HETEROSPECIFIC SOCIAL INTERACTIONS OF THE INVASIVE GUPPY (POECILIA RETICULATA): A POTENTIAL TRAIT TO ENHANCE INVASIVE SUCCESS

Morelia Camacho-Cervantes

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews

2014

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Morelia Camacho-Cervantes

September 2014

University of
St Andrews

Declaration

I, Morelia Camacho-Cervantes, hereby certify that this thesis, which is approximately 30,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

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Abstract

From all the species that arrive to a novel environment, very few manage to form a viable population. The guppy, a very successful invader, is a highly social species that performs some of its vital tasks (*e.g.*, foraging, avoiding predators) in groups. This thesis aimed to quantify heterospecific association benefits that enhance invasion success. Interactions between invaders and natives could be one of the environmental characteristics of a place that increase its risk of invasion. I evaluated the tendency of an invasive species to associate with native individuals with similar ecological requirements. I tested the hypothesis that invaders gain exploring, acquisition of information and foraging benefits when socializing with natives. In these experiments I used the guppy as the invasive model species and endangered native Mexican topminnows (*Poeciliopsis infans, Skiffia bilineata, Ameca splendens, Zoogoneticus tequila, Xenotoca eiseni and Girardinichthys viviparous*). I found that guppies shoal with other species in Trinidad (*Poecilia picta* and *Poecilia sphenops*), where they are native (Chapter 2) and that this trait remains when they are invasive (Chapter 3). Guppies are equally willing to explore novel environments when accompanied by heterospecifics or conspecifics. Guppies are more willing to explore complex environments than simple ones. Moreover, when exploring simple environments they have a higher association tendency, regardless of the partner' species (Chapter 4), which could lead them to acquire the benefits of grouping behaviour and avoid Allee effects - the disadvantages of being part of a small group. In the contexts in which they were tested guppies gained as much information by associating with heterospecifics as with conspecifics (Chapter 5). Finally, I found that when shoaling in bigger shoals guppies are able to locate food faster and spent more time foraging. The benefits of increased shoal size were maintained when the additional guppies were replaced with heterospecifics. However, they derive more benefits from the species they are more willing to associate with (Chapter 6). These results uncover a mechanism enabling founding individuals to survive during the most vulnerable phase of an invasion and help explain why guppies have established viable populations in many parts of Mexico as well as in every continent except Antarctica.

Acknowledgements

I would like to thank Prof. Anne Magurran for her excellent supervision throughout my time at the University of St Andrews. I always came out of her office with better perspectives and more interesting ideas.

I could not have completed this thesis without the support of Dr. Alfredo Ojanguren, who acted as a co-supervisor and had his door open for me and my questions at all times.

Thanks to my tutor Prof. Thomas Meagher and my internal examiners Dr. Carl Smith and Dr. Jeff Graves for their helpful comments and discussions.

To Al Reeve and Dr. Miguel Barbosa thanks for the endless discussions, the fieldwork and lab support.

Thanks to Dr. Hideyasu Shimadzu, Dr. María Dornelas, Faye Moyes and the rest of the Fish Lunch discussion group members for sharing ideas and helping me better understand my data. And to my friends in México and St Andrews who helped me proofread chapters and articles, listened to my talk rehearsals and discussed with me scientific ideas or simply bureaucracy burdens.

The work in México was possible thanks to the support of Dr. Constantino Macías at UNAM and Dr Omar Domínguez at UMSNH. Thanks to both and to the lab members of their groups, Laboratorio de Biología Acuática and Laboratorio de Conducta Animal respectively, especially to Edgar Luna and Diego Chechenquillo. Work in Trinidad was possible thanks to the support of Dr. Amy Deacon, Kharran Deonarienesingh and Dr. Indar Ramnarinne. In St Andrews, Dr. Andrew Whiston, Steve, Cara and the St Andrews Aquarium provided me with a population of goodeidae fish, thanks to them as well.

Hugo Camacho and Cristian Cortés, thanks for driving me around so much to place data loggers and collect fish. María and Sofía Zirión, Hernando Rodríguez and Adrián Ortega, thanks for help me collect fish. Sorry about the sunken phone and Petrush’s injuries.

Ian MacGregor-Fors, this whole doing-a-PhD idea started sitting next to you. Thanks for your help and support though the entire process.

A very special thanks to my parents for their support in all the possible ways; from listening to everything I had to say and complaint about life abroad, science stuff or just anything, to financially support my trips to México and some of the fieldwork there. (Un agradecimento muy especial a mis padres por su apoyo en todas las formas posibles; desde escuchar todo lo que tuve que decir y quejarme de la vida fuera de México, ciencia o cualquier cosa, hasta su apoyo económico para pagar mis viajes a México y parte del trabajo de campo ahí. Gracias Oracio y Lupita por ayudarme incondicionalmente.)

Finally, I want to thank my funding bodies CONACyT, COECyT and the Research Committee of the School of Biology.

Para Duk y Abeja

Heterospecific social interactions of the invasive guppy (*Poecilia reticulata*): a potential trait to enhance invasive success

Contents

Chapter 1

General Introduction

Invasive species are one of the greatest threats to biodiversity (Lodge 1993, Wilcove et al. 1998, Ehrenfeld 2010) and ecosystem services (Vitousek et al. 1997, Arim et al. 2006, Pejchar and Mooney 2009). Invaders are those species that establish populations outside their native range and manage to proliferate, spread and persist (Elton 1958, Mack et al. 2000). Invasive species are transforming the natural world at an accelerating rate and are considered one of the most influential factors of global change (Vitousek et al. 1997).

The establishment and population growth of invasive species damage native communities and ecosystem functions (Sanders et al. 2003). Invaders are responsible for structural and compositional changes in ecological communities, through predation, competition, disease transmission and habitat degradation (Simon and Townsend 2003, Arim et al. 2006). These changes lead to a disruption in the productivity and nutrient availability cycles within the habitat, which influences trophic structure and population dynamics (Parker et al. 1999). In some cases invaders cause a radical alteration in the species composition of the place they had invaded (Rhymer and Simberloff 1996).

All the changes that invasive species cause in the places where they establish may ultimately lead to native species loss and wildlife homogenization (Vitousek et al. 1997, Arim et al. 2006). Indeed, according to Clavero and Garcia-Berthou (2005) invasive species are the main cause of bird species going extinct and the second main cause of the extinctions of fish and mammals. A classic example of this is that of the brown tree snake (*Boiga irregularis*), which was introduced to Guam by accident on a military cargo ship (Savidge 1987). This accidental introduction resulted in the extinction of nine forest birds, fifty per cent of the lizard species and at least one of the three island’s bat species (Fritts and Rodda 1998). Another dramatic example of invasive species causing biodiversity loss is the extinction of more than a hundred endemic cichlid species in Lake Victoria since the introduction of the Nile perch (*Lates niloticus*) in the1950s (Witte et al. 1992).

