442-013-2745-1

Peckarsky BL, Taylor BW, McIntosh AR, McPeek MA, Lytle DA (2001) Variation in mayfly size at metamorphosis as a developmental response to risk of predation. Ecology 82:740–757

Plath M, Parzefall J, Schlupp I (2003) The role of sexual harassment in cave and surface dwelling populations of the Atlantic molly, *Poecilia mexicana* (Poeciliidae, Teleostei). Behav Ecol Sociobiol 54:303–309

Relyea RA (2001) Morphological and behavioral plasticity of larval anurans in response to different predators. Ecology 82:523–540

Relyea RA (2003) How prey respond to combined predators: a review and an empirical test. Ecology 84:1827–1839

Reznick D (1982) The impact of predation on life history evolution in Trinidadian guppies: genetic basis of observed life history patterns. Evolution 36:1236–1250

Reznick D, Endler JA (1982) The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). Evolution 36:160–177. doi:10.2307/2407978

Riesch R, Plath M, Schlupp I (2010) Toxic hydrogen sulfide and dark caves: life-history adaptations in a livebearing fish (*Poecilia mexicana*, Poeciliidae). Ecology 91:1494–1505. doi:10.1890/09-1008.1

Riesch R, Martin RA, Langerhans RB (2013) Predation's role in life-history evolution of a livebearing fish and a test of the Trexler-DeAngelis model of maternal provisioning. Am Nat 181:78–93

Rosenheim JA, Kaya H, Ehler L, Marois JJ, Jaffee B (1995) Intraguild predation among biological-control agents: theory and evidence. Biol Control 5:303–335

Sakai AK et al (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305–332. doi:10.1146/annurev.ecolsys.32.081501.114037

Salo P, Korpimäki E, Banks PB, Nordström M, Dickman CR (2007) Alien predators are more dangerous than native predators to prey populations. Proc R Soc Lond B Biol Sci 274:1237–1243

Schneider RF, Li Y, Meyer A, Gunter HM (2014) Regulatory gene networks that shape the development of adaptive phenotypic plasticity in a cichlid fish. Mol Ecol 23:4511–4526. doi:10.1111/mec.12851

Sih A (1986) Antipredator responses and the perception of danger by mosquito larvae. Ecology 67:434–441

Snell-Rood EC, Van Dyken JD, Cruickshank T, Wade MJ, Moczek AP (2010) Toward a population genetic framework of developmental evolution: the costs, limits, and consequences of phenotypic plasticity. BioEssays 32:71–81

Sommer RJ, Ogawa A (2011) Hormone signaling and phenotypic plasticity in nematode development and evolution. Curr Biol 21:R758–R766

Spencer M, Blaustein L, Cohen JE (2002) Oviposition habitat selection by mosquitoes (*Culiseta longiareolata*) and consequences for population size. Ecology 83:669–679

Stearns SC (1989) Trade-offs in life-history evolution. Funct Ecol 3:259–268

Stevens DJ, Hansell MH, Freel JA, Monaghan P (1999) Developmental trade–offs in caddis flies: increased investment in larval defence alters adult resource allocation. Proc R Soc Lond B Biol Sci 266:1049–1054

Stoks R, Block MD, Van De Meutter F, Johansson F (2005) Predation cost of rapid growth: behavioural coupling and physiological decoupling. J Anim Ecol 74:708–715. doi:10.1111/j.1365-2656.2005.00969.x

Twohy DW, Rozeboom LE (1957) A comparison of food reserves in autogenous and anautogenous *Culex pipiens* populations. Am J Epidemiol 65:316–324

van Uitregt VO, Hurst TP, Wilson RS (2012) Reduced size and starvation resistance in adult mosquitoes, *Aedes notoscriptus*, exposed to predation cues as larvae. J Anim Ecol 81:108–115. doi:10.1111/j.1365-2656.2011.01880.x

Vidal O, Garcia-Berthou E, Tedesco PA, Garcia-Marin J-L (2010) Origin and genetic diversity of mosquitofish (*Gambusia holbrooki*) introduced to Europe. Biol Invasions 12:841–851. doi:10.1007/s10530-009-9505-5

Vinogradova EB (2000) *Culex pipiens pipiens* mosquitoes: taxonomy, distribution, ecology, physiology, genetic, applied importance and control. Pensoft Publishers, Sofia, Bulgaria

Walker JA (1997) Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. Biol J Linn Soc 61:3–50

Wellborn GA (1994) Size-biased predation and prey life histories: a comparative study of freshwater amphipod populations. Ecology 75:2104–2117

Wellborn GA, Bartholf SE (2005) Ecological context and the importance of body and gnathopod size for pairing success in two amphipod ecomorphs. Oecologia 143:308–316

Werner EE (1974) The fish size, prey size, handling time relation in several sunfishes and some implications. J Fish Res Bd Can 31:1531–1536

Wilbur HM (1980) Complex life cycles. Annu Rev Ecol Syst 11:67–93

2.3 The effect of invasive species on ecosystem services

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ORIGINAL PAPER

Pronounced species turnover, but no functional equivalence in leaf consumption of invasive amphipods in the river Rhine

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Abstract A growing body of literature focuses on the adverse effects of biological invasions, e.g., on the decline of indigenous biodiversity, while studies on the consequences of invasions on components of ecosystem functioning are comparatively rare. Owing to their leaf shredding activity, amphipods play a fundamental role in determining energy flow dynamics in Central European freshwater ecosystems, but whether the dramatic change in species composition after the invasion of Ponto–Caspian taxa affects this process has not been addressed in a comprehensive study. In a laboratory experiment we determined consumption rates of three leaf types (*Alnus glutinosa*, *Betula pendula*, *Quercus robur*) from common riparian arboreal vegetation in the Rhine drainage—one of the most heavily invaded river systems worldwide—by the most common native (*Gammarus fossarum*, *G. pulex*, *G. roeselii*) and invasive amphipods (*Dikerogammarus villosus*, *Echinogammarus ischnus*). Leaf-shredding activity was significantly lower in invasive than in native amphipods across leaf types, and a subsequent analysis ruled out an effect of different metabolic rates as an explanation. Another experiment was motivated by the observation that native amphipods are nowadays restricted to smaller tributaries to the Rhine, while invasive taxa are dominant in the main channel. As leaf litter shredding may be more important in headwaters than in lower parts of streams, we sought for a signature of within-species variation in the feeding ecology of amphipods and thus compared two different populations of *G. pulex*, but found very similar leaf consumption rates in upstream and downstream populations, suggesting that food preferences in amphipods could be species-specific with little potential for microevolution or environmentally induced plasticity. In conclusion, the rapid replacement of native amphipod species in the Rhine drainage likely affects vital ecosystem services,

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with the potential to change the aquatic food web (e.g., through reduced shredding activity and hence, reduced resource availability for particle-feeding detritivores), unless other taxonomic groups compensate for those functional alterations.

Keywords *Dikerogammarus villosus* · *Echinogammarus ischnus* · Ecosystem functioning · *Gammarus* · Shredding

Introduction

Biological invasions are a major component of global change (Millennium Ecosystem Assessment 2005), challenging the conservation of native biodiversity, and can have profound impacts on ecological processes and evolutionary dynamics (Olden et al. 2004; Pejchar and Mooney 2009; Schlaepfer et al. 2011). Some species are keystone species (sensu Mills et al. 1993) in their native ecosystems. Thus, their replacement by invasive species has more far-reaching consequences for ecosystem functioning than would be expected from individual numbers or biomass alone, leading, for example, to altered resource availability or energy flow dynamics (Power et al. 1996; Vitousek et al. 1996; Chapin et al. 2000). However, while biological invasions are a widely documented phenomenon, predicting the ecological consequences of invasion events is often difficult (Ehrenfeld 2010). Walker (1992) highlighted the importance of maintaining the integrity of ecosystem functioning despite species invasions to prevent further species losses.

A central question emerging from those considerations is which of the indigenous and invasive faunal elements are significant to ecosystem functioning. Amphipods of the superfamily Gammaroidea are widespread and constitute an important functional element in European freshwater habitats (MacNeil et al. 1997; Väinölä et al. 2008). In terms of abundance, gammarid species often dominate littoral communities, particularly in rivers and streams (Marchant and Hynes 1981; Dangles et al. 2004). Organic matter from terrestrial sources—mostly leaves and twigs of riparian shrubs and trees—is usually the primary carbon and energy source in small streams of temperate regions (Allan 1995; Wallace et al. 1997), and so the decomposition of leaf litter by amphipods represents an essential process in the headwaters of stream ecosystems (Vannote et al. 1980; Gessner et al. 1999). Indeed, amphipods can be considered keystone species in aquatic ecosystems, as their shredding activity accelerates leaf fragmentation, produces fecal pellets, and transfers nutrients into secondary production, all of which are vital for maintaining diverse aquatic food webs (Petersen and Cummins 1974; Wallace et al. 1997; Graça 2001; Dangles and Malmqvist 2004; Navel et al. 2010). Hence, species replacement in amphipod communities following the introduction of invasive species could adversely affect ecosystem functioning and energy flow in freshwater ecosystems.

The river Rhine is a prime example of a dynamic and heavily invaded freshwater ecosystem due to the passive introduction of exotic species (e.g., in ballast water) as a consequence of intense industrial shipping, as well as active invasions along man-made canals connecting Central and Eastern European freshwater biomes (Bij de Vaate et al. 2002; Bernauer and Jansen 2006; Leuven et al. 2009; Panov et al. 2009). Since the early 20th century, the number of invasive species in the Rhine increased continuously, and nowadays invasive species constitute up to 90 % of macroinvertebrates in terms of biomass and numerical abundance (Van Riel et al. 2006; Leuven et al. 2009). Until the 1970s, pollution from municipal and industrial wastewater promoted faunal exchange (Leuven et al. 2009); for instance, one of the first amphipod invaders, the North American *Gammarus tigrinus* Sexton, 1939 exhibited a higher tolerance against organophosphate agrochemicals, which were accidentally released into the upper Rhine in large quantities in 1986 and affected native *Gammarus fossarum* Koch, 1835 and *G. pulex* Linnaeus, 1758 more than the invasive *G. tigrinus* (Streit and Kuhn 1994). Following the opening of the Rhine–Main–Danube canal in 1992, new invaders from the Ponto–Caspian region arrived, and *G. tigrinus* was quickly replaced by *Dikerogammarus villosus* Sowinsky, 1894 after its first appearance in 1994 (Bij de Vaate et al. 2002; Rewicz et al. 2015). At present, two invasive amphipod species are dominant in the Rhine: *D. villosus* and *Echinogammarus ischnus* Stebbing, 1899, while *G. tigrinus* populations are on steep decline and nearly disappeared again (Dick and Platvoet 2000; Bij de Vaate et al. 2002; Kley and Maier 2003).

The question arises of whether (and to what degree) invasive amphipods differ in their leaf shredding activity from their native relatives and thus, whether invasive taxa provide equivalent ecosystem services. Some studies have started to address this question: Piscart et al. (2011) quantified the breakdown of alder [*Alnus glutinosa* (Linnaeus) Gaertn., 1791] leaves by the most common native and invasive amphipods in France, MacNeil et al. (2011) compared the consumption of sycamore (*Acer pseudoplatanus* L. 1753) leaves by amphipods common in coastal waterbodies in the Netherlands, and a recent study by Boeker and Geist (2015) compared feeding rates of *G. roeselii* and *D. villosus* on alder leaves. All studies found reduced consumption rates of invasive amphipods (*D. villosus* and *G. tigrinus*) compared to their native relatives. A comprehensive study comparing leaf shredding activity of several common invasive and indigenous amphipods in the Rhine, however, is as yet lacking, and no study to date has compared different leaf types occurring in riparian forests. For example, in contrast to *D. villosus*, *E. ischnus* has been largely neglected in experimental studies, even though the latter species became abundant or even dominant in the Rhine drainage over the past years (Leuven et al. 2009; Chen et al. 2012a; Emde et al. 2014). Therefore, one aim of our present study was to compare consumption rates of different leaf litter types by indigenous and invasive amphipod species in a controlled laboratory experiment to quantify to what extent invasive amphipods might show reduced leaf litter decomposition. We chose three different leaf types from common shrub and tree species in Central European riparian forests that differ in several physico-chemical features, as the chemical composition and toughness of leaf tissues influence decomposition rates (Webster and Benfield 1986). For example, alder (*A. glutinosa*) leaves have a high nitrogen content due to nitrogen-fixing bacterial symbionts of alder, and so breakdown rates ought to be higher compared to the much tougher and less nitrogen-rich oak (*Quercus robur* L., 1753) leaves (Webster and Benfield 1986).

An additional aspect we considered in this study was that local resource availability might induce phenotypic (morphological and/or behavioral) plasticity of amphipods, or could even lead to microevolutionary diversification. This could have an effect on the leaf litter shredding activity of amphipods, explaining differences between species, or even among populations of the same species. For example, Stoler and Relyea (2013) reared wood frog (*Lithobates sylvaticus*) tadpoles on different leaf types that differed in the composition of chemical compounds and found different rearing conditions to induce dramatic morphological changes in intestine length, mouth size and tail depth. This aspect is of interest as the occurrence of amphipods in the Rhine drainage typically follows a zonation, with *G. fossarum* being restricted to headwater habitats and other native species occurring further downstream, in smaller tributaries of the Rhine (Meijering 1991; Chen et al. 2012a), while invasive amphipods mainly inhabit the main stream channel (Bij de Vaate et al. 2002; Kley and Maier 2006; Chen et al. 2012a). In a first attempt to shed light on this question, we compared two populations of indigenous *G. pulex*, our prediction being higher consumption rates in a population from a small (third order) stream compared to a population occurring in a downstream (~ seventh order) habitat, as leaf litter is a major energy source especially in headwater regions (Vannote et al. 1980).

Even though we expressed leaf decomposition as mass-corrected consumption rates, amphipod taxa studied in our feeding experiments differed in body size (e.g., the mean dry weight of *D. villosus* exceeded the dry weight of *E. ischnus* by more than threefold; Table 1), and so we asked if any differences in leaf litter consumption would be attributable to differences in mass-related energetic requirements among species. On the other hand, even if the allometric relationship between body mass and metabolic rates did not differ between species, differences in leaf consumption rates detected in our first experiment might still be caused by major species differences in (mass-corrected) metabolic rates. We addressed those questions in our third experiment by measuring oxygen consumption rates of all studied amphipod species (e.g., Rowe et al. 2001; Allan et al. 2006).

Altogether, our study aims to provide a comprehensive overview on consumption rates of three leaf types (*Alnus glutinosa*, *Betula pendula*, *Quercus robur*) from common riparian arboreal vegetation in the Rhine drainage by the most common native (*Gammarus fossarum*, *G. pulex*, *G. roeselii*) and invasive amphipods (*Dikerogammarus villosus*, *Echinogammarus ischnus*). The subsequent analysis of metabolic rates enables the interpretation of different consumption rates in light of potential differences in mass-related energetic requirements among species.

Table 1 Mean (±SE) dry weight of amphipods used in the leaf shredding experiment and numbers of replicates (*n*) for each amphipod species and leaf type

Species	Mean dry weight (mg)	*n* (all/alder/birch/oak)
G. fossarum	4.54 ± 0.16	93/34/33/26
G. pulex	6.24 ± 0.29	140/72/40/28
G. roeselii	5.77 ± 0.28	73/27/22/24
D. villosus	6.42 ± 0.54	109/37/32/40
E. ischnus	1.98 ± 0.31	78/36/26/16

Materials and methods

Sample collection and maintenance of test animals

Freshly fallen alder (*A. glutinosa*), birch (*Betula pendula* Roth, 1788) and oak (*Q. robur*) leaves were collected from a single site per tree species during abscission in fall 2012, air-dried, and stored in the laboratory until they were used. We collected amphipods using a kick-and-sweep sampling technique from several sites in the Rhine drainage between February and May 2013. The two formerly common native amphipod species *G. fossarum* and *G. pulex* are nowadays completely displaced by invasive species in the main channel of the Rhine and in its navigable tributaries (Leuven et al. 2009), and so we obtained *G. fossarum* and *G. pulex* from a small Rhine tributary, the Urselbach (50.216143°N/8.531292°E and 50.168740°N/8.621106°E, respectively). Another sample of *G. pulex* came from the river Lahn (50.391259°N/8.184974°E), and we used those animals to test for potential differences in leaf shredding (using alder leaves) between an upstream (Urselbach) and a downstream (Lahn) population of the same species. *Gammarus roeselii* Gervais, 1835 is a common non-native species from the Balkan area that likely reached Germany during the nineteenth century, after which the species established stable populations and is considered to have been naturalized in parts of Central Europe (Jażdżewski 1980; Josens et al. 2005; Piscart et al. 2010). Specimens were collected from the river Nidda (50.176023°N/8.693425°E). The two most abundant invasive amphipod species in the Rhine drainage, *D. villosus* and *E. ischnus*, were collected in the main channel of the Rhine (*D. villosus*: 50.079110°N/8.521854°E; *E. ischnus*: 49.999345°N/8.381787°E). We transported test animals in aerated, water-filled coolers to the animal care facility at the University of Frankfurt/Main and kept them separated by species in 20-L tanks in ADaM medium (Klüttgen et al. 1994) for 5–7 days before the experiment started. Temperature was maintained at 10 °C throughout, and the light/dark cycle was 14:10 h per day. We provided food in the form of diverse leaf litter taken from the collection sites.

Leaf litter consumption

Air-dried leaves were placed in fine mesh bags and secured in a small stream (located near the campus of the University of Frankfurt am Main) for 14 days to achieve a natural microbial community (Abelho 2001; Graça 2001). We transferred the conditioned leaves to the laboratory and cut the wet leaves into discs of 15 mm diameter using a cork borer while avoiding the central veins. Since the initial weight of the leaf discs could be measured only as wet weight, we established a calculation factor for estimating dry weight from wet weight (alder = 0.163; birch = 0.246; oak = 0.240). The factor was calculated by dividing the dry weight by the respective wet weight of $n = 20$ disks per leaf type before and after drying (60 °C, 48 h), weighted to the nearest 0.1 mg on a Sartorius H110 micro-scale.

For each amphipod species, we randomly chose test individuals from the holding tanks and placed them individually into aerated 100 mL plastic cups, filled with ADaM medium and containing three discs of the same leaf type (mean ± SE dry weight of the three discs, alder: 15.9 ± 0.4 mg; birch: 24.2 ± 0.5 mg; oak: 40.4 ± 0.9 mg; for sample sizes see Table 1) at 10 °C under a 14:10 h light: dark illumination cycle. The ADaM medium was not changed during the experiment in any of the treatments; water quality was not tested, but our experimental protocol likely provided equal conditions for the different amphipod species. We selected only healthy and visibly non-parasitized adults as test animals. We terminated trials after 10 days, and test animals were preserved in 96 % ethanol and weighted (after drying at 60 °C for 48 h), along with the remaining leaf material, to the nearest 0.1 mg. Mass loss due to feeding activity was then determined as the difference between the dry weight of the leaf-discs before and after the experiment, and consumption rates were expressed as mass loss per day. We furthermore ran at least $n = 28$ trials for each leaf type without an amphipod present to control for mass loss from leaching and microbial activity (for microbial contributions to leaf mass loss see Hieber and Gessner 2002; Graça et al. 2015). Daily leaf mass loss in the control treatments was strongest in birch (mean ± SE dry weight: 0.94 ± 0.04 mg/day) but far lower in alder (0.04 ± 0.01 mg) and oak (0.01 ± 0.01 mg).

Oxygen consumption

We investigated oxygen consumption rates as an indicator of metabolic rates. Oxygen consumption was determined in an open-flow respirometry system consisting of a cylindrical respiratory chamber (diameter 14 mm, length 75 mm, volume 11.5 mL), placed in a plastic tank (56 × 25 × 15.5 cm) filled with water and attached to a tubing system with O_2-saturated ADaM medium of 21.6 ± 0.1 °C (mean ± SE) running at a constant flow rate of $0.5\ L\ h^{-1}$. Preliminary tests found lower, more natural temperatures to result in lower oxygen consumption, but also an increased measurement error associated with the measurements. Flow rate was continuously monitored and controlled by a valve and a flow-meter [ROTA Type K12/G1831/77 (WEHR–2), accuracy ±1.6 %]. Directly before and after the respirometry chamber, O_2-concentration was measured by two oxygen electrodes (Oxi 315i, WTW; accuracy: ±0.5 %), which were built into the system in sealed, custom-made cuvettes. Oxygen electrodes were calibrated before each measurement against water vapor saturated air by inserting the sensor in the OxiCal-SL container provided by the manufacturer. Flow conditions in the measuring cuvettes were kept constant by magnetic stirrers. The whole set-up was covered with brown cardboard, such that any disturbance from the outside was reduced to a minimum; however, light was provided by overhead fixtures (room illumination).

Because species differed strongly in body weight (Table 1) and the biomass of a single *E. ischnus* was not sufficient for an adequate measurement in our system, we measured O_2-consumptions of small groups of amphipods. Groups consisted of 2–5 individuals and had a cumulative total wet weight of 0.79 ± 0.46 g (mean ± SD). They were placed into the respirometry chamber, and before any measurement started, amphipods were allowed to settle for 20 min. We recorded oxygen consumption rates over a period of 10 min, provided steady-state readings from the electrodes. We tested $n = 16$ groups for each species. Once a test was completed, groups of amphipods were weighed to the nearest 0.01 g.

Statistical analysis

Overall differences in leaf consumption were tested using a generalized linear model (GLM). The adjusted consumption rate (consumption–microbial activity) per day was log-transformed and used as the dependent variable. We ran the GLM with the data from all leaf types combined, using 'amphipod species' and 'leaf type' as fixed factors, and log-transformed 'amphipod dry weight' as a covariate. For the GLM, we specified a normal error distribution and an identity link function. Inspection of model residuals did not indicate violations of model assumptions (i.e., normal error distribution and homoscedasticity). All possible second and third order interactions of the fixed factors were included in the initial model. However, we chose the model with the lowest Akaike Information Criterion (AIC) value (ΔAIC to the next likely model = 5.89), which excluded the non-significant three-way interaction, while retaining all two-way interactions. Estimated marginal means (EMMs) of consumption rates, adjusted for the covariate body weight, were calculated from the GLM and were used to visualize differences among species. In the case of significant effects, we employed post hoc pairwise comparisons of EMMs (LSD tests).

We further compared the two different populations of *G. pulex* using another GLM, with 'population' as fixed factor and log-transformed 'amphipod dry weight' as a covariate. Again, we chose the model with the lowest AIC (ΔAIC to the next likely model = 1.61), which included the (marginally non-significant) two-way interaction 'population × amphipod dry weight'.

Oxygen consumption rates were compared in a third GLM. We used log-transformed O_2-consumption rates [mL/h] as the dependent variable; 'species' served as fixed factor and log-transformed 'cumulative body weight' as a covariate to account for the correlation between body mass and O_2-consumption. We chose the model with the lowest AIC (ΔAIC to the next likely model = 2.59), which excluded the non-significant two-way interaction 'species × cumulative body weight'. For display purpose, data were depicted in the conventional way of plotting log-transformed O_2-consumption against log-transformed body mass (Fig. 3a) and as mass specific oxygen consumption for each species (Fig. 3b). All analyses were conducted in IBM SPSS Statistics for Windows, Version 22 (IBM Corp., Armonk, NY).

Results

Leaf litter consumption

We found pronounced differences in leaf consumption rates between amphipod species (GLM across all three leaf types: $\chi^2 = 33.66$, $P < 0.001$; Table 2a; Fig. 1a), while 'leaf type' had no significant effect ($\chi^2 = 4.54$, $P = 0.10$). Overall, native *G. pulex* and *G. fossarum* as well as *G. roeselii* were efficient shredders, while invasive *D. villosus* and *E. ischnus* were far less efficient (Fig. 1). Invasive *D. villosus* consumed 56.6 % less leaf material than *G. fossarum* and 63.0 % less than *G. pulex*; *E. ischnus* consumed 67.1 % less than *G. fossarum* and 71.9 % less than *G. pulex* (calculated across all leaf types; Fig. 1a).

Amphipod body mass (i.e., dry weight) had a strong effect on leaf consumption rates ($\chi^2 = 30.34$, $P < 0.001$; Pearson correlation across leaf types and amphipod species: $r_P = +0.28$, $P < 0.001$, $R^2 = 0.08$), but this relationship differed between leaf types (interaction effect of 'leaf type × amphipod dry weight': $\chi^2 = 16.39$, $P < 0.001$), and separate Pearson correlations revealed stronger relationships for nutrient-rich and soft leaf types (alder: $r_P = +0.48$, $P < 0.001$, $R^2 = 0.23$; birch: $r_P = +0.41$, $P < 0.001$, $R^2 = 0.17$) compared to the much harder oak leaves ($r_P = +0.08$, $P = 0.38$, $R^2 = 0.01$; Fig. S1). Moreover, the interaction term of 'species × amphipod dry weight' had a significant effect ($\chi^2 = 15.84$, $P = 0.003$), suggesting differences among species in size/age-related leaf consumption. Consumption rates increased with increasing body mass in four species (*G. pulex*: $r_P = +0.30$, $P = 0.002$, $R^2 = 0.09$; *G. roeselii*: $r_P = +0.45$, $P < 0.001$, $R^2 = 0.20$; *D. villosus*: $r_P = +0.27$, $P = 0.005$, $R^2 = 0.07$; *E. ischnus*: $r_P = +0.22$, $P = 0.053$, $R^2 = 0.05$), but not in *G. fossarum* ($r_P = -0.003$, $P = 0.98$, $R^2 < 0.01$), reflecting that, across leaf types, *G. fossarum* were efficient shredders at small size stages, but did not increase shredding with increasing body size (Fig. S2).

Finally, the interaction effect of 'species × leaf type' suggests different consumption rates depending on leaf type and amphipod species ($\chi^2 = 24.43$, $P = 0.002$): with the exception of *E. ischnus*, all amphipod species showed the highest consumption rates when feeding on alder, followed by birch and oak (Fig. 1b–d).

When we compared alder leaf consumption between two populations of *G. pulex*, we found very similar consumption rates ($\chi^2 = 2.29$, $P = 0.13$; Table 2b; Fig. 2).

Oxygen consumption

The GLM revealed a significant effect of body mass on oxygen consumption ($\chi^2 = 235.79$, $P < 0.001$) while oxygen consumption did not differ between species ($\chi^2 = 7.28$, $P = 0.12$; Fig. 3). The interaction term 'species × body mass' had no significant effect ($\chi^2 = 5.60$, $P = 0.23$) and was removed from the final model. *Post hoc* Pearson correlations between log-transformed oxygen consumption and log-transformed body mass confirmed that oxygen consumption increased in a linear fashion with increasing body mass (*G. fossarum*: $r_P = +0.92$, $P < 0.001$, $R^2 = 0.84$; *G. pulex*: $r_P = +0.89$, $P < 0.001$, $R^2 = 0.80$; *G. roeselii*: $r_P = +0.90$, $P < 0.001$, $R^2 = 0.82$; *D. villosus*: $r_P = +0.89$, $P < 0.001$, $R^2 = 0.79$; *E. ischnus*: $r_P = +0.34$, $P = 0.20$, $R^2 = 0.16$), with no apparent differences in this relationship between species (Fig. 3).

Discussion

We examined leaf litter consumption rates and detected pronounced differences between native and invasive amphipod species from the Rhine drainage, with invasive species showing significantly lower decomposition rates. Our results are congruent with recent studies on *D. villosus* (MacNeil et al. 2011; Piscart et al. 2011; Boeker and Geist 2015) and demonstrate that also the co-occurring invasive *E. ischnus* exhibits strongly reduced shredding activity. We further demonstrate that reduced shredding activity of both invasive species is a consistent outcome when comparing three different leaf types from common riparian arboreal vegetation in Central Europe. Accordingly, we argue that invasive amphipods are not functionally equivalent to their native

Table 2 Results from two generalized linear models (GLMs) on consumption rates

Treatment	Source	χ^2	*df*	*P*
(a) Species comparison	**Species**	**33.66**	**4**	**<0.001**
	Leaf type	4.54	2	0.103
	Amphipod dry weight	**30.34**	**1**	**<0.001**
	Species × leaf type	**24.43**	**8**	**0.002**
	Species × amphipod dry weight	**15.84**	**4**	**0.003**
	Leaf type × amphipod dry weight	**16.39**	**2**	**<0.001**
(b) Population comparison	Population	2.29	1	0.130
	Amphipod dry weight	**39.75**	**1**	**<0.001**
	Population × amphipod dry weight	3.70	1	0.054

Statistically significant effects are highlighted in bold

Models were selected using the Akaike Information Criterion. (a) Analysis across amphipod species and leaf types; (b) analysis comparing alder consumption rates of two populations of *G. pulex*

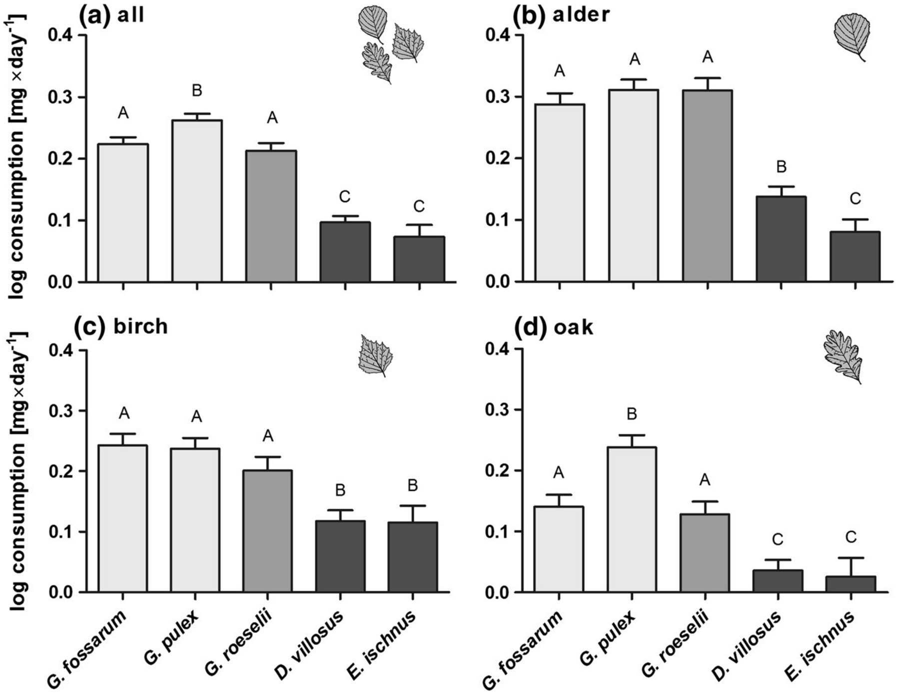

Fig. 1 Mean (±SE) leaf consumption rates **a** across all three tree types and determined for each leaf type separately, i.e., with leaves of **b** alder, **c** birch, and **d** oak. Depicted are estimated marginal means derived from the GLM, where amphipod body mass was adjusted to 0.0022 log(g). Significant between-species differences in breakdown rates (derived from pairwise post hoc comparisons of EMM using LSD tests) are indicated by *different letters*. Native species are depicted in *light gray*, the 'naturalized' species in intermediate *gray* and invasive species in *dark gray*

relatives they replaced in the main channel of the Rhine, and the species turnover observed in recent decades is likely accompanied by alterations in energy-flow dynamics and ecosystem functioning. In theory, lower metabolic rates could explain reduced leaf consumption rates, but such an effect seems unlikely as we found rather similar (or even slightly higher) metabolic rates in *D. villosus* and *E. ischnus* compared to native *Gammarus* species. We also tested for within-species variation, hypothesizing that

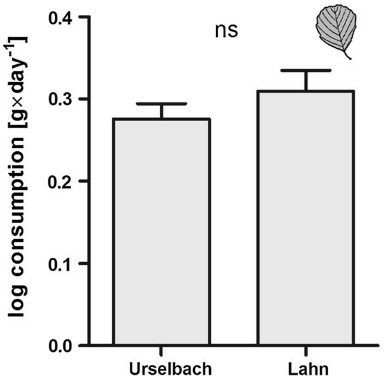

Fig. 2 Comparison of mean (±SE) consumption rates of alder leaves between two populations of *G. pulex.* Amphipods stemmed from a low-order stream (Urselbach) and the middle part of the river Lahn. Depicted are estimated marginal means derived from a GLM, where amphipod body mass was adjusted to 0.0018 log(g)

contrasting ecological conditions in low-order parts of streams (where leaf litter is the most important energy source; Vannote et al. 1980) will result in higher shredding activity compared to populations from higher-order regions. Our comparison of two native *G. pulex* populations revealed no difference in consumption rates, and so traits determining the trophic ecology of amphipods (like mouth-part morphology and behavioral traits) could be species-specific characteristics with little potential for microevolution or phenotypic plasticity. The latter result, however, needs to be interpreted with caution, as true downstream populations of native amphipods (i.e., inhabiting the Rhine main channel) went extinct during the past decades.

Species differences in leaf litter consumption

Higher leaf consumption rates of native amphipods compared to *D. villosus* could be explained by a more predaceous life-style of *D. villosus* (Dick and Platvoet 2000; Kley and Maier 2003; MacNeil and Platvoet 2005), an interpretation that would be consistent with earlier studies on trophic relationships using stable isotope and gut content analyses (Van Riel et al. 2006; Bacela-Spychalska and van der Velde 2013). Recent studies using stable isotope analysis, however, did not reveal differences in the trophic position of *D. villosus* compared to native amphipods (Koester and Gergs 2014; Rothhaupt et al. 2014). Likewise, Koester and Gergs (2014) analyzed gut contents using taxon-specific molecular primers for gammarid amphipods, but found no evidence for intra-guild predation by *D. villosus*—a characteristic often attributed to *D. villosus* in previous studies (e.g., Dick and Platvoet 2000; MacNeil and Platvoet 2005). Similar observations were made by Truhlar et al. (2014), who found no predation by *D. villosus* on *G. pulex* in leaf consumption experiments. It seems, therefore, that *D. villosus* is an omnivore with flexible feeding strategies rather than a strict predator (Platvoet et al. 2006, 2009). This raises the question as to whether *D. villosus* actually possess the functional mouthpart morphology required for efficient leaf shredding. Mayer et al. (2008) addressed this question and concluded that the incisors

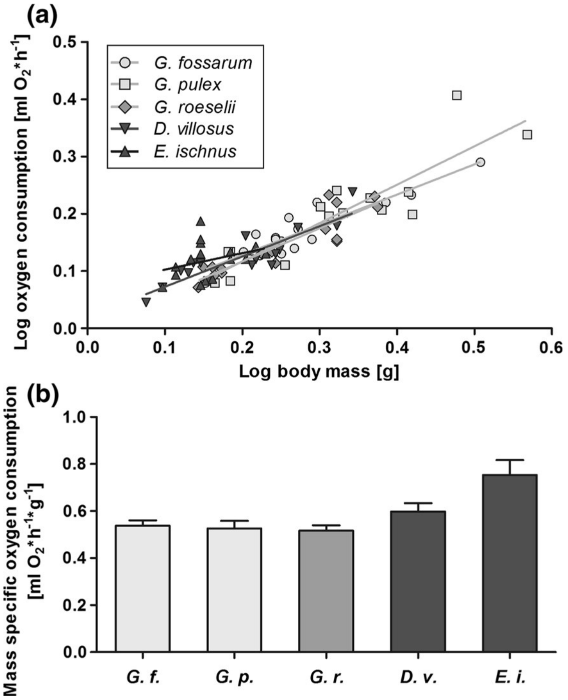

Fig. 3 Graphic illustration of the significant effect of body mass on metabolic rates of amphipods during the respirometric measurements (GLM, see main text); **a** shows the relationship between metabolic rates [log(mL $O_2 \times h^{-1}$)] and body mass [log(g)] for each species separately to visualize the non-significant interaction effect of 'species × body mass' (i.e., slope homogeneity), while **b** shows mean (±SE) mass-specific O_2-consumption rates of *Gammarus fossarum* (*G.f*). *G. pulex* (*G.p.*), *G. roeslii* (*G.r.*), *Dikerogammarus villosus* (*D.v.*) and *Echinogammarus ischnus* (*E.i.*), visualizing absence of a significant 'species'-effect. Native species are depicted in *light gray*, the 'naturalized' species in intermediate *gray* and invasive species in *dark gray*

of the mandibles appear robust and sharp enough to cut off pieces of leaf material. At least from a morphological perspective *D. villosus* should, therefore, have been able to consume similar amounts of leaf litter as native amphipods in our feeding experiment. Still, anatomical and physiological differences of the gastrointestinal tract, as well as behavioral differences are potential explanations for why leaf consumption rates were lower than those of native amphipods.

The trophic role of *E. ischnus* in the river Rhine has not been addressed, and previous studies in the Vistula river drainage in Poland assigned *E. ischnus* to a comparable trophic position as *G. fossarum* (Bacela-Spychalska and van der Velde 2013). In our present study, however, the shredding activity of *E. ischnus* was significantly lower than that of native amphipods. While studies on the trophic ecology of *E. ischnus* are rare, the species is thought to exhibit nonspecific food preferences (Bij de Vaate et al. 2002). Laboratory experiments also reported a stronger predatory behavior of *E. ischnus* compared to native *Gammarus* species, including the observation of intra-guild predation (Kinzler and Maier 2003; Krisp and Maier 2005). Kley and Maier (2006) explained the frequent sympatric occurrence of *E. ischnus* and *D. villosus* with different reproductive strategies: the reproductive life-history strategy of *E. ischnus* is characterized by the production of few, but large eggs to increase juvenile survival in reaches with harsh conditions, enabling juveniles to switch to microhabitats with low food availability/quality in the presence of *D. villosus*. Since studies on the mouthparts of *E. ischnus* are missing, the question of whether their mouthparts may be unsuitable to consume large amounts of leaf litter remains unanswered.

All *Gammarus* species showed, on average, similarly high leaf consumption rates. *Gammarus roeselii* is usually regarded as native or naturalized (e.g., Josens et al. 2005), even though it was most likely one of the first invasive amphipods in the Rhine drainage. The construction of the Ludwigskanal connected the Rhine and Danube systems in 1845 likely facilitated the spread of *G. roeselii* from the Danube to the Rhine drainage (Jażdżewski 1980). *Gammarus roeselii* seems to adopt a similar trophic position as its native relatives, which could be a signature of phylogenetic relatedness (Hou et al. 2007). An alternative possibility would be that early invaders adopted a different trophic niche, but present-day *G. roeselii* could have evolved towards a more leaf-consuming life style. Our limited data implying absence of within-species variation in two contrasting *G. pulex* populations, however, do not support such a scenario (but see discussion below).

We found different relationships between body mass and leaf consumption among amphipod species: While *G. pulex*, *G. roeselii* and *D. villosus* increased their consumption rates with increasing body mass, consumption rates were not as strongly dependent on body mass in *G. fossarum*. Also in *E. ischnus* no body mass effect was detected, even though this was likely due to very low overall consumption rates in that species. Hence, small specimens of *G. fossarum* efficiently shred leaf litter, but do not increase leaf litter consumption when reaching a higher body mass (and higher energy demand). We are lacking a compelling explanation for this finding, but tentatively propose that it might reflect a limited capacity of the digestive tract or mouth parts, which may not increase iso-allometrically with increasing body size (i.e., hypoallometry). Assuming that older (and heavier) individuals of *G. fossarum* show a predilection for additional (possibly more energy-rich) food sources, our study may serve as a starting point to investigate potential ontogenetic shifts in trophic niches in native (and invasive) amphipods (see Summers et al. 1997).

Since leaves from different tree species degrade at different rates (see different mass loss of our control samples), this could lead to a pattern where resources become available to the different amphipod species at different points in time during the leaf degradation cycle. However, our results indicated that both invasive amphipods did not show the consumption rates of their native relatives even when rapidly degrading birch leaves were offered. Still, in contrast to the other amphipod species, *E. ischnus* showed the highest consumption rates when birch leaves were provided (followed by alder and oak), indicating a preference for fast-degrading leaves.

Effects of invasive amphipods on ecosystem functioning

A major challenge for ecologists is to predict further range expansions of invasive taxa and their consequences for ecosystem services. The origin of amphipod populations that invaded Central Europe most likely are the downstream portions of the Danube river (Bij de Vaate et al. 2002; Rewicz et al. 2015), and so invasive populations in the Rhine drainage may be adapted to downstream conditions. Apparently, the range expansions of invasive amphipods in the Rhine drainage slows down where navigability ends (e.g., in the river Lahn: Chen et al. 2012a, b), and smaller tributaries are typically not invaded. However, the factors that might prevent a further spread are as yet unknown. We propose that the progressive shift in basic food resources along the river continuum may be a delimiting factor for further range expansions, since native amphipods are better able to exploit the major energy source (leaf litter) in upstream regions, where they might out-compete invading taxa.