To mitigate the negative consequences of these introductions, it is essential to understand the processes leading to invasion success (Simberloff et al. 2013). In this chapter, I will explore the invasion steps and the characteristics that are related to species success as invaders. I will mainly focus in freshwater ecosystems, particularly interactions between the invasive guppy and the Mexican Goodeidae family. I will discuss how behaviour promotes invasion success and help individuals overcome disadvantages of being part of a small population (Allee effects).

1.1 Invasion process

The arrival of invasive species in environments where they had not previously occurred is not necessarily dependent on human activities. Indeed, most common vectors of non-human introductions are wind, water currents and wild animals (Alpert 2006). For example, when a natural disaster alters a habitat, species may be forced to emigrate and find other suitable places to live (Lodge 1993). Species migrating from one place to another is a common ecological process (di Castri 1989). However, although invasive species are not only due to anthropogenic activities, human activities promote them (Kolar and Lodge 2001). The number of species leaving their native environments is increasing as transport and commerce is becoming more global (di Castri 1989, Mack et al. 2000).

The way in which invasive species are introduced due to human activities can be categorized as: (1) deliberate, which result from active introduction by humans for their benefit; (2) unofficial introductions, which are made by individuals who do not gain any benefit and are unaware of consequences; and three (3) accidental or by-product introductions, which are the result of human introducing species, without noticing, while they are doing activities such as fishing or trading alive fish stocks (Moyle and Light 1996b, Alpert 2006). A striking example of human activities leading to biological invasions is the release of fish from trade of aquarium and ornamental fish. One third of 100 worst invasive species of the International Union for the Conservation of Nature (IUCN) list are fish that were traded by aquarists (Padilla and Williams 2004). Table 1.1 provides examples of the likely vectors associated with species introductions. Through these vectors, the number of species that are introduced in areas outside their native range is increasing (D'Antonio et al. 2004). For instance, a study carried out by Cohen and Carlton (1998) in the San Francisco Bay showed that between 1851 and 1960 a non-native species would establish itself every 55 weeks. This estimate increased to one non-native species establishing in the bay every 14 weeks between 1961 and 1995. This increment of invasive species establishing outside their native range is associated to an increase in global trade and commerce (Mack et al. 2000).

Table 1.1. Routes through which species are introduced into new habitats and examples of likely vectors associated with them. Modified from (Alpert 2006).

Way of entry	**Likely vector**
Deliberate	Mosquito control measures, transport of agricultural, horticultural, pet, game, and study species.
Unofficial	Pet releases, bait releases
Accidental or by-product	Packing, commercial goods, ship ballast, exteriors and interiors of vehicles and vessels, clothing, luggage

Most of the species that arrive to a novel environment do not succeed as invaders (Lodge 1993). In fact, it is believed that only 10% of the species that exit its native range will survive, and form those only another 10% will manage to establish a viable population (Williamson 1996). This calculation is known as the Tens Rule, in each of the invasion stages (Figure 1.1) only 10% will manage to survive to the next due to the unknown conditions, such as novel predators or lack of mutualisms (Williamson 1999). However, this percentage is likely to vary across the different stages. Nevertheless, the Tens Rule highlights the difficulty of predicting which or the many species that are translocated from one place to another will become invasive. According to Sakai et al. (2001), an understanding of the specific stages of invasion is useful for the management and mitigation of its consequences. Indeed, they suggest a specific type of management for the different stages of invasion. Figure 1.1 shows a diagram of the general steps a species follows to become invasive and the type of management strategies proposed by Sakai et al. (2001).

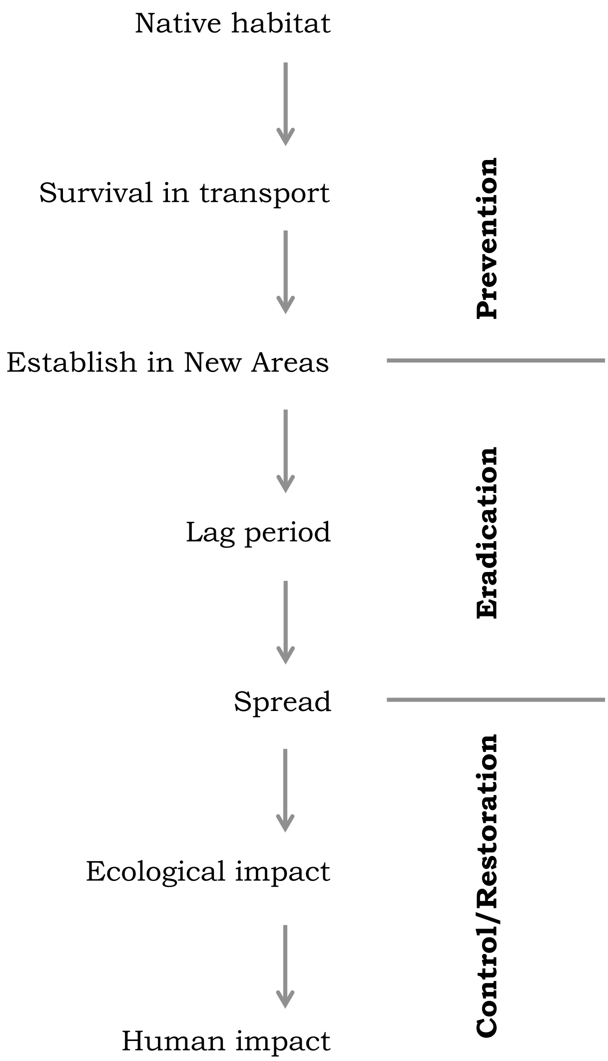


Figure 1.1. Diagram of general invasion steps and their relation to management strategies; modified from (Sakai et al. 2001).

There are many factors that determine the probability that an invader will become successful in establishing a viable population. According to Crawley (1989) the main causes of failure during the establishment stage of invasion are climate and predation, followed by the impact of competition, disease and lack of mutualisms. Invaders success in novel environments is delimited by four main factors: resources (Davis et al. 2000, Jefferies 2000), natural enemies (Mack et al. 2000), the physical environment (Moyle and Light 1996b), and the species ability to cope with these unknown conditions (Kolar and Lodge 2001).