One could argue that through continuous adaptation invasive amphipods might become increasingly competitive by relying more on leaf litter as a food source and in the future could even colonize tributaries and headwaters. *Echinogammarus ischnus*, for example, also occurs in headwaters within its native distribution range (Jażdżewski 1980; Bij de Vaate et al. 2002; Konopacka et al. 2014). On the other hand, inducible phenotypic responses to variation in resource quantity and quality (i.e., phenotypic plasticity) might play a role in exploitation of leaf litter as a food resource (see Stoler and Relyea 2013). Our present study compared two contrasting *G. pulex* populations to test for population differences along the river continuum and found no difference in their leaf shredding activity. This result suggests absence of microevolutionary or environmentally induced (i.e., plastic) trophic diversification in gammarids, with the reservation that we did not include true downstream-adapted populations of *G. pulex*, as these went extinct in recent decades. Hence, future studies should make an attempt to investigate trophic characters of Ponto–Caspian amphipod populations occurring from headwaters to higher-order regions of streams within their native ranges and thus provide a more detailed test for a potential signature of local adaptation and possible gradient evolution. Furthermore, phenotypically plastic responses to resource availability might result in morphological differences between populations and hence result in different consumption rates, an aspect that should also be considered in more detail in future studies.

Due to physical and biological degradation processes, the particle size of leaf material becomes progressively smaller in downstream areas (Vannote et al. 1980). However, also higher-order streams have riparian arboreal vegetation and receive bio-energetic input from leaf material, especially during water high-stands (Cuffney 1988)—even though damming and stream regulation reduced this form of allochthonous energy input in many Central European streams (Wetzel 2001), which could further explain the invasion success of taxa that rely less on leaf material as a food source. Since invasive amphipods fail to maintain the shredding activity of native amphipods in higher-order streams, the question arises if other taxonomic groups can compensate and maintain this function. Diverse communities tend to provide more stable ecosystem services than less diverse communities, and a compensatory community response may maintain ecosystem functions after the loss of certain taxonomic groups (Kremen 2005). Indeed, experimental and theoretical studies suggest that other species can buffer ecosystem functioning against changing environmental conditions (for reviews see: Hooper et al. 2005; Balvanera et al. 2006), but only few studies have empirically tested the potential for other species to compensate the functional role of displaced taxa in freshwater ecosystems (reviewed in Covich et al. 2004). For example, many aquatic insect larvae (e.g., several families of caddisflies and stoneflies) are trophic generalists (Friberg and Jacobsen 1994; Graça 2001), and such omnivores could exploit leaf litter in absence of more specialized shredders and thus, sustain the key process of decomposition despite the absence of the (former) keystone species. Long-term investigations on the trophic ecology (including stable isotope and gut content analyses, as well as feeding experiments) of a broader range of native and introduced aquatic taxa in the Rhine drainage would provide valuable insights into the temporal dynamics of possible compensatory mechanisms of leaf litter decomposition.

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References

Abelho M (2001) From litterfall to breakdown in streams: a review. Sci World 1:656–680

Allan JD (1995) Stream ecology. Chapman & Hall, London

Allan E, Froneman P, Hodgson A (2006) Effects of temperature and salinity on the standard metabolic rate (SMR) of the caridean shrimp *Palaemon peringueyi*. J Exp Mar Biol Ecol 337(1):103–108

Bacela-Spychalska K, van der Velde G (2013) There is more than one 'killer shrimp': trophic positions and predatory abilities of invasive amphipods of Ponto–Caspian origin. Freshw Biol 58(4):730–741. doi:10.1111/fwb.12078

Balvanera P, Pfisterer AB, Buchmann N, He JS, Nakashizuka T, Raffaelli D, Schmid B (2006) Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecol Lett 9(10):1146–1156

Bernauer D, Jansen W (2006) Recent invasions of alien macroinvertebrates and loss of native species in the upper Rhine River, Germany. Aquat Invasions 1(2):55–71

Bij de Vaate A, Jazdzewski K, Ketelaars HA, Gollasch S, Van der Velde G (2002) Geographical patterns in range extension of Ponto–Caspian macroinvertebrate species in Europe. Can J Fish Aquat Sci 59(7):1159–1174

Boeker C, Geist J (2015) Effects of invasive and indigenous amphipods on physico-chemical and microbial properties in freshwater substrates. Aquat Ecol 49(4):467–480. doi:10.1007/s10452-015-9539-y

Chapin FSI, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, Hooper DU, Lavorel S, Sala OE, Hobbie SE (2000) Consequences of changing biodiversity. Nature 405(6783):234–242

Chen W, Bierbach D, Plath M, Streit B, Klaus S (2012a) Distribution of amphipod communities in the Middle to Upper Rhine and five of its tributaries. Bioinvasions Rec 1(4):263–271

Chen W, Lipkowski K, Jourdan J, Plath M, Streit B, Klaus S (2012b) *Dikerogammarus haemobaphes* in the River Lahn, Germany, with notes on the dynamics of Amphipoda assemblages. Lauterbornia 75:87–93

Covich AP, Austen MC, Bärlocher F, Chauvet E, Cardinale BJ, Biles CL, Inchausti P, Dangles O, Solan M, Gessner MO (2004) The role of biodiversity in the functioning of freshwater and marine benthic ecosystems. Bioscience 54(8):767–775

Cuffney TF (1988) Input, movement and exchange of organic matter within a subtropical coastal black water river-flood plain system. Freshw Biol 19(3):305–320

Dangles O, Malmqvist B (2004) Species richness–decomposition relationships depend on species dominance. Ecol Lett 7(5):395–402

Dangles O, Gessner MO, Guerold F, Chauvet E (2004) Impacts of stream acidification on litter breakdown: implications for assessing ecosystem functioning. J Appl Ecol 41(2):365–378

Dick JT, Platvoet D (2000) Invading predatory crustacean *Dikerogammarus villosus* eliminates both native and exotic species. Proc R Soc Lond B Biol Sci 267(1447):977–983

Ehrenfeld JG (2010) Ecosystem consequences of biological invasions. Annu Rev Ecol Evol Syst 41:59–80

Emde S, Kochmann J, Kuhn T, Plath M, Klimpel S (2014) Getting what is served? feeding ecology influencing parasite-host interactions in invasive round goby *Neogobius melanostomus*. PLoS ONE 9(10):e109971

Friberg N, Jacobsen D (1994) Feeding plasticity of two detritivore-shredders. Freshw Biol 32(1):133–142

Gessner MO, Chauvet E, Dobson M (1999) A perspective on leaf litter breakdown in streams. Oikos 85:377–384

Graça MAS (2001) The role of invertebrates on leaf litter decomposition in streams—a review. Int Rev Hydrobiol 86(4–5):383–393

Graça MAS, Ferreira V, Canhoto C, Encalada AC, Guerrero-Bolaño F, Wantzen KM, Boyero L (2015) A conceptual model of litter breakdown in low order streams. Int Rev Hydrobiol 100(1):1–12. doi:10.1002/iroh.201401757

Hieber M, Gessner MO (2002) Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology 83(4):1026–1038

Hooper DU, Chapin Iii F, Ewel J, Hector A, Inchausti P, Lavorel S, Lawton J, Lodge D, Loreau M, Naeem S (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecol Monogr 75(1):3–35

Hou Z, Fu J, Li S (2007) A molecular phylogeny of the genus *Gammarus* (Crustacea: Amphipoda) based on mitochondrial and nuclear gene sequences. Mol Phylogenetics Evol 45(2):596–611

Jażdżewski K (1980) Range extensions of some gammaridean species in European inland waters caused by human activity. Crustaceana Suppl 6:84–107

Josens G, de Bij Vaate A, Usseglio-Polatera P, Cammaerts R, Cherot F, Grisez F, Verboonen P, Bossche J-PV (2005) Native and exotic Amphipoda and other Peracarida in the River Meuse: new assemblages emerge from a fast changing fauna. Hydrobiologia 542:203–220

Kinzler W, Maier G (2003) Asymmetry in mutual predation: possible reason for the replacement of native gammarids by invasives. Arch Hydrobiol 157(4):473–481

Kley A, Maier G (2003) Life history characteristics of the invasive freshwater gammarids *Dikerogammarus villosus* and *Echinogammarus ischnus* in the river Main and the Main-Donau canal. Arch Hydrobiol 156(4):457–470

Kley A, Maier G (2006) Reproductive characteristics of invasive gammarids in the Rhine–Main–Danube catchment, South Germany. Limnologica 36(2):79–90

Klüttgen B, Dülmer U, Engels M, Ratte H (1994) ADaM, an artificial freshwater for the culture of zooplankton. Water Res 28(3):743–746

Koester M, Gergs R (2014) No evidence for intraguild predation of *Dikerogammarus villosus* (Sowinsky, 1894) at an invasion front in the Untere Lorze, Switzerland. Aquat Invasions 9(4):489–497

Konopacka A, Hupalo K, Rewicz T, Graboswki M (2014) Species inventory and distribution patterns of freshwater amphipods in Moldova. North-West J Zool 10(1):4

Kremen C (2005) Managing ecosystem services: what do we need to know about their ecology? Ecol Lett 8(5):468–479

Krisp H, Maier G (2005) Consumption of macroinvertebrates by invasive and native gammarids: a comparison. J Limnol 64(1):55–59

Leuven RS, van der Velde G, Baijens I, Snijders J, van der Zwart C, Lenders HR, Bij de Vaate A (2009) The river Rhine: a global highway for dispersal of aquatic invasive species. Biol Invasions 11(9):1989–2008

MacNeil C, Platvoet D (2005) The predatory impact of the freshwater invader *Dikerogammarus villosus* on native *Gammarus pulex* (Crustacea: Amphipoda); influences of differential microdistribution and food resources. J Zool 267(1):31–38

MacNeil C, Dick JT, Elwood RW (1997) The trophic ecology of freshwater *Gammarus* spp. (Crustacea: Amphipoda): problems and perspectives concerning the functional feeding group concept. Biol Rev Camb Philos Soc 72(03):349–364

MacNeil C, Dick JT, Platvoet D, Briffa M (2011) Direct and indirect effects of species displacements: an invading freshwater amphipod can disrupt leaf-litter processing and shredder efficiency. J North Am Benthol Soc 30(1):38–48

Marchant R, Hynes H (1981) The distribution and production of *Gammarus pseudolimnaeus* (Crustacea: Amphipoda) along a reach of the Credit River, Ontario. Freshw Biol 11(2):169–182

Mayer G, Maier G, Maas A, Waloszek D (2008) Mouthparts of the Ponto–Caspian invader *Dikerogammarus villosus* (Amphipoda: Pontogammaridae). J Crustac Biol 28(1):1–15

Meijering MP (1991) Lack of oxygen and low pH as limiting factors for *Gammarus* in Hessian brooks and rivers. Hydrobiologia 223(1):159–169

Millennium Ecosystem Assessment (2005) Ecosystems and human well-being: synthesis. Island Press, Washington

Mills LS, Soulé ME, Doak DF (1993) The keystone-species concept in ecology and conservation. Bioscience 43:219–224

Navel S, Mermillod Blondion F, Montuelle B, Chauvet E, Simon L, Piscart C, Marmonier P (2010) Interactions between fauna and sediment control the breakdown of plant matter in river sediments. Freshw Biol 55(4):753–766

Olden JD, Poff NL, Douglas MR, Douglas ME, Fausch KD (2004) Ecological and evolutionary consequences of biotic homogenization. Trends Ecol Evol 19(1):18–24

Panov VE, Alexandrov B, Arbačiauskas K, Binimelis R, Copp GH, Grabowski M, Lucy F, Leuven RS, Nehring S, Paunović M (2009) Assessing the risks of aquatic species invasions via European inland waterways: from concepts to environmental indicators. Integr Environ Assess Manag 5(1):110–126

Pejchar L, Mooney HA (2009) Invasive species, ecosystem services and human well-being. Trends Ecol Evol 24(9):497–504

Petersen RC, Cummins KW (1974) Leaf processing in a woodland stream. Freshw Biol 4(4):343–368

Piscart C, Bergerot B, Laffaille P, Marmonier P (2010) Are amphipod invaders a threat to regional biodiversity? Biol Invasions 12(4):853–863

Piscart C, Mermillod-Blondin F, Maazouzi C, Merigoux S, Marmonier P (2011) Potential impact of invasive amphipods on leaf litter recycling in aquatic ecosystems. Biol Invasions 13(12):2861–2868

Platvoet D, Dick JT, Konijnendijk N, van der Velde G (2006) Feeding on micro-algae in the invasive Ponto–Caspian amphipod *Dikerogammarus villosus* (Sowinsky, 1894). Aquat Ecol 40(2):237–245

Platvoet D, van der Velde G, Dick JT, Li S (2009) Flexible omnivory in *Dikerogammarus villosus* (Sowinsky, 1894)(Amphipoda) Amphipod Pilot Species Project (AMPIS) Report 5. Crustaceana 82(6):703–720

Power ME, Tilman D, Estes JA, Menge BA, Bond WJ, Mills LS, Daily G, Castilla JC, Lubchenco J, Paine RT (1996) Challenges in the quest for keystones. Bioscience 46:609–620

Rewicz T, Wattier R, Grabowski M, Rigaud T, Bącela-Spychalska K (2015) Out of the Black Sea: Phylogeography of the Invasive Killer Shrimp *Dikerogammarus villosus* across Europe. PLoS ONE 10(2):e0118121. doi:10.1371/journal.pone.0118121

Rothhaupt K-O, Hanselmann AJ, Yohannes E (2014) Niche differentiation between sympatric alien aquatic crustaceans: an isotopic evidence. Basic Appl Ecol 15:453–463

Rowe CL, Hopkins WA, Zehnder C, Congdon JD (2001) Metabolic costs incurred by crayfish (*Procambarus acutus*) in a trace element-polluted habitat: further evidence of similar responses among diverse taxonomic groups. Comp Biochem Physiol Part C Toxicol Pharmcol 129(3):275–283. doi:10.1016/s1532-0456(01)00204-6

Schlaepfer MA, Sax DF, Olden JD (2011) The potential conservation value of non-native species. Conserv Biol 25(3):428–437

Stoler AB, Relyea RA (2013) Leaf litter quality induces morphological and developmental changes in larval amphibians. Ecology 94(7):1594–1603

Streit B, Kuhn K (1994) Effects of organophosphorous insecticides on autochthonous and introduced *Gammarus* species. Water Sci Technol 29(3):233–240

Summers RB, Delong M, Thorp J (1997) Ontogenetic and temporal shifts in the diet of the amphipod *Gammarus fasciatus*, in the Ohio River. Am Midl Nat 137:329–336

Truhlar AM, Dodd JA, Aldridge DC (2014) Differential leaf-litter processing by native (*Gammarus pulex*) and invasive (*Dikerogammarus villosus*) freshwater crustaceans under environmental extremes. Aquat Conserv Mar Freshw 24(1):56–65

Väinölä R, Witt J, Grabowski M, Bradbury JH, Jazdzewski K, Sket B (2008) Global diversity of amphipods (Amphipoda; Crustacea) in freshwater. Hydrobiologia 595(1):241–255

Van Riel M, Van der Velde G, Rajagopal S, Marguillier S, Dehairs F, Bij de Vaate A (2006) Trophic relationships in the Rhine food web during invasion and after establishment of the Ponto–Caspian invader *Dikerogammarus villosus*. Hydrobiologia 565:39–58

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE (1980) The river continuum concept. Can J Fish Aquat Sci 37(1):130–137

Vitousek PM, D'Antonio CM, Loope LL, Westbrooks R (1996) Biological invasions as global environmental change. Am Sci 84(5):468–478

Walker BH (1992) Biodiversity and ecological redundancy. Conserv Biol 6(1):18–23

Wallace JB, Eggert SL, Meyer JL, Webster JR (1997) Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277(5322):102–104

Webster J, Benfield E (1986) Vascular plant breakdown in freshwater ecosystems. Annu Rev Ecol Syst 17:567–594

Wetzel RG (2001) Limnology: lake and river ecosystems, 3rd edn. Academic Press, San Diego

2.4 Behavioural differences between native and invasive species

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Highly variable, unpredictable activity patterns in invasive, but not native amphipod species

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Abstract Behavioral differences between native and introduced species may contribute to the invasiveness of certain species. This includes differences at the species level, consistent variation among individuals ("personality") and within-individual variation (e.g., behavioral plasticity). Here, we investigated swimming activity of individuals from four different amphipod species occurring in the river Rhine system, three of which were native or naturalized (>100 years present) while one is a recent invader (*Dikerogammarus villosus*, <25 years present). At the species level, *D. villosus* did not show higher average swimming activity than the three non-invasive species. However, the non-invasive species, on average, changed their behavior predictably over the course of the experiment ("average behavioral plasticity"), while *D. villosus* did not exhibit any consistent change in activity. At the individual level, *D. villosus* exhibited greater among- and within-individual variation in activity levels than all non-invasive species. The non-invasive species further showed significant individual differences in plasticity, that is, individuals of these species differed consistently in how they changed their activity over time. The high within-individual variation in *D. villosus* translated into a lack of consistent individual differences in plasticity in this species. We hypothesize that the highly variable and unpredictable patterns of

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individual activity variation in *D. villosus* might help this successful invader to cope with new environmental conditions encountered in the river Rhine system.

Keywords Behavioral plasticity · *Dikerogammarus villosus* · *Gammarus pulex* · Animal personality · Invasion

Introduction

Biological invasions are a common feature in a globalizing world (Davis 2009; Lockwood et al. 2013). However, to reach the stage of “full invasiveness” (Blackburn et al. 2011), a species has to pass successfully through several stages, including transport and introduction outside their natural distribution range, population establishment and further spread (reviewed in Chapple et al. 2012). At each stage, variation in physiological, morphological, life history, behavioral and other traits may hamper or promote invasion success (Kolar and Lodge 2001; Hayes and Barry 2008; Canestrelli et al. 2015). Hence, dispersing individuals likely represent a non-random sample of the initial population, selected on the basis of interindividual variation in multiple traits (Hayes and Barry 2008; Chapple et al. 2012; Canestrelli et al. 2015). Behavior may be especially relevant in determining how successful individuals of a given population are at invading a novel environment (Chapple et al. 2012; Sih et al. 2012; Wolf and Weissing 2012; Carere and Gherardi 2013; Canestrelli et al. 2015). Empirical studies for a range of taxa have documented differences between dispersers and residents (reviewed in Cote et al. 2010a; Canestrelli et al. 2015); for instance, dispersers tend to be, on average, more aggressive (Duckworth and Badyaev 2007) and less sociable than residents (Cote et al. 2010b).

Given the recent upsurge of research on animal personality (e.g., Magurran 1986; Sih et al. 2004, 2012; Réale et al. 2007), the question arises as to whether native and invading populations/species differ systematically in other components of behavioral variation beside mean differences between population/species (e.g., Carvalho et al. 2013; Canestrelli et al. 2015). Animal personality research emphasizes that individuals tend to differ consistently in their behavioral tendencies (among-individual variation). Among-individual behavioral variation within a subset of dispersing individuals may influence invasion success (Wright et al. 2010; Phillips and Suarez 2012), and two conflicting predictions can be formulated: (1) First, greater among-individual variation may be expected in successful invaders due to insurance effects (a.k.a. “portfolio effect,” see Schindler et al. 2010; Anderson et al. 2013), whereby the presence of different behavioral types buffers a population against harsh or novel environmental conditions encountered in the novel distribution range (see similar argumentations in Fogarty et al. 2011; Wolf and Weissing 2012). (2) Alternatively, invasive species/populations may exhibit lower among-individual variation in behavior if the population recently underwent a genetic bottleneck (Tsutsui et al. 2000, Canestrelli et al. 2015), or as a consequence of directional selection (Cote et al. 2010a).

Animal personality research also highlights the need to investigate components of within-individual variation (Fogarty et al. 2011; Dingemanse and Wolf 2013). Within-individual variation in behavior refers to any behavioral changes an individual makes over time (in behavioral experiments: with repeated testing) or in different situations (Dingemanse et al. 2010). In some cases, this variation can be considered noise in the behavioral signal, but unpredictable and changing behavior can be advantageous, for example, when avoiding predators (Dingemanse et al. 2010). A particular form of within-individual variation is behavioral plasticity, which can be defined as predictable changes in behavior over an environmental gradient (Dingemanse et al. 2010). Well-known examples are habituation (where individuals consistently decrease a given behavior over time) and sensitization (where individuals increase a given behavior; Brown 2001). High levels of within-individual variation allow individuals to quickly change their behavior in response to a novel environment (Gross et al. 2010), and so invasive populations are predicted to show greater within-individual behavioral variation than native populations (Chapple et al. 2012).

In our present study, we investigated behavioral differences at the species level, as well as patterns of among- and within-individual variation between four different amphipod species (2 natives, 1 naturalized and 1 invasive) occurring in the river Rhine system (Germany). Amphipods offer an excellent system to investigate how behavior influences invasion success (see

Truhlar and Aldridge 2015), as there are several cases of invasive and native populations occurring syntopically or in close proximity, minimizing potentially confounding effects of ecological differences when comparing invasive and indigenous populations (Grabowski et al. 2007; Chen et al. 2012). One of the most successful amphipod invaders that entered the Rhine in 1994 is *Dikerogammarus villosus* Sowinsky, 1894 (Dick and Platvoet 2000; Bij de Vaate et al. 2002; Kinzler et al. 2009). *Dikerogammarus villosus* has the potential to affect several native species such as *Gammarus fossarum*, *G. pulex* and the well-established (naturalized) early invader *G. roeseli* (Dick and Platvoet 2000; Kinzler et al. 2009; Platvoet et al. 2009), which are the species we investigated in our present study.

A recent study investigated behavioral types of *D. villosus* and *G. pulex* in the UK, where both species are invasive, and *G. pulex* was found to be, on average, more active and more explorative than *D. villosus* (Truhlar and Aldridge 2015). Likewise, when tested in groups, more individuals that are moving were found in *G. pulex* compared to *D. villosus* (Maazouzi et al. 2011). In another study, the naturalized *G. roeseli* spent more time swimming freely than *D. villosus* and thus had an increased likelihood to fall victim to fish predation (Kley et al. 2009). However, neither have average activity levels been compared between invasive *D. villosus* and co-occurring native amphipods in Germany, nor have components of among- and within-individual variation been investigated in this framework.

Here, we compared the two native species *G. fossarum* and *G. pulex*, the naturalized *G. roeseli* and the invasive *D. villosus* from the Rhine system in Germany and repeatedly measured individual activity levels over the course of one week (5 days). This allowed us to quantify average behavioral differences between species, as well as among- and within-individual components of behavioral variation. We predicted that invasive *D. villosus* should exhibit (*a*) no higher overall levels of activity, but (*b*) greater among- and within-individual variation compared to the three other species (*G. fossarum*, *G. pulex* and *G. roeseli*). (*c*) Finally, we predicted that all species should exhibit habituation (e.g., decreases in activity with repeated testing; a form of behavioral plasticity), but again, the strongest among-individual variation in this plasticity should be seen in invasive *D. villosus*.

Materials and methods

Study system

The Rhine is one of the most dynamic and most heavily invaded freshwater ecosystems worldwide (Bij de Vaate et al. 2002; Leuven et al. 2009) due to man-made canals connecting Central and Eastern Europe freshwater biomes. Although canal construction started in the early eighteenth century, the Rhine-Main-Danube canal, which was completed in 1992, only recently opened invasion corridors that resulted in a massive influx of Ponto-Caspian amphipod species into Central and Western Europe (Bij de Vaate et al. 2002). They are key players in aquatic ecosystems, because their shredding activity of coarse particulate organic matter is crucial in many aquatic ecosystems, and detritus is an essential resource for maintaining diverse food webs (Wallace et al. 1997; Hunting et al. 2012; Jourdan et al. 2016). Invasive amphipods constitute up to 90 % of macroinvertebrates in terms of biomass and numerical abundance in the Rhine (Van Riel et al. 2006; Leuven et al. 2009), and so they can be considered ecosystem engineers (sensu Cassey et al. 2004) determining the functional diversity and food web structure (MacNeil et al. 2012).

Animal collection and maintenance

We collected amphipods between May 1, 2013 and August 28, 2013 with a “kick and sweep” technique (Barbour et al. 1999) at four nearby sites in the vicinity of Frankfurt am Main (Fig. 1) using a pond net with an opening of 25 × 25 cm, depth of 60 cm and mesh width of 500 µm (Bioform Entomology & Equipment, Germany). We identified species using the keys provided by Eggers and Martens (2001) in water-filled petri dishes under a stereomicroscope (Microscopes Nikon SMZ 1500 and Nikon AZ 100, Japan). Each sampling site was almost exclusively dominated (>90 % of collected individuals) by a single amphipod species (see Chen et al. 2012; Jourdan et al. 2016; Fig. 1). This eliminates potential effects of site-specific differences in community structures and species interactions on the amphipods’ behavior.

We transported animals in aerated, water-filled coolers to the animal care facilities at the University of Frankfurt/Main and kept them in 20-L tanks containing ADaM (Aachener Daphnien Medium; Klüttgen

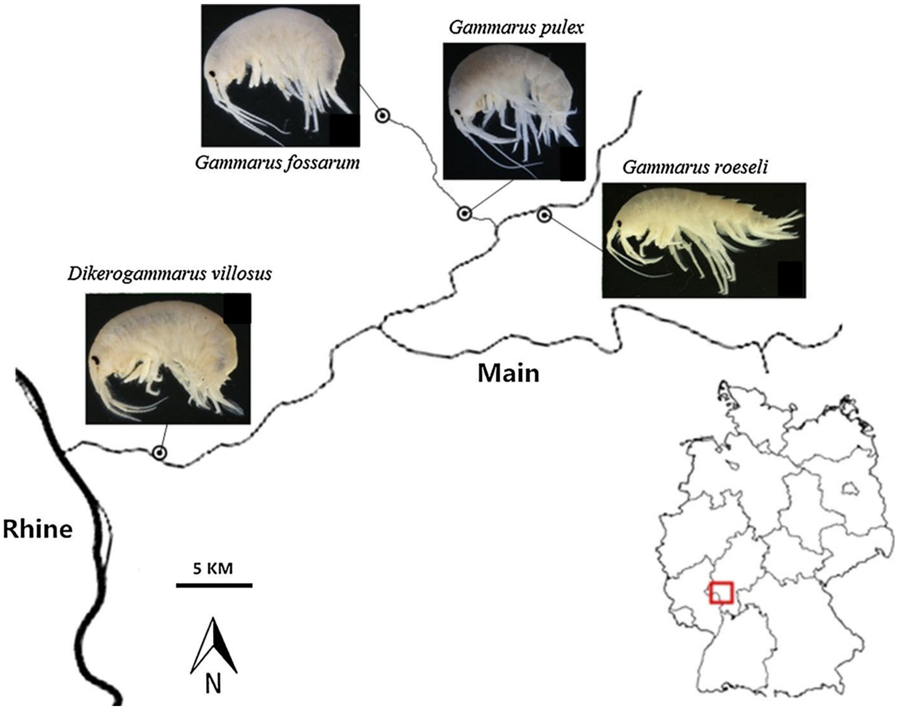

Fig. 1 Sampling sites that were dominated by one of our four study species, invasive *Dikerogammarus villosus* (49°59′55.49″N, 8°23′8.35″E) from the city of Rüsselsheim, and indigenous *Gammarus roeseli* (50°10′33.68″N, 8°41′36.33″E), *Gammarus pulex* (50°10′7.46″N, 8°37′15.98″E) and *Gammarus fossarum* (50°12′58.11″N, 8°31′52.65″E) from the city of Frankfurt/Main, Germany

et al. 1994). Temperature was maintained at 10 °C throughout, and light/dark periods were adjusted to 12:12 h per day. We provided food in the form of leaf litter taken from the collection sites. Animals were maintained in these holding tanks for 5–7 days before the experiment started.

Experimental setup

To enable individual identification throughout the testing phase, amphipods were transferred into individual perforated conical Falcon tubes. Tubes were fixed within a filter sponge in 80-L tanks containing ADaM (max. 25 tubes per tank) in a way that two-third of the tube was submerged. Water quality and food provisioning was identical to their holding tanks. After a 3-day acclimation period in the tubes, we measured each individual's activity in an open field test. We transferred individual test subjects into a glass petri dish (20 cm in diameter) filled to 3 cm height with ADaM, allowed them to acclimate for 5 min and then recorded their activity for 1 min using a webcam (Microsoft LifeCam VX-2000). A 2 × 2 cm grid on the bottom of the petri dish allowed us to count the number of squares crossed as a measure of activity. All recordings took place in a climate chamber maintaining a temperature of 10 °C throughout and minimizing disturbances of the focal animal. We measured each individual's activity on five consecutive days and afterward sacrificed and stored amphipods individually in 70 % ethanol to determine body size and sex under a stereomicroscope. Sample sizes were $N = 49$ (*D. villosus*), $N = 75$ (*G. roeseli*), $N = 107$ (*G. fossarum*) and $N = 45$ (*G. pulex*).

Data analysis

Activity scores were square root transformed to normalize the distribution and stabilize variance; transformed scores were then centered on their grand mean to ease interpretation across species. To test our first prediction about average activity differences between all four species, we used linear mixed models (LMMs) with activity as the response variable and species as our fixed effect of interest. We also included sex, trial and body size as additional fixed effects and individual ID as a random effect to account for the multiple observations per individual.

Our second research question investigated the patterns of consistent individual variation in activity among the four species. To address this, we ran LMMs on each species separately. We included sex, size and trial as fixed effects and individual ID as a random effect. This allowed us to compare the total behavioral variance not accounted for by the fixed effects, as well as the among- and within-individual variance components across species (see Dingemanse et al. 2012). We also used these variance estimates to calculate a repeatability estimate. The repeatability of a behavior is defined as the proportion of the behavioral variance attributable to differences among individuals (Nakagawa and Schielzeth 2010). A significant repeatability estimate is interpreted as evidence of consistent individual differences. Significant differences in variance components and repeatability between the four species can be assumed when 95 % CI of the estimates do not overlap.

Our third and final research question was whether there was evidence for differences in individual behavioral plasticity over time (trials) between the four species. To test this, we fit separate LMMs for each species in which we included a random intercept and slope (across trials) for each individual and also included a covariance term between intercept and slope.

For all analyses, we used LMMs with Gaussian error distribution and Markov Chain Monte Carlo estimation, using MCMCglmm (Hadfield 2010) in R v3.0 (R_Core_Team, 2013). MCMC estimation offers a particularly powerful method for partitioning variance among random effects. MCMC also returns 95 % credibility intervals for all model effects, allowing for easy comparison of effects. For fixed effects, if the 95 % CI of an effect does not overlap zero, we interpret this as evidence for a significant effect of that factor. We additionally tested for the significance of each effect by comparing the deviance information criteria (DIC) of a model including the factor to a model without the factor. If including the effect reduces the DIC value by greater than 2, we considered this support that the effect improved the model. This was especially important to test the significance of the random effects, as variance estimates, by definition, are constrained to be non-negative. For all models, we used non-informative proper priors and 500,000 iterations, while discarding 1000 iterations as burn-in and sampling every 100 iterations. We ensured model convergence and proper mixing by running five independent chains for each model and visually inspecting the resulting autocorrelation and posterior distributions of model effects.

Results

Invasive species are not more active than non-invasive species

As predicted, the invasive *D. villosus* did not exhibit higher average activity levels compared to the three non-invasive species (Table 1). We found that across all four species, individuals decreased their activity across the five trials (but see single-species analyses below). We also found that larger individuals tended to be more active (the 95 % CI of "size" did not overlap zero), even though this effect was weak, as evidenced by the marginal decrease in DIC and small effect size.

Greater behavioral variation in invasive compared to non-invasive species

We found strong support for the presence of consistent individual differences (i.e., repeatability) in activity levels in all species, which accounted for at least one-third of the total behavioral variation (Table 2). In support of our second prediction, both the among- and within-individual variance components were significantly larger in the invasive *D. villosus* compared to any of the three non-invasive species (95 % CIs for both variance components did not overlap, Table 2), indicating that individuals of the invasive species were more different from each other and more variable in their behavior over repeated testing (Fig. 2). Although total behavioral variance was greater in *D. villosus*, a similar proportion of this variance was due to individual differences (i.e., repeatability) as in the non-invasive species, as 95 % CIs of the repeatability estimates all overlapped.

Native and naturalized, but not invasive species exhibit individual variation in plasticity

We found that individuals in the three non-invasive species, on average, changed their behavior in the same way (average behavioral plasticity) over the course of the experiment (effect of "trial" in Table 2). However, in direct contrast to our prediction, not all

Table 1 Differences among species in average activity levels

Effect	*B* [95 % CI]	ΔDIC
Species: *D. villosus*	−0.03 [−0.73, 0.67]	+1.05
G. roeseli	0.14 [−0.37, 0.69]	
G. fossarum	−0.19 [−0.83, 0.35]	
G. pulex	−0.17 [−0.86, 0.46]	
Sex: females	0.49 [−0.05, 0.99]	−0.27
Size	5.1×10^{-5} [1.8×10^{-5}, 9.8×10^{-5}]	−0.76
Trial	−0.12 [−0.19, −0.04]	−9.19

Results are from linear mixed models (MCMC estimation) with activity levels as dependent variable and “species,” “sex” (male or female), “size” and “trial” as fixed factors. “Individual ID” was included as a random factor to account for multiple observations per animal. Depicted are effect estimates (*b*, with 95 % CI) and the change in deviance information criteria (DIC). If 95 % CIs do not overlap zero and ΔDIC is below −2.0, an effect can be considered significant (see main text)

three non-invasive species showed the same direction of effect: *G. roeseli* and *G. fossarum* both decreased activity with repeated trials (i.e., habituation), whereas *G. pulex* increased activity (i.e., sensitization). In line with our prediction, the invasive *D. villosus* did not exhibit any consistent effect of trial (Table 2). This does not indicate that this species is invariable in its behavior over time; rather, the lack of a significant trial effect appears to be due to the high levels of within-individual variation in activity in this species. There was no evidence for differences between the sexes or an effect of body size in any species.

We predicted that the invasive species should show stronger among-individual variation in habituation effects (behavioral plasticity) over repeated testing compared to the non-invasive species. Indeed, in the three non-invasive species, there was considerable support for the inclusion of an “individual × trial” interaction term (individual slopes), indicating the presence of individual variation in behavioral plasticity in those three species (Table 3; Fig. 2). Not unexpectedly, we also found support for negative covariance between individual slopes and intercepts in the three non-invasive species, suggesting that individuals with higher initial activity levels showed the greatest decrease in activity with repeated testing. Interestingly, we did not find support that including either an individual slope or covariance term improved the

Table 2 Individual behavioral variation across four species of amphipods

Effects	*D. villosus*		*G. roeseli*		*G. fossarum*		*G. pulex*	
	b [95 % CI]	ΔDIC	*b* [95 % CI]	ΔDIC	*b* [95 % CI]	ΔDIC	*b* [95 % CI]	ΔDIC
Fixed								
Sex	1.38 [−0.54, 3.51]	+0.09	0.98 [−0.03, 1.67]	−0.48	0.30 [−0.51, 1.13]	+0.41	0.44 [−0.59, 1.57]	+0.23
Size	−0.02 [−0.04, −0.01]	−1.36	0.001 [−0.007, 0.01]	+0.46	−0.001 [−0.005, 0.002]	+0.32	−0.003 [−0.01, 0.001]	+0.40
Trial	0.05 [−0.21, 0.34]	+1.96	−0.28 [−0.40, −0.16]	−20.9	−0.23 [−0.32, −0.12]	−19.1	0.22 [0.06, 0.33]	−7.80
Random								
Among-individual	6.79 [3.62, 11.02]	−94.01	1.38 [0.94, 2.35]	−108.6	1.58 [1.04, 2.28]	−155.7	1.06 [0.52, 1.86]	−59.3
Within-individual	9.40 [7.78, 11.63]		2.83 [2.36, 3.26]		2.87 [2.52, 3.31]		2.02 [1.71, 2.58]	
R	0.43 [0.28, 0.56]		0.36 [0.24, 0.47]		0.37 [0.26, 0.45]		0.34 [0.20, 0.49]	

Results are from linear mixed models (MCMC estimation, separate models for each species) with activity levels as dependent variable and “sex” (male or female), “size” and “trial” as fixed factors. “Individual ID” was included as a random factor to decompose the total variation into its among- and within-individual variance components. Depicted are effect estimates (*b*, with 95 % CI), the change in deviance information criteria (DIC) as well as variance estimates along with the behavioral repeatability (*R*). If 95 % CIs do not overlap zero and ΔDIC is below −2.0, an effect can be considered significant (see main text)

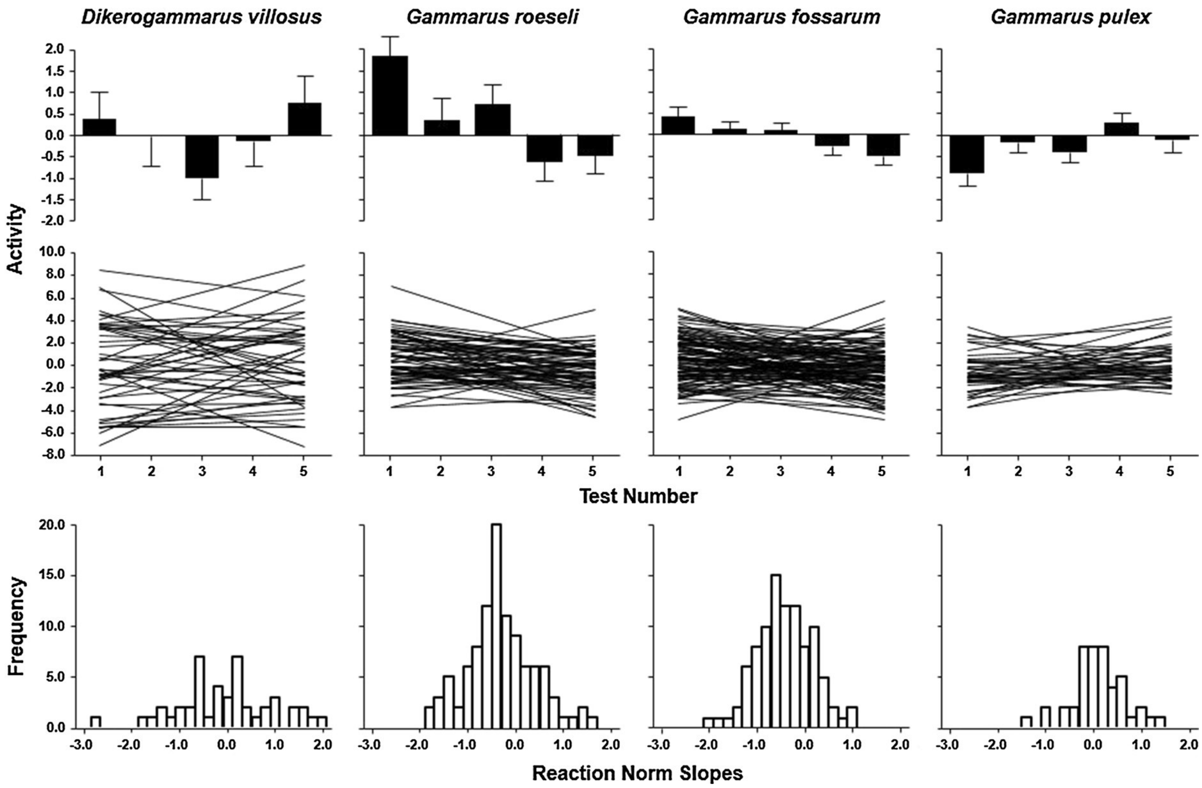

Fig. 2 Population-level average activity levels (*top*, centered around grand mean), individual reaction norm slopes between the five repeated measures (*middle*, centered around grand mean) and frequency distribution of the reaction norm slopes (*bottom*) of invasive and native/naturalized amphipod species in the Rhine system, *Dikerogammarus villosus* representing the invasive species

model for the invasive *D. villosus* (see Table 3). Again, this finding was likely driven by the high levels of within-individual variation in behavior: There was no predictable pattern in how individuals of *D. villosus* would behave with repeated testing.

Discussion

In congruence with our first prediction and previous research (Truhlar and Aldridge 2015), the invasive amphipod *D. villosus* did not show higher mean activity levels compared to the three non-invasive species. This contrasts with the results of studies on other invasive species (e.g., Cote et al. 2010b; Canestrelli et al. 2015). We argue that higher activity rates increase the likelihood of invasions only in actively dispersing species like Western mosquitofish (*Gambusia affinis*; Cote et al. 2010b), while *D. villosus* depends on passive drift (van Riel et al. 2011), or ships function as dispersal vectors (Leuven et al. 2009; see also Chen et al. 2012 for discussion). However, we found *D. villosus* to exhibit greater among-individual and within-individual variation in activity levels (prediction 2). The high within-individual variation in this species was striking and, in essence, swamped out any signal of consistent changes over the repeated testing (average- or individual-level plasticity). In contrast, within-individual behavioral variation was more structured in the three other species, in which individuals exhibited predictable changes in activity with repeated testing. Not all of the three species changed their behavior in the same way over time though, indicating that activity habituation may not be a universal characteristic of more established (naturalized) and native populations. The dramatic differences in the patterns of behavioral variation between native (or naturalized) and invasive species suggest that differences in within-species behavioral variation could indeed play a role in determining the invasion

Table 3 Individual variation in plasticity across four species of amphipods

(Co-) variance	*D. villosus*		*G. roeseli*		*G. fossarum*		*G. pulex*	
	Estimate [95 % CI]	ΔDIC	Estimate [95 % CI]	ΔDIC	Estimate [95 % CI]	ΔDIC	Estimate [95 % CI]	ΔDIC
Intercept	7.31 [2.24, 17.69]		3.18 [1.52, 5.69]		3.81 [2.66, 6.49]		2.33 [0.83, 4.68]	
Slope	0.33 [0.15, 0.71]	+6.45	0.25 [0.13, 0.41]	−5.29	0.32 [0.22, 0.53]	−39.46	0.24 [0.12, 0.43]	−8.14
Covariance	−0.22 [−2.04, 0.55]	−0.55	−0.53 [−1.11, 0.14]	−18.19	−0.92 [−1.47, −0.48]	−56.78	−0.41 [−1.02, −0.04]	−12.06

Results are from linear mixed models (MCMC estimation, separate models for each species) treating activity levels as the dependent variable and including individual random slopes and intercepts. We also included a covariance term between intercept and slope. Depicted are variance estimates (with 95 % CI) and the change in deviance information criteria (DIC). If 95 % CIs do not overlap zero and ΔDIC is below −2.0, an effect can be considered significant (see main text)

success of a species. This result is in congruence with the "insurance hypothesis" (see Wolf and Weissing 2012), which assumes invading species to benefit from a higher behavioral variability (see "Discussion" section below).