Successful invasion involve interactions between invaders and the biotic and abiotic characteristics of the novel environment (Hayes and Barry 2008). Many studies have focused on trying to identify the characteristics in species that predicts which would be successful invaders and which would not; they are mainly based on the idea that successful invaders share characteristics that explain their success (Williamson 1999, Kolar and Lodge 2001, Lockwood et al. 2006). Characteristics that are associated with invasion success include genetic traits (Bazin et al. 2014), behavioural plasticity (Sol et al. 2002), taxonomy (Karatayev et al. 2009), and life history traits (Lodge 1993, Alcaraz et al. 2005).

Life history traits are of particular interest as they are believed to be one of the most important characteristics predicting invasion success (Ghalambor et al. 2007). According to Lodge (1993), these traits include: “fast” life histories (*r*-selected, rapid maturation and production of many small offspring; Kolar and Lodge 2001), high dispersal rate, vegetative or single-parent reproduction, high genetic variability, phenotypic plasticity, large native range and human commensalism. Nevertheless, although scientists have identified traits that are common in invaders, these traits point to explain invasion success and might be limited to predict it (Williamson 2006). Biological invasions that become successful involve complex interactions between invaders and biotic and abiotic characteristics of the recipient environment (see Table 1.2), Hayes and Barry (2008) suggest that analysis that are specific to each site and taxa are needed to provide insights that are of better use for conservation.

Table 1.2. Examples of phenomena that influence interactions between invasive species and biotic and abiotic conditions of the recipient environment. Updated from (Hayes and Barry 2008).

Phenomena	**Examples**
Positive feedback mechanisms	(Noble 1989)
Allee effects	(Taylor and Hastings 2005, Angulo et al. 2013, Cassey et al. 2014)
Behavioural changes	(Holway and Suarez 1999, Sol et al. 2013)
Genetic variability	(Holdgate 1986, Joly 2000)
Adaptation	(Rosecchi et al. 2001, Moran and Alexander 2014)
Phenotypic plasticity	(Hulme 2008, Reeve et al. 2014)
Potential lag time between invasion and establishment.	(Sakai et al. 2001, Cassey et al. 2014)
Cryptogenic species	(Carlton 1996, Avery et al. 2013)

1.2 Freshwater systems under threat

Freshwater ecosystems are amongst the most altered and invaded in the world (Garcia-Berthou et al. 2005, Strayer 2010). From all the North American fish extinctions of the 20th century two thirds are associated with introduced species (Miller et al. 1989). Like islands, freshwater ecosystems are vulnerable due to their geographic isolation and high rates of endemicity (Moyle 1996). Fish is the vertebrate group with, by far, the most estimated number of species; from these around half are found in lakes, rives, and other freshwater systems (Nelson 2006).

Common routes of fish invasion include introductions of biological control agents (Englund 1999), releases designed to provide food and sport, or discards of aquarium fish and bait buckets (Strayer 2010). Although in some cases freshwater fish invasions may have a positive outcome for the local fish communities and on human economy (Gozlan 2008), in most their effects are catastrophic (Vitule et al. 2009). Freshwater invaders are responsible for effects that range from local extinctions to alterations in nutrient and energy fluxes (Simon and Townsend 2003). For example, the Asian Silver Carp (*Hypophthalmichthys molitrix*) has invaded much of the Great Lakes of North America. Invasion started with the intentional release of a few individuals to control algae growth in aquaculture and waste water treatment facilities; now Asian Silver Carp has outcompeted the native fish species and has become a major problem for the fishery industry in the area (Lohmeyer and Garvey 2009, Hinterthuer 2012).

When fish first arrive in a new environment, their life history traits will have some influence in establishment success but there are more components that are important as well (Gozlan 2008, Gozlan et al. 2010). Traits that characterize freshwater fish invaders include tolerance to a broad range of environmental conditions, rapid dispersal and colonization, aggressive behaviour and competitiveness, and desirability to humans (edibility, sporting qualities, aesthetic characteristics, etc.)(Moyle and Light 1996a, Sakai et al. 2001). Early experience and learning can be crucial when animals initially encounter a novel environment (Magurran 1999). For example, fish may have to change the allocation of their time from feeding or mating to avoiding predators, or might have to shift their shoaling behaviour.

1.3 Benefits of living in groups during invasion

Social behaviour plays a key role in enhancing survival (Krause and Ruxton 2002). Many species depend on social interactions to forage, avoid predators or raise their young (Tobin et al. 2011). Behavioural adaptations influence the competitive ability and spread of invasive species and can underpin successful invasions (Holway and Suarez 1999). Behaviour is flexible and can be modified more quickly than life history or morphology, it is more likely to promote survival following stressful situations (Magurran 1999). This flexibility is thought to be an important component of success for invaders (Sol et al. 2002). For example, red swamp crayfish (*Procambarus clarkii*) change their pattern of shelter occupancy when invading the native habitat of the signal crayfish (*Pacifastacus leniusculus*). Shelter occupancy of red swamp crayfish when alone is moderate and increases significantly when invading signal crayfish, because for them shelter occupancy is relatively important (Hanshew and Garcia 2012). Flexibility and adjustment of behaviour, whether non-aggressive or agonistic, can promote establishment and success of species (Sol et al. 2013). Invasive guppies thus might be keen to shift from the ideal scenario of associating with heterospecifics to a second best by interacting with native species.

Social networks influence access to resources and information (Croft et al. 2009b). Being part of a group delivers crucial benefits to individuals, such as: protection from predators (Hamilton 1971, Pavlov and Kasumyan 2000, Couzin and Krause 2003), increased foraging efficiency (Day et al. 2001), the possibility to interact with potential mates (Guevara-Fiore et al. 2010a), and reducing of energetic costs of movement (Chapman et al. 2008). It is believed that the two main environmental influences of group size are food and predation; individuals join or leave groups that maximize the benefits they gain (Krebs and Davies 1987). Locating food is one of the primary benefits of joining groups to forage; individuals follow others that have more information than them. The later reduces trial and error costs, which sometimes can be lethal (Krause and Ruxton 2002). However, foraging in groups comes at a cost, for example, the bigger the group the faster the food patch depletes. Table 1.3 shows some examples of benefits and costs associated to group foraging.

Table 1.3. Advantages and disadvantages of foraging in groups.