Modeling frameworks suggest that individual differences in plasticity may arise when the benefits of showing a plastic response depend on the frequency of individuals that show either plastic or non-plastic responses (i.e., negative frequency-dependent selection; Wolf et al. 2008; Dubois et al. 2010). Such a mechanism could explain the observed composition of reaction norms in the native or naturalized species, in which we found consistent individual differences in behavioral plasticity. The pattern for the invasive *D. villosus*, however, seems to follow a different logic with no detectable plasticity differences among individuals due to high and non-predictable within-individual variation in behavior. There are several (not necessarily mutually exclusive) potential explanations for this finding:

1. First, some authors propose a predatory lifestyle for *D. villosus* (Dick and Platvoet 2000). Individuals may frequently move between microhabitats in search of prey, and selection could favor those individuals that are highly flexible in their responses to ecological gradients (Dingemanse and Wolf 2013). Moreover, predatory behavior might include alternating sit-and-wait and active search phases (Dick and Platvoet 2000), leading to highly variable activity patterns. However, native species are also known to exhibit variation in foraging behaviors, including both leaf-shredding and predatory foraging like cannibalism (Dick and Platvoet 1996), and so it seems unlikely that differences in foraging behavior are a major explanation here.
2. Recent invaders could face relaxed natural selection pressures (Hayes and Barry 2008; Davis 2009; Blackburn et al. 2011), e.g., due to neophobia of their potential predators (Greenberg 1990). Thus, even otherwise maladapted individuals may survive during the initial stages of a biological invasion, leading to higher behavioral variation in invasive populations. Likewise, it was shown that the Ponto-Caspian racer goby (*Babka gymnotrachelus*) preferred *G. fossarum* over *D. villosus* as prey in Poland where *B. gymnotrachelus* is invasive (Błońska et al. 2015). Using immobilized amphipods, the authors demonstrated that this is not due to differences in behavior but to a harder exoskeleton of *D. villosus*. If *D. villosus* is indeed less palatable, it might be able to express more variable swimming behavior while still being protected from predation. High predation rates on *D. villosus* by the highly abundant invasive piscine predator *Neogobius melanostamus* (Emde et al. 2014), however, render this scenario unlikely. Also, native fish species like European eel (*Anguilla anguilla*) and perch (*Perca fluviatilis*) are known to readily include *D. villosus* in their diets when available (Eckmann et al. 2008).
3. Piscine predation could rather play an active role in generating the greater and more unpredictable behavioral variation found in *D. villosus*. The now very common invasive round goby (*N. melanostomus*) was found to predominantly feed

on *D. villosus* in the Rhine, ignoring the locally much more abundant amphipod *Echinogammarus trichiatus* that invaded the Rhine even more recently (Emde et al. 2014). The observed unpredictable activity levels in the invasive *D. villosus*—with alternating periods of high activity and resting/hiding—may in fact be an antipredator response, precluding learning about their prey's activity patterns by *N. melanostomus*.

4. Truhlar and Aldridge (2015) proposed differences in parasite loads as one factor that might affect individual differences in amphipod behavior. For example, parasites are known to affect photophobic behavior in *G. pulex* (Bethel and Holmes 1973), with infected individuals showing a weaker photonegative response (see also Perrot-Minnot et al. 2012). Furthermore, the influence of some parasites on their host's behavior seems to be species-specific (Bauer et al. 2000). However, measuring parasitization rates was beyond the scope of our current study, and we strongly advise inclusion of those measurements in future studies.

Interestingly, average activity levels changed predictably over time in the native and naturalized species, albeit not into the same direction. Even though we are lacking a compelling explanation for the observed differences, predictability seems to be a feature of populations that have been established for long enough in a certain habitat. This view is underpinned by the study of Truhlar and Aldridge (2015) who found no repeatable behavior in *G. pulex* and *D. villosus* collected in Great Britain, where both species are invasive. Possibly, colonization of new habitats leads to an initial loss of predictable individual differences in behavior. Future studies should pursue this topic further and should also ask whether ecological or life history features (Bollache et al. 2006; Grabowski et al. 2007) might explain the direction of the observed average behavioral plasticity.

In conclusion, our results indicate pronounced differences in patterns of individual behavioral variation between non-native and invasive amphipod species from river Rhine and support hypotheses that predict invading species to benefit from high levels of among- and between-individual variation (e.g., "insurance hypothesis" sensu Wolf and Weissing 2012). As our current study presents data from only one population per species, we encourage sampling populations along the entire distribution range of *D. villosus*, that is, native and invasive populations, including invasive populations that are at different stages of the invasion process [see Truhlar and Aldridge (2015) for a similar approach on *G. pulex* and *D. villosus* in Great Britain]. Such an approach will allow determining whether differences between non-invasive and invasive species as reported here might be washed out over time through natural selection.

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References

Anderson SC, Cooper AB, Dulvy NK (2013) Ecological prophets: quantifying metapopulation portfolio effects. Methods Ecol Evol 4:971–981

Barbour MT, Gerritsen J, Snyder BD, Stribling JB (1999) Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, 2nd edn. U.S. Environmental Protection Agency, Washington

Bauer A, Trouvé S, Grégoire A, Lc Bollache, Cézilly F (2000) Differential influence of *Pomphorhynchus laevis* (Acanthocephala) on the behaviour of native and invader gammarid species. Int J Parasitol 30:1453–1457

Bethel WM, Holmes JC (1973) Altered evasive behaviour and responses to light in amphipods harboring acanthocephalan cystacanths. J Parasitol 59:945–956

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. Can J Fish Aquat Sci 59:1159–1174. doi:10.1139/f02-098

Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, Wilson JRU, Richardson DM (2011) A proposed unified framework for biological invasions. Trends Ecol Evol 26:333–339. doi:10.1016/j.tree.2011.03.023

Błońska D, Grabowska J, Kobak J, Jermacz Ł, Bącela-Spychalska K (2015) Feeding preferences of an invasive Ponto-Caspian goby for native and non-native gammarid prey. Freshw Biol 60:2187–2195. doi:10.1111/fwb.12647

Bollache L, Kaldonski N, Troussard J-P, Lagrue C, Rigaud T (2006) Spines and behaviour as defences against fish predators in an invasive freshwater amphipod. Anim Behav 72:627–633. doi:10.1016/j.anbehav.2005.11.020

Brown C (2001) Familiarity with the test environment improves escape responses in the crimson spotted rainbowfish, *Melanotaenia duboulayi*. Anim Cogn 4:109–113. doi:10.1007/s100710100105

Canestrelli D, Bisconti R, Carere C (2015) Bolder takes all? The behavioral dimension of biogeography. Trends Ecol Evol 31:35–43. doi:10.1016/j.tree.2015.11.004

Carere C, Gherardi F (2013) Animal personalities matter for biological invasions. Trends Ecol Evol 28:5–6. doi:10.1016/j.tree.2012.10.006

Carvalho CF, Leitão AV, Funghi C, Batalha HR, Reis S, Mota PG, Lopes RJ, Cardoso GC (2013) Personality traits are related to ecology across a biological invasion. Behav Ecol 24:1081–1091. doi:10.1093/beheco/art034

Cassey P, Blackburn TM, Jones KE, Lockwood JL (2004) Mistakes in the analysis of exotic species establishment: source pool designation and correlates of introduction success among parrots (Aves: Psittaciformes) of the world. J Biogeogr 31:277–284. doi:10.1046/j.0305-0270.2003.00979.x

Chapple DG, Simmonds SM, Wong BBM (2012) Can behavioral and personality traits influence the success of unintentional species introductions? Trends Ecol Evol 27:57–64. doi:10.1016/j.tree.2011.09.010

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. BioInvasion Rec 1:263–271

Cote J, Clobert J, Brodin T, Fogarty S, Sih A (2010a) Personality-dependent dispersal: characterization, ontogeny and consequences for spatially structured populations. Philos Trans R Soc B 365:4065–4076. doi:10.1098/rstb.2010.0176

Cote J, Fogarty S, Weinersmith K, Brodin T, Sih A (2010b) Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). Proc R Soc B 277:1571–1579. doi:10.1098/rspb.2009.2128

Davis MA (2009) Invasion biology. Oxford University Press, Oxford

Dick J, Platvoet D (1996) Intraguild predation and species exclusions in amphipods: the interaction of behaviour, physiology and environment. Freshw Biol 36:375–383. doi:10.1046/j.1365-2427.1996.00106.x

Dick JTA, Platvoet D (2000) Invading predatory crustacean *Dikerogammarus villosus* eliminates both native and exotic species. Proc R Soc B 267:977–983. doi:10.1098/rspb.2000.1099

Dingemanse NJ, Wolf M (2013) Between-individual differences in behavioural plasticity within populations: causes and consequences. Anim Behav 85:1031–1039. doi:10.1016/j.anbehav.2012.12.032

Dingemanse NJ, Kazem AJN, Réale D, Wright J (2010) Behavioural reaction norms: animal personality meets individual plasticity. Trends Ecol Evol 25:81–89. doi:10.1016/j.tree.2009.07.013

Dingemanse NJ, Bouwman KM, van de Pol M, van Overveld T, Patrick SC, Matthysen E, Quinn JL (2012) Variation in personality and behavioural plasticity across four populations of the great tit *Parus major*. J Anim Ecol 81:116–126. doi:10.1111/j.1365-2656.2011.01877.x

Dubois F, Morand-Ferron J, Giraldeau L-A (2010) Learning in a game context: strategy choice by some keeps learning from evolving in others. Proc R Soc B 277:3609–3616. doi:10.1098/rspb.2010.0857

Duckworth RA, Badyaev AV (2007) Coupling of dispersal and aggression facilitates the rapid range expansion of a passerine bird. Proc Natl Acad Sci USA 104:15017–15022. doi:10.1073/pnas.0706174104

Eckmann R, Mörtl M, Baumgärtner D, Berron C, Fischer P, Schleuter D, Weber A (2008) Consumption of amphipods by littoral fish after the replacement of native *Gammarus roeseli* by invasive *Dikerogammarus villosus* in Lake Constance. Aquat Invasion 3:184–188. doi:10.3391/ai.2008.3.2.9

Eggers T, Martens A (2001) Bestimmungsschlüssel der Süßwasser-Amphipoda (Crustacea) Deutschlands. Lauterbornia 42:1–68

Emde S, Kochmann J, Kuhn T, Plath M, Klimpel S (2014) Getting what is served? Feeding ecology influencing parasite–host interactions in invasive round goby *Neogobius melanostomus*. PLoS ONE 9:e109971. doi:10.1371/journal.pone.0109971

Fogarty S, Cote J, Sih A (2011) Social personality polymorphism and the spread of invasive species: a model. Am Nat 177:273–287

Grabowski M, Bacela K, Konopacka A (2007) How to be an invasive gammarid (Amphipoda: Gammaroidea)—comparison of life history traits. Hydrobiologia 590:75–84. doi:10.1007/s10750-007-0759-6

Greenberg R (1990) Feeding neophobia and ecological plasticity: a test of the hypothesis with captive sparrows. Anim Behav 39:375–379. doi:10.1016/S0003-3472(05)80884-X

Gross K, Pasinelli G, Kunc HP (2010) Behavioral plasticity allows short-term adjustment to a novel environment. Am Nat 176:456–464

Hadfield JD (2010) MCMC methods for multi-response generalised linear mixed models: the MCMCglmm R package. J Stat Softw 33:1–22

Hayes K, Barry S (2008) Are there any consistent predictors of invasion success? Biol Invasion 10:483–506. doi:10.1007/s10530-007-9146-5

Hunting ER, Whatley MH, Geest HGvd, Mulder C, Kraak MHS, Anton MB, Admiraal W (2012) Invertebrate footprints on detritus processing, bacterial community structure, and spatiotemporal redox profiles. Freshwat Sci 31:724–732. doi:10.1899/11-134.1

Jourdan J, Westerwald B, Kiechle A, Chen W, Streit B, Klaus S, Oetken M, Plath M (2016) Pronounced species turnover, but no functional equivalence in leaf consumption by invasive amphipods in the river Rhine. Biol Invasion 18:763–774. doi:10.1007/s10530-015-1046-5

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. Aquat Ecol 43:457–464. doi:10.1007/s10452-008-9206-7

Kley A, Kinzler W, Schank Y, Mayer G, Waloszek D, Maier G (2009) Influence of substrate preference and complexity on co-existence of two non-native gammarideans (Crustacea: Amphipoda). Aquat Ecol 43:1047–1059. doi:10.1007/s10452-009-9242-y

Klüttgen B, Dülmer U, Engels M, Ratte H (1994) ADaM, an artificial freshwater for the culture of zooplankton. Water Res 28:743–746

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol 16:199–204. doi:10.1016/S0169-5347(01)02101-2

Leuven REW, van der Velde G, Baijens I, Snijders J, van der Zwart C, Lenders HJR, Bij de Vaate A (2009) The river Rhine: a global highway for dispersal of aquatic invasive species. Biol Invasion 11:1989–2008. doi:10.1007/s10530-009-9491-7

Lockwood JL, Hoopes MF, Marchetti MP (2013) Invasion ecology. Wiley, New York

Maazouzi C, Piscart C, Legier F, Hervant F (2011) Ecophysiological responses to temperature of the “killer shrimp” *Dikerogammarus villosus*: is the invader really stronger than the native *Gammarus pulex*? Comp Biochem Phys A 159:268–274. doi:10.1016/j.cbpa.2011.03.019

MacNeil C, Boets P, Platvoet D (2012) ‘Killer Shrimps’, dangerous experiments and misguided introductions: how freshwater shrimp (Crustacea: Amphipoda) invasions threaten biological water quality monitoring in the British Isles. Freshw Rev 5:21–35. doi:10.1608/frj-5.1.457

Magurran AE (1986) Individual differences in fish behaviour. In: Pitcher TJ (ed) The behaviour of teleost fishes. Croom Helm, London, pp 338–365

Nakagawa S, Schielzeth H (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biol Rev 85:935–956. doi:10.1111/j.1469-185X.2010.00141.x

Perrot-Minnot M-J, Maddaleno M, Balourdet A, Cézilly F (2012) Host manipulation revisited: no evidence for a causal link between altered photophobia and increased trophic transmission of amphipods infected with acanthocephalans. Funct Ecol 26:1007–1014. doi:10.1111/j.1365-2435.2012.02027.x

Phillips BL, Suarez A (2012) The role of behavioural variation in the invasion of new areas. In: Candolin U, Wong BBM (eds) Behavioural responses to a changing world: mechanisms and consequences. Oxford University Press, Oxford

Platvoet D, Dick JTA, MacNeil C, van Reil M, van der Velde G (2009) Invader-invader interactions in relation to environmental heterogeneity leads to zonation of two invasive amphipods, *Dikerogammarus villosus* (Sowinsky) and *Gammarus tigrinus* Sexton: amphipod pilot species project (AMPIS) report 6. Biol Invas 11:2085

R_Core_Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. Biol Rev 82:291–318. doi:10.1111/j.1469-185X.2007.00010.x

Schindler DE, Hilborn R, Chasco B, Boatright CP, Quinn TP, Rogers LA, Webster MS (2010) Population diversity and the portfolio effect in an exploited species. Nature 465:609–612

Sih A, Bell A, Johnson JC (2004) Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol Evol 19:372–378. doi:10.1016/j.tree.2004.04.009

Sih A, Cote J, Evans M, Fogarty S, Pruitt J (2012) Ecological implications of behavioural syndromes. Ecol Lett 15:278–289. doi:10.1111/j.1461-0248.2011.01731.x

Truhlar A, Aldridge D (2015) Differences in behavioural traits between two potentially invasive amphipods, *Dikerogammarus villosus* and *Gammarus pulex*. Biol Invasion 17:1569–1579. doi:10.1007/s10530-014-0816-9

Tsutsui ND, Suarez AV, Holway DA, Case TJ (2000) Reduced genetic variation and the success of an invasive species. Proc Natl Acad Sci USA 97:5948–5953. doi:10.1073/pnas.100110397

van Riel MC, van der Velde G, Rajagopal S, Marguillier S, Dehairs F, Bij de Vaate A (2006) Trophic relationships in the Rhine food web during invasion and after establishment of the Ponto-Caspian invader *Dikerogammarus villosus*. In: Leuven RSEW, Ragas AMJ, Smits AJM, van der Velde G (eds) Living rivers: trends and challenges in science and management. Springer, Netherlands, pp 39–58. doi:10.1007/1-4020-5367-3_3

van Riel MC, van der Velde G, Bij de Vaate A (2011) Dispersal of invasive species by drifting. Curr Zool 57:818–827

Wallace JB, Eggert SL, Meyer JL, Webster JR (1997) Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277:102–104. doi:10.1126/science.277.5322.102

Wolf M, Weissing FJ (2012) Animal personalities: consequences for ecology and evolution. Trends Ecol Evol 27:452–461

Wolf M, van Doorn GS, Weissing FJ (2008) Evolutionary emergence of responsive and unresponsive personalities. Proc Natl Acad Sci USA 105:15825–15830. doi:10.1073/pnas.0805473105

Wright TF, Eberhard JR, Hobson EA, Avery ML, Russello MA (2010) Behavioral flexibility and species invasions: the adaptive flexibility hypothesis. Ethol Ecol Evol 22:393–404. doi:10.1080/03949370.2010.505580

3. NATURAL BIOLOGICAL INVASIONS

3.1 Distribution and phenotypic trait divergence along environmental gradients

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Shared and unique patterns of phenotypic diversification along a stream gradient in two congeneric species

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Abstract

Stream ecosystems show gradual variation of various selection factors, which can result in a zonation of species distributions and gradient evolution of morphological and life-history traits within species. Identifying the selective agents underlying such phenotypic evolution is challenging as different species could show shared and/or unique (species-specific) responses to components of the river gradient. We studied a stream gradient inhabited by two mosquitofishes (genus *Gambusia*) in the Río Grijalva basin in southern Mexico and found a patchy distribution pattern of both congeners along a stretch of 100 km, whereby one species was usually dominant at a given site. We uncovered both shared and unique patterns of diversification: some components of the stream gradient, including differences in piscine predation pressure, drove shared patterns of phenotypic divergence, especially in females. Other components of the gradient, particularly abiotic factors (max. annual temperature and temperature range) resulted in unique patterns of divergence, especially in males. Our study highlights the complexity of selective regimes in stream ecosystems. It exemplifies that even closely related, congeneric species can respond in unique ways to the same components of the

river gradient and shows how both sexes can exhibit quite different patterns of divergence in multivariate phenotypic character suites.

Introduction

Environmental gradients provide a unique opportunity to study natural selection[1]. They allow investigating whether and how gradual variation in ecologically-based selection affects adaptive phenotypic differentiation[2]. Evidence for adaptive diversification along environmental gradients stems from studies of latitudinal[3-5] and altitudinal (i.e., thermal) gradients[6], as well as gradients formed by environmental stressors like salinity[7,8] or acidification[9,10]. A widespread environmental gradient is found in stream ecosystems, in which various abiotic and biotic selection factors vary systematically from source regions over smaller tributaries to slow-flowing lowland rivers[11-13]. Low-diversity headwater communities are often subjected to strongly variable abiotic conditions and recurrent catastrophic flooding (e.g., after snow melt), while abiotic conditions are more stable in downstream river portions, where multiple tributaries interconnect to form an extensive wetland system and ecological communities become more speciose[11,14-16].

Evolutionary diversification along repeated stream gradients has been particularly well investigated in northern Trinidad, where populations of the livebearing freshwater fish *Poecilia reticulata* (the guppy; family Poeciliidae) occur from the mountainous source regions to lowland portions of river systems. Fast-flowing lower-order creeks are characterized by dense canopy cover, low algal primary production and thus, low food availability for the algivorous guppies[17] (but see also[18]). This results in low population densities of guppies and an absence of larger predatory fishes[19-22]. Lowland rivers are slow-flowing, accumulate more nutrients, have higher photosynthetic primary production and thus, higher densities of guppies, and harbour an array of predatory species[17,20-24]. Guppies show a repeated and predictable pattern of life-history divergence along this gradient, which was mainly interpreted as a consequence of differences in predation risk: under high predation (i.e., increased extrinsic mortality rates), guppy females produce more, but smaller offspring and allocate more resources to reproduction[21,23,25], while males mature at an earlier age and develop less conspicuous secondary sexual ornamentation[26,27]. Several studies investigated guppy populations that are separated by waterfalls (allowing for a rather clear distinction between low- and high-predation habitats[21,23]), but similar patterns of phenotypic differentiation were also found along a continuous gradient of predation[28].

River systems comprise complex environmental gradients, and so it often remains unclear, which components of the river gradient drive patterns of phenotypic divergence in fishes. Circumstantial evidence for the role of stream velocity governing morphological evolution stems from studies on the effects of impoundments (dams), where water reservoirs reduce flow velocity and thus create artificial 'downstream conditions'[29-31]. Physical characteristics of reservoirs (i.e., altered flow characteristics) appear to drive changes in a set of morphological traits: fish are usually deeper-bodied and have smaller heads in reservoirs[29,31]. This likely increases manoeuvrability when feeding on prey suspended in the water column, while more streamlined body contours increase locomotor performance in lotic environments[32]. Moreover, morphological diversification in fishes is linked to predation regimes[33-35]. Specifically, fish are predicted to evolve an enlarged caudal region (body region stretching from the dorsal and anal fins to the caudal fin base) and to have smaller anterior body/head regions under high predation pressure, which improves predator escape performance through an increased burst speed[33,34,36].

Studying gradient evolution—gradual phenotypic divergence in multiple character suites, including evolved differences and adaptive phenotypic plasticity—becomes possible when populations of the same species have adapted to divergent conditions along environmental gradients. In reality, however, different species tend to compete along those gradients, and site-specific competitive advantages of ecologically similar taxa (i.e., competitive exclusion) structure local species compositions[14,37,38]. Various abiotic factors are known to determine the distribution limits of species along environmental gradients[39,40]. In stream ecosystems, salinity, water velocity, temperature regimes, and dissolved oxygen are of particular importance[11,14]. Other studies found interactions between biotic and abiotic variables to predict species distributions along environmental gradients[37,41], as exemplified by the study of Torres-Dowdall *et al.*[42], who examined ecological factors explaining the parapatric distribution of the congeners *P. reticulata* and *P. picta* in the lowlands of Trinidad. It appears as if the distribution of *P. reticulata* is limited by an abiotic factor (increasing salinity), whereas that of *P. picta* is limited by a biotic interaction (interspecific competition with *P. reticulata*)[42].

In our present study, we examined patterns of phenotypic (i.e., morphological and life-history) diversification along the river gradient of the southern Mexican Río Grijalva[43,44]. We focused on members of mosquitofishes (genus *Gambusia*; Poeciliidae[45-47]), a widespread group of freshwater fishes in Central and North America[45,46]. In the Grijalva basin, three species of mosquitofishes have been described: widemouth gambusia (*G. eurystoma*) are endemic to hydrogen sulphide-rich spring complexes at the Baños del Azufre[48-50], while two other species occur throughout the Río Grijalva basin: the teardrop mosquitofish (*G. sexradiata*; Fig. 1A, B) and the Yucatan gambusia (*G. yucatana*; Fig. 1C, D)[43]. Even though both species can reach high local abundances, only few studies reported aspects of their ecology, including trophic ecology[51-53] and microhabitat preferences[53], as well as morphological characteristics within and among species[45,54,55]. Previous reports on Mexican and Belizean ichthyofauna suggest that *G. yucatana* may occur more in coastal waters; however, the species is occasionally also found in inland waters[55,56]. The opposite pattern was reported for *G. sexradiata*[45,55,57]. This distribution pattern is reflected by different salinity tolerances: *G. sexradiata* exhibits a lower tolerance to sea water compared to *G. yucatana*[57], while *G. yucatana* is even known from some marine habitats[55,57]. However, both species co-occur at some sites[55,56], raising the question of what (additional) factors predict their distribution.

The co-occurrence of two morphologically similar congeners in the Grijalva basin prompted a set of questions regarding both their distribution patterns and patterns of phenotypic divergence along the stream gradient (Fig. 2). Even though the existing literature suggests otherwise ([55,56]; see above), both species might co-occur at least in low frequencies along the entire river gradient (Fig. 2A-C). Alternatively, one species might occupy the up-, and the other the downstream portions of the stream. Given that hybridization has been demonstrated to occur even between more distantly related poeciilids[58,59], this type of distribution could result in a hybrid zone where both distributions meet (Fig. 2D-F). Finally, only some components of the river gradient might predict species distribution patterns, leading to a patchy distribution along the river gradient (Fig. 2G-I). In all three cases, phenotypic differences between and within species could be due to the following factors: (1) differences could reflect a phylogenetic signal that is independent of the river gradient (statistically, this would result in a significant main effect of the factor ‘species’ in multivariate analyses of variance; see Fig. 2A, D, G), (2) both species could show the same (shared) pattern of gradient evolution (i.e., a significant effect of the covariate ‘environmental gradient’; Fig. 2B, E, H), or (3) both species could respond differently to components of the river gradient (reflected by a significant interaction effect; Fig. 2C, F, I). (4) Finally, if both species co-occur along the entire stream gradient or if a zonation in species distributions is accompanied by an overlap zone in which both species co-occur, then presence or absence of the respective other, ecologically competing species could be another driver of phenotypic divergence (see Supplementary Fig. S3). This ecological character displacement (ECD) has been described for various systems in which congeneric and ecologically similar taxa form secondary contact zones[60-62].

In summary, we used an integrated analytical framework to tackle several questions related to the coexistence of both species, as well as phenotypic divergence along an environmental gradient in the Río Grijalva. Specifically, we assessed several abiotic and biotic environmental variables at ten sites across a stretch of approximately 100 km in the Río Grijalva, established fish community structures, and assessed morphological and life-history variation in *Gambusia* spp. to answer the following questions: (1) What environmental factors predict the distribution of *G. sexradiata* and *G. yucatana*? (2) Do we find gradient evolution in life histories and morphology in line with *a priori* predictions (life-history variation[21]; body-shape variation[33,36]), and, if so, do both species show shared or unique patterns of phenotypic divergence? (3) Which component(s) of the river gradient (including differences in temperature and water depth, predation, etc.) drive divergence in different trait suites?

Results

Molecular and phenotypic species identification

Phylogenetic analysis

Bayesian phylogenetic analysis of the cyt*b* fragment for two individuals from each population confirmed the presence of both species, *G. sexradiata* and *G. yucatana*, in our dataset. Phylogenetic relationships to representatives of other *Gambusia* species were in line with previously published phylogenies[46,47], even though the rather short cyt*b* fragment yielded only minor support for several divergence events (Fig. 3B). Our analysis confirmed the close relationship between *G. puncticulata* and *G. yucatana*, with the latter often being treated as a subspecies of *G. puncticulata*[63]. Interestingly, the hydrogen sulphide-spring endemic *G. eurystoma* clustered within the sampled specimens of *G. sexradiata.*

Population genetic analyses

In a second step we amplified nuclear microsatellites and conducted population genetic analyses to verify species identity of the $n = 239$ genotyped individuals. We detected $K = 2$ as the uppermost hierarchical level of population structure according to Evanno *et al.*[64]. Considering those individuals included in both analysis we found that the two major genetic clusters in the STRUCTURE analysis correspond to *G. sexradiata* (orange) and *G. yucatana* (green; Fig. 3C). The second highest ΔK was found for $K = 3$, followed by $K = 6$ (see Supplementary Fig. S1). The pattern of individual assignment into two and six subpopulations, respectively, revealed population genetic structure within *G. sexradiata*, but not in *G. yucatana*.

Descriptive statistics for site-specific means of standard indicators of genetic variability are provided in Supplementary Tab. S4. We found significantly higher allelic richness (A), expected (H_E) and observed heterozygosity (H_O) in *G. sexradiata* ($A = 3.8$, $H_E = 0.61$, $H_O = 0.50$) compared to *G. yucatana* ($A = 3.1$, $H_E = 0.49$, $H_O = 0.37$; Wilcoxon signed-rank tests comparing both species across loci, in all cases: $z < -2.49$, $p < 0.05$; see Supplementary Tab. S4).

Test for hybridization

When we tested for a potential hybrid zone between both species (Fig. 2D-F), the software NEWHYBRIDS identified most individuals (98.7 %) to be either ‘pure’ ($Q \geq 0.95$) *G. sexradiata* (169 individuals, 70.7 %) or ‘pure’ *G. yucatana* (67 individuals, 28.0 %). Only three individuals (1.3 %) were of putative mixed ancestry (i.e. $Q < 0.95$). Those individuals, however, could not be unambiguously identified as F_1- or F_2-hybrids, or backcrosses with either parental species ($Q \leq 0.49$). For example, the individual with lowest probability of being a ‘purebred’ (female no. 10 from site 5; Q-value for assignment to *G. yucatana* = 0.47; Q-value for being an F_2-hybrid = 0.04; Q-value for being a backcross to *G. yucatana* = 0.49; marked by an asterisk in Fig. 3C) was heterozygous at locus Mf13, with two alleles of 167 and 177 bp length, and the 177 bp allele was otherwise exclusive to *G. sexradiata* in our dataset. Nevertheless, it seems most likely that this individual was a migrant from an adjacent population of *G. yucatana* rather than a hybrid, since it not only showed several alleles not found in other individuals of this population (locus Gaaf22: both the 283 bp and the 304 bp allele; locus Gafu1: 105 bp allele), but also had one allele that was unique within the entire dataset (locus Mf13: 167 bp allele).

Species identification based on external characteristics

Application of different criteria described in identification keys[55] found only pigmentation patterns to accurately distinguish *G. sexradiata* from *G. yucatana*: lateral black spots are arranged in rows on the dorsal half of the body in *G. sexradiata*, while *G. yucatana* has scattered black spots at the dorsal half of the body (Fig. 1). Using this criterion we verified species assignment for all individuals to either the *G. sexradiata* (n = 169) or the *G. yucatana* cluster (n = 70), i.e., according to the most likely assignment in the STRUCTURE analysis (see above). By comparison, when we used caudal fin spots as a criterion to distinguish species (described as 'heavily peppered' in *G. sexradiata*, while caudal fins were described as 'usually crossed by 1-3 rows of spots' in *G. yucatana*[55]), only 83.3 % of individuals could be correctly assigned. We, therefore, used lateral black spot colour patterns for species delimitation in case of individuals not included in the molecular analyses but included in subsequent analyses.

Species distributions patterns

We tested three different predictions regarding the distribution of both congeners (Fig. 2) and found the majority of sites (seven out of ten) to harbour only one *Gambusia* species, while both species occurred syntopically at sites 2, 3, and 5 (Fig. 3A). There was no obvious pattern of zonation (Fig. 3A), whereby *G. sexradiata* might be restricted to upstream, and *G. yucatana* to downstream portions along the stream gradient (Fig. 2D-F).

Canonical correspondence analysis: community compositions

We asked whether environmental factors explain the distribution of the two congeners. Our first CCA using presence/absence data of all teleost species per site explored the effects of environmental factors on local fish community compositions (Fig. 4). A permutation test (p = 0.04) suggested that a significant portion of the variance in community compositions could be ascribed to variation along the three environmental PCs. The first two axes of the CCA ordination map explained 91.5% of the cumulative (constrained) variance (axis 1, eigenvalue = 0.65, 59.6% variance explained; axis 2, eigenvalue = 0.35, 31.9% variance explained; Fig. 4). The first axis ordered sample sites along a gradient from large, deep and more coastal water bodies with high predation pressure to shallow inland habitats with low predation pressure (Fig. 4). The second axis ordered sample sites from deep inland waters to shallow, more coastal water bodies. Fish communities changed from species-rich coastal assemblages including marine species, to inland communities with a lower α-diversity, often characterized by the presence of platyfish (*Xiphophorus maculatus*) and dogtooth rivulus (*Cynodonichthys tenuis*; Fig. 4; see Supplementary Tab. S2).

Canonical correspondence analysis: distribution of *Gambusia* spp.

Both *Gambusia* species clustered closely together in our first CCA, and we found only a minor shift of *G. sexradiata* towards a more negative position along environmental PC 2, suggesting a somewhat higher likelihood of occurrence at sites with lower water depth, reduced predation pressure, and lower salinity and conductivity (Fig. 4). Furthermore, compared to *G. yucatana*, *G. sexradiata* adopted a position along more positive values of environmental PC 3, suggesting that the latter species occurs at sites with higher maximum temperatures and a higher annual temperature range.

Caution is required when interpreting the results from our first CCA in light of the outcome of our second CCA, which analysed the influence of environmental factors on the local abundance of both *Gambusia* species. In this analysis, we did not find any evidence that the environmental factors would explain the occurrence of both congeners. The first and only axis of the CCA (eigenvalue = 0.16) explained 19.6 % of the cumulative (constrained) variance, and we found no significant effect of the three environmental PCs on species distribution patterns (permutation test, $p = 0.71$).

Phenotypic divergence

Based on the observed distribution patterns (see above), we proceeded with analyses of phenotypic trait divergence according to the predictions outlined in Fig. 2A-I. Tests of a signature of ECD (see Supplementary Fig. S3) were not possible because only few sites harboured both species. The results from MANCOVAs testing conflicting predictions of gradient evolution (Fig. 2A-I) found support for (*a*) major species differences (significant 'species' effects on male and female life histories and body length in both sexes, but notably only on female, but not male body shape variation; Tab. 3), (*b*) shared responses to at least some components of the river gradient (main effects of environmental PCs 1 through 3, which were found in all analyses), and (*c*) unique (species-specific) responses, as indicated by significant interaction terms of 'species' and environmental PCs 1 through 3 (Tab. 3). In the following we will discuss the results from trait-specific univariate ANCOVAs (see Supplementary Tab. S5-S8) that were conducted *post hoc* for all significant effects in our main MANCOVA models (Tab. 3).

Species differences in morphology and life histories

In the MANCOVAs, we found only a small proportion of phenotypic variance to be explained by species identity [relative variance explained (V_{rel}) for female body shape = 16.6%, male life histories = 8.9%, females life histories = 13.6%; Tab. 3]. In the subsequent trait-wise ANOVAs, species differences became apparent in three cases: (see Supplementary Tab. S6-S8): *G. sexradiata* females had a deeper body and smaller head size compared to *G. yucatana* females (V_{rel} = 11.3%; Fig. 4A). Furthermore, female fat content was lower in *G. sexradiata* than in *G. yucatana* (V_{rel} = 8.6%; Fig. 5B), and male GSI was higher in *G. sexradiata* than in *G. yucatana* (V_{rel} = 6.6%; Fig. 5C).

Shared patterns of divergence

We found shared patterns of body-shape and life-history divergence only in female (but not male) *Gambusia* spp. (see Supplementary Tab. S6 and S8). The MANCOVAs revealed that the shared component of female body-shape and life-history divergence could be explained by environmental PC 2 (V_{rel} = 50.5% and V_{rel} = 36.5%, respectively) and environmental PC 3 (V_{rel} = 43.0% and V_{rel} = 13.0%, respectively). Furthermore, the ANCOVA examining body length in both sexes showed a strong effect of environmental PC 2 on shared patterns of body length divergence (V_{rel} = 34.4%).

Environmental PC 2: Environmental PC 2 describes the gradient from shallow water bodies, with low oxygen levels and low predation risk, lower pH, salinity and conductivity towards deeper water bodies, with high oxygen content and increased predation risk (Table 2). We found a strong effect of environmental PC 2 on components of female body morphology, namely relative warp (RW) 1 (V_{rel} = 25.5%). This effect can be interpreted as females of both species evolving a deeper body and relatively smaller heads in bigger, deeper water bodies and under increased predation risk, while females were more slender-bodied in shallow water bodies with low predation risk (Fig. 6A). Moreover, we found a slight decrease in RW 2 along environmental PC 2 (V_{rel} = 9.5%) across species, which can be interpreted as caudal peduncle lengths becoming smaller with higher values of environmental PC 2 (Fig. 6B). Regarding female life-histories we found a strong shared response of increasing fecundity along environmental PC 2 (V_{rel} = 19.2%), suggesting that females produced more offspring per clutch when exposed to higher predation risk in deeper water bodies (Fig. 6C). Furthermore, RA increased significantly across species along environmental PC 2 (V_{rel} = 19.2%; Fig. 6D). Our analysis of body length uncovered a strong increase of body length along environmental PC 2 (V_{rel} = 34.4%; Fig. 6E).

Environmental PC 3: Environmental PC 3 describes the gradient from coastal waters, with high salinity and conductivity, towards more inland waters with higher maximum temperatures and a high annual temperature range. Along this gradient, females of both species showed strong divergence in RW 4 (V_{rel} = 28.2%), suggesting deeper bodies with increasing salinity and conductivity (Fig. 6F). Additionally, a weak shared response of increasing fecundity along environmental PC 3 was detected (V_{rel} = 5.1%; Fig. 6G).

Unique patterns of body-shape and life-history divergence

In both sexes, we found several cases of unique (species-specific) responses to environmental variables in body-shape and life-history divergence (Tab. 3; see Supplementary Tab. S5 – S8). The MANCOVAs revealed that male and female shape variation was influenced by the interaction terms ‘species × environmental PC 1’ (V_{rel} = 22.9% and V_{rel} = 21.1%, respectively) and ‘species × environmental PC 3’ (V_{rel} = 28.8% and V_{rel} = 17.4%, respectively). For male life-history divergence, we found significant interaction effects of ‘species × environmental PC 1’ (V_{rel} = 20.0%), ‘species × environmental PC 2’ (V_{rel} = 9.5%) and ‘species × environmental PC 3’ (V_{rel} = 6.1%), while for female life-history divergence, only the interaction terms ‘species × environmental PC 1’ (V_{rel} = 15.8%) and ‘species × environmental PC 2’ (V_{rel} = 14.7%) were significant.

Environmental PC 1: Environmental PC 1 describes the gradient from colder waters at high altitudes with high annual precipitation towards lowland water bodies with higher temperatures. We found a strong species-specific pattern of divergence of male RW 3 along environmental PC 1 (V_{rel} = 34.9%). This effect can be interpreted as male *G. sexradiata* being more slender-bodied at higher altitudes, while *G. yucatana* males showed the opposite pattern, with more slender-bodied males found at lowland sites (Fig. 7A). Male RW 1 increased slightly with environmental PC 1 in *G. sexradiata* but increased strongly in *G. yucatana* (V_{rel} = 14.2%). The position of the gonopodium changed only little in *G. sexradiata*, while it adopted a more anterior position on the body at sites with higher mean annual temperature in *G. yucatana* (Fig. 7B). Female RW 2 decreased with environmental PC 1 in *G. sexradiata* but increased in *G. yucatana* (V_{rel} = 17.3%), suggesting that *G. sexradiata* females decreased caudal peduncle length and increased head size along environmental PC 1, while *G. yucatana* showed the opposite pattern (Fig. 7C). Male fat content decreased slightly with environmental PC 1 in *G. sexradiata* but decreased strongly in *G. yucatana* (V_{rel} = 17.4%; Fig. 7D). Female fat content did not change along environmental PC 1 in *G. sexradiata*, but strongly decreased in *G. yucatana* (V_{rel} = 7.9%; Fig. 7E). Embryo fat content decreased slightly along environmental PC 1 in *G. sexradiata*, but strongly in *G. yucatana* (V_{rel} = 4.1%, Fig. 7F). However, *G. yucatana* was largely restricted to sites in the upper part of the distribution range along environmental PC 1, and so the significant interaction effects must be interpreted with caution in all cases.

Environmental PC 2: We found only few cases of unique responses along environmental PC 2, which received positive axis loadings, among other factors, from water depth and predation risk (Table 2). Male fat content increased slightly with environmental PC 2 in *G. sexradiata* while the increase was more pronounced in *G. yucatana* (V_{rel} = 8.8%; Fig. 7G). A similar pattern was found for female fat content, for which *G. sexradiata* showed no response along environmental PC 2, while fat content increased in *G. yucatana* females (V_{rel} = 4.4%; Fig. 7H).

Environmental PC 3: We found strong patterns of species-specific divergence along environmental PC 3, which describes the gradient from coastal waters, with high salinity and conductivity, towards more inland waters with higher maximum temperatures and a high annual temperature range. Along this gradient we found unique patterns of variation in male body shape (V_{rel} = 25.1%), with *G. sexradiata* being more slender-bodied at coastal sites and deeper-bodied in inland habitats, while *G. yucatana* displayed the opposite pattern, with deeper-bodied specimens being found at coastal sites and slender-bodied inland populations (Fig. 7I). In male *G. sexradiata* we found a decrease of RW 2 along environmental PC 3, while RW 2 increased in *G. yucatana* (V_{rel} = 23.8%). This effect can be interpreted as male *G. sexradiata* decreasing head size from coastal to inland waters, while *G. yucatana* showed the opposite pattern of divergence (Fig. 7J). Females showed species-specific shape divergence in RW 2 (V_{rel} = 6.8%), reflecting that caudal peduncle lengths became smaller with increasing environmental PC 3, with a less pronounced decrease in *G. sexradiata* compared to a much stronger decrease in *G. yucatana* (Fig. 7K). Finally, male fat content increased in *G. sexradiata* along environmental PC 3 while it decreased in *G. yucatana* (V_{rel} = 6.1%; Fig. 7L).

Maternal provisioning (matrotrophy index)

To evaluate the mode of maternal provisioning, we calculated matrotrophy indices (MI)[65,66]. The GLM detected no effect of species identity ($F_{1,3}$ = 0.32, P = 0.61, V_{rel} = 13.5%), and estimated MI-values (across populations) were similar between *G. sexradiata* (MI = 0.941) and *G. yucatana* (MI = 0.889; see Supplementary Fig. S5). We detected some degree of variation among sites, whereby some populations showed little to no maternal provisioning (0.5 > MI < 0.75), while others showed moderate amounts of maternal provisioning (0.8 > MI < 1.2; Supplementary Fig. S6). When statistically comparing whether or not there was maternal provisioning after fertilization, MI was significantly greater than 0.7 in two populations (Population 2: $z_{16} = 2.16$, $p = 0.031$; Population 3: $z_{15} = 2.09$, $p = 0.036$), and there was a non-significant trend in population 7 ($z_{10} = 1.77$, $p = 0.077$). However, our GLM found no predictable pattern of diversification along the three environmental PCs (environmental PC 1: $F_{1,3} = 0.45$, $p = 0.55$, $V_{\mathrm{rel}} = 18.3\%$; environmental PC 2: $F_{1,3} = 0.14$, $p = 0.74$, $V_{\mathrm{rel}} = 6.1\%$; environmental PC 3: $F_{1,3} = 0.16$, $p = 0.71$, $V_{\mathrm{rel}} = 7.2\%$).

Discussion

Phylogenetic relationships uncovered in our present study supported our assessment of species identity inferred on the basis of colour patterns and confirmed the proposed close relationship between *Gambusia yucatana* and *Gambusia puncticulata*[47]. *Gambusia eurystoma*—a species that is endemic to a hydrogen sulphide-rich spring complex[48-50]—clustered within *G. sexradiata*, which mirrors the results of a previous study[67]. This further highlights the need for additional investigations to assess gene flow and the degree of population genetic differentiation in this system[45-47], which was beyond the scope of this study. Specifically, it appears as if '*G. eurystoma*' could represent a case of incipient ecological speciation as a locally-adapted ecotype of the widespread *G. sexradiata*. Similar patterns were described for sulphide-adapted populations in the *Poecilia mexicana*-species complex, with phylogenetically old lineages (*P. sulphuraria*, *P. thermalis*) occurring in some sulphide springs, while others are inhabited by phylogenetically young sulphide-adapted ecotypes of *P. mexicana*[68].

Compared to *G. sexradiata*, we found a lower genetic variability and no population genetic structure in *G. yucatana*, which could be an indicator of a more recent invasion of the Río Grijalva system by the latter species. *Gambusia yucatana* is a member of the *Gambusia puncticulata*-species group, the members of which are distributed across the Greater

Antilles[45-47]. Given its tolerance to sea water[57], it is well conceivable that the species invaded Mexican watersheds through marine long-distance dispersal, e.g., during hurricanes[69]. Such a close Caribbean–Central American relationship of freshwater biota also becomes evident for other groups of organisms, e.g., freshwater crabs[70]. *Gambusia sexradiata* is part of the *Gambusia nobilis*-species group, all extant members of which occur on the North American mainland. *Gambusia sexradiata* is the only member with a wide distribution range, and it is the only member of the clade that is not entirely restricted to freshwater environments[45,46]. Altogether, the evolutionary histories of both species suggest a competitive advantage of *G. yucatana* at coastal sites, while *G. sexradiata* should be better adapted to inland conditions; which would also be consistent with previous reports on their distributions[45,55,57].

Contrary to prediction, we found a patchy occurrence of *G. sexradiata* and *G. yucatana* along the investigated stream gradient, with syntopic occurrences at only few sites. Both species occupied a wide range of environmental conditions regarding pH, dissolved oxygen and conductivity, and we did not identify any ecological factor that might limit the distributions of both congeners. *Gambusia sexradiata* and *G. yucatana* are adapted to slow-flowing or stagnant conditions[45,56] and are probably more affected by floods compared to species like *Poecilia mexicana* and *Astyanax aeneus*, both of which are adapted to higher stream velocities[16,56]. Contrary to other river systems of Mexico, damming projects were not as extensive in the Grijalva basin, and so the stream still experiences regular catastrophic flooding[44]. This may partly determine the seemingly stochastic distribution of both species along the river gradient, since massive dislocations during floods leave few individuals at a given site and subsequently allow either species to build up local populations again.

With the exception of three doubtful cases, we found no indication for extensive hybridization between species, suggesting reproductive isolation by means of behavioural (pre-mating) isolation, mechanical mating incompatibilities (for different gonopodial structures see[53]), or gametic incompatibilities (for review see[71]). For example, a unidirectional post-reproductive barrier by means of different chromosomal sex-determination has evolved between the closely related *G. affinis* (heteromorphic WZ–ZZ system) and *Gambusia holbrooki* (XX–XY)[72,73]. Future studies will need to shed light on the mechanisms of reproductive isolation at play in the system studied here.

We found shared phenotypic responses in components of body-shape and life-history divergence that were driven mainly by variation in predation regimes (included in 'environmental PC 2'), and which followed patterns of phenotypic diversification along ecological gradients of predation risk described for other poeciliids (shape divergence[33,35,74], life history divergence[21,23,66]). Interestingly, we found shared patterns of body-shape and life-history divergence only in female (but not male) *Gambusia* spp. It appears as if female body shape and life-history traits are a common target of selection, while males are responding in a species-specific way (see below). As described for other mosquitofishes[56,75], male *Gambusia* spp. (SL, mean ± SE: 23.26 ± 0.30 mm) in our study were substantially smaller than females (28.47 ± 0.43 mm), possibly translating into a much stronger predation risk for females, as avian, piscine and invertebrate predators may favour large-bodied poeciliids[21,76]. Furthermore, males of most *Gambusia* species do not show flamboyant secondary sexual colour ornaments like male guppies (*P. reticulata*), which therefore suffer higher mortality from predation compared to female guppies[19,77] (but see[78] for sexual ornamentation in *Gambusia hubbsi*).

We found females of both species to evolve relatively smaller heads (RW 1) and a deeper caudal peduncle (which was, however, not longer, as reported for other poeciliids[33,36,74]) in bigger, deeper water bodies and under increased predation risk, while females were more slender-bodied in shallow water bodies with low predation risk. Similar observations were made by Langerhans and Makowicz[79], who reported deeper bodies, but no increased caudal peduncle length in *G. caymanensis* females in response to increased predation risk. The deeper caudal region and the relatively small head are trait values associated with improved fast-start performance, while the short, but deep caudal peduncle is associated with increased steady-swimming performance and likely increases manoeuvrability in bigger water bodies[74]. Likewise, increased fecundity and RA under high predation risk was reported for several poeciliids, including *P. reticulata*[21,23], *Brachyrhaphis rhabdophora*[80], and *G. hubbsi* [66]. This is in congruence with life-history theory, which predicts that when adult mortality rates are high relative to juvenile mortality rates, females increase reproductive effort, investing in as many offspring per clutch as possible to maximize fitness, even if this investment comes at the cost of producing smaller offspring[25,81].

Previous studies mostly considered divergence of single traits (i.e., either shape or life history divergence) along stream gradients. Our analytical framework enabled us to consider the interplay of multivariate variation of several character suites. For example, body-shape divergence between predator regimes in females might reflect a trade-off between selection on locomotion and selection on reproduction[82-84]. Selection might act primarily on reproductive traits (fecundity, RA) that indirectly affect body shape. Hence, a possible explanation for the unexpectedly short, but deep caudal region under increased predation risk could be that selection favours large abdominal regions that provide space for an increased number of embryos. Those changes, however, may come at a cost as they could be developmentally linked to a shorter caudal region, which inhibits higher burst-swimming capabilities—normally favoured when prey species coexist with piscine predators[33,74]. An alternative explanation would be that selection favours females with good steady-swimming abilities in bigger water bodies and these traits might become manifest also under increased predation pressure due to the greater energetic requirements of increased RA and fecundity (i.e. more time spent foraging). This would explain short caudal regions, a trait associated with increased steady-swimming performance[74].

Furthermore, we found increased body length in both sexes in deeper water bodies and under higher predation risk. Lowland waters are characterized by higher accumulation of nutrients, higher photosynthetic primary production, more stable conditions and higher conspecific densities[17,20-24]. Bisazza and Marin[85] found bigger *G. holbrooki* males to have higher reproductive success under high population densities, since they monopolize access to females, while small males had an advantage when interactions with other males were rare. Therefore, high intraspecific competition should favour increased body size and lateral projection areas, especially in deep water bodies with more stable population densities[85,86]. This explanation is also congruent with the increased body depth in females of both species along environmental PC 3, suggesting deeper bodies with increasing salinity and conductivity (Fig. 6F). Deep-bodied populations were previously described for *G. yucatana* from localities close to the sea[54], and we could confirm those patterns not only for *G. yucatana* (Fig. 6F, 7I) but also for *G. sexradiata* (Fig. 6F). However, in *G. sexradiata* only females were more deep-bodied at coastal sites, while males showed the opposite pattern with deeper bodies at inland sites (Fig. 7I). It is tempting to argue that this reflects more stable population densities (and higher competition) in *G. sexradiata* at inland sites, a scenario supported by other findings from our present study (see below). Instead, body depth of females does not seem to be density-dependent and rather reflects higher manoeuvrability (deep body, short caudal regions; Fig. 7F) in bigger water bodies[74].

We found unique (species-specific) phenotypic responses in components of body-shape and life-history divergence that were driven mainly by abiotic conditions (climatic conditions, salinity, conductivity), and were particularly strong in males. Unique patterns of diversification were most pronounced along environmental PC 3, which describes the gradient from coastal waters, with high salinity and conductivity, towards more inland waters with higher maximum temperatures and a high annual temperature range. This is further evidence that patterns of local adaptation in poeciliid fishes are often sex-specific[87-89]. While we do not have a compelling explanation for the patterns uncovered here, we propose that they might be based on differences in intra- and intersexual selection between these two species, which is known to act strongly on males[90,91] and is often population-specific[92-94]. This combination might lead to slightly idiosyncratic patterns in males, which would be absent in females, which are more strongly affected by natural selection.

Differences in potentially fitness-related traits like fat content as a function of environmental PCs 1, 2, and 3 were slightly contradictory with respect to some environmental variables, as they would suggest almost opposite patterns of fat content along the gradient from high-altitude inland populations to low-altitude and more coastal populations. However, when investigating patterns of fat content specifically in response to ‘distance to the sea’ (see supplementary Fig. S4), it became apparent that in males, patterns were opposite for both species, with fat content in *G. sexradiata* increasing with increasing distance to the sea, while fat content decreased in *G. yucatana*. For females, patterns of fat content were similar in both species, so that fat content decreased with increasing distance from the sea. However, this pattern was again much stronger in *G. yucatana* than in *G. sexradiata* (Fig. S4). If such patterns were persistent and not just a temporary pattern caught in our sampling scheme, then this would be congruent with previous reports of their respective distributions[45,55,57], which suggested that *G. yucatana* might be better adapted to coastal regions relative to *G. sexradiata*. Future studies will have to investigate this further.

Deeper bodies (Fig. 7I) and bigger bodies (compared to head size, Fig. 7J) correlated with increased fat content (Fig. 7L) and might be a by-product of higher fat content, and thus indicative of a better body condition[95]. Furthermore, more stable population densities of *G. sexradiata* in inland waters are likely to cause higher mate competition among males and favour larger bodies with increased lateral projection area (i.e. body depth; RW 2, RW 3), which is a target of female preferences[85,86] (see above).

Finally, caution is required when interpreting gradual phenotypic divergence in multiple character suites as a signal of evolutionary divergence, as the observed phenotypic divergence could reflect both evolved differences and adaptive phenotypic plasticity. For example, Hendry *et al.*[34] found pronounced differences in body shape between wild-caught *P.*

reticulata from two consecutive years of sampling at the same locality, suggesting that plasticity may have contributed to the observed variation. On the other hand, Riesch *et al.*[88] found strong site-specific life-history divergence in cave-dwelling *P. mexicana* compared to surface-dwelling populations in traits such as fat content, standard length and gonadosomatic index, whereby divergence between these traits was retained after several generations under common garden conditions, suggesting a strong heritable component to the observed divergence.

In conclusion, we compared two closely related, congeneric species, which responded by a combination of shared and unique ways to the same components of a river gradient. Our findings therefore demonstrate the complexity of phenotypic responses even in relatively closely related, and supposedly ecologically very similar, species, when exposed to the same environmental variation along a river-system gradient. Our results also emphasise how the interplay between natural and sexual selection seems to affect both sexes in different, sometimes even opposing, ways (both on the level of populations and along the river gradient). Finally, our study was conducted in a river drainage that has, until now, not been affected much by damming projects[44], which have the potential to affect the evolutionary trajectories of populations[29,31]. Our current study demonstrates complex patterns of microevolutionary phenotypic diversification that are threatened by the widespread practice to produce hydroelectric power from stream impoundments[96].

Material and Methods

For the collection of these data, the authors have adhered to the Guidelines for the Use of Animals in Research. The current study does not include experiments involving living animals. All collections of specimens from natural populations in Mexico were approved by the Mexican Federal Agency (CONAPESCA: PRMN/DGOPA-003/2014 and PRMN/DGOPA-009/2015).

Study sites

We collected *Gambusia* spp. (Fig. 1) from 10 sites in the Grijalva Basin in Tabasco, Estados Unidos de México, ranging from coastal lagoons to stagnant waters in the foothills of the Sierra Madre de Chiapas (Fig. 3a, Tab. 1). We caught fish with dip nets (35×35 cm, mesh size 3 mm) during the dry season in April 2014 and 2015, when several stagnant water bodies are separated from the various affluent streams draining the Río Grijalva. All captured specimens were sacrificed with an overdose of clove oil and preserved in 96% ethanol until they were processed in the laboratory for molecular, morphological, or life-history analyses.

Environmental characteristics in the Grijalva Basin change markedly along the longitudinal gradient from the mountainous Sierra Madre de Chiapas to the coastal plains of Tabasco. The upper Grijalva has two large hydroelectric dams that dampen the stream flow regime and remove large amounts of sediment from the system[44]. *Gambusia* spp. typically do not occur in headwaters[43,56], and so our study sites were located below the dams at the lower reaches of the Grijalva basin, where the Grijalva interconnects with the Usumacinta Basin through multiple bifurcating distributaries that form an extensive wetland system. The lower Grijalva is strongly affected by human activities, and the petroleum industry has probably created the most substantial change[44].

Environmental and climate data

We examined a range of environmental variables that show gradual variation along the examined stretch of the Río Grijalva. At each site, we measured salinity using a Hanna HI 96822 refractometer, and dissolved oxygen (mg L^{-1}) and pH using a HACH®, HQ40d multimeter (see Supplementary Tab. S1). We estimated average water depth at our study sites as belonging to one of four categories (<1 m, 1–3 m, 3–5 m, >5 m). We were not able to quantify predation risk but made an attempt to provide an overview of co-occurring (predatory and non-predatory) fishes. First, we conducted predator surveys by slowly walking the survey areas along the shoreline and in shallow parts of the water for at least 30 min and noted all predatory species we sighted (see Supplementary Tab. S2). Afterwards, four persons collected fishes for at least 30 min per site using a seine (3 m long, 3 mm mesh with). We expressed predation risk as one of three categories (*low*: *Gambusia* spp. co-occurred with the killifish

Cynodonichthys tenuis or other small, omnivorous poeciliids that do not prey on adult *Gambusia*; *medium*: *Gambusia* spp. co-occurred with cichlid predators or the poeciliid *Belonesox belizanus* that regularly preys on *Gambusia*; *high*: the presence of large-bodied predators of marine origin, like *Centropomus undecimalis*, in combination with large-bodied piscivorous cichlids suggested high predation pressure on all size classes of *Gambusia* spp.).

We downloaded climatic data (averaged from 1950–2000) for each study site from the Worldclim database[97] at a 2.5 arc-minutes resolution. We included five climatic variables: (*i*) mean annual temperature, (*ii*) maximum temperature of the warmest month, (*iii*) minimum temperature of the coldest month, (*iv*) annual temperature range, and (*v*) annual precipitation. Altitude was extracted from Google Earth (http://earth.google.com/).

We condensed environmental variables through a factor reduction (principal components analysis, PCA) with varimax rotation. The three resulting principle components (PCs; henceforth called 'environmental PCs') with an eigenvalue > 1.0, explaining 89.1% of environmental variation, were used to characterize the stream gradient along the examined stretch of the Río Grijalva, and were used as covariates in the statistical analyses (Table 2).

Molecular species identification

For species identification we initially used the keys provided by Greenfield *et al.*[55]. However, *ad hoc* determination of both *Gambusia* species may be difficult, since morphological characteristics, like numbers of fin rays or pigmentation patterns are variable across populations[55]. To verify our assessment of species identity, we therefore used the following approach: First, we generated phylogenetic information for a subset of individuals based on sequence variation of mitochondrial DNA. Second, we amplified nuclear microsatellites for a larger number of individuals (including those for which phylogenetic information was available) and conducted population genetic analyses to verify species identity and to test for potential hybridization. After genotyping, we found pigmentation patterns to be the most accurate criterion to distinguish *G. sexradiata* from *G. yucatana*: lateral black spots are arranged in rows on the dorsal half of the body in *G. sexradiata*, while *G. yucatana* has scattered black spots at the dorsal half of the body (Fig. 1). Individuals classified using this criterion obtained the highest probability of correct assignment in our population genetic analyses (see Results). Therefore, we used this criterion to identify species for all further analyses. Note, however, that this trait appears not to distinguish both species over their entire distribution range[55].

Phylogenetic analysis

We sequenced a 387 bp segment of the mitochondrial cytochrome *b* gene for two specimens from each population ($n = 20$). We extracted DNA from fin-clips using the Nucleo Spin Tissue kit (Macherey-Nagel). PCR mixes (total volume: 14 µl) included 8.5 µl ultrapure water, 1.25 µl PCR buffer, 1.15 µl $MgCl_2$ (50 mM), 0.25 µl of each primer (10 µM) (forward: L14724: 5′-CGAAGCTTGATATGAAAAACCATCGTTG-3′, reverse: H15149: 5′-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3′[47,98]), 0.02 µl of each dNTP (12.5 µM), 0.8 µl Taq (5 U/µl) polymerase and 1.0 µl DNA template. Thermocycling conditions were as follows: initial denaturation for 3 min at 95°C, followed by 35 cycles of denaturation at 95°C for 40 s, primer annealing at 52°C for 40 s, and extension at 72°C for 30 s; and a final extension step at 72°C for 5 min. PCR products were cleaned using the Bioline SureClean kit according to the manufacturer's protocol. Sequencing was outsourced to GATC Biotech AG (Konstanz, Germany). We sequenced fragments in both directions.

Sequences were checked for stop codons and aligned manually (as there were no insertions/deletions that might have caused ambiguous alignment alternatives), including reference sequences and sequences of closely related *Gambusia* spp. from Genbank, as well as one sequence of *Belonesox belizanus* that served as an outgroup[47]. Bayesian phylogenetic inference was conducted in MrBayes 3.2.2[99] under a GTR+G substitution model with four chains and two independent runs for 7M iterations, sampling every 5,000th iteration. Potential autocorrelation (effective sample size for all parameters >950) and stationarity of the Marcov chain were checked in Tracer 1.6[100]. A maximum clade credibility tree (Fig. 3*b*) was calculated in TreeAnnotator 1.8.2 (part of the BEAST package[101]), with a burn-in of 350 trees.

Population genetic analysis

We used 15 nuclear microsatellite loci to genotype $n = 239$ fish from all 10 sites (Tab. 1; Fig. 3a). This allowed verification of species identity and testing for potential signs of hybridization by using a population genetic approach. We used primer pairs established for *G. affinis*[102,103], which were arranged in three separate multiplex reactions (*reaction 1*: Gaaf10, Gaaf11, Gaaf13, Gafu3; *reaction 2*: Gaaf7, Gaaf9, Gaaf15, Gaaf16, Gaaf22, Gafu2, Gafu6; *reaction 3*: Gafu1, Gafu4, Gafu7, Mf13). Microsatellites were amplified with the Type-it Microsatellite PCR kit from Qiagen (Hilden, Germany). The PCR protocol included an initial denaturation step for 5 min at 95°C, 28 cycles at 95°C for 30 s (denaturation), 57°C for 90 s (primer annealing), and 72°C for 30 s (extension), followed by a final extension step for 30:00 min at 60°C. The 5 µl reaction mix included 2.5 µl Type-it master mix, 0.4 µl primer mix, 0.4 µl Q-solution, 0.9 µl RNase-free water, and 0.8 µl template DNA. PCR products were analyzed on a CEQ2000 sequencer (Beckman Coulter; denaturation at 90°C for 2 min, injection at 2.0 kV for 30 s, separation at 6.0 kV for 45 min) along with the manufacturer's internal size standard. We screened the resulting fragment length data using Genome Lab GeTX 10.2 software (Beckman Coulter) and performed allele-calling manually.

We used the software STRUCTURE 2.3.4[104] to calculate individual assignment probabilities (Q-values) to varying numbers of genetically distinct clusters (K). For each value of $K = 1–12$, ten iterations were run using the admixture model with a burn-in period of 250,000 generations, followed by a sampling phase of 750,000 iterations. We detected the uppermost level of population differentiation with the method presented by Evanno *et al.*[64] using the web-based tool STRUCTURE HARVESTER 0.6.93[105]. To detect potential hybrids, we used the software NEWHYBRIDS[106]. This approach also provided Q-values, which describe the probability that an individual belongs to each of six different genotypes (i.e., parental 'purebreds', F_1-hybrids, F_2-hybrids and backcrosses with either parental genotype). We ran NEWHYBRIDS with a burn-in period of 250,000 generations, followed by a sampling phase of 750,000 iterations. We considered individuals to be 'purebreds' if $Q \geq 0.95$.

To calculate standard indicators of genetic variability and pairwise F_{ST}-values between populations, we conducted species-wise analyses, thus excluding single individuals of the respective other species if a site was dominated by one of the two *Gambusia* species. For example, in case of site 2 we excluded the single individual that was identified as *G. yucatana*. However, in the case of site 5, where both species occurred at sufficiently high frequencies, we analyzed the subset of *G. sexradiata* ($n = 5$) and *G. yucatana* ($n = 19$), separately. We used ARLEQUIN v 3.5[107] to calculate pairwise F_{ST}-values and used FSTAT[108] to calculate standard population genetic metrics, namely observed (H_{O}) and expected heterozygosity (H_{E}) as well as allelic richness (A) and to conduct a probability test for deviations from Hardy-Weinberg equilibrium (HWE).

Species distributions patterns

We used canonical correspondence analysis CCA; [109], to analyze patterns of variation of fish assemblage composition along our sample sites in the Río Grijalva basin in relation to environmental characteristics and to evaluate whether and how environmental variables explain the occurrence of both congeneric *Gambusia* species. The first CCA was conducted using presence/absence data of all species as the dependent data matrix, and the three environmental PCs (see above) as independent variables. For the second CCA we used numbers of both *Gambusia* species at each site (obtained from seining) as the dependent data matrix, and the three environmental PCs as independent variables. Both CCAs were conducted with a Monte Carlo permutation test using XLSTAT 2016[110].

Phenotypic divergence

Geometric morphometric analyses

One multivariate measure of phenotypic trait divergence along the stream gradient was body shape variation, which we assessed for 32 to 40 individuals per population ($n = 384$ individuals altogether; Tab. 1) using landmark-based geometric morphometric analysis. Photographs were taken in lateral view using a Canon eos 600D camera mounted on a stand. We digitized 15 lateral landmark coordinates using the software program tpsDig2[111]. Landmarks were selected to provide adequate coverage of the lateral body profile and largely followed previous studies on *Gambusia* spp.[33,112] with additional landmark points defined at the eyes and pectoral fins (see Supplementary Fig. 2). To account for bending of specimens owing to preservation, we unbent landmarks using the landmarks at the tip of the mouth and middle of the tail fin and two additional temporary landmarks set at the lateral line (but removed in the final analyses) using the 'unbend specimens' function in tpsUtil[113]. Procrustes fits were obtained using a full Procrustes fits procedure implemented in the software morphoJ[114] that orthogonally projects landmark data to a tangent space and automatically excludes variation that is not caused by true shape-variation (e.g., differences due to scaling and positioning of the test subjects; for a detailed description of geometric morphometrics see[115]). We subjected procrustes coordinates to a factor reduction and retained the first 5 relative warps (RWs), which are principal components of shape variation, explaining 73.6% (males) and 70.3% (females) of the morphological variance, respectively. We analyzed males and females separately since mature *Gambusia* species show pronounced sexual dimorphism in an array of morphological traits[75].

We tested our predictions regarding shared and unique patterns of gradient evolution (Fig. 2) by assessing the relative contributions of species identity (reflecting a phylogenetic signal) and environmental conditions at each sample site to shape variation. We used the 'morphological PCs' as dependent variables in two multivariate GLMs (MANCOVAs, one for each sex), in which 'species' was specified as fixed factor. Log-transformed 'body centroid size' and the three 'environmental PCs' were included as covariates. We included all two-way interactions of covariates with the fixed factor. Inspection of model residuals did not indicate violations of model assumptions (i.e., normal error distribution and homoscedasticity). To quantify the relative importance of model terms, we estimated effect sizes using Wilk's partial eta squared (η_p^2) and calculated relative variances as the partial variance for a given term divided by the maximum partial variance value in that model.

Life history measurements

Dissections to collect male, female, and offspring-related life-history traits followed well established protocols [21,87]. We collected the following male and female life-history traits from 21 to 38 individuals per population (n = 301 altogether; Tab. 1): standard length (SL [mm]), dry weight [g], lean weight [g], fat content [%], and reproductive investment ([%]; for males: testis dry weight divided by the sum of reproductive and somatic tissue dry weight, i.e., gonadosomatic index (GSI); for females: offspring dry weight divided by the sum of offspring plus somatic dry weight, i.e., reproductive allocation (RA)). In case of females, we further determined offspring lean weight [mg], offspring fat content [%]

and fecundity (number of offspring per clutch). During dissections performed on gravid females, all developing offspring were removed and their stage of development determined according to the classification scheme outlined in Riesch *et al.*[116].

As predicted[21,66,87], a preparatory GLM revealed an effect of embryo stage on embryo lean weight, and so we used residuals for further analysis. Prior to statistical analyses we $\log_{10}$-transformed (male and female SL, male and female lean weight, and embryo lean weight), square root-transformed (fecundity), or arcsine (square root)-transformed (male, female, and embryo fat content, male GSI and female RA) all life-history variables, and used subsequent *z*-transformation to meet assumptions of statistical analyses (i.e., these transformations greatly facilitated normality of model residuals).

To evaluate the mode of maternal provisioning, we calculated the matrotrophy index (MI) using the slopes and intercepts from linear regressions by analyzing the relationship between log-transformed embryonic dry mass and stage of development[65,66]. If the eggs were fully provisioned by yolk before fertilization (lecithotrophy), then we would expect the embryos to lose 25%–40% of their dry mass during development (MI between 0.60 and 0.75; see[117]). On the other hand, in the case of continuous maternal provisioning after fertilization (matrotrophy), one would expect the embryos to lose less weight (MI between 0.75 and 1.00) or to even gain weight during development (MI 1 1.00; e.g., Reznick et al.[65]).

We excluded site 5 and 8 from the analyses of maternal provisioning due to a low number of pregnant females of each species ($n \leq 4$). We calculated the MI for species separately, that means in the case of site 3, we used the subset of *G. sexradiata* ($n = 16$) and omitted pregnant females of *G. yucatana* ($n = 2$). We tested for differences in MI under different environmental conditions in a GLM, in which we included 'MI' as the dependent variable 'environmental PCs' as covariates and 'species' a fixed factor. We then tested each population separately for significant divergence from an MI of 0.7 (a cutoff, below which represents lecithotrophy and above which represents at least some level of incipient matrotrophy) by testing each population against that population's hypothetical slope for an MI of 0.7 in one-sample *z*-tests (following[65]).

The main statistical analysis of life-history traits followed our approach described for the morphometric analysis (see above). We used the *z*-transformed life-history variables as dependent variables in multivariate GLMs for both sexes separately, with ‘species’ specified as fixed factor, and ‘SL’ and ‘environmental PCs’ as covariates. Again, we included all two-way interactions of covariates with the fixed factor. The evaluate differences among populations in body size, we conducted a univariate GLM for both sexes combined, in which we used log transformed ‘SL’ as the dependent variable, ‘species’ and ‘sex’ as fixed factors, and ‘environmental PCs’ as covariates. Inspection of model residuals did not indicate violations of model assumptions (i.e., normal error distribution and homoscedasticity) in all models described in this article.

Competing financial interests

The authors declare that they have no competing financial interests.

Authors’ contributions

Conceived and designed the experiments: JJ, MP. Collected data: JJ, SKr, VML, CZ, CS-T, LA-R. Analysed the data: JJ, SKl, RR, MP. Contributed analysis tools: LA-R, SKl. Wrote the paper: JJ, RR, SKl, MP. All authors contributed to and approved the final manuscript version.

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Tables

Table 1. Overview of sample sites and numbers of individuals (*n*) used for the morphometric, life history and population genetic analyses.

Site ID	Site name	Latitude, longitude	*n* population genetics (males/females)	*n* body shape (males/females)	*n* life-history (males/females)
1	Laguna Mecoacán	18.342, -93.125	12/12	20/20	28/9
2	Simon Sarlat	18.337, -92.779	12/12	17/20	14/17
3	Laguna de Las Ilusiones	18.019, -92.931	12/12	20/20	20/18
4	Laguna Ranchería 1ra Lázaro Cárdenas	18.003, -93.022	12/12	20/20	19/18
5	Campus DACBiol-UJAT	17.989, -92.975	12/12	17/17	15/8
6	Ismate Chilapilla	17.913, -92.545	12/12	20/20	18/17
7	San Antonio I	17.749, -92.896	12/12	14/20	9/13
8	San Antonio II	17.734, -92.878	12/11	18/21	16/5
9	Laguna Canto Rodado	17.589, -92.981	12/12	20/20	15/19
10	Pond near Teapa	17.556, -92.952	12/12	20/20	19/15

Table 2: Results of principal components analysis of the 12 environmental variables measured at 10 study sites located within the Río Grijalva basin. PC loadings ≥ |0.6| in bold type.

	Environmental PC		
	1	2	3
Predation risk	0.196	**0.862**	-0.378
Water depth	0.190	**0.934**	0.037
pH	0.581	**0.722**	0.151
DO [%]	0.047	**0.872**	0.226
Conductivity [µS/cm]	0.091	**0.628**	**-0.607**
Salinity [PPT]	0.056	**0.635**	**-0.611**
Mean annual temperature	**0.829**	0.229	0.453
Max. temperature of the warmest month	0.088	0.099	**0.928**
Temperature of the coldest month	**0.977**	0.131	-0.137
Annual temperature range	-0.444	0.014	**0.852**
Annual precipitation	**-0.951**	-0.167	0.164
Altitude	**-0.919**	-0.103	0.249

Table 3. Results of MANCOVAs examining (*a*) female and (*b*) male shape variation as well as (*c*) female and (*d*) male life history differentiation along environmental gradients. (e) Results of ANCOVA examining body length in both sexes. *F* ratios were approximated using Wilk's λ values. Partial variance explained was estimated using Wilk's partial η^2 (for details see main text).

Model	Source	*F*	d.f.	*P*	Partial variance explained (%)
(*a*) male body shape	Species	1.778	5,172	0.120	14.31
	Centroid size	**17.903**	**5,172**	**< 0.001**	**99.69**
	Environmental PC 1	**3.126**	**5,172**	**0.010**	**24.26**
	Environmental PC 2	0.905	5,172	0.479	7.46
	Environmental PC 3	1.547	5,172	0.178	12.53
	Species × Centroid size	1.924	5,172	0.093	15.43
	Species × Environmental PC 1	**2.935**	**5,172**	**0.014**	**22.89**
	Species × Environmental PC 2	1.623	5,172	0.156	13.12
	Species × Environmental PC 3	**3.773**	**5,172**	**0.003**	**28.79**
(*b*) female body shape	**Species**	**2.387**	**10, 168**	**0.040**	**16.56**
	Centroid size	**20.208**	**10, 168**	**< 0.001**	**96.34**
	Environmental PC 1	1.722	10, 168	0.132	12.15
	Environmental PC 2	**8.396**	**10, 168**	**< 0.001**	**50.49**
	Environmental PC 3	**6.917**	**10, 168**	**< 0.001**	**43.00**
	Species × Centroid size	2.245	10, 168	0.052	15.63
	Species × Environmental PC 1	**3.094**	**10, 168**	**0.010**	**21.08**
	Species × Environmental PC 2	1.033	10, 168	0.400	7.42
	Species × Environmental PC 3	**2.520**	**10, 168**	**0.031**	**17.42**
(*c*) male life histories	**Species**	**4.542**	**3. 161**	**0.004**	**8.90**
	Length	**379.008**	**3. 161**	**< 0.001**	**100.00**
	Environmental PC 1	**14.791**	**3. 161**	**< 0.001**	**24.66**
	Environmental PC 2	1.311	3. 161	0.273	2.74
	Environmental PC 3	**4.674**	**3. 161**	**0.004**	**9.13**
	Species × Length	1.053	3. 161	0.371	2.17
	Species × Environmental PC 1	**11.419**	**3. 161**	**< 0.001**	**19.98**
	Species × Environmental PC 2	**4.862**	**3. 161**	**0.003**	**9.47**
	Species × Environmental PC 3	**3.022**	**3. 161**	**0.031**	**6.05**
(*d*) female life histories	**Species**	**2.366**	**6, 113**	**0.034**	**13.58**
	Length	**88.980**	**6, 113**	**< 0.001**	**100.00**
	Environmental PC 1	**3.062**	**6, 113**	**0.008**	**16.97**
	Environmental PC 2	**8.125**	**6, 113**	**< 0.001**	**36.48**
	Environmental PC 3	**2.264**	**6, 113**	**0.042**	**12.97**
	Species × Length	0.685	**6, 113**	0.662	4.24
	Species × Environmental PC 1	**2.820**	**6, 113**	**0.014**	**15.76**
	Species × Environmental PC 2	**2.603**	**6, 113**	**0.021**	**14.67**
	Species × Environmental PC 3	2.136	6, 113	0.055	12.36
(*e*) SL both sexes	**Species**	**23.292**	**1, 289**	**< 0.001**	**7.72**
	Sex	**119.811**	**1, 289**	**< 0.001**	**72.90**
	Environmental PC 1	3.724	1, 289	0.055	3.16
	Environmental PC 2	**46.357**	**1, 289**	**0.000**	**34.39**
	Environmental PC 3	1.814	1, 289	0.179	1.55
	Species × Sex	2.933	1, 289	0.088	2.50
	Sex × Environmental PC 1	**5.146**	**1, 289**	**0.024**	**4.35**
	Sex × Environmental PC 2	3.340	1, 289	0.069	2.84
	Sex × Environmental PC 3	**4.257**	**1, 289**	**0.040**	**3.61**
	Species × Environmental PC 1	0.105	1, 289	0.747	0.09
	Species × Environmental PC 2	0.004	1, 289	0.948	0.00
	Species × Environmental PC 3	2.971	1, 289	0.086	2.53

Figures

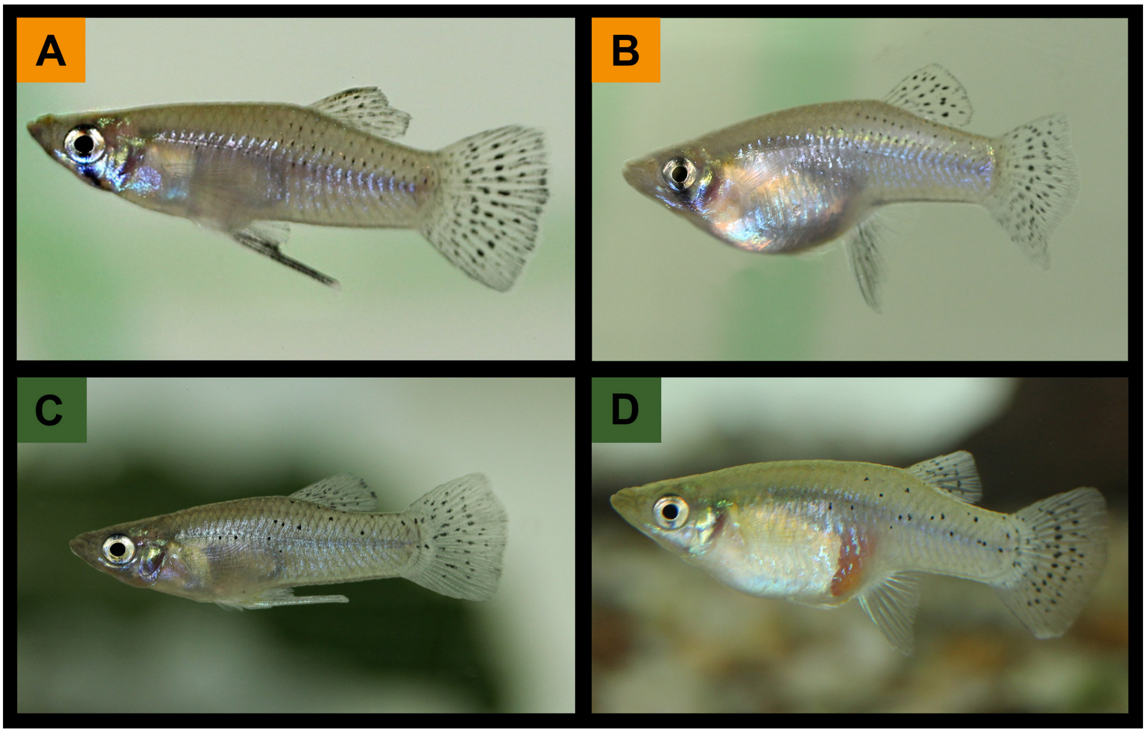

Figure 1. Representative photographs of aquarium-reared *Gambusia* spp. (A) male, and (**B**) female *G. sexradiata* (orange) from site 7 and *G. yucatana* (green), (**C**) male and (**D**) female from site 8. Note different pigmentation patterns that allowed us to unambiguously distinguish both species: in *G. sexradiata* lateral black spots are arranged in rows on the dorsal half of the body, while *G. yucatana* displays scattered black spots on the dorsal half of the body.