	Species example	**References**
Advantages		
Acquire information about food sources	Bumblebees (*Bombus terrestris*)	(Leadbeater and Florent 2014)
	Brown trout (*Salmo trutta*)	(White and Gowan 2014)
Acquire information on foraging tools	Bottlenose dolphins (*Tursiops sp.*)	(Kopps et al. 2014)
Predator vigilance	Nutmeg mannikins (*Lonchura punctulata*)	(Rieucau and Giraldeau 2009)
	Peaceful dove (*Geopelia striata*)	(Tang and Schwarzkopf 2013)
	Meerkats (*Suricata suricatta*)	(le Roux et al. 2009)
Cooperative hunting	Wolves (*Canis lupus*)	(Escobedo et al. 2014)
	Crab spider (*Diaea ergandros*)	(Ruch et al. 2014)
More time devoted to forage	Guanacos *(Lama guanicoe*)	(Cappa et al. 2014)
Disadvantages		
Resource sharing	Japanese macaque (*Macaca fuscata*)	(Kazahari 2014)
	Spice finches (Lonchura punctulata)	(Livoreil and Giraldeau 1997)
Foraging in suboptimal habitats	Sandpipers (*Charadrii sp.*)	(Gavrilov 2014)

1.3.1 Shoaling behaviour

Fish associate with other individuals during a variety of activities including mating, hibernation, sleeping and foraging (Bleakley et al. 2007). Shoaling is a behavioural characteristic of fish that refers to any social grouping of individuals, including schooling, which is a specific type of shoal in which fish move together in a synchronized way (Pitcher 1983). Consequences of group behaviour are generally positive, nevertheless, there are cases when being part of a shoal represents a disadvantage for individuals, such as an increase of competitive interactions, depletion of resources or infection by parasites and other disease organisms (Magurran and Seghers 1991, Cote and Poulin 1995, Krause and Ruxton 2002). The decision of joining a shoal or not is always made by assessing the costs and benefits of doing so (Croft et al. 2003). Individuals tend to adjust their behaviour in response to the social context that their groups provide them (Bleakley et al. 2006).

Shoals are non-random assemblages of conspecifics. Because benefits from joining a shoal will depend on its composition, individuals are choosy as to which one they will join (Griffiths and Magurran 1998). Generally, benefits of grouping increase when individuals are similar phenotypically and behaviourally (Dyer et al. 2009) and when the group size is bigger (Hager and Helfman 1991, Hoare et al. 2004). For example, when group size increases, vigilance behaviour per individual decreases (Bleakley et al. 2007) and predator success may decrease (Griffiths and Magurran 1997b, Botham et al. 2005).

Female and male preferences for shoaling have been documented in various studies, examples include: males prefer to shoal with receptive females (Guevara-Fiore et al. 2010b), females prefer to shoal with larger females (Jones et al. 2010), males and females prefer to associate with familiar individuals (Magurran et al. 1994, Bhat and Magurran 2006) and bolder individuals (Brosnan et al. 2003). A particular example is that of the European minnows (*Phoxinus phoxinus*), they have been proven to show a distinct preference for shoaling with fish of low competitive ability than with others like them or better (Metcalfe and Thomson 1995). When invading, fish might encounter native species alike them, this could promote their willingness to interact with them and thus gain the advantages of being social. In the first two chapters of this thesis, I explore guppies' response to the possibility of associating with individuals of different species.

1.4 Allee effects

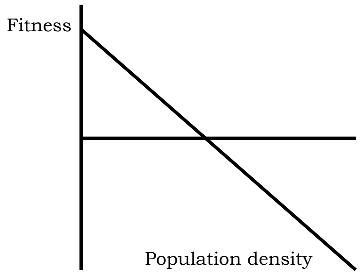
In the 1930s, Warder Clyde Allee proposed that cooperation between conspecifics could lead to inverse density dependence (Allee 1939). This idea was first named by his collaborator, Eugene P. Odum (1953), as 'Allee's Principle'. Nowadays, this principle is generally known as the Allee effect. The recent emphasis put on the relevance of studying behavioural ecology to promote conservation has highlighted the interest and importance of social interactions for the viability of populations (Stephens and Sutherland 1999).

An Allee effect is a positive relationship between fitness and density of a population, i.e. an individual that is part of a population experiencing this effect will have lower viability when conspecific density is low (Stephens and Sutherland 1999, Taylor and Hastings 2005). These effects can be caused through a variety of mechanisms, including mate finding limitation, limited reproductive facilitation in colonial breeders, or reduction of antipredator tactics (Courchamp et al. 2008). Allee effects can be 'component' when some components of individual fitness decreases when conspecific density is low, or 'demographic' if the overall population fitness decrease when conspecifics are in low numbers. In many cases, when ‘component’ Allee effect are strong above a threshold level, they lead to ‘demographic’ effects (Stephens et al. 1999).

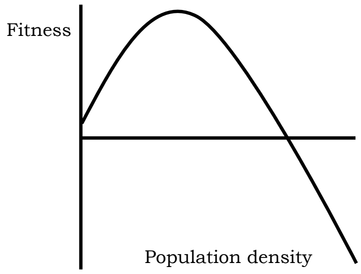
Allee effects have been studied largely in recent years, partially because they may play a role in extinction of already endangered species; nevertheless, by the same token, they affect the dynamics of invasive species (Taylor and Hastings 2005). Invaders are often introduced at low densities, making them vulnerable to Allee effects (Taylor and Hastings 2005). The most general consequence of these is the creation of a critical population density (Allee effect threshold), below which negative per capita growth can occur (Taylor and Hastings 2005). This threshold varies among populations and taxa, but it is defined as the minimum number of individuals in a population to ensure fitness maintenance or increase among its members (Figure 2.2 c). Allee effect thresholds could provide an explanation for lack of range expansion by invasive species even when arriving in a suitable habitat (Keitt et al. 2001). Figure 2.2 shows the relation between individual fitness and population density when Allee effects are not present, and are present and are either weak or strong. Allee effects result a way of explaining why a rare species might have a lower density threshold to ensure the permanence of its populations as well as a tool when designing conservation plans (Courchamp et al. 2008).