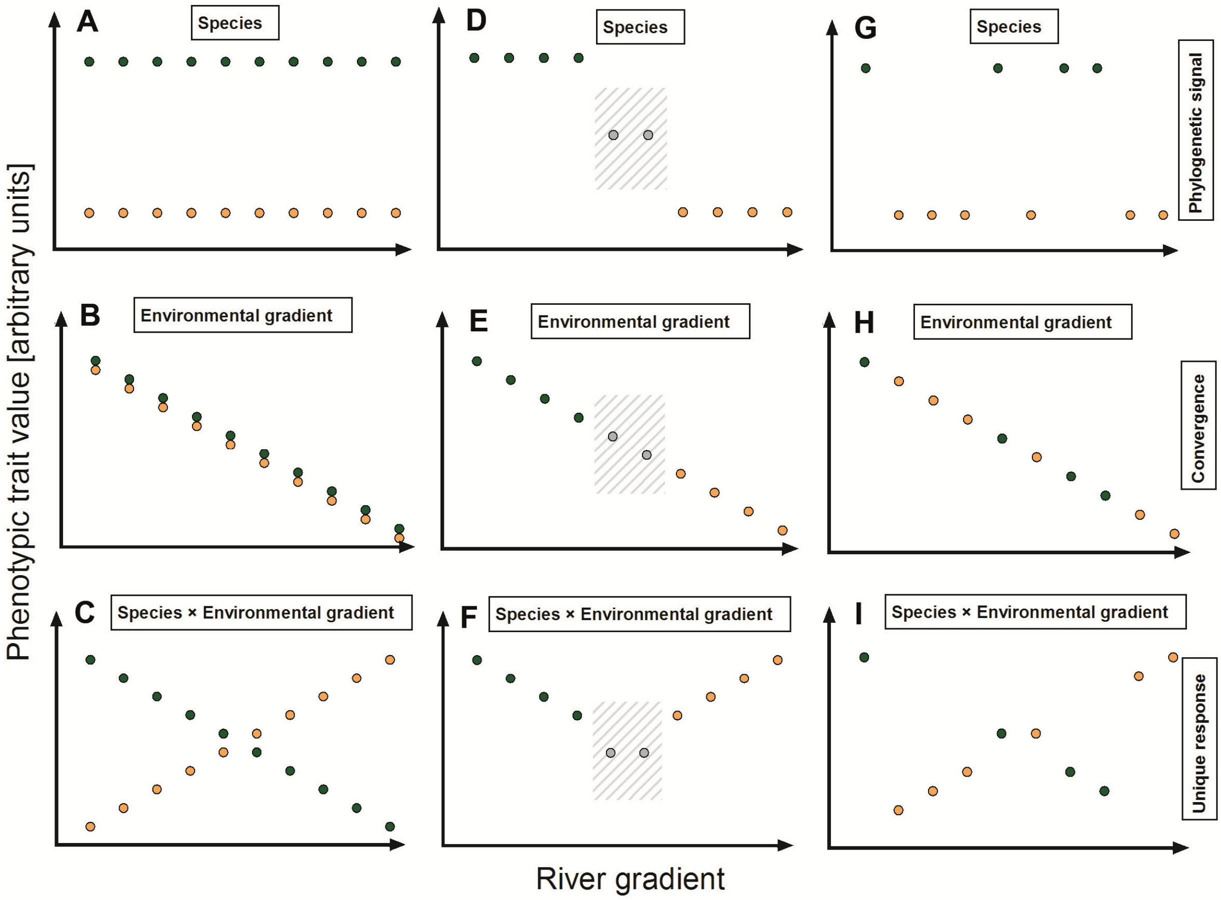

Figure 2. Illustration of hypothetical effects of shared and unique phenotypic trait divergence along a stream gradient in two ecologically competing species (green and orange). (**A-C**) Both species occur syntopically along the river gradient. (**A**) Unique responses could arise from different evolutionary histories of both species (i.e., represent a phylogenetic signal) and are thus independent of the river gradient, (**B**) selective forces could result in convergent (shared) patterns of divergence in both species, or (**C**) phenotypic diversification could be due to unique (species-specific) responses to components of the river gradient. (**D-F**) Alternatively, the river gradient altogether could determine species distributions, with a potential overlap zone in between, in which hybridization could occur (grey). Again, phenotypic differences could reflect (**D**) a phylogenetic signal, (**E**) shared patterns of gradient evolution, or (**F**) species-specific responses (not illustrated here is the potential outcome of ecological character displacement, where both species diverge in opposing directions in the overlap zone; for illustration see Supplementary Fig. S3). (**G-I**) Moreover, certain components of the river gradient could determine small-scale species distribution patterns, leading to a patchy occurrence of both species. Also under this scenario, the same general patterns of gradient evolution can be predicted. Boxes indicate significant effects of the main factor (‘species’), the covariate (‘environmental gradient’), or their interaction in analyses of covariance [(M)ANCOVA] using phenotypic trait values as the dependent variable.

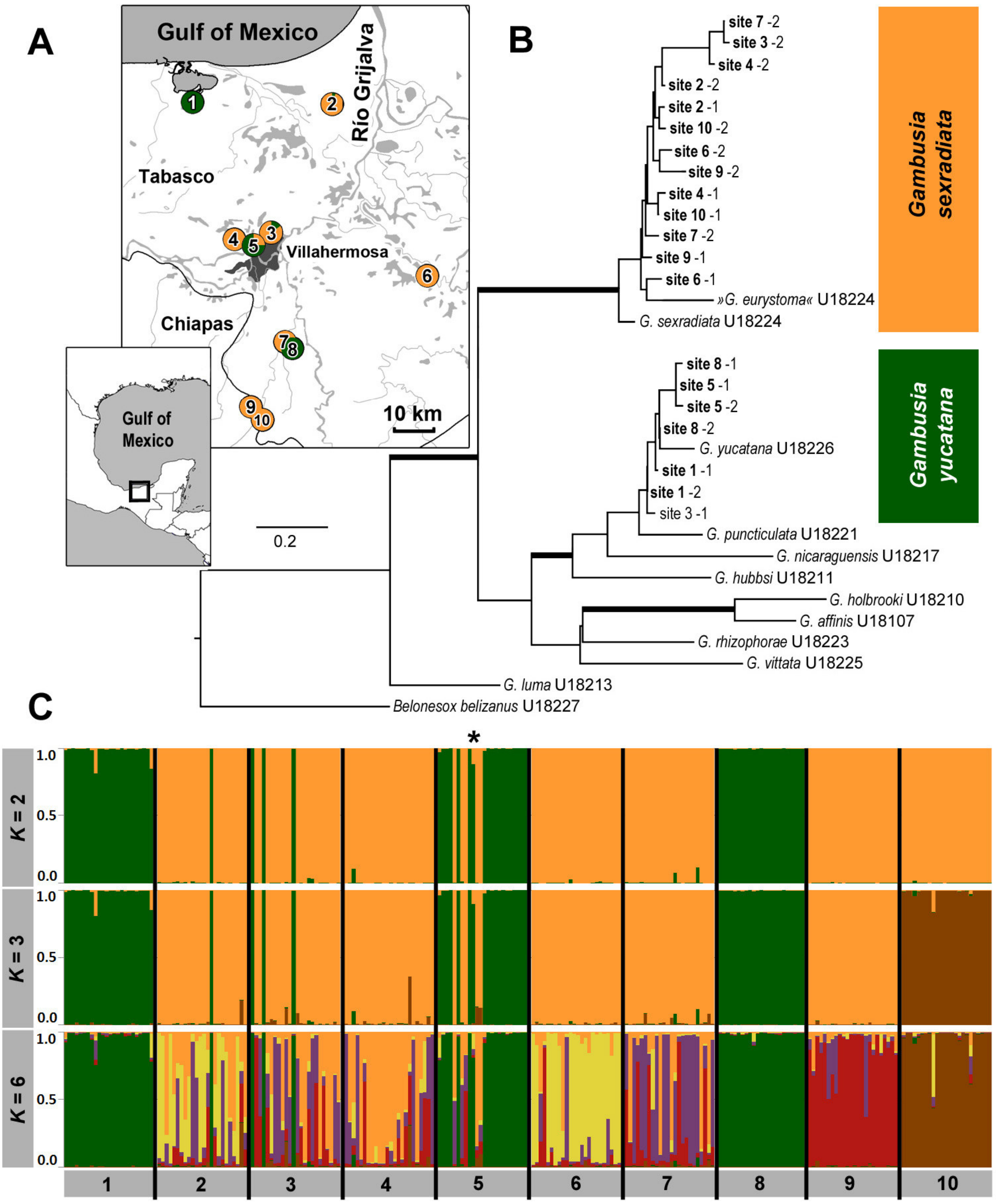

Figure 3. Sample sites and molecular species identification. (**A**) Sampling sites at which *Gambusia sexradiata* (orange) and *G. yucatana* (green) were collected in the Río Grijalva basin. The insert shows the location of our study area in Mexico. At sites 2, 3, and 5 both species were found to occur syntopically, and proportions of occurrence are indicated by different colour coding. The map was generated using DIVA-GIS 7.5[118]. (**B**) Phylogenetic relationships between exemplary individuals from all sampling sites and reference samples from GenBank[47] inferred using a Bayesian phylogenetic approach based on cyt*b* sequences (maximum clade credibility tree). Branches with posterior probability > 0.95 are given in bold. (**C**) Results from STRUCTURE[104] based on fragment length polymorphisms of 15 nuclear microsatellites. $K = 2$ was the most likely number of genetically distinct clusters according to the method provided by Evanno *et al.*[64], followed by $K = 3$ and $K = 6$ (see Supplementary Fig. S1). Each individual is represented by a vertical bar, which is partitioned into K-coloured segments representing its estimated likelihood of membership (Q) to each of the identified clusters. The asterisk marks an individual of putative hybrid origin according to the NEWHYBRIDS analysis (for details see main text).

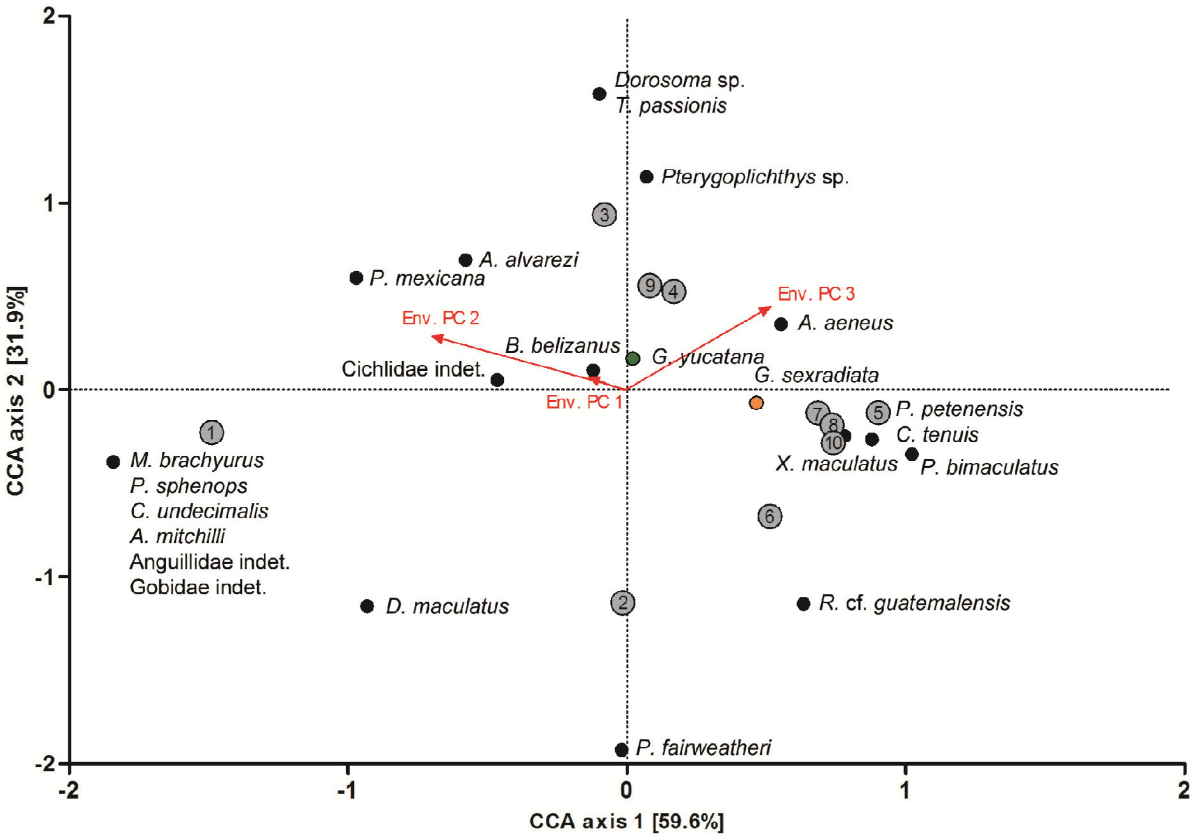

Figure 4. Fish community structure across sample sites. Results from canonical correspondence analysis (CCA) showing the effects of environmental variables ('environmental PCs', see main text) on fish community structures using occurrence data (present/absent) of different teleosts as the dependent data matrix (see Supplementary Tab. S2). Species are marked by black circles; grey circles indicate the position of sample sites. Length and direction of arrows indicate the relative importance and direction of the environmental variables.

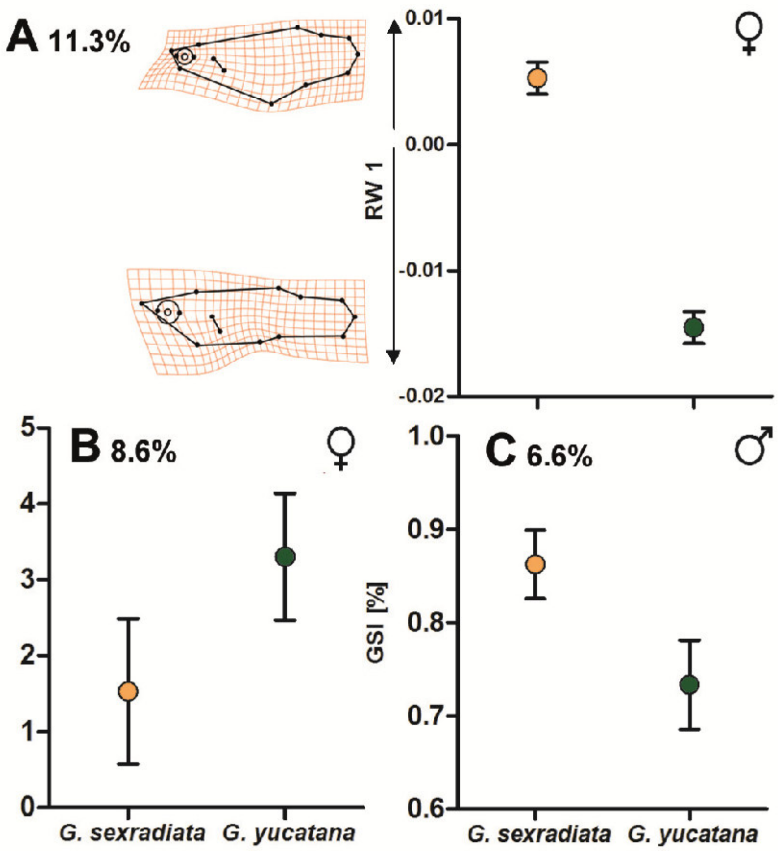

Figure 5. Graphic illustration of three traits for which a main effect of species identity was uncovered. This suggests that differences arose from different evolutionary histories of both taxa (i.e., represent a phylogenetic signal) and are independent of the environmental gradient (Tab. 3): (**A**) *G. sexradiata* females had a deeper body and smaller head size compared to *G. yucatana*, as indicated by a significant effect on relative warp (RW) 1, (**B**) female fat content was lower in *G. sexradiata* than in *G. yucatana*, and (**C**) male GSI was higher in *G. sexradiata* than in *G. yucatana*. Relative variance explained (in percent) is shown for each factor.

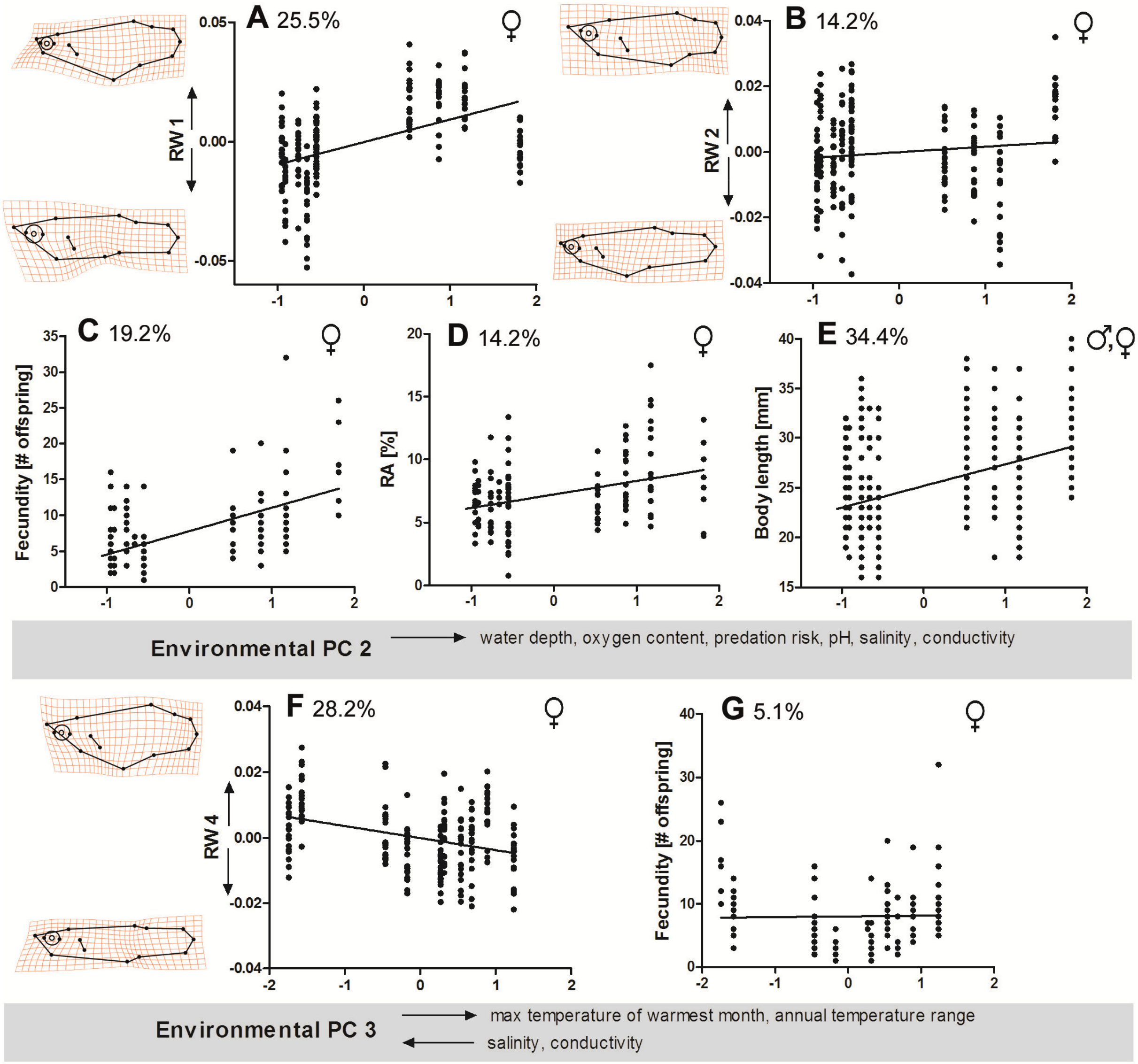

Figure 6. Graphic illustration of shared patterns of phenotypic divergence, suggesting that selective forces along the stream gradient result in convergent patterns of differentiation in both species. With increasing values of environmental PC 2, (**A**) female *Gambusia* spp. showed deeper bodies and smaller heads [as indicated by a significant effect on relative warp (RW) 1], (**B**) females slightly decreased caudal peduncle length and increased head size (RW 2), (**C**) females increased fecundity as well as (**D**) RA, and (**E**) both sexes increased body length. Increasing values of environmental PC 3 resulted in (**F**) deeper bodies in females (RW 4) and (**G**) a slightly higher female fecundity. Relative variance (in percent) is given for each variable (see also Supplementary Tab. S5-S8).

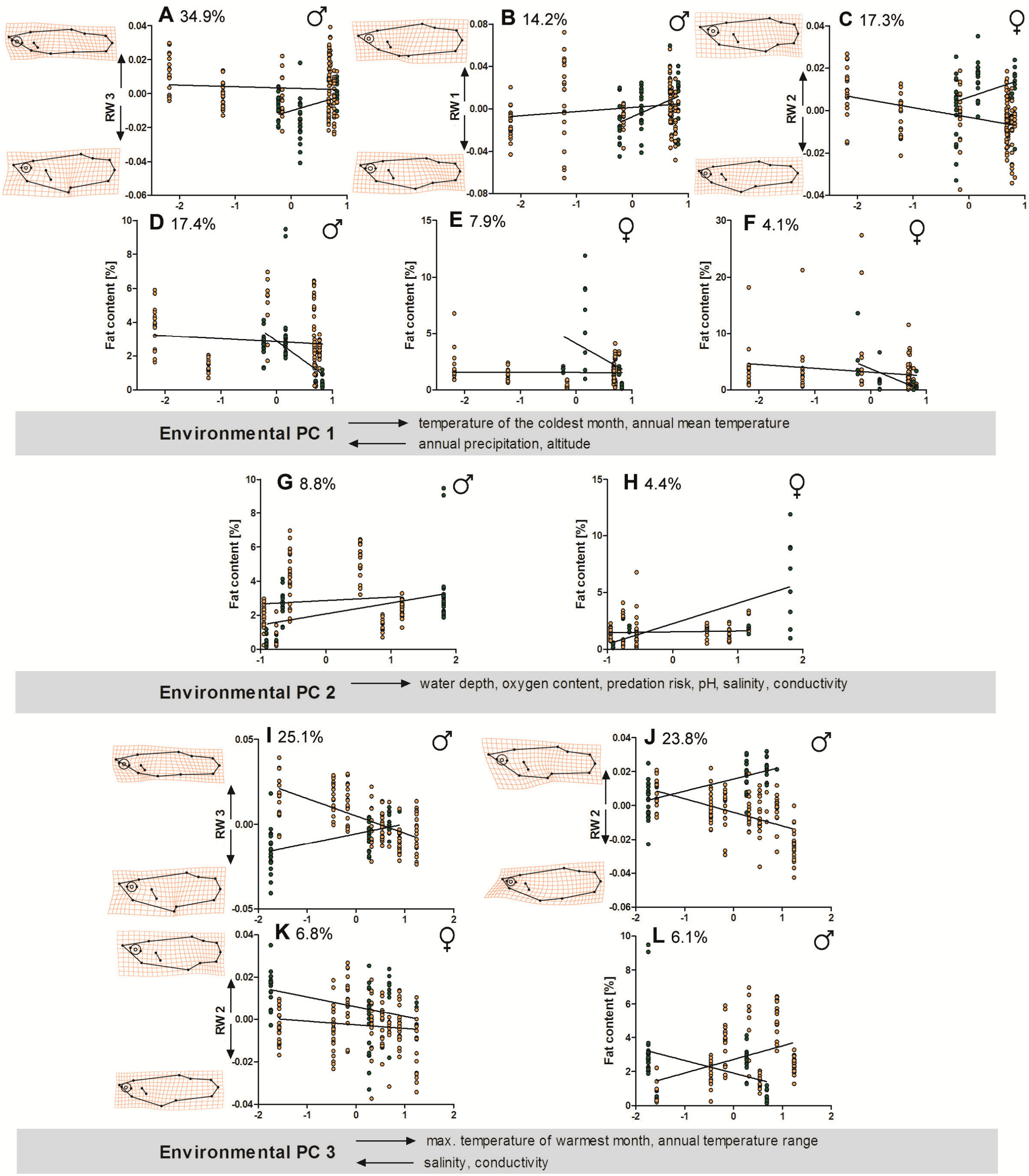

Figure 7. Graphic illustration of unique (species-specific) patterns of phenotypic divergence in *Gambusia sexradiata* (orange) and *G. yucatana* (green). Increasing values along 'environmental PC 1' (Table 2) resulted in (**A**) slightly decreased body depth (RW 3) of male *G. sexradiata*, while *G. yucatana* increased body depth, (**B**) slight (*G. sexradiata*) versus strong (*G. yucatana*) shifts towards a more anterior position of the gonopodium (RW 1), (**C**) decreased caudal peduncle length and increased head size in female *G. sexradiata*, but opposing patterns in *G. yucatana* (RW 2), (**D**) slightly (*G. sexradiata*) or strongly (*G. yucatana*) decreased male fat content, (**E**) virtually no change in female fat content (*G. sexradiata*) versus strongly decreased fat content (*G. yucatana*), and (**F**) slightly (*G. sexradiata*) versus strongly (*G. yucatana*) decreased embryo fat content. With increasing values along 'environmental PC 2', (**G**) male fat content slightly increased in *G. sexradiata* while the increase was more pronounced in *G. yucatana*, and (**H**) female fat content showed an increase only in *G. yucatana*. Increasing values along 'environmental PC 3' resulted in: (**I**) *G. sexradiata* males being deeper-bodied, while *G. yucatana* males were more slender-bodied (RW 3), (**J**) male *G. sexradiata* decreasing head size, while *G. yucatana* increased head size (RW 2), (**K**) *G. sexradiata* females slightly and *G.*

yucatana females strongly decreasing caudal peduncle length and increasing head size (RW 2), and (**L**) male fat content increasing in *G. sexradiata*, whereas it decreased in *G. yucatana.* Relative variance (in percent) is given for each interaction term (see also Supplementary Tab. S5 – S8).

References

1 Endler, J. A. *Natural selection in the wild*. (Princeton University Press, 1986).

2 Keller, I. & Seehausen, O. Thermal adaptation and ecological speciation. *Mol. Ecol.* **21**, 782-799 (2012).

3 Palo, J. *et al.* Latitudinal divergence of common frog (*Rana temporaria*) life history traits by natural selection: evidence from a comparison of molecular and quantitative genetic data. *Mol. Ecol.* **12**, 1963-1978 (2003).

4 Heibo, E., Magnhagen, C. & Vøllestad, L. A. Latitudinal variation in life-history traits in Eurasian perch. *Ecology* **86**, 3377-3386 (2005).

5 Watt, C., Mitchell, S. & Salewski, V. Bergmann's rule; a concept cluster? *Oikos* **119**, 89-100 (2010).

6 Hodkinson, I. D. Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews* **80**, 489-513 (2005).

7 Gomez-Mestre, I. & Tejedo, M. Contrasting patterns of quantitative and neutral genetic variation in locally adapted populations of the natterjack toad, *Bufo calamita*. *Evolution* **58**, 2343-2352 (2004).

8 Laverty, G. & Skadhauge, E. in *Extremophile Fishes* (eds Rüdiger Riesch, Michael Tobler, & Martin Plath) 85-106 (Springer, 2015).

9 Hangartner, S., Laurila, A. & Räsänen, K. Adaptive divergence of the moor frog (*Rana arvalis*) along an acidification gradient. *BMC Evol. Biol.* **11**, 366 (2011).

10 Nelson, J. A. in *Extremophile Fishes* (eds Rüdiger Riesch, Michael Tobler, & Martin Plath) 193-215 (Springer, 2015).

11 Allan, J. D. *Stream ecology*. 388 (Chapman & Hall, 1995).

12 Matthews, W. J. *Patterns in freshwater fish ecology*. (Springer Science & Business Media, 1998).

13 Wetzel, R. G. *Limnology: lake and river ecosystems, third edition*. (Academic Press, 2001).

14 Jackson, D. A., Peres-Neto, P. R. & Olden, J. D. What controls who is where in freshwater fish communities the roles of biotic, abiotic, and spatial factors. *Can. J. Fish. Aquat. Sci.* **58**, 157-170 (2001).

15 Winemiller, K. O. & Leslie, M. A. Fish assemblages across a complex, tropical freshwater/marine ecotone. *Environ. Biol. Fishes* **34**, 29-50 (1992).

16 Plath, M. *et al.* Locally adapted fish populations maintain small-scale genetic differentiation despite perturbation by a catastrophic flood event. *BMC Evol. Biol.* **10**, doi:25610.1186/1471-2148-10-256 (2010).

17 Grether, G. F., Millie, D. F., Bryant, M. J., Reznick, D. N. & Mayea, W. Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology* **82**, 1546-1559 (2001).

18 Zandonà, E., Auer, S. K., Kilham, S. S. & Reznick, D. N. Contrasting Population and Diet Influences on Gut Length of an Omnivorous Tropical Fish, the Trinidadian Guppy (*Poecilia reticulata*). *PLoS One* **10**, e0136079, doi:10.1371/journal.pone.0136079 (2015).

19 Magurran, A. E. *Evolutionary ecology: the Trinidadian guppy*. (Oxford University Press, 2005).

20 Endler, J. A. A predator’s view of animal color patterns. *Evolutionary Biology* **11**, 319-364 (1978).

21 Reznick, D. & Endler, J. A. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* **36**, 160-177, doi:10.2307/2407978 (1982).

22 Seghers, B. H. Schooling behavior in the guppy (*Poecilia reticulata*): an evolutionary response to predation. *Evolution* **28**, 486-489 (1974).

23 Reznick, D., Iv, M. J. B. & Rodd, H. Life-History Evolution in Guppies. VII. The Comparative Ecology of High- and Low- Predation Environments. *The American Naturalist* **157**, 126-140, doi:10.1086/318627 (2001).

24 Gilliam, J. F., Fraser, D. F. & Alkins-Koo, M. Structure of a tropical stream fish community: a role for biotic interactions. *Ecology* **74**, 1856-1870 (1993).

25 Moore, M. P., Riesch, R. & Martin, R. A. The predictability and magnitude of life-history divergence to ecological agents of selection: a meta-analysis in livebearing fishes. *Ecol. Lett.* **9**, 435-442 (2016).

26 Endler, J. A. Natural selection on color patterns in *Poecilia reticulata*. *Evolution* **34**, 76-91 (1980).

27 Reznick, D. A., Bryga, H. & Endler, J. A. Experimentally induced life-history evolution in a natural population. *Nature* **346**, 357-359, doi:10.1038/346357a0 (1990).

28 Torres-Dowdall, J. *et al.* Fine-scale local adaptation in life histories along a continuous environmental gradient in Trinidadian guppies. *Funct. Ecol.* **26**, 616-627 (2012).

29 Franssen, N. R., Harris, J., Clark, S. R., Schaefer, J. F. & Stewart, L. K. Shared and unique morphological responses of stream fishes to anthropogenic habitat alteration. *Proceedings of the Royal Society B-Biological Sciences* **280**, doi:10.1098/rspb.2012.2715 (2013).

30 Haas, T. C., Blum, M. J. & Heins, D. C. Morphological responses of a stream fish to water impoundment. *Biology Letters* **6**, 803-806 (2010).

31 Franssen, N. R. Anthropogenic habitat alteration induces rapid morphological divergence in a native stream fish. *Evolutionary Applications* **4**, 791-804 (2011).

32 Langerhans, R. B. Predictability of phenotypic differentiation across flow regimes in fishes. *Integrative and Comparative Biology* **48**, 750-768 (2008).

33 Langerhans, R. B., Layman, C. A., Shokrollahi, A. & DeWitt, T. J. Predator-driven phenotypic diversification in *Gambusia affinis*. *Evolution* **58**, 2305-2318 (2004).

34 Hendry, A., Kelly, M., Kinnison, M. & Reznick, D. Parallel evolution of the sexes? Effects of predation and habitat features on the size and shape of wild guppies. *J. Evol. Biol.* **19**, 741-754 (2006).

35 Langerhans, R. B., Gifford, M. E. & Joseph, E. O. Ecological speciation in *Gambusia* fishes. *Evolution* **61**, 2056-2074 (2007).

36 Langerhans, R. B. Morphology, performance, fitness: functional insight into a post-Pleistocene radiation of mosquitofish. *Biology Letters* **5**, 488-491 (2009).

37 Taniguchi, Y. & Nakano, S. Condition-specific competition: implications for the altitudinal distribution of stream fishes. *Ecology* **81**, 2027-2039 (2000).

38 Rauchenberger, M., Kallman, K. D. & Morizot, D. C. Monophyly and geography of the Rio Panuco basin swordtails (genus *Xiphophorus*) with descriptions of four new species. *Am. Mus. Novit.* **2975**, 1-41 (1990).

39 Sexton, J. P., McIntyre, P. J., Angert, A. L. & Rice, K. J. Evolution and ecology of species range limits. *Annual Review of Ecology, Evolution and Systematics* **40**, 415-436 (2009).

40 Wiens, J. J. *et al.* Niche conservatism as an emerging principle in ecology and conservation biology. *Ecol. Lett.* **13**, 1310-1324 (2010).

41 Arif, S., Adams, D. C. & Wicknick, J. A. Bioclimatic modelling, morphology, and behaviour reveal alternative mechanisms regulating the distributions of two parapatric salamander species. *Evol. Ecol. Res.* **9**, 843-854 (2007).

42 Torres-Dowdall, J., Dargent, F., Handelsman, C. A., Ramnarine, I. W. & Ghalambor, C. K. Ecological correlates of the distribution limits of two poeciliid species along a salinity gradient. *Biol. J. Linn. Soc.* **108**, 790-805 (2013).

43 Gómez-González, A. E., Velázquez-Velázquez, E., de Jesús Anzueto-Calvo, M. & Maza-Cruz, M. F. Fishes of the Grijalva River basin of Mexico and Guatemala. *Check List* **11**, 1726 (2015).

44 Hudson, P. H. *et al.* in *Rivers of North America* (eds A. C. Benke & C. E. Cushing) 1031–1085 (Elsevier, 2005).

45 Rauchenberger, M. *Systematics and biogeography of the genus Gambusia (Cyprinodontiformes, Poecilidae)*. (American Museum of Natural History, 1989).

46 Lydeard, C., Wooten, M. C. & Meyer, A. Molecules, morphology, and area cladograms: a cladistic and biogeographic analysis of *Gambusia* (Teleostei: Poeciliidae). *Syst. Biol.* **44**, 221-236 (1995).

47 Lydeard, C., Wooten, M. C. & Meyer, A. Cytochrome b sequence variation and a molecular phylogeny of the live-bearing fish genus *Gambusia* (Cyprinodontiformes: Poeciliidae). *Can. J. Zool.* **73**, 213-227 (1995).

48 Tobler, M., Riesch, R., Garcíade León, F., Schlupp, I. & Plath, M. Two endemic and endangered fishes, *Poecilia sulphuraria* (Alvarez, 1948) and *Gambusia eurystoma* Miller, 1975 (Poeciliidae, Teleostei) as only survivors in a small sulphidic habitat. *J. Fish Biol.* **72**, 523-533 (2008).

49 Tobler, M. & Plath, M. Threatened fishes of the world: *Gambusia eurystoma* Miller, 1975 (Poeciliidae). *Environ. Biol. Fishes* **85**, 251-251 (2009).

50 Culumber, Z. W. *et al.* Habitat use by two extremophile, highly endemic, and critically endangered fish species (*Gambusia eurystoma* and *Poecilia sulphuraria*; Poeciliidae). *Aquat. Conserv. Mar. Freshw.*, doi:10.1002/aqc.2640 (in press).

51 Horstkotte, J. & Strecker, U. Trophic differentiation in the phylogenetically young *Cyprinodon* species flock (Cyprinodontidae, Teleostei) from Laguna Chichancanab (Mexico). *Biol. J. Linn. Soc.* **85**, 125-134 (2005).

52 Rakocinski, C. F. & Greenfield, D. W. Diet comparisons between savannah reed-pond *Gambusia sexradiata* and *Gambusia puncticulata yucatana* (deep-bodied form) (Pisces: Poeciliidae) on two dates during the dry season. *Stud. Neotrop. Fauna Environ.* **20**, 157-165 (1985).

53 Greenfield, D. W., Greenfield, T. A. & Brinton, S. L. Spatial and trophic interactions between *Gambusia sexradiata* and *Gambusia puncticulata yucatana* (Pisces: Poeciliidae) in Belize, Central America. *Copeia* **1983**, 598-607 (1983).

54 Greenfield, D. W. Review of the *Gambusia yucatana* complex (Pisces: Poeciliidae) of Mexico and Central America. *Copeia* **1985**, 368-378 (1985).

55 Greenfield, D. W., Greenfield, T. A. & Wildrick, D. M. The taxonomy and distribution of the species of *Gambusia* (Pisces: Poeciliidae) in Belize, Central America. *Copeia* **1982**, 128-147 (1982).

56 Miller, R., Minckley, W. & Norris, S. Freshwater fishes of Mexico. *University of Chicago, Chicago* (2005).

57 Carter, H. J. Aspects of the physiological ecology of species of *Gambusia* from Belize, Central America. *Copeia* **1981**, 694-700 (1981).

58 Meyer, A., Salzburger, W. & Schartl, M. Hybrid origin of a swordtail species (Teleostei: *Xiphophorus clemenciae*) driven by sexual selection. *Mol. Ecol.* **15**, 721-730 (2006).

59 Culumber, Z. *et al.* Replicated hybrid zones of *Xiphophorus* swordtails along an elevational gradient. *Mol. Ecol.* **20**, 342-356 (2011).

60 Jansen, M., Plath, M., Brusquetti, F. & Ryan, M. J. Asymmetric frequency shift in advertisement calls of sympatric frogs. *Amphibia-Reptilia*, doi:10.1163/15685381-00003038 (2016).

61 Dayan, T. & Simberloff, D. Ecological and community-wide character displacement: the next generation. *Ecol. Lett.* **8**, 875-894 (2005).

62 Stuart, Y. E. *et al.* Rapid evolution of a native species following invasion by a congener. *Science* **346**, 463-466 (2014).

63 Fink, W. L. A revision of the *Gambusia puncticulata* complex (Pisces: Poeciliidae). *Publ. Gulf Coast Res. Lab. Mus* **2**, 11-46 (1971).

64 Evanno, G., Regnaut, S. & Goudet, J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**, 2611-2620 (2005).

65 Reznick, D. N., Mateos, M. & Springer, M. S. Independent origins and rapid evolution of the placenta in the fish genus *Poeciliopsis*. *Science* **298**, 1018-1020 (2002).

66 Riesch, R., Martin, R. A. & Langerhans, R. B. Predation's role in life-history evolution of a livebearing fish and a test of the Trexler-DeAngelis model of maternal provisioning. *Am. Nat.* **181**, 78-93 (2013).
67 Riesch, R., Plath, M., Schlupp, I., Tobler, M. & Brian Langerhans, R. Colonisation of toxic environments drives predictable life-history evolution in livebearing fishes (Poeciliidae). *Ecol. Lett.* **17**, 65-71 (2014).
68 Palacios, M. *et al.* The rediscovery of a long described species reveals additional complexity in speciation patterns of poeciliid fishes in sulfide springs. *PLoS One* **8**, e71069 (2013).
69 Gillespie, R. G. *et al.* Long-distance dispersal: a framework for hypothesis testing. *Trends Ecol. Evol.* **27**, 47-56 (2012).
70 Rodríguez, G. Centers of radiation of freshwater crabs in the Neotropics. *Crustac. Issues* **4**, 51-67 (1986).
71 Coyne, J. A. & Orr, H. A. *Speciation*. Vol. 37 (Sinauer Associates Sunderland, MA, 2004).
72 Pyke, G. H. A Review of the Biology of *Gambusia affinis* and *G.holbrooki*. *Rev. Fish Biol. Fish.* **15**, 339-365, doi:10.1007/s11160-006-6394-x (2005).
73 Black, D. A. & Howell, W. M. The North American mosquitofish, *Gambusia affinis*: a unique case in sex chromosome evolution. *Copeia* **1979**, 509-513 (1979).
74 Langerhans, R. B. Trade-off between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*. *J. Evol. Biol.* **22**, 1057-1075 (2009).
75 Krumholz, L. A. Reproduction in the Western Mosquitofish, *Gambusia affinis affinis* (Baird & Girard) and its use in mosquito control. *Ecol. Monogr.* **18**, 1-43, doi:10.2307/1948627 (1948).
76 Plath, M., Parzefall, J. & Schlupp, I. The role of sexual harassment in cave and surface dwelling populations of the Atlantic molly, *Poecilia mexicana* (Poeciliidae, Teleostei). *Behav. Ecol. Sociobiol.* **54**, 303-309 (2003).
77 Seghers, B. H. *An analysis of geographic variation in the antipredator adaptations of the guppy, Poecilia reticulata*, Ph.D. Thesis, University of British Columbia, (1973).
78 Martin, R. A., Riesch, R., Heinen-Kay, J. L. & Langerhans, R. B. Evolution of male coloration during a post-Pleistocene radiation of Bahamas mosquitofish (*Gambusia hubbsi*). *Evolution* **68**, 397-411 (2014).
79 Langerhans, R. & Makowicz, A. Shared and unique features of morphological differentiation between predator regimes in *Gambusia caymanensis*. *J. Evol. Biol.* **22**, 2231-2242 (2009).
80 Johnson, J. B. & Belk, M. C. Predation environment predicts divergent life-history phenotypes among populations of the livebearing fish *Brachyrhaphis rhabdophora*. *Oecologia* **126**, 142-149 (2001).
81 Stearns, S. C. *The evolution of life histories*. Vol. 249 (Oxford University Press Oxford, 1992).
82 Ghalambor, C. K., Reznick, D. N. & Walker, J. A. Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). *The American Naturalist* **164**, 38-50 (2004).
83 Belk, M. C. & Tuckfield, R. C. Changing costs of reproduction: age-based differences in reproductive allocation and escape performance in a livebearing fish. *Oikos* **119**, 163-169 (2010).
84 Wesner, J. S., Billman, E. J., Meier, A. & Belk, M. C. Morphological convergence during pregnancy among predator and nonpredator populations of the livebearing fish *Brachyrhaphis rhabdophora* (Teleostei: Poeciliidae). *Biol. J. Linn. Soc.* **104**, 386-392 (2011).
85 Bisazza, A. & Marin, G. Sexual selection and sexual size dimorphism in the eastern mosquitofish *Gambusia holbrooki* (Pisces Poeciliidae). *Ethol. Ecol. Evol.* **7**, 169-183 (1995).
86 MacLaren, R. D. & Fontaine, A. Female preference for male lateral projection area in *Poecilia reticulata*. *Environ. Biol. Fishes* **93**, 105-119 (2012).
87 Riesch, R., Plath, M. & Schlupp, I. Toxic hydrogen sulfide and dark caves: life-history adaptations in a livebearing fish (*Poecilia mexicana*, Poeciliidae). *Ecology* **91**, 1494-1505, doi:10.1890/09-1008.1 (2010).
88 Riesch, R., Plath, M. & Schlupp, I. Toxic hydrogen sulphide and dark caves: pronounced male life-history divergence among locally adapted *Poecilia mexicana* (Poeciliidae). *J. Evol. Biol.* **24**, 596-606, doi:10.1111/j.1420-9101.2010.02194.x (2011).
89 Riesch, R., Reznick, D. N., Plath, M. & Schlupp, I. Sex-specific local life-history adaptation in surface- and cave-dwelling Atlantic mollies (*Poecilia mexicana*). *Scientific Reports* **6**, 22968 (2016).
90 Farr, J. in *Ecology & Evolution of Livebearing Fishes (Poeciliidae)* (eds G K Meffe & F F Jr Snelson) 91-123 (Prentice Hall, 1989).
91 Rios-Cardenas, O. & Morris, M. R. in *Ecology and Evolution of Poeciliid Fishes* (eds J P Evans, A Pilastro, & I Schlupp) 188-196 (University of Chicago Press, 2011).
92 Jennions, M. D. & Kelly, C. D. Geographical variation in male genitalia in *Brachyrhaphis episcopi* (Poeciliidae): is it sexually or naturally selected? *Oikos* **97**, 79-86 (2002).
93 Endler, J. A. & Houde, A. E. Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* **49**, 456-468 (1995).
94 Lindholm, A. K. *et al.* Causes of male sexual trait divergence in introduced populations of guppies. *J. Evol. Biol.* **27**, 437-448 (2014).
95 Plath, M., Heubel, K. U., De León, F. J. G. & Schlupp, I. Cave molly females (*Poecilia mexicana*, Poeciliidae, Teleostei) like well-fed males. *Behav. Ecol. Sociobiol.* **58**, 144-151 (2005).
96 Bunn, S. E. & Arthington, A. H. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environ. Manag.* **30**, 492-507 (2002).
97 Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. & Jarvis, A. Very high resolution interpolated climate surfaces for global land areas. *International journal of climatology* **25**, 1965-1978 (2005).
98 Kocher, T. D. *et al.* Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences* **86**, 6196-6200 (1989).