The existence of Allee effects in an invasive population could be exploited in the management of invasive species. In fact, management of invasive species suffering from ‘demographic’ or ‘component’ Allee effects should probably be different from those without them (Taylor and Hastings 2005). Indeed, to eradicate an invasive species, it is only necessary to reduce its population below the Allee effect threshold (Liebhold and Bascompte 2003). In the same direction, if an invasive population is suffering from ‘component’ Allee effects, these could be enhanced until they result in ‘demographic’ (Tobin et al. 2011).

a) **No** Allee effect



b) **Weak** Allee effect



c) **Strong** Allee effect

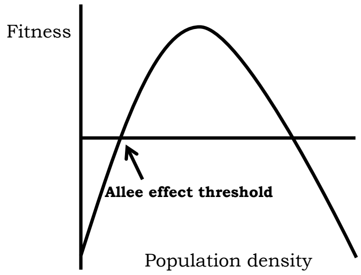


Figure 2.2. Demographic Allee effect. a) when there is no Allee effect present individual fitness decreases as the population density increases, b) when Allee effect is weak individual fitness increases as the population increases until density reaches a threshold after which the relation between fitness and density is as in a), c) when Allee effect is strong a minimum density threshold appears, bellow which individual fitness decreases when population fitness decreases, leading even to local extinction. Redrawn from (Taylor and Hastings 2005).

1.5 Heterospecific social interactions

Animal groups are usually composed of individuals of the same species (Morse 1970). Nevertheless, associations between individuals of different species have been found in many taxa. Heterospecific aggregations (also called: interspecific, polyspecific or mixed species) are the ones with two or more species associating at the same time and space (Morse 1970). These associations range from closely related species to species from different orders and occur across a wide range of taxa (Stensland et al. 2003), including reef fish (Sazima et al. 2007), primates (Stensland et al. 2003), and birds (Powell 1989, Campobello et al. 2012).

The benefits of grouping have been confirmed in heterospecific associations as well as in conspecific ones (Morse 1977, Ward et al. 2002). As a result, when individuals of different species are similar in their phenotype and obtain benefits, they may form heterospecific groups (Landeau and Terborgh 1986). For example, killifish (*Fundulus heteroclitus* and *F. diaphanus*) in Morice Lake have been found to associate with golden shiners (*Notemigonus crysoleucas*), white suckers (*Catostomus commersoni*), threespine sticklebacks (*Gasterosteus aculeatus*) and fourspine sticklebacks (*Apeltes quadracus*) to get protection from predators, through an enhanced confusion effect and early predator warning (Krause et al. 2005).

1.6 The guppy, an ideal study species for invasion

The guppy is a poeciliid with a natural range of distribution in Trinidad, Guyana, Venezuela and Surinam. It inhabit shallow rivers and ponds (Magurran et al. 1995, Magurran 2005). Guppies exhibit strong sexual dimorphism; females have a beige colouration and continue to grow throughout their lifetime, reaching a body length of 3 cm or more. Males, on the other hand, grow until they reach sexual maturity and are usually not bigger than 2.5 cm. Males are individually distinctive, their colour pattern, mainly consisting of yellow, orange and black spots (Figure 1.3). They are primary benthic feeders and their sexual dimorphic phenotype leads to differences in the allocate of time to daily activities such as mating or feeding (Magurran 2005). It is a remarkably opportunistic species with reproductive adaptations that enable a few individuals or even a single pregnant female to found a viable population (Deacon et al. 2011).

Figure 1.3. Male and female guppies. Females are larger, with a drab beige colouration (bottom right); males are smaller and display individual colour patterns (picture taken by Sean Earnshaw).

Guppies possess many of the physiological, behavioural and life-history characters that are associated with extreme adaptability (Magurran 2005) – traits associated with increased invasion success (Hellmann et al. 2008). They are capable of undergoing fast evolutionary adaptations under changing environmental conditions (Reznick et al. 1997). Table 1.4 summarises the characteristics that make guppies a successful invasive species.

Guppies are able to establish a population in a wide range of conditions (Gibson and Hirst 1955, Chervinski 1984, Chung 2001). They manage to survive and establish at temperatures (Chung 2001, Reeve et al. 2014) and salinities (Chervinski 1984) that are distant from those of their native environment. The guppy is a notoriously successful species when invading new environments, introductions of just a few animals or even a single individual can develop into thriving populations (Deacon et al. 2011). Their dispersion across the globe is a good example of their adaptability (Magurran 2005). For instance, guppies can be found in unusual locations such as the Moscow sewage works (Zhuikov 1993) and the River Lee in Essex, England, (Wheeler 1998); in these places artificial heating effluent keeps the water temperature high enough for them to survive.

Table 1.4. Summary of the traits that may influence the invasive success of the guppy, *Poecilia reticulata*; from (Deacon 2010) with updates.

Trait	Origin*	Evidence	Reference
Ecology & Life History			
High dispersal rate	Wild (Trinidad)	There is considerable gene flow between natural populations	(Crispo et al. 2006), (Sievers et al. 2012)
Ability to establish a population from a single individual	All	Sperm storage means that a single female is able to found a population.	(Winge 1937)
	Wild (Trinidad)	J. S. Kenny's introduction in Trinidad persists despite being founded by a single female.	(Carvalho et al. 1996)
Highly specialised reproduction	All	Ovoviviparity. No seasonal cycles, young born well-developed, minimizing mortality.	(Courtenay and Meffe 1989)
		Multiple mating strategies: polyandry often leads to multiple paternities within a brood	(Barbosa and Magurran 2011)
Abundant in native range	Wild	The guppy is abundant within its native range.	(Magurran 2005)
Eurytopy	Laboratory	Laboratory guppies tolerant to wide range of salinities.	(Chervinski 1984)
		Laboratory and wild guppies display considerable thermal adaptability	(Chung 2001), (Reeve et al. 2014)
	Feral (Brazil)	Guppies associated with stream degradation, indicating broad tolerance to physical and chemical environmental stressors.	(Casatti et al. 2006)
Phenotypic plasticity in life history traits	Wild (Trinidad)	Size at maturity and number of offspring differ according to resource ability.	(Reznick 1990)
		Sperm storage period longer in more isolated populations.	(Carvalho et al. 1996)
Human commensalism	Feral (India & worldwide)	The guppy is a popular ornamental aquarium fish. It is also introduced in human-inhabited areas to control malaria.	(Ghosh et al. 2005, Froese and Pauly 2013)
Genetics			
High genetic variability/resistance to loss of genetic variability	Introduced (Japan)	Evidence for multiple introductions enhancing variation	(Shoji et al. 2007)
	Introduced (Australia)	Invasive despite loss of neutral genetic diversity through bottleneck. High additive genetic variation in some cases. Possibly reflecting speed of population size increase after founding.	(Lindholm et al. 2005)
Behavioural			
Phenotypic plasticity in behaviour	Wild (Trinidad)	The guppy employs social learning to improve predation evasion.	(Kelley et al. 2003)
Antipredator behaviour modified by selection	Wild (Trinidad)	Schooling and predator inspection behaviours are modified by selection in a short period of time.	(Magurran et al. 1992)

* 'Wild': guppies studied in their natural habitat or caught and observed in the laboratory.
'Laboratory': those bred for several generations in the laboratory.
'Feral': those introduced and established outside of their natural range.