99 Ronquist, F. *et al.* MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**, 539-542 (2012).
100 Rambaut, A., Drummond, A. & Suchard, M. (2013).
101 Drummond, A. J., Suchard, M. A., Xie, D. & Rambaut, A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **29**, 1969-1973 (2012).
102 Purcell, K. M., Lance, S. L., Jones, K. L. & Stockwell, C. A. Ten novel microsatellite markers for the western mosquitofish *Gambusia affinis*. *Conservation Genetics Resources* **3**, 361-363, doi:10.1007/s12686-010-9362-7 (2011).
103 Spencer, C. C. *et al.* Polymorphic microsatellite markers in the western mosquitofish, *Gambusia affinis*. *Mol. Ecol.* **8**, 157-158 (1999).
104 Pritchard, J. K., Stephens, M. & Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **155**, 945-959 (2000).
105 Earl, D. A. & von Holdt, B. M. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation genetics resources* **4**, 359-361 (2012).
106 Anderson, E. & Thompson, E. A model-based method for identifying species hybrids using multilocus genetic data. *Genetics* **160**, 1217-1229 (2002).
107 Excoffier, L. & Lischer, H. E. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular ecology resources* **10**, 564-567 (2010).
108 Goudet, J. FSTAT (version 1.2): a computer program to calculate F-statistics. *J. Hered.* **86**, 485-486 (1995).
109 Ter Braak, C. J. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology* **67**, 1167-1179 (1986).
110 XLSTAT. Addinsoft, http://www.xlstat.com. (2016).
111 Rohlf, F. J. tpsDig2 version 2.16. Computer program and documentation distributed through http://life.bio.sunysb.edu/morph/. . (2010).
112 Riesch, R. *et al.* Extremophile Poeciliidae: multivariate insights into the complexity of speciation along replicated ecological gradients. (submitted).
113 Rohlf, F. tpsUtil version 1.58. Computer program and documentation distributed through http://life.bio.sunysb.edu/morph/. (2013).
114 Klingenberg, C. P. MorphoJ: an integrated software package for geometric morphometrics. *Molecular ecology resources* **11**, 353-357 (2011).
115 Zelditch, M. L., Swiderski, D. L. & Sheets, H. D. *Geometric morphometrics for biologists: a primer*. (Academic Press, 2012).
116 Riesch, R., Schlupp, I., Langerhans, R. B. & Plath, M. Shared and unique patterns of embryo development in extremophile poeciliids. *PLoS One* **6**, e27377 (2011).
117 Wourms, J. P. Viviparity: the maternal-fetal relationship in fishes. *Am. Zool.* **21**, 473-515 (1981).
118 Hijmans, R., Guarino, L. & Mathur, P. DIVA-GIS version 7.5. Published by the authors. Software and shapefiles available at http://www.diva-gis.org (2004).

Supplementary Information

Shared and unique patterns of phenotypic diversification along a stream gradient in two congeneric species

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Environmental conditions

Table S1. Spatial variation of environmental factors among the sample sites.

Site ID	pH	Dissolved oxygen [mg/L]	Dissolved oxygen [%]	Conductivity [µs/cm]	Dissolved salt concentration [ppt]	Mean annual temperature	Predation risk	water depth [m]
1	8.25	7.79	112.5	8700	7	26.4	high	> 5
2	7.4	1.19	16.5	540	0	26.2	medium	1-3
3	9.2	10.67	161	209	0	27.0	medium	> 5
4	8.05	7.64	112.2	482	0	26.9	medium	3-5
5	6.92	0.59	7.8	757	0	26.9	low	< 1
6	7.34	3.76	52.4	175	0	26.4	low	< 1
7	7.22	0.41	5.3	350	0	26.4	low	1-3
8	6.61	0.3	3.7	256	0	26.4	low	1-3
9	7.94	8.24	115.2	441	0	26.1	medium	3-5
10	5.44	3.7	47.6	72	0	25.7	low	< 1

Table S2: Summary of the fish communities in the different habitats.

Site ID	Site name	Predation risk category	Accompanying fauna
1	Laguna Mecoacán	High	*Atherinella alvarezi* (Atherinopsidae) *Centropomus undecimalis* (Centropomidae) *Anchoa mitchilli* (Engraulidae) *Dormitator maculatus* (Eleotridae) *Belonesox belizanus* (Poeciliidae) *Poecilia mexicana* (Poeciliidae) *Poecilia sphenops* (Poeciliidae) *Microphis brachyurus* (Syngnathidae) Anguillidae indet., larvae Cichlidae indet. Goobidae indet.
2	Simon Sarlat	Medium	*Dormitator maculatus* (Eleotridae) *Belonesox belizanus* (Poeciliidae) *Phallichthys fairweatheri* (Poeciliidae) Cichlidae indet.

3	Laguna de Las Ilusiones	Medium	*Atherinella alvarezi* (Atherinopsidae) *Astyanax aeneus* (Characidae) *Thorichthys passionis* (Cichlidae) *Dorosoma* sp. (Clupeidae) *Pterygoplichthys* sp. (Loricariidae) *Belonesox belizanus* (Poeciliidae) *Poecilia mexicana* (Poeciliidae) Cichlidae indet.
4	Laguna Ranchería 1ra Lázaro Cárdenas	Medium	*Atherinella alvarezi* (Atherinopsidae) *Pterygoplichthys* sp. (Loricariidae) *Belonesox belizanus* (Poeciliidae) *Xiphophorus maculates* (Poeciliidae) *Cynodonichthys tenuis* (Rivulidae)
5	Campus DACBiol-UJAT	Low	*Pseudoxiphophorus bimaculatus* (Poeciliidae) *Xiphophorus maculatus* (Poeciliidae) *Cynodonichthys tenuis* (Rivulidae)
6	Ismate Chilapilla	Low	*Rhamdia* cf. *guatemalensis* (Heptapteridae) *Xiphophorus maculatus* (Poeciliidae) *Cynodonichthys tenuis* (Rivulidae)
7	San Antonio I	Low	*Astyanax aeneus* (Characidae) *Poecilia petenensis* (Poeciliidae) *Xiphophorus maculatus* (Poeciliidae) *Cynodonichthys tenuis* (Rivulidae)
8	San Antonio II	Low	*Astyanax aeneus* (Characidae) *Poecilia petenensis* (Poeciliidae) *Xiphophorus maculatus* (Poeciliidae) *Cynodonichthys tenuis* (Rivulidae)
9	Laguna Canto Rodado	Medium	*Pterygoplichthys* sp. (Loricariidae) *Belonesox belizanus* (Poeciliidae) Cichlidae indet.
10	Pond near Teapa	low	*Pseudoxiphophorus bimaculatus* (Poeciliidae) *Xiphophorus maculatus* (Poeciliidae) *Cynodonichthys tenuis* (Rivulidae)

Population genetic analysis

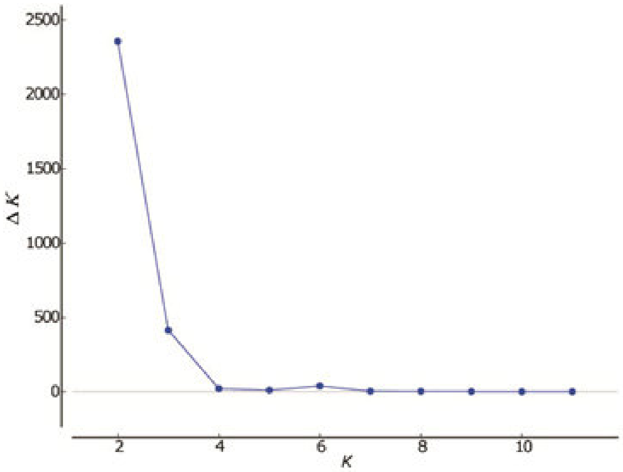

Figure S1: Bayesian inference of the number of genetically distinct clusters (*K*) among the 10 sampled populations using Δ*K* (Evanno et al. 2005).

Table S3: Genetic differentiation (estimated by pairwise F_{ST}) among study sites. To calculate F_{ST} values we removed individuals which did not assign to the major cluster from each site, except study site 5 which was divided in a *G. yucatana* (5_*yuc*.) and a *G. sexradiata* (5_*sex*.) group.

	1	**2**	**3**	**4**	**5_*yuc*.**	**5_*sex*.**	**6**	**7**	**8**	**9**	**10**
1	0										
2	**0.337**	0									
3	**0.373**	0.032	0								
4	**0.372**	0.036	0.006	0							
5_*yuc*.	0.023	**0.365**	**0.403**	**0.396**	0						
5_*sex*.	**0.430**	**0.069**	0.032	0.032	**0.470**	0					
6	**0.354**	0.008	**0.066**	**0.066**	**0.381**	**0.082**	0				
7	**0.335**	0.017	0.014	0.035	**0.361**	0.050	0.043	0			
8	0.034	**0.351**	**0.388**	**0.386**	0.044	**0.450**	**0.368**	**0.346**	0		
9	**0.386**	0.049	0.041	0.043	**0.409**	**0.088**	**0.069**	0.039	**0.392**	0	
10	**0.461**	**0.214**	**0.223**	**0.220**	**0.488**	**0.252**	**0.223**	**0.210**	**0.484**	**0.271**	0

Table S4: For each population and locus, observed (*H*o) and expected (*H*E) heterozygosities as well as allelic richness (*A*) are given. Zero values indicate that the locus is monomorphic in this population. Ranges of allele sizes are given for the entire data set.

Locus	**Number of alleles**	**Range of allele size**	**Test**	**1** *n* = 24	**2** *n* = 23	**3** *n* = 21	**4** *n* = 24	**5_*yuc*** *n* = 19	**5_*sex*** *n* = 5	**6** *n* = 24	**7** *n* = 24	**8** *n* = 23	**9** *n* = 24	**10** *n* = 24	**Mean across populations**
Gaaf10	17	218-282	H_O	0.22	0.67	0.53	0.65	0.10	0.40	0.55	0.43	0.14	0.57	0.39	0.43
			H_E	0.34	0.80	0.83	0.73	0.81	0.64	0.86	0.81	0.82	0.72	0.39	0.74
			A	4.34	4.67	4.82	4.15	4.57	3.58	5.01	4.51	4.45	3.69	2.17	4.18
Gaaf11	23	87-187	H_O	0.18	0.80	0.71	0.58	0.38	0.80	0.91	0.88	0.35	0.35	0.38	0.57
			H_E	0.17	0.87	0.76	0.65	0.33	0.84	0.90	0.83	0.30	0.47	0.51	0.60
			A	1.57	5.51	4.57	3.97	2.18	5.18	5.99	5.06	1.94	2.78	2.95	3.79
Gaaf13	49	111-327	H_O	0.17	0.82	0.84	0.87	0.06	0.40	0.47	0.75	0.00	0.88	0.48	0.52
			H_E	0.17	0.97	0.97	0.97	0.06	0.82	0.96	0.96	0.00	0.94	0.45	0.66
			A	1.70	7.10	7.11	7.12	1.25	4.56	7.04	7.07	1.00	6.56	2.86	4.85
Gafu3	39	145-285	H_O	0.75	0.74	0.89	0.83	0.82	0.80	0.83	0.88	0.74	1.00	0.67	0.81
			H_E	0.89	0.95	0.96	0.93	0.89	0.80	0.93	0.95	0.89	0.94	0.79	0.90
			A	5.52	6.78	6.96	6.31	5.55	4.40	6.51	6.85	5.64	6.57	4.20	5.94
Gaaf7	6	125-141	H_O	0.22	0.05	0.10	0.00	0.28	0.00	0.04	0.21	0.52	0.04	0.00	0.13
			H_E	0.34	0.05	0.10	0.00	0.25	0.00	0.13	0.20	0.52	0.04	0.00	0.15
			A	2.20	1.20	1.40	1.00	1.88	1.00	1.50	1.74	2.60	1.17	1.00	1.52
Gaaf9	2	224-228	H_O	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
			H_E	0.09	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
			A	1.32	1.00	1.00	1.33	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.06
Gaaf15	7	130-154	H_O	0.39	0.50	0.45	0.45	0.50	0.80	0.43	0.42	0.45	0.63	0.30	0.48
			H_E	0.64	0.59	0.52	0.60	0.61	0.69	0.42	0.56	0.46	0.64	0.26	0.54
			A	3.47	3.00	2.19	2.78	3.48	2.98	2.30	2.46	2.80	2.78	1.76	2.73
Gaaf16	14	207-279	H_O	0.62	0.95	0.60	0.83	0.37	0.80	0.91	0.67	0.67	0.74	0.35	0.68
			H_E	0.59	0.83	0.82	0.85	0.47	0.80	0.85	0.68	0.75	0.65	0.49	0.71
			A	3.00	4.65	4.71	5.00	2.64	4.40	5.14	3.62	3.87	3.56	2.83	3.95
Gaaf22	41	211-388	H_O	1.00	1.00	0.80	0.91	0.74	0.40	0.96	0.91	0.95	1.00	0.50	0.83
			H_E	0.91	0.96	0.94	0.94	0.80	0.67	0.94	0.95	0.89	0.94	0.65	0.87
			A	5.97	6.96	6.62	6.50	4.88	4.20	6.49	6.72	5.78	6.64	3.54	5.84
Gafu2	24	110-186	H_O	0.17	0.86	0.50	0.96	0.16	0.60	0.83	0.67	0.18	0.83	0.35	0.56
			H_E	0.17	0.91	0.79	0.87	0.20	0.78	0.91	0.85	0.22	0.86	0.45	0.64
			A	1.62	6.05	4.64	5.40	1.84	3.78	5.92	4.96	1.91	4.98	1.97	3.92
Gafu6	15	137-205	H_O	0.13	0.50	0.05	0.17	0.16	0.00	0.48	0.13	0.20	0.43	0.45	0.25
			H_E	0.79	0.75	0.14	0.27	0.60	0.00	0.79	0.49	0.70	0.60	0.61	0.52
			A	4.36	4.03	1.50	1.98	3.27	1.00	4.25	2.63	3.46	3.24	2.87	2.96
Gafu1	8	103-119	H_O	0.09	0.18	0.05	0.00	0.00	0.00	0.30	0.00	0.04	0.08	0.00	0.07
			H_E	0.20	0.62	0.49	0.39	0.11	0.36	0.62	0.49	0.04	0.54	0.31	0.38
			A	1.79	2.71	2.16	1.93	1.42	1.98	2.74	2.28	1.17	2.30	2.03	2.05
Gafu4	17	173-199	H_O	0.61	0.14	0.43	0.30	0.79	0.50	0.25	0.17	0.65	0.00	0.42	0.39
			H_E	0.77	0.13	0.36	0.37	0.80	0.43	0.23	0.41	0.83	0.00	0.51	0.44
			A	4.32	1.52	2.15	2.29	4.26	2.00	1.78	2.33	4.62	1.00	2.16	2.58
Gafu7	34	155-237	H_O	0.83	0.82	0.81	0.87	0.50	1.00	0.75	0.67	0.74	0.48	0.25	0.70
			H_E	0.87	0.94	0.92	0.94	0.76	0.93	0.91	0.94	0.83	0.87	0.45	0.85
			A	5.27	6.57	6.31	6.45	3.84	6.00	5.98	6.52	4.67	5.51	2.65	5.43
Mf-13	17	165-191	H_O	0.29	0.73	0.62	0.67	0.26	0.50	0.79	0.75	0.18	0.67	0.58	0.55
			H_E	0.45	0.85	0.74	0.74	0.46	0.68	0.83	0.76	0.18	0.69	0.68	0.64
			A	2.76	5.00	3.84	3.74	2.91	3.00	4.72	4.01	1.73	3.57	3.28	3.50
Mean across loci			H_O	0.38	0.58	0.49	0.54	0.34	0.47	0.57	0.50	0.39	0.51	0.34	
			H_E	0.52	0.68	0.62	0.62	0.48	0.56	0.68	0.66	0.50	0.59	0.44	
			A	3.28	4.45	4.00	4.00	3.00	3.27	4.42	4.12	3.11	3.69	2.48	

Geometric morphometric analyses

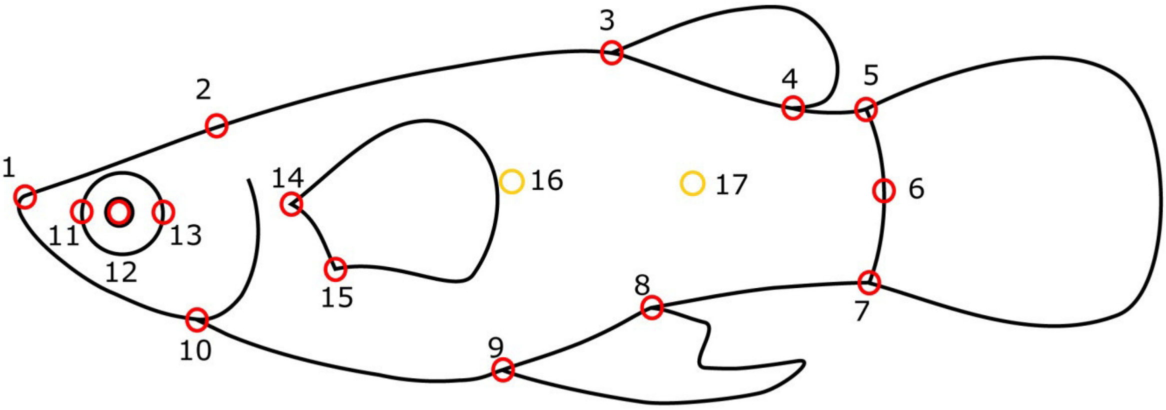

Figure S2: The 15 landmarks for the morphological analyses included (1) the tip of the upper jaw, (2) the posterodorsal tip of the supraoccipital crest, (3) the anterior and (4) posterior insertions of the dorsal fin, (5) the dorsal, (6) the central and (7) the ventral insertions of the caudal fin, (8) the posterior and (9) anterior junctions of the anal fin, (10) the bottom of the head where the operculum breaks away from the body outline, (11) the anterior, (12) the centre and the posterior (13) margin of the eye, as well as (14) the dorsal and (14) ventral insertions of the pectoral fin. Two additional temporary landmarks (16, 17) were placed at the lateral line and used to account for bending of specimens owing to preservation (but removed in the final analyses).

Patterns of phenotypic diversification

Table S5:

Results from ANCOVAs on variables assessed in morphometric GLM on males using ‘species’ as fixed factor, ‘centroid size’ and environmental variables as covariates.

Source	Dependent variable	d.f.	*F*	*p*	Relative variance explained (%)
Species	Male RW 1	1, 176	1.244	0.266	5.74
	Male RW 2	1, 176	0.192	0.662	0.89
	Male RW 3	1, 176	6.662	0.011	29.82
	Male RW 4	1, 176	0.297	0.586	1.38
	Male RW 5	1, 176	1.032	0.311	4.77
Centroid size	Male RW 1	1, 176	14.873	< 0.001	63.71
	Male RW 2	1, 176	0.674	0.413	3.12
	Male RW 3	1, 176	7.537	0.007	33.58
	Male RW 4	1, 176	24.525	< 0.001	100.00
	Male RW 5	1, 176	7.096	0.008	31.69
Environmental PC 1	Male RW 1	1, 176	6.165	0.014	27.67
	Male RW 2	1, 176	0.711	0.400	3.29
	Male RW 3	1, 176	6.039	0.015	27.12
	Male RW 4	1, 176	2.063	0.153	9.47
	Male RW 5	1, 176	1.699	0.194	7.82
Environmental PC 2	Male RW 1	1, 176	0.954	0.330	4.41
	Male RW 2	1, 176	0.761	0.384	3.52
	Male RW 3	1, 176	0.088	0.767	0.41
	Male RW 4	1, 176	3.302	0.071	15.06
	Male RW 5	1, 176	0.355	0.552	1.65
Environmental PC 3	Male RW 1	1, 176	4.267	0.040	19.36
	Male RW 2	1, 176	0.002	0.968	0.01
	Male RW 3	1, 176	1.714	0.192	7.89
	Male RW 4	1, 176	1.224	0.270	5.65
	Male RW 5	1, 176	1.084	0.299	5.01
Species × Centroid size	Male RW 1	1, 176	1.536	0.217	7.08
	Male RW 2	1, 176	0.017	0.897	0.08
	Male RW 3	1, 176	7.616	0.006	33.91
	Male RW 4	1, 176	0.528	0.468	2.45
	Male RW 5	1, 176	0.826	0.365	3.82
Species × Environmental PC 1	Male RW 1	1, 176	5.400	0.021	24.34
	Male RW 2	1, 176	0.608	0.437	2.81
	Male RW 3	1, 176	7.858	0.006	34.94
	Male RW 4	1, 176	1.464	0.228	6.75
	Male RW 5	1, 176	0.063	0.802	0.29
Species × Environmental PC 2	Male RW 1	1, 176	2.815	0.095	12.87
	Male RW 2	1, 176	0.237	0.627	1.10
	Male RW 3	1, 176	0.876	0.351	4.05
	Male RW 4	1, 176	2.613	0.108	11.96
	Male RW 5	1, 176	1.551	0.215	7.14
Species × Environmental PC 3	Male RW 1	1, 176	2.913	0.090	13.31
	Male RW 2	1, 176	5.267	0.023	23.76
	Male RW 3	1, 176	5.577	0.019	25.11
	Male RW 4	1, 176	2.849	0.093	13.02
	Male RW 5	1, 176	0.003	0.960	0.01

Table S6:

Results from ANCOVAs on variables assessed in morphometric GLM on females using ‘species’ as fixed factor, ‘centroid size’ and environmental variables as covariates.

Source	Dependent variable	d.f.	*F*	*p*	Relative variance explained (%)
Species	Female RW 1	1, 188	6.720	0.010	11.27
	Female RW 2	1, 188	3.078	0.081	5.26
	Female RW 3	1, 188	1.744	0.188	3.00
	Female RW 4	1, 188	2.367	0.126	4.06
	Female RW 5	1, 188	0.664	0.416	1.15
Centroid size	Female RW 1	1, 188	78.629	< 0.001	96.31
	Female RW 2	1, 188	6.093	0.014	10.25
	Female RW 3	1, 188	3.287	0.071	5.61
	Female RW 4	1, 188	0.105	0.747	0.18
	Female RW 5	1, 188	3.333	0.070	5.69
Environmental PC 1	Female RW 1	1, 188	2.439	0.120	4.18
	Female RW 2	1, 188	1.323	0.252	2.28
	Female RW 3	1, 188	0.428	0.514	0.74
	Female RW 4	1, 188	4.196	0.042	7.13
	Female RW 5	1, 188	0.741	0.391	1.28
Environmental PC 2	Female RW 1	1, 188	15.919	< 0.001	25.49
	Female RW 2	1, 188	5.644	0.019	9.52
	Female RW 3	1, 188	3.425	0.066	5.84
	Female RW 4	1, 188	0.530	0.468	0.92
	Female RW 5	1, 188	0.013	0.909	0.02
Environmental PC 3	Female RW 1	1, 188	15.903	< 0.001	25.47
	Female RW 2	1, 188	3.121	0.079	5.33
	Female RW 3	1, 188	0.165	0.685	0.29
	Female RW 4	1, 188	17.775	< 0.001	28.21
	Female RW 5	1, 188	2.271	0.133	3.90
Species × Centroid size	Female RW 1	1, 188	4.495	0.035	7.63
	Female RW 2	1, 188	3.593	0.060	6.12
	Female RW 3	1, 188	1.705	0.193	2.93
	Female RW 4	1, 188	3.084	0.081	5.27
	Female RW 5	1, 188	0.595	0.442	1.03
Species × Environmental PC 1	Female RW 1	1, 188	0.136	0.713	0.24
	Female RW 2	1, 188	10.500	0.001	17.28
	Female RW 3	1, 188	0.790	0.375	1.37
	Female RW 4	1, 188	0.170	0.681	0.29
	Female RW 5	1, 188	2.135	0.146	3.67
Species × Environmental PC 2	Female RW 1	1, 188	0.203	0.653	0.35
	Female RW 2	1, 188	0.537	0.464	0.93
	Female RW 3	1, 188	0.978	0.324	1.69
	Female RW 4	1, 188	0.073	0.787	0.13
	Female RW 5	1, 188	3.216	0.075	5.49
Species × Environmental PC 3	Female RW 1	1, 188	2.257	0.135	3.87
	Female RW 2	1, 188	3.891	0.050	6.62
	Female RW 3	1, 188	3.625	0.058	6.18
	Female RW 4	1, 188	2.785	0.097	4.77
	Female RW 5	1, 188	0.092	0.762	0.16

Table S7:

Results from ANCOVAs on male life history variables using ‘species’ as fixed factor, ‘SL’ and environmental variables as covariates. All values were z-transformed.

Source	Dependent variable	d.f.	*F*	*p*	Relative variance explained (%)
Species	Male lean weight	1, 163	0.928	0.337	0.65
	Male fat content	1, 163	0.904	0.343	0.64
	GSI	1, 163	9.943	0.002	6.62
SL	Male lean weight	1, 163	1078.215	< 0.001	100.00
	Male fat content	1, 163	0.428	0.514	0.30
	GSI	1, 163	5.412	0.021	3.70
Environmental PC 1	Male lean weight	1, 163	0.019	0.890	0.01
	Male fat content	1, 163	40.941	< 0.001	23.11
	GSI	1, 163	0.667	0.415	0.47
Environmental PC 2	Male lean weight	1, 163	0.176	0.676	0.12
	Male fat content	1, 163	1.222	0.271	0.86
	GSI	1, 163	2.912	0.090	2.02
Environmental PC 3	Male lean weight	1, 163	0.028	0.868	0.02
	Male fat content	1, 163	10.640	0.001	7.05
	GSI	1, 163	1.764	0.186	1.23
Species * SL	Male lean weight	1, 163	2.026	0.157	1.41
	Male fat content	1, 163	0.615	0.434	0.43
	GSI	1, 163	0.008	0.930	0.01
Species × Environmental PC 1	Male lean weight	1, 163	0.821	0.366	0.58
	Male fat content	1, 163	28.991	< 0.001	17.38
	GSI	1, 163	3.322	0.070	2.30
Species × Environmental PC 2	Male lean weight	1, 163	0.272	0.603	0.19
	Male fat content	1, 163	13.516	< 0.001	8.81
	GSI	1, 163	0.529	0.468	0.37
Species × Environmental PC 3	Male lean weight	1, 163	0.165	0.685	0.12
	Male fat content	1, 163	9.158	0.003	6.12
	GSI	1, 163	0.038	0.845	0.03

Table S8:

Results from ANCOVAs on female life history variables using ‘species’ as fixed factor, ‘SL’ and environmental variables as covariates. Embryo lean weight was corrected for embryo stage. All values were z-transformed.

Source	Dependent variable	d.f.	*F*	*p*	Relative variance explained (%)
Species	Female lean weight	1, 118	3.315	0.071	3.35
	Female fat content	1, 118	8.919	0.003	8.63
	Fecundity	1, 118	0.481	0.489	0.50
	Embryo fat content	1, 118	0.870	0.353	0.90
	Embryo lean weight	1, 118	0.622	0.432	0.64
	RA	1, 118	2.179	0.143	2.23
SL	Female lean weight	1, 118	518.554	< 0.001	100.00
	Female fat content	1, 118	7.152	0.009	7.01
	Fecundity	1, 118	22.452	< 0.001	19.62
	Embryo fat content	1, 118	0.010	0.920	0.01
	Embryo lean weight	1, 118	0.150	0.700	0.16
	RA	1, 118	0.820	0.367	0.85
Environmental PC 1	Female lean weight	1, 118	1.666	0.199	1.71
	Female fat content	1, 118	7.341	0.008	7.19
	Fecundity	1, 118	0.119	0.730	0.12
	Embryo fat content	1, 118	6.862	0.010	6.75
	Embryo lean weight	1, 118	0.445	0.506	0.46
	RA	1, 118	0.445	0.506	0.46
Environmental PC 2	Female lean weight	1, 118	0.000	0.990	0.00
	Female fat content	1, 118	23.701	< 0.001	20.53
	Fecundity	1, 118	21.828	< 0.001	19.16
	Embryo fat content	1, 118	0.357	0.551	0.37
	Embryo lean weight	1, 118	1.610	0.207	1.65
	RA	1, 118	15.448	0.000	14.21
Environmental PC 3	Female lean weight	1, 118	1.224	0.271	1.26
	Female fat content	1, 118	2.368	0.126	2.42
	Fecundity	1, 118	5.137	0.025	5.12
	Embryo fat content	1, 118	0.249	0.619	0.26
	Embryo lean weight	1, 118	0.062	0.803	0.06
	RA	1, 118	3.438	0.066	3.48
Species × SL	Female lean weight	1, 118	0.199	0.656	0.21
	Female fat content	1, 118	3.770	0.055	3.80
	Fecundity	1, 118	0.182	0.671	0.19
	Embryo fat content	1, 118	0.006	0.938	0.01
	Embryo lean weight	1, 118	0.289	0.592	0.30
	RA	1, 118	0.592	0.443	0.61
Species × Environmental PC 1	Female lean weight	1, 118	0.073	0.787	0.08
	Female fat content	1, 118	8.156	0.005	7.94
	Fecundity	1, 118	0.020	0.888	0.02
	Embryo fat content	1, 118	4.107	0.045	4.13
	Embryo lean weight	1, 118	0.548	0.461	0.57
	RA	1, 118	0.141	0.708	0.15
Species × Environmental PC 2	Female lean weight	1, 118	0.376	0.541	0.39
	Female fat content	1, 118	4.400	0.038	4.41
	Fecundity	1, 118	3.702	0.057	3.73
	Embryo fat content	1, 118	0.238	0.626	0.25
	Embryo lean weight	1, 118	1.007	0.318	1.04
	RA	1, 118	1.078	0.301	1.11
Species × Environmental PC 3	Female lean weight	1, 118	1.130	0.290	1.16
	Female fat content	1, 118	0.712	0.401	0.74
	Fecundity	1, 118	9.056	0.003	8.75
	Embryo fat content	1, 118	0.685	0.410	0.71
	Embryo lean weight	1, 118	0.374	0.542	0.39
	RA	1, 118	9.390	0.003	9.05

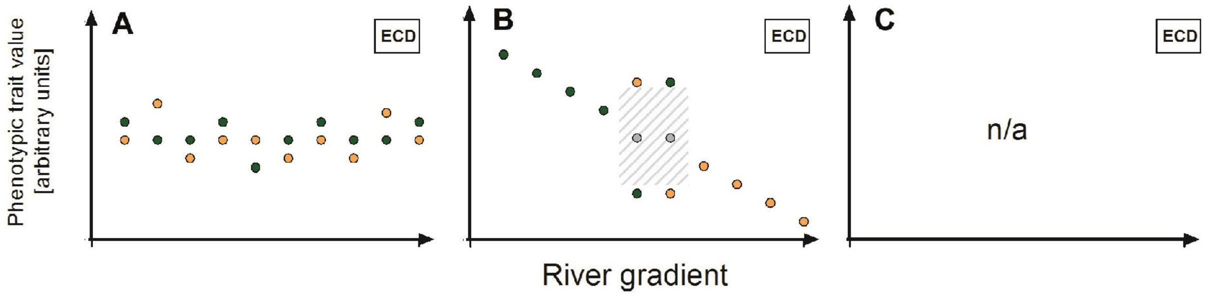

Figure S3: Appendix to Fig. 2 (see main text). Illustration of potential outcome of ecological character displacement, where both species would diverge in opposing directions, when (**A**) both species occur syntopically along the river gradient, or (**B**) in the overlap zone. Note that we depict non-directed ECD here, whereby both species variably unpredictably in both directions. If (**C**) certain components of the river gradient determine small-scale species distribution patterns and prevent syntopic occurrence of both species, ECD is not applicable.

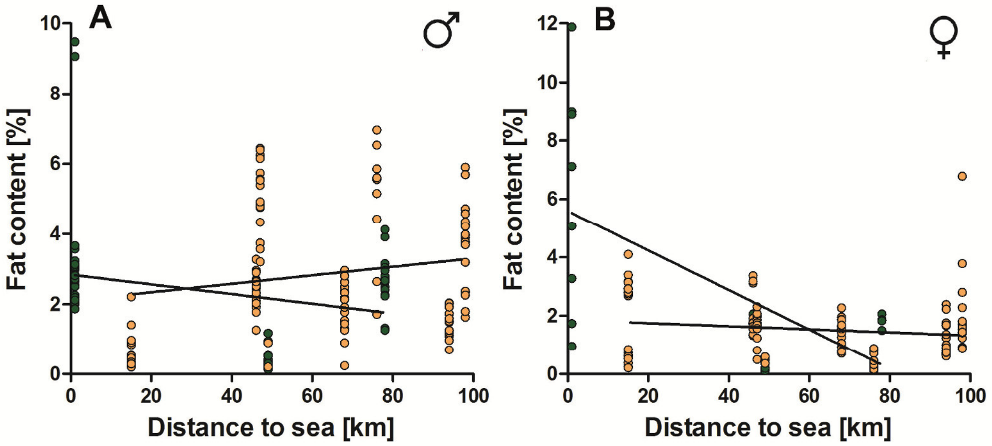

Figure S4: Relationship of fat content with distance to sea of (**A**) male and (**B**) female *Gambusia sexradiata* (orange) and *Gambusia yucatana* (green).

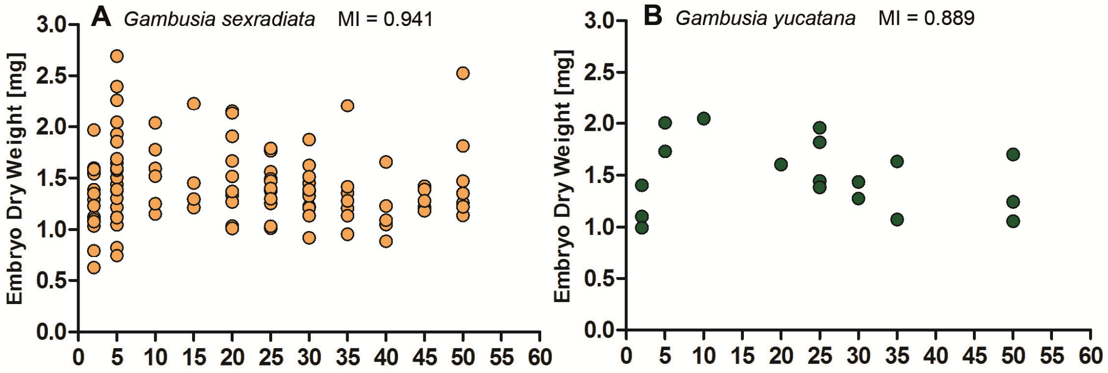

Figure S5: Scatterplots of mean embryo dry mass versus stage of development of (**A**) *Gambusia sexradiata* (orange) and (**B**) *Gambusia yucatana* (green). The matrotrophy index (MI) is the estimated dry mass at birth divided by dry mass at fertilization.

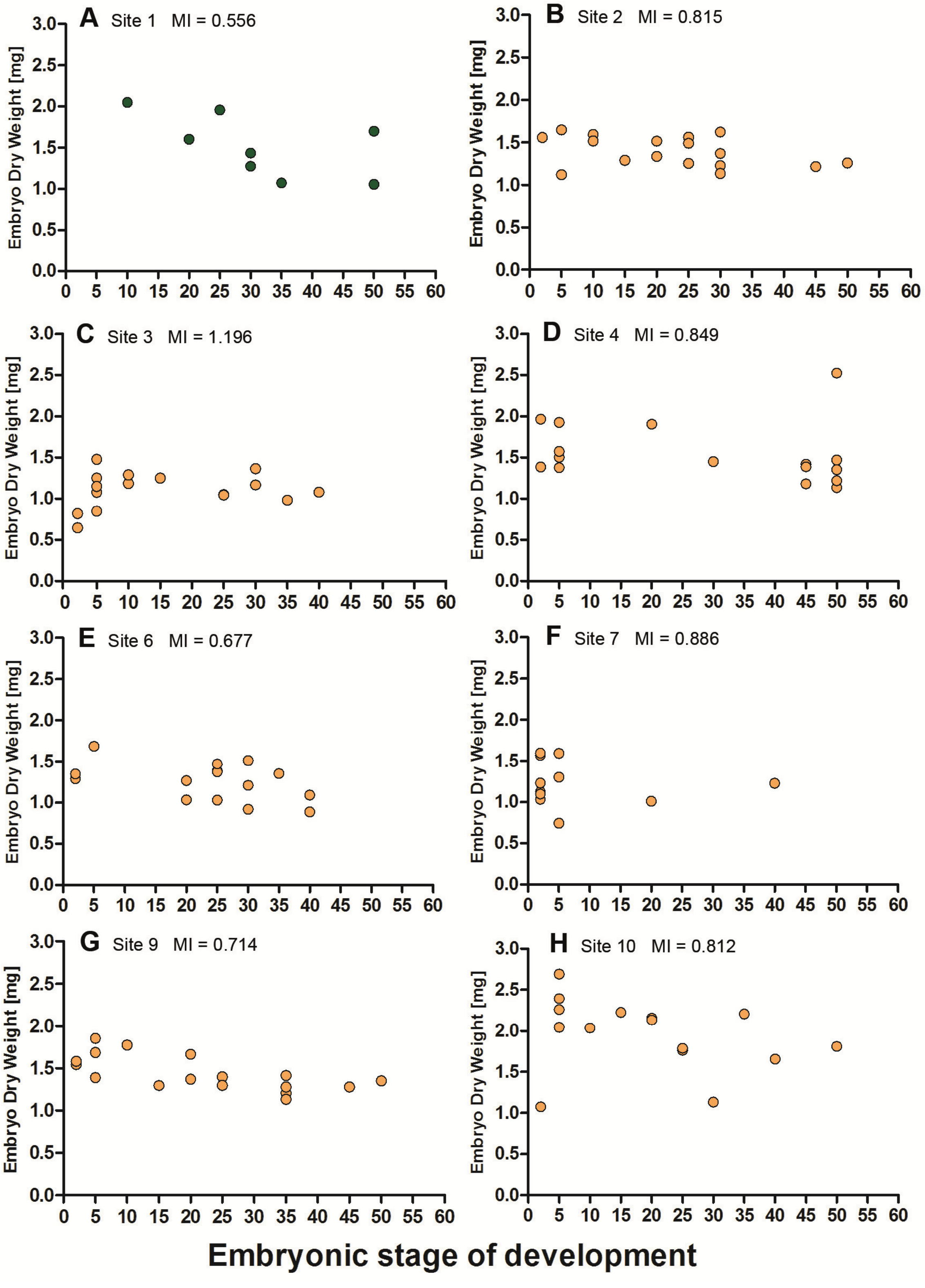

Figure S6: Scatterplots of mean embryo dry mass versus stage of development of *Gambusia sexradiata* (orange) and *Gambusia yucatana* (green) at each sampling site. The matrotrophy index (MI) is the estimated dry mass at birth divided by dry mass at fertilization. The MI was calculated for each species separately and only if $n \geq 8$ pregnant females were available; due to low sample sizes sites 5 and 8 are not shown and neither are data for *G. yucatana* from site 3.