Guppies are known to feed on mosquito larvae and promote the depletion of their populations (Manna et al. 2008). This has made them a useful control tool for Malaria in places like India or México, among many others (Ghosh and Dash 2007, Deacon et al. 2011). As well, the guppy is a popular aquarium display species, which makes them subject of continuous trade (Magurran 1999). Guppies have been released from home aquariums after pet owners decide they do not want or can keep them (Carvalho et al. 1996). During the past century guppies have been released into environments outside their native range and have now established populations in at least 72 different countries across the globe (Deacon et al. 2011).

1.7 Vulnerable Mexican fish communities

Mexican fresh water fish diversity is particularly rich with about 506 species distributed in 47 families; this represents about 6% of all the species known in the planet (De la Vega-Salazar 2006). The majority of these species are concentrated in the Mexican Central Plateau (Dominguez-Dominguez et al. 2006). Guppies are reported to have invaded the Mexican Central Plateau and seem to be expanding the range of their invasive populations (Contreras-MacBeath et al. 1998). It is believed that they were introduced in an attempt to control mosquito larvae (De La Vega-Salazar et al. 2003).

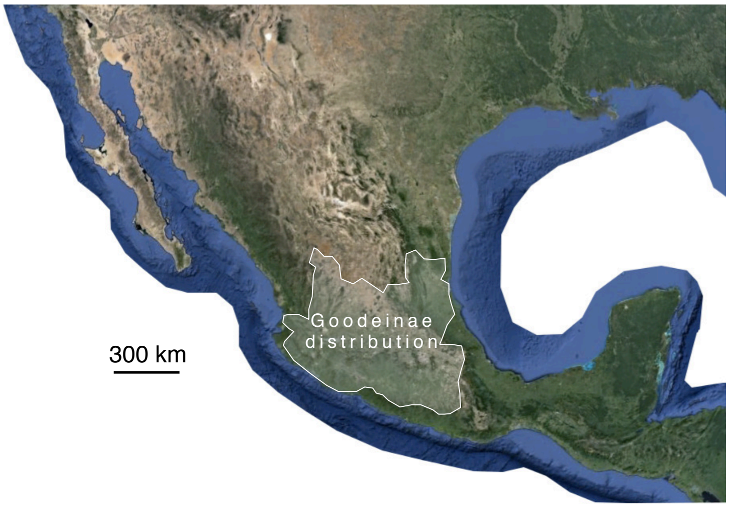
The main basins of this plateau are the Ameca, Balsas, Coahuayana, Mezquital, Lerma-Chapala-Santiago, and Pánuco, which are currently inhabited by species that have a strong endemic component, among which the Goodeinae family is one of the most threatened (De La Vega-Salazar et al. 2003, Dominguez-Dominguez et al. 2006). They are a clade consisting of *ca.* 55 species of small livebearing fish (Froese and Pauly 2013). Goodeinae populations are declining in the wild; habitat destruction, water pollution and invasive species are some of the factors influencing this decline

(Lyons 2011). Of the 17 species included in the IUCN Red List of Threatened Species, two are reported to be already extinct (Froese and Pauly 2013). Table 1.5 shows the list of goodeinae species and Map 1.1 shows the polygon where the Goodeinae family inhabits.

Table 1.5. Species of goodeidae registered in México (Froese and Pauly 2013).

Scientific name	**English common name**	**Author**
Allondontichthys hubbsi	Whitepatched splitfin	Miller & Uyeno, 1980
Allondontichthys polylepis	Finescale splitfin	Rauchenberger, 1988
Allondontichthys tamazulae	Tuxpan splitfin	Turner, 1946
Allondontichthys zonistius	Bandfin splitfin	Hubbs, 1932
Alloophorus robustus	Bulldog goodeid	Bean, 1892
Allotoca catarinae	Catarina allotoca	de Buen, 1942
Allotoca diazi	Patzcuaro allotoca	Meek, 1902
Allotoca dugesii	Opal allotoca	Bean, 1887
Allotoca goslinei	Banded allotoca	Smith and Miller, 1987
Allotoca maculate	Blackspot allotoca	Smith and Miller, 1980
Allotoca meeki	Zirahuen allotica	Álvarez, 1959
Allotoca regalis	Allotoca regalis	Álvarez, 1959
Alotoca zacapuensis	Zacapu allotoca	Meyer, Radda and Dominguez-Dominguez, 2001
Ameca splendens	Butterfly splitfin	Miller and Fitzsimons, 1971
Ataenioubius toweri	Bluetail goodea	Meek, 1904
Chapalichthys encaustus	Barred splitfin	Jordan and Snyder, 1899
Chapalichthys pardalis	Polka-dot splitfin	Álvarez, 1963
Chapalichthys peraticus	Alien splitfin	Álvarez, 1963
Characodon audax	Bold characodon	Smith and Miller, 1986
Characodon garmani	Parras characodon	Jordan and Evermann, 1898
Characodon lateralis	Rainbow characodon	Günther, 1866
Crenichthys baileyi albivallis	Preston White River springfish	Williams and Wilde, 1981
Crenichthys baileyi baileyi	White River springfish	Gilbert, 1893
Crenichthys baileyi grandis	Hiko White River springfish	Williams and Wilde, 1981
Crenichthys baileyi moapae	Moapa White River springfish	Williams and Wilde, 1981