3.2 The natural invasion of extreme habitats

Erklärung zu den Autorenanteilen

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Microhabitat use, population densities, and size distributions of sulfur cave-dwelling *Poecilia mexicana*

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ABSTRACT

The Cueva del Azufre in Tabasco, Mexico, is a nutrient-rich cave and its inhabitants need to cope with high levels of dissolved hydrogen sulfide and extreme hypoxia. One of the successful colonizers of this cave is the poeciliid fish *Poecilia mexicana*, which has received considerable attention as a model organism to examine evolutionary adaptations to extreme environmental conditions. Nonetheless, basic ecological data on the endemic cave molly population are still missing; here we aim to provide data on population densities, size class compositions and use of different microhabitats. We found high overall densities in the cave and highest densities at the middle part of the cave with more than 200 individuals per square meter. These sites have lower H_2S concentrations compared to the inner parts where most large sulfide sources are located, but they are annually exposed to a religious harvesting ceremony of local Zoque people called La Pesca. We found a marked shift in size/age compositions towards an overabundance of smaller, juvenile fish at those sites. We discuss these findings in relation to several environmental gradients within the cave (i.e., differences in toxicity and lighting conditions), but we also tentatively argue that the annual fish harvest during a religious ceremony (La Pesca) locally diminishes competition (and possibly, cannibalism by large adults), which is followed by a phase of overcompensation of fish densities.

Subjects Aquaculture, Fisheries and Fish Science, Biodiversity, Conservation Biology, Ecology, Zoology
Keywords Cave fish, Extremophile teleosts, Fisheries, Rotenone, Overcompensation

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INTRODUCTION

Cave fishes are emerging as model systems to study regressive evolutionary processes like the reduction of eyes and pigmentation that typically accompany the colonization of caves by previously surface-dwelling species (*Romero & Green, 2005*; *Jeffery, 2009*). For example, the characid *Astyanax mexicanus* is a model organism for EvoDevo studies of cave evolution (*Wilkens, 1988*; *Jeffery, 2001*; *Jeffery, 2009*). The cave form of a Mexican

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live-bearing fish, the so-called cave molly (*Poecilia mexicana*; *Gordon & Rosen, 1962*) has adapted to the vastly divergent ecological conditions inside a South Mexican sulfide cave, the Cueva del Azufre (also referred to as Cueva Villa Luz or Cueva de las Sardinas; *Parzefall, 1993*; *Parzefall, 2001*) Cave environments are usually energy limited compared to photosynthetically based epigean habitats (*Hüppop, 2005*) and fish densities reported for several different cave systems are low, with often less than one individual per m^2 (*Trajano, 2001*). In contrast, the Cueva del Azufre is a sulfidic, nutrient-rich habitat due to chemoautotrophic primary production through sulfide oxidizing bacteria that utilize the abundant hydrogen sulfide in the cave (*Hose & Pisarowicz, 1999*; *Colaço, Dehairs & Desbruyeres, 2002*; *Summers Engel, 2005*). Hydrogen sulfide is acutely toxic to most metazoans and leads to extreme hypoxia in the water (*Evans, 1967*; *Bagarinao, 1992*). Beside the Cueva del Azufre, few other sulfurous chemoautotrophic cave-ecosystem are described, such as Movile in Romania (*Sarbu, Kane & Kinkle, 1996*), Frasassi in Italy (*Flot, Wörheide & Dattagupta, 2010*) and Ayyalon in Israel (*Por, 2007*). All of these caves are inhabited by invertebrates—many of them endemic to the caves—that exploit this unusual food web. The Cueva del Azufre is the only known chemoautotrophic cave ecosystem which is inhabited by a vertebrate species (*Plath & Tobler, 2010*). However, due to its toxicity, hydrogen sulfide requires energetically costly behavioral (i.e., actively avoiding microhabitats with high levels of toxicity) and physiological adaptations (various forms of detoxification) by animals exposed to it (*Tobler et al., 2009*; *Riesch, Plath & Schlupp, 2010*). As a result of the simultaneous action of two strong selective forces (permanent darkness and hydrogen sulfide), locally adapted *P. mexicana* populations in the Cueva del Azufre system have received considerable scientific interest. The cave molly differs from its surface-dwelling ancestors in a distinct set of morphological, physiological, behavioral, and life-history traits; e.g., cave mollies have reduced eye size and reduced pigmentation, and females have a reduced fecundity combined with an increase in individual offspring size (*Parzefall, 2001*; *Tobler et al., 2008a*; *Riesch, Plath & Schlupp, 2010*; *Tobler et al., 2011b*). Although the cave molly has been established as a model to examine evolutionary adaptations to extreme environmental conditions, population densities have not yet been quantified in the Cueva del Azufre system, which makes interpretation of some of the ecological and evolutionary data difficult with regards to how they influence long-term stability of the systems and population dynamics.

The Cueva del Azufre drains into the El Azufre, a sulfidic surface creek, which eventually joins the Río Oxolotán. The Cueva del Azufre and El Azufre differ dramatically in the composition of fish communities compared to adjacent non-sulfidic surface habitats. *Poecilia mexicana* occurs as the single dominant species in both systems. Only one further fish species, the predatory cichlid '*Cichlasoma*' *salvini* occurs in the upper parts of the El Azufre, but only in small numbers. In downstream areas of the El Azufre where H_2S in not measurable, *Heterandria bimaculata* and *Xiphophorus hellerii* (Poeciliidae), *Astyanax aeneus* (Characidae) as well as '*Cichlasoma*' *salvini* and *Thorichtys helleri* (Cichlidae) occur (*Plath & Tobler, 2010*). In surrounding non-sulfidic surface habitats, diverse fish communities can be found, often dominated by cichlid and poeciliid species

(*Tobler et al., 2006*; *Plath & Tobler, 2010*). In the Clear Creek, a small stream that is directly connected to El Azufre, *H. bimaculata* occurs at a high abundance together with small numbers of *X. hellerii* and *P. mexicana.* A reduced species diversity and dominance of a few specialists have been documented from other caves (*Trajano, 2001*) and other sulfidic habitats (*Tobler et al., 2008c*).

Little is known about anthropogenic disturbances on the population ecology of *P. mexicana* inhabiting the Cueva del Azufre. Today, the system is increasingly influenced by a growing number of visitors which reach their peak during a traditional annual ceremony of the local indigenous Zoque people named 'La Pesca'. The Cueva del Azufre is sacred to the Zoque people, and once a year, on the first Sunday of Easter week, the Zoque enter the cave and introduce rotenone- and deguelin-containing barbasco roots (*Lonchocarpus* sp., Fabaceae) into the water. Rotenone is an inhibitor of the mitochondrial complex-I of the respiratory chain, causing reduced cellular respiration (*Singer & Ramsay, 1994*). Barbasco is introduced into the water in the middle portion of the cave, therefore only downstream cave chambers are affected (Fig. 1). Capture of poisoned cave fish is facilitated by the anesthetic effect of barbasco, as narcotized fish are flushed out of the cave, where they are harvested using wooden baskets, and afterwards cooked and eaten as part of a religious ceremony honoring the Rain Gods (*Tobler et al., 2011a*). The yield of the annual harvest is considered to be indicative of the quality of the subsequent crop harvest (*Hose & Pisarowicz, 1999*; *Tobler et al., 2011a*). Annual harvests amount to several thousand individuals, and the ceremony is likely to have taken place for centuries (*Hose & Pisarowicz, 1999*), so it is likely to act as a strong selective force on *P. mexicana* populations annually exposed to it.

In the present study, we provide first data of local densities within different chambers of the Cueva del Azufre and adjacent El Azufre and discuss our findings with regard to environmental conditions and annual harvesting of cave mollies. We used a non-invasive technique to repeatedly assess fish densities and size-distribution patterns (as a proxy for age) inside the Cueva del Azufre (up- and downstream of the barbasco-release site) and in the sulfidic creek leaving the cave (El Azufre). Moreover, given the high structural heterogeneity of the water course inside the Cueva del Azufre with respect to water depth and flow velocity (*Hose & Pisarowicz, 1999*), and because *Croft, Botham & Krause (2004)* reported on size-specific preferences regarding water depth in another poeciliid, the Trinidadian guppy (*P. reticulata*), we combined our assessment of fish densities with an investigation of microhabitat use by different size classes of cave mollies.

MATERIAL AND METHODS

Study system

Locally adapted subterranean populations of *P. mexicana* (Fig. 1A) can be found in at least two different limestone caves in the vicinity of the southern Mexican city of Tapijulapa (state of Tabasco, México): the Cueva del Azufre (*Gordon & Rosen, 1962*) and the much smaller, non-sulfidic Cueva Luna Azufre (*Tobler et al., 2008b*). The sulfidic Cueva del Azufre is about 500–600 m deep and divided into 13 different cave chambers (I-XIII), with the innermost chamber being XIII (*Gordon & Rosen, 1962*). Several springs discharge

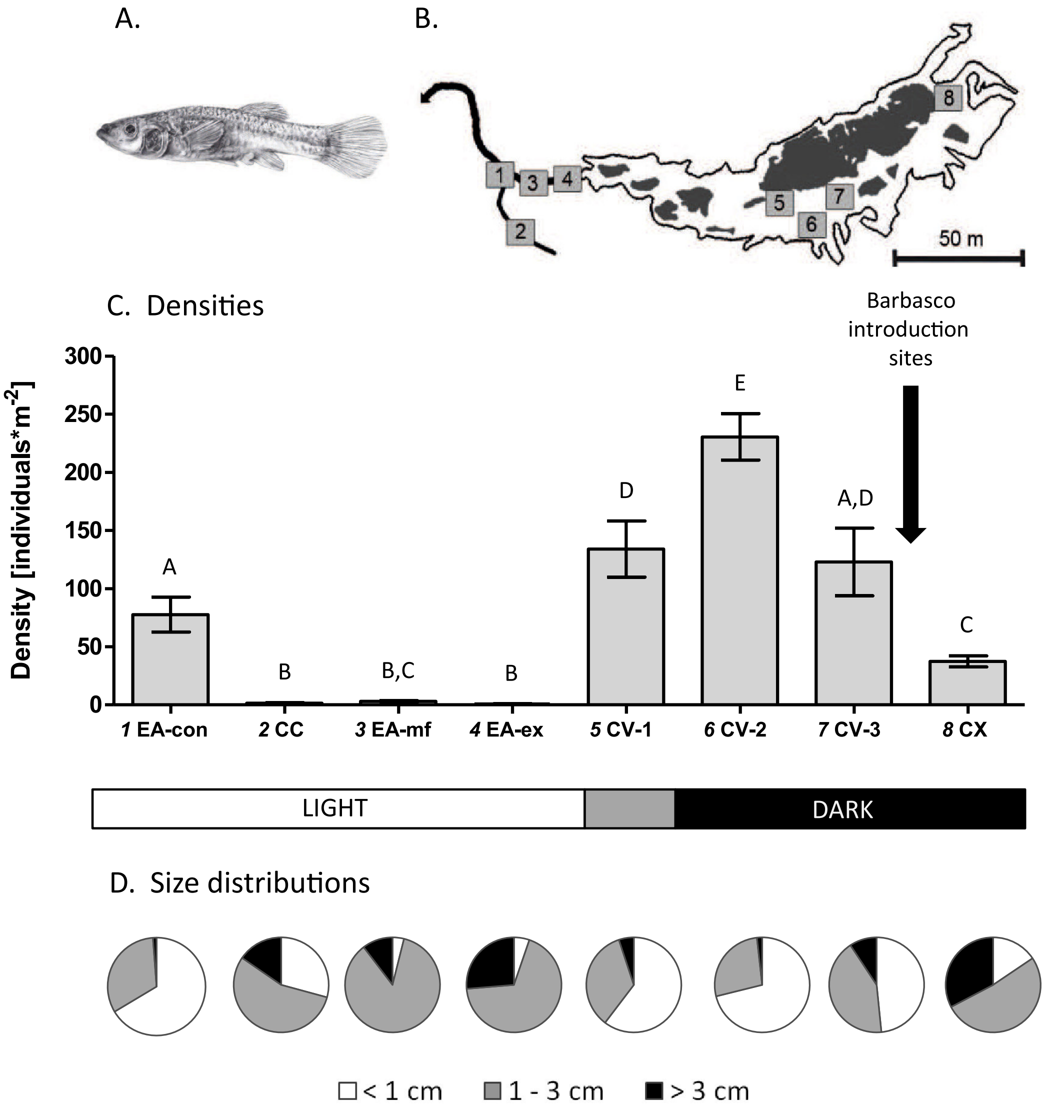

Figure 1 **Study system and population densities.** (A) Drawing of a female cave molly. (B) Map of the study area showing the different sampling sites (*numbers*) where white areas represent water within the cave (Cueva del Azufre) and dark areas indicate dry land and bedrock. *1* EA-con, *2* CC, *3* EA-mf, *4* EA-ex, *5* CV-1, *6* CV-2, *7* CV-3, *8* CX. With the exception of sampling site CV-1 all sampling sites inside the Cueva del Azufre are completely dark. Barbasco is released annually between chamber V (CV) and chamber X (CX). Three sampling sites inside chamber V were defined (CV-1 to CV-3). Downstream of the exit of the Cueva del Azufre (EA-ex), a rather homogeneous mudflat (EA-mf) was sampled. Further sampling sites were a small non-sulfidic creek (Clear Creek; CC) and its confluence with El Azufre (EA-con). (C) Mean (±SE) densities of mollies at each sampling site. Letters above the error bars signify statistically different groups (Fisher's LSD tests). (D) Size class compositions of mollies at the different sampling sites.

water with high concentrations of hydrogen sulfide (H_2S) into the creek draining the cave (*Tobler et al., 2006*). The cave creek forms a highly heterogeneous mosaic of shallow pools and backwaters that are partially divided by swift flowing riffle passages (*Gordon & Rosen, 1962*; *Hose & Pisarowicz, 1999*). While the front cave chambers receive some dim light through cracks in the ceiling, the inner parts of the cave are lightless. Consequently, (sub-)populations experience divergent selection regimes regarding light exposure, with populations from the innermost chambers living under perpetually dark conditions, whereas those from front chambers are exposed to dim sunlight through a number of cracks in the cave ceiling, so-called sky lights (*Fontanier & Tobler, 2009*).

Upon leaving the underground, the sulfidic creek draining the Cueva del Azufre is called 'El Azufre'. After meandering for approximately 1.5 km, it eventually drains into the Río Oxolotán, which is part of the Río Grijalva drainage system. Despite the gradual oxidation of H_2S to sulfate and elemental sulfur with increasing distance from the sulfide sources, which increases the water turbidity, and despite the influx of some smaller clear water affluents, El Azufre still has a remarkably high H_2S concentration of up to ∼40 μMol (*Tobler et al., 2006*; *Schlupp et al., 2013*).

Study sites and data collection

We compared the abundance and distribution of different size classes of *P. mexicana* among different sampling sites along a transect starting at chamber X in the Cueva del Azufre, and following the water flow outside the cave to the confluence of El Azufre with the first freshwater influx from the Clear Creek. This transect, therefore, covered sample sites located upstream of the release point of barbasco during La Pesca (sample point in chamber X) and sites directly downstream of the release point of barbasco that are strongly affected by the annual ceremony (three sites in chamber V; CV-1, CV-2, and CV-3; Figs. 2A and 2B). Surface sites of El Azufre are also annually exposed to barbasco due to downstream effects (EA-ex, EA-mf), even though concentrations are probably considerably lower than inside the cave (Table 1). Clear creek (CC) and its confluence with EA (EA-con; Fig. 2C), on the other hand, are not influenced by barbasco.

Field work was conducted in January 2010, i.e., about 9 month after the latest La Pesca ceremony in 2009 (L Arias-Rodriguez, pers. obs., 2009). At each of the eight sample sites, we defined sampling grids consisting of 50 × 50 cm quadrants with wooden sticks fixed in the ground (or stones where a grid angle fell on the shore). The number of quadrants was mostly 25 per sampling grid (i.e., 5 × 5 quadrants). In the narrow non-sulfidic surface creek (CC), however, the arrangement of quadrants was more longitudinal ($4 \times 7 = 28$ quadrants), and in chamber V, where a particularly high degree of structural heterogeneity precluded defining larger grids, one sampling site of 5 × 5 quadrants and two smaller ones (15 and 8 quadrants, respectively) were defined (Table 1). The grids reflected the natural variation in water depth, flow velocity, and substrate types, thus covering the range of different microhabitats inhabited by mollies (an example is shown in Fig. 3).

Daily measurements took place between 11:00 a.m. and 4:30 p.m. Each site was visited at least 5 times (mean ± SD = 6.25 ± 1.16) on consecutive days. During the counts, we slowly

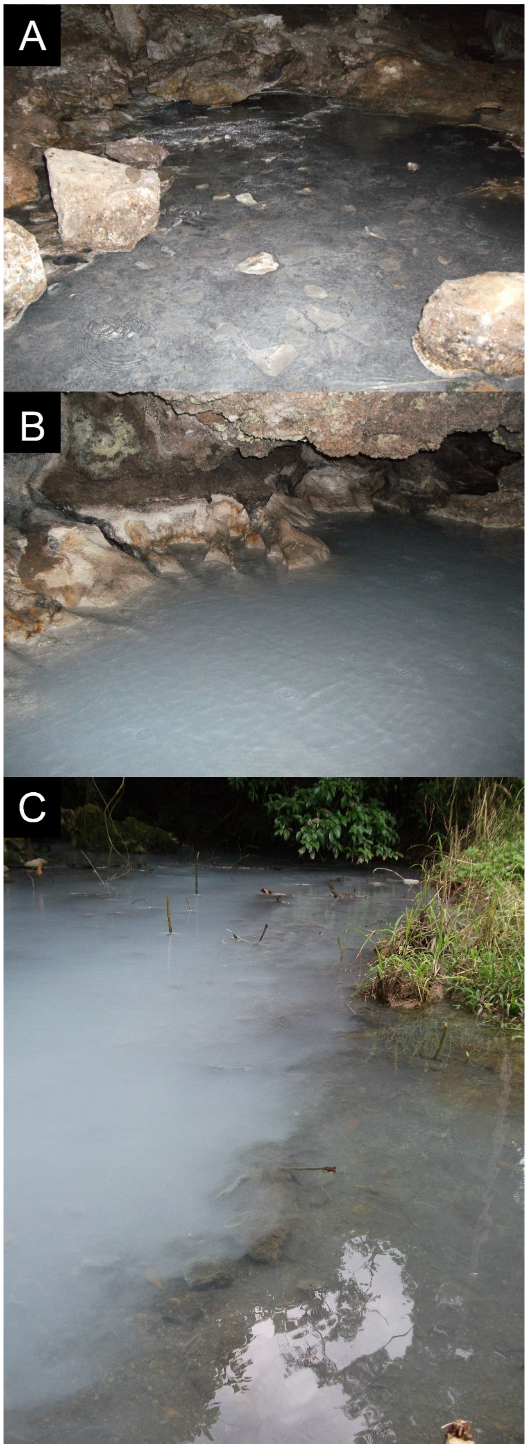

Figure 2 **Pictures of study sites.** (A) Cueva del Azufre chamber V (*6* CV-2) and (B) site *7* CV-3. (C) El Azufre confluence with Clear Creek (*1* EA-con).

Table 1 Sampling sites, their abbreviation code as used throughout the article, numbers of quadrants examined, and details regarding barbasco release.

Site code	Site	Number of quadrants	Affected by deposition of rotenone?	Approximate distance to upstream rotenone release site [m]
1 EA-con	El Azufre, confluence with clear Creek	25	No (only partly)	150
2 CC	Clear Creek	28	No	—
3 EA-mf	El Azufre, mudflat	25	Yes	120
4 EA-ex	El Azufre, exit of the Cueva del Azufre	25	Yes	110
5 CV-1	Cueva del Azufre, Chamber V, site 1	25	Yes	0
6 CV-2	Cueva del Azufre, Chamber V, site 2	15	Yes	0
7 CV-3	Cueva del Azufre, Chamber V, site 3	8	Yes	0
8 CX	Cueva del Azufre, Chamber X	25	No	—

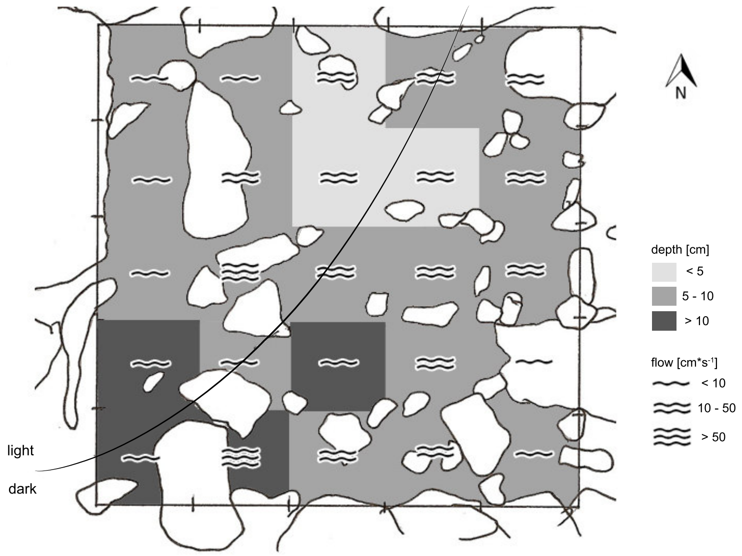

Figure 3 **Exemplary sketch of site 5 CV-1.** Showing the high degree of heterogeneity in flow regimes, water depth, substrate types, and (in this case) light regime.

approached a site while trying to avoid any movements that would cause the resident fish to flee, and we counted juveniles (<10 mm standard length (SL)), sub-adults (10–30 mm) and adults (>30 mm) in each quadrant. The observer was standing motionless at least 1.5 m downstream from the respective quadrant. Sizes were estimated qualitatively, aided by a prior training session that used wooden sticks of known size as a reference. Our definition of adults roughly followed *Riesch, Plath & Schlupp (2010)*, who determined the mean (±SD) standard length of reproducing females to be 31.44 ± 4.40 mm (El Azufre) and 36.97 ± 4.59 mm (Cueva del Azufre, chambers V and X).

Habitat parameters were assessed after the last fish count. For each quadrant, we determined water depth using a wooden ruler stuck vertically into the water at five random locations and calculating the mean from those five measurements. Flow velocity was measured on the water surface by scoring the time a small wooden stick of about 3 cm length took to float through the whole length of a quadrant (measurement was repeated five times per quadrant and averaged across the five observations per quadrant). Mean surface flow velocity was then expressed as $\mathrm{cm} * \mathrm{s}^{-1}$. Research followed the authorizations from CONAPESCA-DGOPA.09004.041111.3088 and Tacotalpa, Tabasco municipality.

Statistical analysis

Our first question was whether population densities differed among sampling sites. We used data for the different quadrants per site (averaged from the repeated measurements) and expressed density as total numbers of individuals per square meter. Density estimates per quadrant were used as the dependent variable in a univariate general linear model (GLM) with 'sampling site' as a fixed factor. We initially entered 'mean water depth' ($F_{1,162} = 0.12$, $P = 0.98$) and 'mean flow rate' ($F_{1,162} = 0.22$, $P = 0.64$) as covariates, but removed them from the final analysis since neither had a significant effect (also none of the interaction terms were significant). We used Fisher's LSD tests for pairwise *post hoc* comparisons among sites.

A further question was whether size-class compositions differed among sample sites and whether distribution patterns would be stable among repeated sampling days. We used the Bray-Curtis index (*Bray & Curtis, 1957*) to estimate pairwise similarities among each sampling point (calculated with the R-package 'ecodist 1.2.7'; *Goslee and Urban, 2007*; *R Development Core Team, 2008*), and used these for non-metric multidimensional scaling ('NMDS PROXSCAL' function in SPSS 21). To detect clusters, we used the 'two step cluster analysis' function based on Euclidian distances and the Bayesian information criterion. For visualization of size class compositions per site, we averaged repeated measurements of different size classes and used a mean value for each quadrant per site. By using these means we calculated the total average size class distribution per site.

Our first analysis detected pronounced variation in population densities and size distributions (see results) and thus, we decided to analyze potential effects of water depth and flow velocity (i.e., microhabitat choice) in a site-wise fashion. We focused on sites inside the Cueva del Azufre (CV-1, CV-3 and CX) where (*a*) fish densities were sufficiently high and (*b*) sufficient variability of those environmental variables was found to allow for a meaningful analysis. All other sites were excluded from this analysis. For each site, fish density per quadrant was entered as the dependent variable in repeated measures (rm) GLMs with 'size class' (three levels) as the repeated measurement. We grouped water depth (<5 cm, 5–10 cm, >10 cm) and flow velocity ($<10\ \mathrm{cm} * \mathrm{s}^{-1}$, $10–50\ \mathrm{cm} * \mathrm{s}^{-1}$, $>50\ \mathrm{cm} * \mathrm{s}^{-1}$) into three classes each and used these habitat parameters as fixed factors. However, neither the main factor 'flow velocity' nor any interaction term involving 'flow velocity' had a significant effect in any of the three site-specific models (CV-1: $F_{4,38} = 1.27$, $P = 0.30$; CV-3: $F_{2,4} = 3.28$, $P = 0.14$; CX: $F_{2,20} = 0.44$, $P = 0.65$), and so we subsequently removed this term from all models.

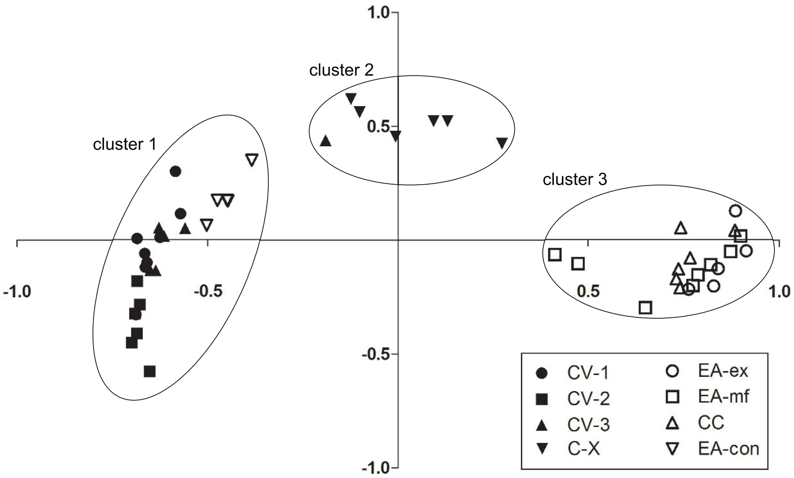

Figure 4 Differences in size-class compositions of *Poecilia mexicana* in the Cueva del Azufre system. Non-metric Multi-Dimensional Scaling (NMDS) plots based on Bray-Curtis similarities for each sampling site and day.

RESULTS

Local population densities

When comparing mean densities per quadrant across sites we detected a significant difference among sampling sites (GLM; $F_{7,164} = 32.49$, $P < 0.001$; Fig. 1B). *Post hoc* pairwise LSD tests found most pairwise comparisons to be statistically significant; qualitatively, densities increased from surface sites (mean ± SE across sites = 21.0 ± 5.0 individuals $* \text{m}^{-2}$) towards the cave (119.5 ± 12.7 individuals $* \text{m}^{-2}$). Also, sites downstream of the barbasco release-site (chamber V; 162.3 ± 16.1 individuals $* \text{m}^{-2}$) had considerably higher densities than the site in chamber X that lies upstream of the release-site (37.4 ± 4.8 individuals $* \text{m}^{-2}$).

Differences in size-class compositions

The NMDS based on Bray-Curtis similarities found data from repeated sampling days to cluster together, suggesting that the observed size-class compositions were stable over the period of this study (Fig. 4). There were three distinct clusters, and in only one case was a single sampling day of a given sampling site assigned to the 'wrong' cluster. The first cluster comprised the three sample sites in cave chamber V and EA-con. Samples had high overall densities and were composed mostly of small individuals. Cluster two comprised the rearmost cave site CX. Samples in this cluster were characterized by intermediate densities but a particularly high proportion of large individuals. Cluster three comprised all surface sites except EA-con and was characterized by overall low densities and mostly intermediate-sized fish (Fig. 4).

Microhabitat use of different size classes

In the rmGLMs treating the different size-classes as the repeated measurement, the interaction term 'size-class × water-depth' had a significant effect for two of the three

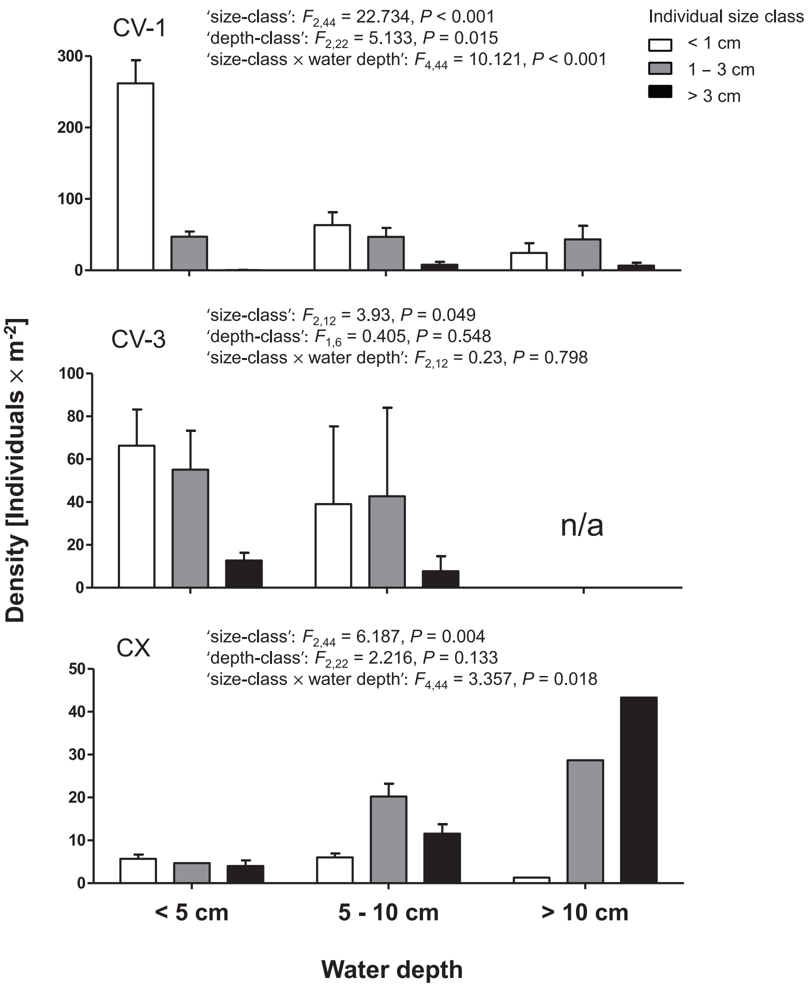

Figure 5 Population densities of cave mollies in the Cueva del Azufre. Mean (±SE) densities of mollies, categorized in three size classes (<1 cm, *white*, 1–3 cm, *gray*, and >3 cm, *black*) in three water depths (<5 cm, 5–10 cm, and >10 cm). Results of rmGLMs are inserted. Note the different y-axis scales. Error bars are given if more than one sampling grid of a given depth class was present within the sampling site.

sampling sites included in this analysis—notably, those sites with the most variation in water depth (Fig. 5). This result is indicative of differences in microhabitat use among different size classes of cave mollies: generally, larger fish were found in deeper areas, whereas smaller fish resided in shallow parts. A significant main effect of the repeated measurement ('size class') in all three analyses confirms the overabundance of small-sized fish in cave chamber V, and of large-bodied fish in chamber X (Fig. 5).

DISCUSSION

We provide detailed information on population densities of cave-adapted *P. mexicana* in the Cueva del Azufre. Repeated measurement in different cave chambers uncovered very stable patterns of high densities, confirming qualitative estimates provided by *Parzefall (1993)*. Density estimates of *P. mexicana* in the cave were extraordinarily high and exceed those of other cave fishes, which are usually low, with often less than one individual per m^2

(*Trajano, 2001*). Furthermore, densities were higher inside the cave compared to adjacent surface populations.

Variation in population densities can be explained by different factors affecting cave molly population dynamics; e.g., environmental heterogeneity may contribute to population differences. The highest H_2S concentrations (>300 μM) are found in parts of chamber X, where most large sulfide sources are located, while concentrations in chamber V are lower (2–32 μM), as H_2S is increasingly bound with oxygen with increasing distance from the sulfide sources (*Tobler et al., 2006*). However, ecotoxicological experiments repeatedly found small adults to have higher H_2S-resistance than large-bodied adults, possibly reflecting senescence effects or size-specific thresholds regarding the rate of sulfide influx to the body to oxidation (*Tobler et al., 2011b*; *Plath et al., 2013*; *Riesch et al., 2014a*). Hence, we would expect fish in chamber X to actually be smaller than fish from chamber V if different H_2S concentrations were the main driver of population differences.

Beside different H_2S concentrations, the sites within the cave differ in the presence of light. Whereas chamber V receives some dim light through cracks in the ceiling, the inner parts of the cave are lightless. Photophobic behavior is a factor that has been proposed to promote the colonization of perpetually dark caves and the choice of microhabitat (*Poulson, 1964*; *Barr Jr, 1968*). While photophobic behavior has been reported in several cavefishes (*Wilkens, 1988*; *Camassa, 2001*; *Wilkens, 2001*; *Timmermann & Plath, 2009*), photophilic behavior was found in both surface and cave forms of *P. mexicana* (*Parzefall et al., 2007*). In theory, this photophilic behavior could lead to an accumulation of fish in chamber V (sites *5–7*) compared to chamber X (site *8*) if fish moved between cave chambers but were less likely to return to dark sites, but this line of argumentation is not compatible with the observation of small-scale genetic structure among different cave chambers (*Plath et al., 2007*).

The different light regimes may also affect trophic interactions since the deep and lightless parts of the cave depend solely on chemoautotrophic primary production, while organic matter can enter through cracks in chamber V, and then provide the basis for detritivore animal communities that constitute an additional food source in other cave systems (*Hüppop, 2005*). Nevertheless, more research is needed on the extent to which these few sky lights might indeed provide significant influx of additional nutrients, because stomach content analysis of cave mollies, for example, does not so far strongly support such a notion (*Tobler et al., 2009*).

Furthermore, cave chambers may differ in predation regimes. Inside the Cueva del Azufre, aquatic water bugs of the genus *Belostoma* prey upon cave mollies and *Belostoma* prefer large over small cave mollies as prey (*Tobler, Schlupp & Plath, 2007*; *Tobler, Franssen & Plath, 2008*; *Plath et al., 2011*). Mark-recapture analysis found individual densities of water bugs to be approximately one individual per m^2 in chamber V (*Tobler, Schlupp & Plath, 2007*), and while empirical data are as yet lacking, observational evidence over several years of field work suggests that densities are much lower in the innermost chambers.

Belostoma predation, however, might explain microhabitat use of different size classes of cave mollies. *Belostoma* are typically found on rocks at the water's edge (*Tobler, Franssen*

& Plath, 2008), and so large cave mollies—being preferred by the water bugs (*Plath et al., 2011*)—could use deeper parts of the water column to avoid predation risk. The preference for large size-classes was confirmed for another belostomatid preying on mosquitofish (*Schumann, Cavallaro & Hoback, 2012*). On the other hand, small fish could avoid filial cannibalism, which is known from other poeciliids (*Loekle, Madison & Christian, 1982*; *Nesbit & Meffe, 1993*), by using shallow parts of the water column that exclude large mature fish.

One factor that most likely influences population dynamics is the annual 'La Pesca' ceremony. The ceremony leads to a strong temporary reduction of local fish densities in those cave chambers that are situated downstream of the barbasco release site (*Tobler et al., 2011a*). Our study was conducted approximately nine months after the last ceremony, but given rather long generation times in *P. mexicana* (roughly 3–6 months for males and 7–10 months for females from birth until reaching maturation under common-garden rearing conditions; *Riesch et al., 2014b*), we predicted to find lower (sub-)population densities and especially fewer large-bodied individuals downstream of the site in the Cueva del Azufre where barbasco is annually released. Instead, while fish densities were generally high in the cave, they were highest downstream of the barbasco release site. However, sample sites affected by the release of barbasco had population structures that were strongly shifted towards an overabundance of the smallest size classes (i.e., juveniles). These patterns were stable when repeated samplings from subsequent days were compared.

Migration within the Cueva del Azufre is unidirectional, out of the cave, and migration among different cave chambers occurs only to a small extent, which results in population genetic differentiation, as shown based on nuclear microsatellites (*Plath et al., 2007*), and is also reflected in morphological differences among fish from different cave chambers (*Fontanier & Tobler, 2009*). Hence, re-colonization of the affected sites from other parts of the cave (i.e., source–sink dynamics) is unlikely, and the observed recovery of the respective populations likely represents an autochthonous effect. After the temporal decline in population density following La Pesca, the surviving individuals benefit from reduced intraspecific resource competition. Detritus and green algae are the dominant food sources of surface-dwelling *P. mexicana* from non-sulfidic streams, while diets of conspecifics in the sulfidic surface and cave streams are dominated by chemoautotrophic (sulfur) bacteria and aquatic invertebrates (like larvae of the dipteran *Goeldichironomus fulvipilus* and small snails; (*Roach, Tobler & Winemiller, 2011*)). In particular, access to invertebrate prey could be favored not only by the absence of competing fish species, but especially by temporarily relaxed competition among the surviving adult *P. mexicana*. Generally, relaxed competition translates into higher growth rates, faster maturation, and increased adult fecundity (*Stearns, 1976*), which may lead to stage-specific biomass overcompensation, thereby compensating for the removal of individuals from the population (*Werner & Gilliam, 1984*; *de Roos et al., 2007*; *Schröder, Persson & de Roos, 2009*). This idea received support from empirical harvesting experiments that found the negative relationship between adult mortality and abundance/density to be reversed if mortality does not affect a certain portion of the population. Experimental studies on laboratory populations of the poeciliid fish *Heterandria formosa* showed that biomass of the juvenile size class increased in response to intermediate adult mortality rates (*Schröder, Persson & de Roos, 2009*). Another study showed that a pathogen outbreak in a wild perch population (*Perca fluviatilis*) was followed by a biomass overcompensation of the juvenile stage as a result of increased adult mortality. Age-specific adult fecundity and body mass of one- and two year old perch increased after the disease outbreak, suggesting that increased adult mortality released perch from competition and cannibalism, thereby increasing somatic and reproductive growth (*Ohlberger et al., 2011*). In the Cueva del Azufre, the stage-specific biomass overcompensation may lead to increasing population densities, based on temporarily increased adult fecundity that leads to high numbers of juvenile fish. This would result in cave molly populations regaining the high densities seen before La Pesca, again leading to increased competition. This is consistent with earlier observations of cave mollies showing reduced body condition (measured, e.g., as fat content) compared to fish from surface sites (*Riesch, Plath & Schlupp, 2010*; *Riesch, Plath & Schlupp, 2011*). Hence, human-induced selection and predation by *Belostoma* ought to have very similar effects on the populations exposed to them. We are inclined to argue, however, that the relative influence of *Belostoma* predation is considerably lower than the effects of the massive annual fish harvest. Previous reports of increased rotenone-resistance in fish from chamber V, but not chamber X (*Tobler et al., 2011a*), confirm that La Pesca undoubtedly has a strong selective influence on populations annually exposed to it.

In summary, we found remarkable fish densities of more than 200 individuals per m^2 in some parts of the cave. While other selective forces certainly also need to be considered, we argue that the annual La Pesca has major effects on the population ecology and evolutionary trajectory of cave mollies. We are aware of potential caveats of this line of argument, as not all differences reported here may be due to the annual La Pesca ceremony. Nevertheless, from a conservational point of view, knowledge about whether and how human activities affect teleost populations is especially pertinent in the case of locally adapted populations that are endemic to a small area. Therefore, we recommend a repeated sampling before and after La Pesca in order to demonstrate the influence of the ritual. While human influences on highly endemic, locally adapted populations (or, in terms of conservation biology, evolutionary significant units; *Moritz, 1994*) generally are to be evaluated as highly problematic, management plans for cave mollies ought to consider the important role La Pesca plays in the religion of the local human population. Carried out in the traditional way, fish populations in downstream cave chambers can obviously recover after the ceremony. However, we wish to highlight the necessity to critically review that those practices do not affect deeper parts of the cave and that no commercially available, more efficient fish toxins will be employed in the future.

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The authors declare there are no competing interests. David Bierbach is an employee of the Leibniz-Institute of Freshwater Ecology and Inland Fisheries; Jonas Jourdan is an employee of the Biodiversity and Climate Research Centre.

Author Contributions

- Jonas Jourdan analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- David Bierbach analyzed the data, reviewed drafts of the paper.
- Rüdiger Riesch reviewed drafts of the paper.
- Angela Schießl and Adriana Wigh performed the experiments.
- Lenin Arias-Rodriguez performed the experiments, contributed reagents/materials/analysis tools, reviewed drafts of the paper.
- Jeane Rimber Indy contributed reagents/materials/analysis tools.
- Sebastian Klaus analyzed the data, contributed reagents/materials/analysis tools.
- Claudia Zimmer performed the experiments, analyzed the data, reviewed drafts of the paper.
- Martin Plath conceived and designed the experiments, performed the experiments, analyzed the data, reviewed drafts of the paper.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

The methodology used in our study was a non-invasive technique. The study system was not influenced, and data recoding was based exclusively on visual observations.

Field Study Permissions

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REFERENCES

Bagarinao T. 1992. Sulfide as an environmental factor and toxicant: tolerance and adaptations in aquatic organisms. *Aquatic Toxicology* **24**:21–62 DOI 10.1016/0166-445X(92)90015-F.

Barr Jr TC. 1968. Cave ecology and the evolution of troglobites. *Evolutionary Biology* **2**:35–102.

Bray JR, Curtis JT. 1957. An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs* **27**:325–349 DOI 10.2307/1942268.

Camassa MM. 2001. Responses to light in epigean and hypogean populations of *Gambusia affinis* (Cyprinodontiformes:Poeciliidae). *Environmental Biology of Fishes* **62**:115–118 DOI 10.1023/A:1011872509199.

Colaço A, Dehairs F, Desbruyeres D. 2002. Nutritional relations of deep-sea hydrothermal fields at the Mid-Atlantic Ridge: a stable isotope approach. *Deep Sea Research Part I: Oceanographic Research Papers* **49**:395–412 DOI 10.1016/S0967-0637(01)00060-7.

Croft DP, Botham MS, Krause J. 2004. Is sexual segregation in the guppy, *Poecilia reticulata*, consistent with the predation risk hypothesis? *Environmental Biology of Fishes* **71**:127–133 DOI 10.1007/s10641-004-0092-5.

de Roos AM, Schellekens T, van Kooten T, van de Wolfshaar KE, Claessen D, Persson L. 2007. Food-dependent growth leads to overcompensation in stage-specific Biomass when mortality increases: the influence of maturation versus reproduction regulation. *American Naturalist* **170**:E59–E76 DOI 10.1086/520119.

Evans CL. 1967. The toxicity of hydrogen sulphide and other sulphides. *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences* **52**:231–248.

Flot J-F, Wörheide G, Dattagupta S. 2010. Unsuspected diversity of *Niphargus* amphipods in the chemoautotrophic cave ecosystem of Frasassi, central Italy. *BMC Evolutionary Biology* **10**:171 DOI 10.1186/1471-2148-10-171.

Fontanier ME, Tobler M. 2009. A morphological gradient revisited: cave mollies vary not only in eye size. *Environmental Biology of Fishes* **86**:285–292 DOI 10.1007/s10641-009-9522-3.

Gordon MS, Rosen DE. 1962. A cavernicolous form of the poeciliid fish *Poecilia sphenops* from Tabasco, Mexico. *Copeia* **2**:360–368 DOI 10.2307/1440903.

Goslee SC, Urban DL. 2007. The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software* **22(7)**:1–19.

Hose LD, Pisarowicz JA. 1999. Cueva de Villa Luz, Tabasco, Mexico: reconnaissance study of an active sulfur spring cave and ecosystem. *Journal of Cave and Karst Studies* **61**:13–21.

Hüppop K. 2005. Adaptation to low food. In: Culver DC, White WB, eds. *Encyclopedia of caves.* Amsterdam: Elsevier, 4–10.

Jeffery WR. 2001. Cavefish as a model system in evolutionary developmental biology. *Developmental Biology* **231**:1–12 DOI 10.1006/dbio.2000.0121.

Jeffery WR. 2009. Regressive evolution in *Astyanax* cavefish. *Annual Review of Genetics* **43**:25–47 DOI 10.1146/annurev-genet-102108-134216.

Loekle DM, Madison DM, Christian JJ. 1982. Time dependency and kin recognition of cannibalistic behavior among poeciliid fishes. *Behavioral and Neural Biology* **35**:315–318 DOI 10.1016/S0163-1047(82)90749-X.

Moritz C. 1994. Defining 'evolutionarily significant units' for conservation. *Trends in Ecology & Evolution* **9**:373–374 DOI 10.1016/0169-5347(94)90057-4.

Nesbit DH, Meffe GK. 1993. Cannibalism frequencies in wild populations of the eastern mosquitofish (*Gambusia holbrooki*: Poeciliidae) in South Carolina. *Copeia* **3**:867–870 DOI 10.2307/1447254.

Ohlberger J, Langangen Ø, Edeline E, Claessen D, Winfield IJ, Stenseth NC, Vøllestad LA. 2011. Stage-specific biomass overcompensation by juveniles in response to increased adult mortality in a wild fish population. *Ecology* **92**:2175–2182 DOI 10.1890/11-0410.1.

Parzefall J. 1993. Behavioral ecology of cave-dwelling fishes. In: Pitcher TJ, ed. *Behavior of teleost fishes*. London: Chapman & Hall, 573–606.

Parzefall J. 2001. A review of morphological and behavioural changes in the cave molly, *Poecilia mexicana*, from Tabasco, Mexico. *Environmental Biology of Fishes* **62**:263–275 DOI 10.1023/A:1011899817764.

Parzefall J, Kraus C, Tobler M, Plath M. 2007. Photophilic behaviour in surface- and cave-dwelling Atlantic mollies *Poecilia mexicana* (Poeciliidae). *Journal of Fish Biology* **71**:1225–1231 DOI 10.1111/j.1095-8649.2007.01581.x.

Plath M, Hauswaldt JS, Moll K, Tobler M, De Leon FJG, Schlupp I, Tiedemann R. 2007. Local adaptation and pronounced genetic differentiation in an extremophile fish, *Poecilia mexicana*, inhabiting a Mexican cave with toxic hydrogen sulphide. *Molecular Ecology* **16**:967–976 DOI 10.1111/j.1365-294X.2006.03212.x.

Plath M, Pfenninger M, Lerp H, Riesch R, Eschenbrenner C, Slattery PA, Bierbach D, Herrmann N, Schulte M, Arias-Rodriguez L, Indy JR, Passow C, Tobler M. 2013. Genetic differentiation and selection against migrants in evolutionarily replicated extreme environments. *Evolution* **67**:2647–2661 DOI 10.1111/evo.12133.

Plath M, Riesch R, Culumber Z, Streit B, Tobler M. 2011. Giant water bug (*Belostoma* sp.) predation on a cave fish (*Poecilia mexicana*): effects of female body size and gestational state. *Evolutionary Ecology Research* **13**:133–144.

Plath M, Tobler M. 2010. Subterranean fishes of Mexico (*Poecilia mexicana*, Poeciliidae). In: Trajano E, Bichuette ME, Kapoor BG, eds. *The biology of subterranean fishes.* Enfield, NH: Science Publishers, 283–332.

Por FD. 2007. Ophel: a groundwater biome based on chemoautotrophic resources. The global significance of the Ayyalon cave finds, Israel. *Hydrobiologia* **592**:1–10 DOI 10.1007/s10750-007-0795-2.

Poulson TL. 1964. Animals in aquatic environments: animals in caves. In: Bill DB, ed. *Handbook of physiology*. Washington, D.C.: American Physiological Society, 749–771.

R Development Core Team. 2008. *R: a language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria.

Riesch R, Plath M, Schlupp I. 2010. Toxic hydrogen sulfide and dark caves: life-history adaptations in a livebearing fish (*Poecilia mexicana*, Poeciliidae). *Ecology* **91**:1494–1505 DOI 10.1890/09-1008.1.

Riesch R, Plath M, Schlupp I. 2011. Toxic hydrogen sulphide and dark caves: pronounced male life-history divergence among locally adapted *Poecilia mexicana* (Poeciliidae). *Journal of Evolutionary Biology* **24**:596–606 DOI 10.1111/j.1420-9101.2010.02194.x.

Riesch R, Plath M, Schlupp I, Tobler M, Langerhans RB. 2014a. Colonisation of toxic environments drives predictable life-history evolution in livebearing fishes (Poeciliidae). *Ecology Letters* **17**:65–71 DOI 10.1111/ele.12209.

Riesch R, Reznick DN, Plath M, Schlupp I. 2014b. The impact of permanent darkness and food availability on life histories of surface- and cave-dwelling Atlantic mollies (*Poecilia mexicana*). Submitted.

Roach KA, Tobler M, Winemiller KO. 2011. Hydrogen sulfide, bacteria, and fish: a unique, subterranean food chain. *Ecology* **92**:2056–2062 DOI 10.1890/11-0276.1.

Romero A, Green S. 2005. The end of regressive evolution: examining and interpreting the evidence from cave fishes. *Journal of Fish Biology* **67**:3–32 DOI 10.1111/j.0022-1112.2005.00776.x.

Sarbu SM, Kane TC, Kinkle BK. 1996. A chemoautotrophically based cave ecosystem. *Science* **272**:1953–1955 DOI 10.1126/science.272.5270.1953.

Schlupp I, Colston TJ, Joachim BL, Riesch R. 2013. Translocation of cave fish (*Poecilia mexicana*) within and between natural habitats along a toxicity gradient. *Ecology of Freshwater Fish* **22**:228–233 DOI 10.1111/eff.12017.

Schröder A, Persson L, de Roos AM. 2009. Culling experiments demonstrate size-class specific biomass increases with mortality. *Proceedings of the National Academy of Sciences of the United States of America* **106**:2671–2676 DOI 10.1073/pnas.0808279106.

Schumann DA, Cavallaro MC, Hoback WW. 2012. Size selective predation of fish by *Hydrophilis triangularis* (Coleoptera: Hydrophilidae) and *Lethocerus americanus* (Hemiptera: Belostomatidae). *Journal of the Kansas Entomological Society* **85**:155–159 DOI 10.2317/JKES120116.2.1.

Singer TP, Ramsay RR. 1994. The reaction sites of rotenone and ubiquinone with mitochondrial NADH dehydrogenase. *Biochimica Et Biophysica Acta-Bioenergetics* **1187**:198–202 DOI 10.1016/0005-2728(94)90110-4.

Stearns SC. 1976. Life-history tactics: a review of the ideas. *Quarterly Review of Biology* **51**:3–47 DOI 10.1086/409052.

Summers Engel A. 2005. Chemoautotrophy. In: Culver DC, White WB, eds. *Encyclopedia of caves*. Amsterdam: Elsevier, 90–102.

Timmermann M, Plath M. 2009. Phototactic response and light sensitivity in an epigean and a hypogean population of a barb (*Garra barreimiae*, Cyprinidae). *Aquatic Ecology* **43**:539–547 DOI 10.1007/s10452-008-9173-z.

Tobler M, Culumber Z, Plath M, Winemiller K, Rosenthal G. 2011a. An indigenous religious ritual selects for resistance to a toxicant in a livebearing fish. *Biology Letters* **7**:229–232 DOI 10.1098/rsbl.2010.0663.

Tobler M, Palacios M, Chapman LJ, Mitrofanov I, Bierbach D, Plath M, Arias-Rodriguez L, de Leon FJG, Mateos M. 2011b. Evolution in extreme environments: replicated phenotypic differentiation in livebearing fish inhabiting sulfidic springs. *Evolution* **65**:2213–2228 DOI 10.1111/j.1558-5646.2011.01298.x.

Tobler M, Franssen CM, Plath M. 2008. Male-biased predation of a cave fish by a giant water bug. *Naturwissenschaften* **95**:775–779 DOI 10.1007/s00114-008-0382-z.

Tobler M, DeWitt TJ, Schlupp I, García de León FJ, Herrmann R, Feulner PG, Tiedemann R, Plath M. 2008a. Toxic hydrogen sulfide and dark caves: phenotypic and genetic divergence across two abiotic environmental gradients in *Poecilia mexicana*. *Evolution* **62**:2643–2659 DOI 10.1111/j.1558-5646.2008.00466.x.

Tobler M, Riesch R, de Leon FJG, Schupp I, Plath M. 2008b. New and morphologically distinct population of cavernicolous *Poecilia mexicana* (Poeciliidae:Teleostei). *Environmental Biology of Fishes* **82**:101–108 DOI 10.1007/s10641-007-9258-x.

Tobler M, Riesch R, Garcíade León F, Schlupp I, Plath M. 2008c. Two endemic and endangered fishes, *Poecilia sulphuraria* (Alvarez, 1948) and *Gambusia eurystoma* Miller, 1975 (Poeciliidae, Teleostei) as only survivors in a small sulphidic habitat. *Journal of Fish Biology* **72**:523–533 DOI 10.1111/j.1095-8649.2007.01716.x.

Tobler M, Riesch RW, Tobler CM, Plath M. 2009. Compensatory behaviour in response to sulphide-induced hypoxia affects time budgets, feeding efficiency, and predation risk. *Evolutionary Ecology Research* **11**:935–948.

Tobler M, Schlupp I, Heubel KU, Riesch R, de Leon FJG, Giere O, Plath M. 2006. Life on the edge: hydrogen sulfide and the fish communities of a Mexican cave and surrounding waters. *Extremophiles* **10**:577–585 DOI 10.1007/s00792-006-0531-2.

Tobler M, Schlupp I, Plath M. 2007. Predation of a cave fish (*Poecilia mexicana*, Poeciliidae) by a giant water-bug (*Belostoma*, Belostomatidae) in a Mexican sulphur cave. *Ecological Entomology* **32**:492–495 DOI 10.1111/j.1365-2311.2007.00892.x.

Trajano E. 2001. Ecology of subterranean fishes: an overview. *Environmental Biology of Fishes* **62**:133–160 DOI 10.1023/A:1011841913569.

Werner EE, Gilliam JF. 1984. The ontogenetic niche and species interactions in size-structured populations. *Annual Review of Ecology and Systematics* **15**:393–425 DOI 10.1146/annurev.es.15.110184.002141.

Wilkens H. 1988. Evolution and genetics of epigean and cave *Astyanax fasciatus* (Characidae, Pisces). *Evolutionary Biology* **23**:271–367.

Wilkens H. 2001. Convergent adaptations to cave life in the *Rhamdia laticauda* catfish group (Pimelodidae, Teleostei). *Environmental Biology of Fishes* **62**:251–261 DOI 10.1023/A:1011897805681.

3.3 Evolution of constructive traits in extreme habitats

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Brief Communication

Response to conspecific chemical cues in surface- and cave-dwelling populations of Atlantic mollies, *Poecilia mexicana*

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Abstract

Cavefishes typically evolve sensory adaptations to compensate for the loss of visual orientation and communication in their naturally dark habitats. We compared the response to chemical cues from conspecifics between surface- and cave-dwelling populations of Atlantic mollies (*Poecilia mexicana*) using dichotomous association preference tests. In one of the two described cave populations, focal females spent significantly more time in the preference zone containing pheromones compared to the preference zone receiving control water, while no discrimination was detected in the second cave population and in two surface populations, suggesting independent evolutionary trajectories in both cave populations.

Keywords:

Chemosensation, cavefish, extremophile teleost, olfaction, pheromones

Introduction

Compared to their surface-dwelling ancestors, cavefishes typically show a combination of regressive traits (reduction of eyes and pigmentation) and constructive traits that facilitate orientation, navigation, food-finding, and communication in absence of visual information (troglomorphic traits; Niemiller and Soares 2015). Constructive traits include improved chemosensation, *e.g.*, trough elongated barbels or an increased number of taste buds (Protas and Jeffery 2012; Niemiller and Soares 2015). In some species, troglomorphic traits evolved independently (*i.e.*, convergently) following the repeated colonization of subterranean habitats by surface-dwelling ancestral forms (Niemiller and Soares 2015). For instance, the Mexican tetra *Astyanax mexicanus* (De Filippi 1853)—currently the most extensively studied cavefish—has evolved at least 29 cave-dwelling populations with different degrees of eye degeneration and loss of body pigmentation (Wilkens 2010; Protas and Jeffery 2012).

The “neutral mutation hypothesis” tries to explain eye reduction in cavefishes. It posits that absence of stabilizing selection to retain the visual apparatus leads to the random accumulation of neutral mutations in genes underlying eye development, leading to an increase in the frequency of ‘blindness alleles’ and a potential fixation of those mutations via genetic drift (Wilkens 2010; Protas and Jeffery 2012). Another hypothesis assumes genes involved in eye development to be linked—via antagonistic pleiotropy— to the development of constructive traits, such that directional selection on the latter brings about a reduction of the former. It was recently suggested that increased expression of embryonic *sonic hedgehog* (*shh*) results in increased numbers of taste buds, but simultaneously hampers eye development in *A. mexicanus* (Yamamoto et al. 2009; Protas and Jeffery 2012). This hypothesis, if true, highlights the role of strong, directional selection for improved chemosensation (in this case: gustation) in cavefishes.

Improved chemosentation (gustation and olfaction) helps cavefish find food in darkness and may be particularly important in resource-limited subterranean environments (Hüppop 2005). Bibliowicz *et al*. (2013) compared food finding abilities of *A. mexicanus* from a cave population with pronounced variation in eye- and pigment-reduction and uncovered a correlation between the degree of eye-reduction and improved non-visual food finding abilities. Moreover, eyeless *A. mexicanus* had significantly larger naris size than individuals with a more surface-like phenotype.

Besides food detection, chemosensation plays a vital role in intraspecific communication. In resource-limited cave environments, it may be advantageous to respond to chemical cues indicative of conspecifics accumulating at food patches, or simply indicating the presence of potential mating partners. General attraction to water containing chemical cues from conspecifics was reported for the non-territorial, little aggressive cavefish *A. mexicanus*, while studies on other cave fishes, like the Somalian barb *Phreatichthys andruzzii* (Vinciguerra 1924) or the Brazilian catfish *Pimelodella kronei* (Miranda Ribeiro 1907) yielded contradictory results, possibly related to differences in their social behavior (reviewed in Parzefall and Trajano 2010; Niemiller and Soares 2015).

Modifications of intraspecific chemical communication were observed in another teleost that received attention in the study of cave adaptations: the Atlantic molly *Poecilia mexicana* (Steindachner 1863) (Tobler et al. 2008b). Populations of this species repeatedly colonized extreme habitats in Tabasco in southern Mexico, including caves and hydrogen sulfide (H_2S)-rich spring complexes, no longer than few hundred thousand years ago (Pfenninger et al. 2014). Locally adapted subterranean populations can be found in at least two limestone caves in this area: the Cueva del Azufre (CdA)—the only known chemoautotrophic (sulfidic) cave ecosystem sustaining a vertebrate population (Parzefall 2001)—and the much smaller, non-sulfidic Cueva Luna Azufre (LA; Tobler et al. 2008a). Both cave populations show reduced eye diameters (a heritable trait that persists in light-reared fish: Eifert et al. 2015) and behavioral changes like reduced shoaling (Plath and Schlupp 2008). In females of the CdA population, the genital region is enlarged to form a gland-rich genital pad, and the number of cephalic taste buds is considerably increased in both sexes, suggesting improved gustation in the CdA population, especially when males ‘nip’ at a female’s genital opening (Parzefall 2001). However, the evolution of olfactory capacities—such as the response to water-borne conspecific chemical cues—has not yet been examined.

Studies on female mate choice found surface, CdA, and LA females to express a visual mating preference for large male body size when mate choice tests were conducted in light; however, while CdA females also exerted mate choice in darkness, LA females failed to do so (Tobler et al. 2008b). Non-visual mate choice involves the perception of mechanosensory cues (via the lateral line) and chemical cues from potential mating partners (Plath et al. 2007). This prompted the question of whether differences exist between CdA and LA females in their ability to perceive chemical cues emanating from conspecifics, while the examination of potential differences in lateral line structure and function will be reserved to future studies. To answer this question, dichotomous association preference tests were conducted in which focal females from surface and both cave populations were given a choice between water containing chemical cues emanating from conspecifics (pheromones and metabolic waste products) and water without pheromones (control).

Materials & Methods

Our present study compared four populations of *P. mexicana* from (*a*) a non-sulfidic stream in proximity to both caves (Río Oxolotán), (*b*) a sulfidic stream connecting both caves (El

Azufre), (*c*) the sulfidic CdA cave, and (*d*) the non-sulfidic LA cave (for details see Tobler et al. 2008a). Three additional species were included: Guppies *Poecilia reticulata* (Peters 1859) were reported to respond to conspecific cues in a similar experimental set-up (Guevara-Fiore et al. 2009) and served as a "positive control" of the experimental approach. Test fish stemmed from a feral population inhabiting a thermally altered tributary to the river Erft in Germany (Jourdan et al. 2014). As another representative of the family Poeciliidae, Western mosquitofish *Gambusia affinis* (Baird & Girard 1853) were collected near Lincoln park in Texas, USA, and as a representative of another cave fish, *Astyanax mexicanus* (Characidae) from Pachón cave was included (Strecker et al. 2012). All test subjects were laboratory-reared descendants of wild caught fish, held in 200-L stock tanks at temperatures between 27°C and 29°C under a 12:12 hours light:dark cycle. Fish were fed at least twice daily with commercially available flake food (TetraMin® Tetra GmbH), frozen chironomid larvae, bosmids, *Artemia salina* shrimps, and frozen spinach (except for mosquito fish).

The experimental setup largely followed Guevara-Fiore *et al*. (2009): as we were interested in general responses to conspecific pheromones (i.e., social attraction), we used mixed-sex stimulus shoal consisting of five conspecifics (2 males, 3 females; cumulative SL ± S.E., *P. reticulata*: 94.7 ± 0.6 mm; *G. affinis*: 160.2 ± 3.1 mm; *P. mexicana*, Río Oxolotán: 208.2 ± 4.5 mm; El Azufre: 176.6 ± 3.8 mm; CdA: 210.7 ± 8.8 mm; LA: 229.1 ± 1.0 mm; *A. mexicanus*: 280.0 ± 6.9 mm) to create stimulus water containing pheromones. Stimulus individuals were kept in aerated 12-L tanks for 24 hours to accumulate chemical cues. Tanks were filled with 5-12 L of water depending on the cumulative length of the stimulus shoals in an attempt to keep the ratio of 'cumulative SL by total water volume' constant (Tab. 1). Stimulus fish were not fed during this period. We transferred focal fish from the stock tank to a temporary 12-L holding-tank at 27°C in direct proximity to the experimental setup and allowed them to acclimate for 24 h, during which regular feeding was continued. Standard length (SL) of focal females ranged from 21.7 to 59.7 mm (Tab. 1). Only females were considered as focal fish to reduce variation arising from potential behavioral differences between sexes.

Association preference tests were carried out using a grey plastic box (56.5 cm length, 36.9 cm width, 29.2 cm height) as the test arena, which was visually divided by brown tethers spanned over the upper side, into two lateral preference zones of 18.8 cm length and an equal-sized, central neutral zone (Fig. 1). The test tank was filled with aged tap water to a height of 5 cm, heated to 27°C, and aerated. We removed heater and air-stone before the behavioral tests.

During the tests, water was slowly dropping from two funnels (250 mL) into the two preference zones at a constant rate of 2 ml/min (Fig. 1). One preference zone received water containing pheromones (see above), while the other received water from an aerated 10-L aquarium without a stimulus shoal (control). After water flow was started, a focal female was placed in the central neutral zone. We observed focal fish via a webcam (Microsoft LifeCam VX-2000™) installed centrally above the test tank at approximately 1.5 m height. Measurement began once the focal female had started to swim freely in the water column. We determined times spent in both preference zones during a 5-min observation period. To detect side biases, focal fish were retested after 15 min with reversed side-assignments. We carefully removed the focal fish using a dip net and transferred it into an aerated 12-L tank next to the test arena. Between the two parts of a test, the tank was cleaned with 98% alcohol, rinsed thoroughly, and refilled with conditioned (aged, aerated, and heated) tap water.

Upon termination of the second part of a test, the standard lengths (SL) of the focal and stimulus fish were measured to the nearest millimeter using a plastic ruler.

We expressed the response to stimulus vs control water as individual strength of preference (SOP)-values. To do so, we summed association times (*i.e.*, times spent in association with stimulus versus control water) from the two trials. Afterwards we calculated SOP as (association time in stimulus water - time spent in control water / sum of both association times). First, we tested against a 'no choice'-expectation of SOP = 0 using one-sample *t*-tests within each test situation. SOP-values could range from -1 (complete avoidance of predator) over 0 (indifferent behavior) to +1 (complete attraction to predator kairomones). To compare the responses of both amphipod species to different predator cues, SOP-values were also used as the dependent variable in a general linear model (GLM) treating the four predator types as independent variable. The covariates 'cumulative SL of the stimulus shoal per liter stimulus water' ($F_{1,128} = 0.13$, $P = 0.72$) and 'SL of focal fish' ($F_{1,132} = 0.61$, $P = 0.44$) were not significant and thus, removed from the final model. All analyses were conducted in IBM SPSS Statistics for Windows, Version 22 (IBM Corp., Armonk, NY).

Results

Poecilia reticulata females and females of the *P. mexicana* population from the CdA cave were attracted to chemical cues from conspecifics as indicated by statistically significant, positive SOP-values, while no significant effects were seen in the other groups of test fish (for results from one-sample *t*-tests see Fig. 2). Females from the LA cave population tended to avoid the preference zone with chemical cues, even though this effect was not statistically significant (Fig. 2).

The GLM confirmed statistically significant behavioral differences among the seven groups of focal fish ($F_{6,136} = 2.88$, $P = 0.01$). LSD tests for *post hoc* pairwise comparisons detected a significant difference between the CdA and LA populations ($P = 0.02$), while no significant differences between other populations of *P. mexicana* were detected ($P \geq 0.18$). *Poecilia* *reticulata* exhibited the strongest preference, which was significantly different from all other groups ($P \leq 0.03$) except the CdA population of *P. mexicana* ($P = 0.15$).

Discussion

In theory, differences between species and especially among the four examined *P. mexicana* populations in their responses to chemical cues from conspecifics can be explained by two hypotheses: populations could differ in (*a*) sensory structures responsible for the detection of pheromones, or (*b*) in their behavioral motivation to associate with conspecifics (i.e., social attraction). In case of guppies (*P. reticulata*) we found a strong attraction to conspecific pheromones, confirming previous studies on this species (e.g., Guevara-Fiore et al. 2009). The strong response in our present study could be explained by the origin of the studied population, which stemmed from a stream with high predation pressure (Jourdan et al. 2014), where shoaling is typically increased (Krause and Ruxton 2002). However, this line of reasoning (*i.e.*, differences in the motivation to associate with conspecifics) fails to explain the finding that *P. mexicana* from the CdA cave showed the strongest response of the examined *P. mexicana* populations, as a previous study reported equally reduced shoaling tendencies in *P. mexicana* from the CdA and LA caves, likely reflecting reduced piscine and avian predation (Plath and Schlupp 2008). Likewise, both cave populations show reduced aggression (Bierbach et al. 2012) and reduced sexual activity (Plath et al. 2003; Plath 2008), thus ruling out differences between both cave populations in their motivations to respond to conspecific cues in other behavioral contexts.

The results of this study do, however, support a hypothesis that arose from previous experimentation on female mate choice in different locally adapted populations of *P. mexicana*, as only females from the CdA cave, but not LA females exhibited mate choice under lightless conditions (Tobler et al. 2008b). Obviously, exerting mate choice in their naturally dark habitat would be advantageous also for LA females, and testing those females under light conditions uncovered an intrinsic preference for large male body size (Tobler *et al*., 2008*b*). Those results, in conjunction with the results from this study, suggest that only the CdA cave population, but not the LA population, has evolved improved chemoreception (*i.e*., olfaction). Absence of an evolutionary response to selection imposed by perpetual darkness in the LA population could be a consequence of lower evolvability due to a small population size (see Willi et al. 2006), or possibly due to a more recent colonization compared to the CdA cave population. The results from this study identify the CdA cave population as a candidate for further investigations into the mechanisms involved in improved olfaction in cave fishes.

Cave-dwelling *A. mexicanus* show an increased taste bud density, along with an enlargement of brain regions that processes chemoreception in the telencephalon (Niemiller and Soares 2015). Likewise, improved olfaction was demonstrated (Bibliowicz et al. 2013). Still, we detected no response to conspecific pheromones in our present study, and so it seems that the examined population was not motivated to associate with conspecifics. Just like the cave forms of *P. mexicana*, cave-dwelling *A. mexicanus* have a reduced tendency to form shoals (Gregson and Burt de Perera 2007), probably because the costs of shoaling (competition for food) outweigh the potential protective benefits in absence of piscine predators (Plath and Schlupp, 2008). It seems plausible to argue that in this case the behavioral motivation to (not) respond to chemical cues from conspecifics conceals the ability of the focal fish to detect pheromones. Whether differences in sensory structures or different behavioral motivations explain absence of a response to pheromones in *G. affinis* remains elusive. This highlights the need to carefully consider both aspects in future studies on chemical communication before reaching conclusions regarding sensory capacities versus different motivations to respond to chemical stimuli.

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References

Bibliowicz J et al. (2013) Differences in chemosensory response between eyed and eyeless *Astyanax mexicanus* of the Rio Subterráneo cave. EvoDevo 4:25

Bierbach D et al. (2012) Divergent evolution of male aggressive behaviour: another reproductive isolation barrier in extremophile poeciliid fishes? Int J Evol Biol 2012:148745

Eifert C et al. (2015) Brain size variation in extremophile fish: local adaptation versus phenotypic plasticity. J Zool 295:143-153

Gregson J, Burt de Perera T (2007) Shoaling in eyed and blind morphs of the characin *Astyanax fasciatus* under light and dark conditions. J Fish Biol 70:1615-1619

Guevara-Fiore P, Skinner A, Watt P (2009) Do male guppies distinguish virgin females from recently mated ones? Anim Behav 77:425-431

Hüppop K (2005) Adaptation to low food. In: Culver DC, White WB (eds) Encyclopedia of caves. Elsevier, Amsterdam, pp 4-10

Jourdan J et al. (2014) On the natural history of an introduced population of guppies (*Poecilia reticulata* Peters, 1859) in Germany. Bioinvasions Rec 3:175–184. doi: 10.3391/bir.2014.3.3.07

Krause J, Ruxton GD (2002) Living in groups. Oxford University Press, Oxford

Niemiller ML, Soares D (2015) Cave Environments. In: Riesch R, Tobler M, Plath M (eds) Extremophile Fishes. Springer, pp 161-191

Parzefall J (2001) A review of morphological and behavioural changes in the cave molly, *Poecilia mexicana*, from Tabasco, Mexico. Environ Biol Fishes 62:263-275. doi: 10.1023/a:1011899817764

Parzefall J, Trajano E (2010) Behavioral patterns in subterranean fishes. In: Trajano E, Bichuette ME, Kapoor BG (eds) Biology of Subterranean Fishes. Science Publishers, Enfield, New Hampshire, USA, pp 83-116

Pfenninger M et al. (2014) Parallel evolution of *cox* genes in H_2S-tolerant fish as key adaptation to a toxic environment. Nat Commun 5:3873

Plath M (2008) Male mating behavior and costs of sexual harassment for females in cavernicolous and extremophile populations of Atlantic mollies (*Poecilia mexicana*). Behaviour 145:73-98

Plath M, Parzefall J, Schlupp I (2003) The role of sexual harassment in cave and surface dwelling populations of the Atlantic molly, *Poecilia mexicana* (Poeciliidae, Teleostei). Behav Ecol Sociobiol 54:303-309

Plath M, Schlupp I (2008) Parallel evolution leads to reduced shoaling behavior in two cave dwelling populations of Atlantic mollies (*Poecilia mexicana*, Poeciliidae, Teleostei). Environ Biol Fishes 82:289-297

Plath M, Schlupp I, Parzefall J, Riesch R (2007) Female choice for large body size in the cave molly, *Poecilia mexicana* (Poeciliidae, Teleostei): influence of species-and sex-specific cues. Behaviour 144:1147-1160

Protas M, Jeffery WR (2012) Evolution and development in cave animals: from fish to crustaceans. Wiley Interdisciplinary Reviews: Developmental Biology 1:823-845

Strecker U, Hausdorf B, Wilkens H (2012) Parallel speciation in *Astyanax* cave fish (Teleostei) in Northern Mexico. Mol Phylogenet Evol 62:62-70

Tobler M et al. (2008a) Toxic hydrogen sulfide and dark caves: phenotypic and genetic divergence across two abiotic environmental gradients in *Poecilia mexicana*. Evolution 62:2643-2659. doi: 10.1111/j.1558-5646.2008.00466.x

Tobler M, Schlupp I, Plath M (2008b) Does divergence in female mate choice affect male size distributions in two cave fish populations? Biology Letters 4:452-454. doi: 10.1098/rsbl.2008.0259

Wilkens H (2010) Genes, modules and the evolution of cave fish. Heredity 105:413-422

Willi Y, Van Buskirk J, Hoffmann AA (2006) Limits to the adaptive potential of small populations. Annual Review of Ecology, Evolution, and Systematics 37:433-458. doi: 10.1146/annurev.ecolsys.37.091305.110145

Yamamoto Y, Byerly MS, Jackman WR, Jeffery WR (2009) Pleiotropic functions of embryonic sonic hedgehog expression link jaw and taste bud amplification with eye loss during cavefish evolution. Dev Biol 330:200-211

Tables

Table 1

Mean (± SE) standard lengths (SL) of the focal and stimulus fish per L and numbers of replicates (*n*) for each of the test fishes used in the experiment.

Species	Mean SL [mm] of focal fish	SL [mm] per L stimulus water	*n*
P. reticulata	21.7 ± 1.7	19.0 ± 0.1	18
G. affinis	39.8 ± 1.3	32.0 ± 0.6	19
P. mexicana ‘Río Oxolotán’	44.9 ± 1.6	20.8 ± 0.5	31
P. mexicana ‘El Azufre’	41.1 ± 3.3	20.0 ± 0.0	17
P. mexicana ‘Cueva del Azufre’	41.6 ± 3.1	20.1 ± 0.4	23
P. mexicana ‘Luna Azufre’	47.1 ± 1.5	19.9 ± 0.1	23
A. mexicanus	59.7 ± 1.8	28.0 ± 0.7	12

Figures

Figure 1

Schematic view of the experimental set-up used for the dichotomous association preference tests; in this exemplary side-assignment, stimulus water is provided on the left, and control water on the right side. The test tank is divided into two lateral preference zones (P1 and P2) and a central neutral zone (N). For details see main text.

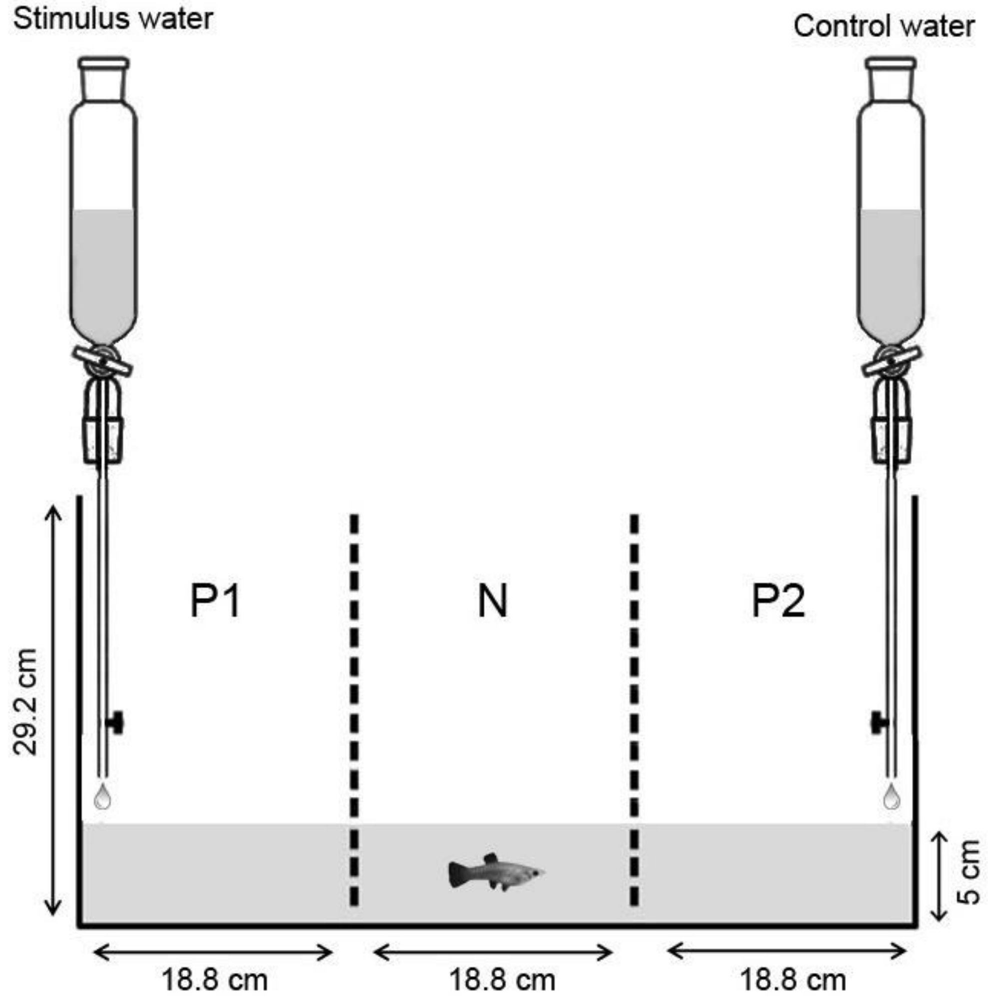
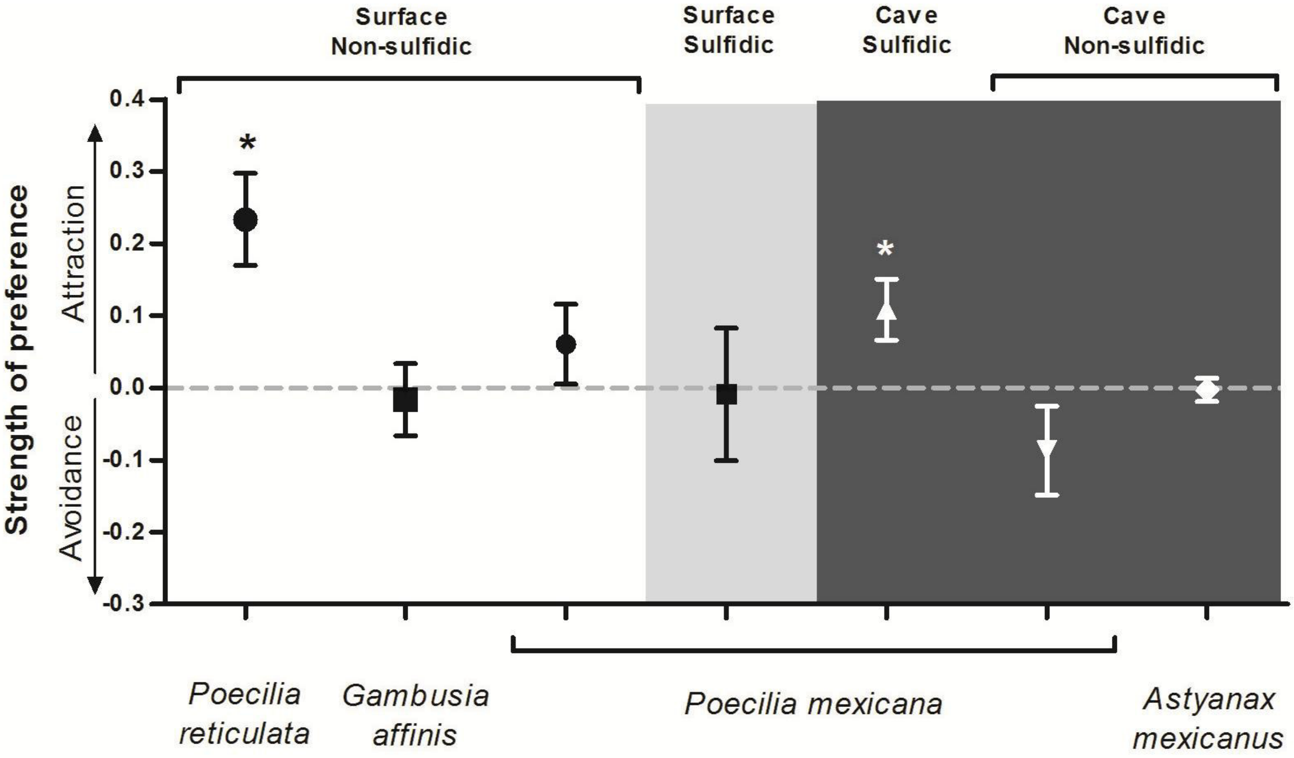


Figure 2

Association preferences of focal females for chemical cues from conspecifics. Positive strength of preference (SOP)-values indicate a preference for water containing pheromones from a mixed-sex stimulus shoal. Results from two-sided one-sample *t*-tests are shown, testing against the null assumption of SOP = 0, whereby * signifies $P < 0.05$ (from left to right: feral guppies, *Poecilia reticulata*: $t_{17} = 3.65$, $P < 0.01$; Western mosquito fish, *Gambusia affinis*: $t_{18} = -0.33$, $P = 0.75$; Atlantic mollies, *Poecilia mexicana*, population from the non-sulfidic Río Oxolotán: $t_{30} = 1.09$, $P = 0.28$; sulfidic El Azufre: $t_{16} = 0.10$, $P = 0.92$; CdA (sulfidic cave): $t_{22} = 2.55$; $P = 0.02$; LA (non-sulfidic cave): $t_{22} = -1.41$, $P = 0.17$; cave-dwelling *Astyanax mexicanus* from the Pachón cave: $t_{11} = -0.16$, $P = 0.87$). Shown are means ± SE.



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