Scientific name	English common name	Author
Crenichthys baileyi thermophilus	Mormon White River springfish	Williams and Wilde, 1981
Crenichthys nevadae	Railroad Valley springfish	Hubbs, 1932
Empetrichthys latos concavus	Raycraft Ranch poolfish	Miller, 1948
Empetrichthys latos latos	Pahrump poolfish	Miller, 1948
Empetrichthys latos pahrump	Pahrump Ranch poolfish	Miller, 1948
Empetrichthys merriami	Ash Meadows killifish	Gillbert, 1893
Girardinichthys ireneae	Girardinichthys ireneae	Radda and Meyer 2003
Girardinichthys multiradiatus	Darkedged splitfin	Meek, 1904
Girardinichthys viviparus	Chapultepec splitfin	Bustamante, 1837
Goodea atripinnis	Blackfin goodea	Jordan, 1880
Goodea gracilis	Dusky splitfin	Hubbs and Turner, 1938
Goodea luitpoldii	Green goodea	Steindachner, 1894
Hubbsina turneri	Highland splitfin	de Buen 1940
Ilyodon cortesae	Freckled splitfin	Paulo-Maya and Trujillo-Jiménez, 2000
Ilyodon furcidens	Goldbreast splitfin	Jordan and Gilbert, 1882
Ilyodon lennoni	Chacambero splitfin	Meyer and Göster, 1983
Ilyodon whitei	Balsas splitfin	Meek, 1904
Ilyiodon xantusi	Limones splitfin	Hubbs and Turner, 1939
Skiffia bilineata	Twoline skiffia	Bean, 1887
Skiffia francesae	Golden skiffia	Kingston, 1978
Skiffia lermae	Olive skiffia	Meek, 1902
Skiffia multipunctata	Spotted skiffia	Pellegrin, 1901
Xenoophorus captivus	Relict splitfin	Hubbs, 1924
Xenotaenia resolanae	Leopard splitfin	Turner, 1946
Xenotoca eiseni	Redtail splitfin	Rutter, 1896
Xenotoca melanosoma	Black splitfin	Fitzsimons, 1972
Xenotoca variata	Jeweled splitfin	Bean, 1887
Zoogoneticus purhepechus	Zoogoneticus purhepechus	Dominguez-Dominguez, Pérez-Rodríguez and Doadrio, 2008
Zoogoneticus quitzeoensis	Picotee goodeid	Bean, 1898
Zoogoneticus tequila	Tequila splitfin	Webb and Miller, 1998



Map 1.1. México. The Goodeinae family (white polygon) are distributed in the Central Mexican Plateau, including the Ameca, Balsas, Coahuayana, Lerma-Chapala-Santiago, Mezquital, and Pánuco basins. Redrawn from (De la Vega-Salazar 2006).

Goodeidaes are freshwater topminnows, which inhabit mostly shallow lakes and rivers, are largely omnivorous and are viviparous. Goodeidae species are sexually selective and there is variation within species in sexual dimorphism; unlike the guppy, they do not have an intromittent gonopodium and thus are incapable of reproducing without females consent to copulate (Ritchie et al. 2005). Males use a modification of their anal fin, called spermatopodium, to eject spermatophora into females when these consent to copulate (Moyaho et al. 2004).

The Mexican Godaidae resemble poeciliids in their size and habitat use (Valero et al. 2008). They are ideal fish for assessing the impacts of invasions as they are morphologically similar to guppies and share same ecological niches. Like guppies, goodeidaes forage on zooplankton, small insects and detritus, th ey both live in vegetated waters and have similar predators; both species are diurnal (Froese and Pauly 2013). Table 1.6 shows some of the characteristics species of goodeidae and poeciliidae used in this experiment share.

Table 1.6. Species of goodeidae and poeciliidae used in the experimental chapters of this thesis and some of tis characteristics (Froese and Pauly 2013, IUCN 2013).

Species	**Size range (cm)**	**Main food**	**Habitat characteristics**	**IUCN Red List Status**
Goodeidae				
Ameca splendens (Chapters 3 and 4)	2-8	mainly plants and detritus, smaller animals	freshwater, tropical, demersal, pH: 6-8, 26º-32ºC, non-migratory	Extinct in the wild*
Zoogoneticus tequila (Chapter 6)	2-6	mainly plants and detritus, smaller animals	freshwater, tropical, benthopelagic, pH: 6.5-7.5, 20º-24ºC, non-migratory	Critically endangered
Xenotoca eiseni (Chapter 6)	2-6	mainly plants and detritus, smaller animals	freshwater, tropical, demersal, pH: 6-8, 15º-32ºC, non-migratory	Not evaluated
Skiffia Bilineata (Chapter 6)	2-6	mainly plants and detritus, smaller animals	freshwater, tropical, demersal, pH: 7-7.5, 22º-28ºC, non-migratory	Not evaluated
Girardinichthys viviparous (Chapter 6)	2-6.5	mainly plants and detritus, smaller animals	freshwater, tropical, benthopelagic, 20º-22ºC, non-migratory	Critically endangered
Poeciliidae				
Poecilia reticualta (all chapters)	2-6	mainly smaller animals, plants, detritus	freshwater, tropical, brackish, benthopelagic, pH: 7-8, 18º-28ºC, non-migratory	Potential pest
Poecilia picta (Chapter 2)	2-5	plants, detritus, smaller animals	freshwater, tropical, brackish, benthopelagic, pH: 7.5-8.2, 26º-28ºC, non-migratory	Not evaluated
Poecilia sphenops (Chapter 2)	2-6	mainly smaller animals, plants, detritus	freshwater, tropical, brackish, benthopelagic, pH: 7.5-8.2, 18º-28ºC, non-migratory	Not evaluated
Poeciliopsis infans (Chapter 3)	2-5	plants, detritus, smaller animals	freshwater, tropical, benthopelagic, 22º-25ºC, non-migratory	Not evaluated

*Although reported in the IUCN Red List as extinct, a few populations still persist in the wild in Jalisco state, México (personal observation, 2013).

Goodeidaes are considered endangered with population declines directly attributed to guppy invasion (Valero et al. 2008, Valero et al. 2009). Unlike poeciliids, both males and females continue growing after reaching maturity and they do not store sperm, thus they need to copulate to produce new broods. Fertilized ova depend on maternally supplied nutrients for development and growth and there is no evidence of maternal care after birth (Macias Garcia and Valero 2010).For instance, goodeidae females have been reported to suffer from guppy males harassment (Valero et al. 2008), which is potentially more deleterious for them than for guppy females (Magurran and Seghers 1994a) as goodeidae males do not have a gonopodium to fertilize females (Macias Garcia and Valero 2010). A well documented example of a goodeidae in the brink of extinction is that of the Picote de Tequila (*Zoogoneticus tequila,* Figure 1.4), it lives in a single, 4-m-wide pool in the Ameca Basin. There are less than 500 individuals and guppies outnumber them six to one (Magurran 2009).

Figure 1.4. Picote de tequila (*Zoogoneticus tequila*). Picture by Roman Slaboch for the Goodeid Working Group website.

1.8 Aims

Arriving to a new habitat is a challenge and an opportunity for invasive species, they will be exposed to new selective forces that can result in adaptation or adjustment to the new conditions ending in phenotypic changes (Simon and Townsend 2003, Nelson et al. 2011). The guppy, is a species known for its ability to adjust to new conditions, like those they find outside their native range in places where they have been able to establish successful populations (Magurran 2005, Deacon et al. 2011). Behavioural traits are more flexible, even reversible and therefore easier to adjust to new environmental conditions than, for example, morphological or life history traits that require more time and tend to be more permanent (Magurran 1999, Sol et al. 2002). Indeed, morphological adaptations may be ineffective if they are not accompanied by the adequate behaviour (Magurran 1999). Information on the interactions between native and invasive species is a useful tool when designing effective strategies to mitigate and prevent invasive species negative outcomes (Sakai et al. 2001, Alcaraz et al. 2005). **The aim of my doctoral research project is to explore what behavioural traits have consequences for invasion success. My specific objective is explore how interactions between invasive and native fish species shape an invader's ability to deal with novel conditions and improve its chances of success by avoiding the disadvantages of being part of a small group (Allee effects)**.

This thesis explores guppies' tendency to associate with conspecifics and heterospecifics when in Trinidad, guppies' native environment (Chapter 2) and in their invasive environment (Chapter 3). As explained before, being social confers benefits that are exploited by invaders to establish viable populations and avoid Allee effects. I ask if a high sociability level is a trait guppies exploit as a native, as an invasive species or both. After discussing guppies' heterospecific sociability, I investigate whether this sociability provides guppies an actual benefit. Chapter 4 and 5 explore guppies' exploratory behaviour (Chapter 4) and adjustment of their swimming patterns in accordance with that of another knowledgeable group of fish (Chapter 5). The last experimental chapter (Chapter 6) discusses the outcomes of an experiment were actual foraging benefits (food location and time spent eating) were tested. In Chapter 6 I discuss guppies gains when increasing shoal size with other guppies or increasing it with the native Mexican goodeidae. As well, I explored if different tendencies towards the various tested species are related to the gains of guppies when shoaling with them. Finally, in Chapter 7 I discuss the outcomes of all the experiments carried out in this project under the invasion context and the gains invaders might have when being social with natives.

Chapter 2

Association tendency and preference for heterospecifics in an invasive species

2.1 Abstract

Animals gain benefits by forming groups with phenotypically and behaviourally similar individuals. The most common groups are homogenous, composed by conspecifics, although in some cases associations of similar organisms of different species have been reported. In this study, I tested the prediction that the Trinidadian guppy, *Poecilia reticulata,* a fish that has successfully invaded at least 70 countries, will shoal with heterospecifics to increase group size and avoid the disadvantages of being part of a small population (Allee effects). I measured shoaling tendency and shoal companion preference in wild-caught female guppies when they encounter two heterospecific species: the native *Poecilia picta* and the non-native *Poecilia sphenops* - a poeciliid recently introduced in Trinidad. Results show that guppies have a higher tendency to shoal with conspecifics but if the alternative to be with them is be alone, they readily shoal with both species even when they have had no previous experience with other poeciliids. Individuals in these associations could benefit from safety in numbers along with other advantages of group living. This predisposition to associate with other species that share similar ecological conditions could help explain the guppy's success as invasive species as it enables them to increase their shoal size during the first stages of invasion and thus avoid Allee effects. [1]

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[1] A version of this chapter was published in February 2014 in the scientific journal *Behaviour*. Additional material and discussion is included in this thesis chapter.

M. Camacho-Cervantes, A. F. Ojanguren, A. E. Deacon, I. Ramnarine, A. E. Magurran (2014) Heterospecific and conspecific shoaling tendency and preference of three native guppy populations. *Behaviour*. DOI:10.1163/1568539X-00003169

2.2 Introduction

2.2.1 Sociability

Gregarious behaviour provides individual benefits, these may include a reduction in predation risk and faster food location, also they benefit from each other during vigilance tasks (Hamilton 1971, Pavlov and Kasumyan 2000, Couzin and Krause 2003). However, being part of a group also comes at a price, for example an increase of competitive interactions and depletion of resources (Magurran and Seghers 1991, Krause and Ruxton 2002). Groups are usually composed of phenotypically similar individuals, who may be genetically related (Griffiths and Magurran 1999). Indeed, some of the benefits of being part of a group, such as the confusion effect, require that all members of the group look the same (Landeau and Terborgh 1986, Croft et al. 2009a).

Many species of fish live in shoals. A shoal is a group of fish that remains together, and in doing so gains benefits such as protection from predators and an increase in foraging efficiency (Pitcher 1983, Magurran and Seghers 1991, Chapman et al. 2008, Croft et al. 2009a, Piyapong et al. 2011). The benefits of grouping increase when individuals are phenotypically and behaviourally similar (Dyer et al. 2009). Thus, individuals typically assort their shoals by size (Ward and Krause 2001) and parasite load (Barber 2003). Since conspecifics are likely to be more similar in appearance, given the option, individuals should show a preference to associate with individuals of the same species.

2.2.2 Heterospecific interactions

Even though social interactions occur mostly within species, associations between individuals of different species have been found in many taxa, including reef fish (Sazima et al. 2007), primates (Stensland et al. 2003), and birds (Powell 1989, Campobello et al.

2012). The benefits of grouping have been confirmed in heterospecific associations as well as in conspecific ones (Morse 1977, Ward et al. 2002). As a result, when individuals of different species are similar in their phenotype and obtain benefits, they may form heterospecific groups (Landeau and Terborgh 1986). For example, killifish (*Fundulus heteroclitus* and *F. diaphanus*) in Morice Lake have been found to associate with golden shiners (*Notemigonus crysoleucas*), white suckers (*Catostomus commersoni*), threespine sticklebacks (*Gasterosteus aculeatus*) and fourspine sticklebacks (*Apeltes .quadracus*) (Krause et al. 2005). In general, when the individuals that conform the group gain shared benefits with a low or none competition cost they will remain together, whethear they are the same species or not (Alexander 1974, Krause and Ruxton 2002, Stensland et al. 2003). Farine (2014) summarized some of the benefits that have been proved in heterospecific associations; these include reduced predation risk (Harrison and Whitehouse 2011), faster food location (Aplin et al. 2012), increased foraging efficiency (Sridhar et al. 2009). The last two were assessed in this thesis (Chapter 5).

2.2.3 The social guppy

The guppy (*Poecilia reticulata*) is a highly social species with a strong shoaling tendency (Magurran 2005). Guppies shoal in a variety of situations. They gather in shoals specially to get protection from predators, during foraging activities and to find mating partners. As the guppy is a dimorphic species, female and male guppies have a different shoaling strategy, being females the core of shoals as they are able to recognize individuals and choose to remain together (Griffiths and Magurran 1998). Guppies occurs naturally in Trinidad, Guyana, Venezuela and Surinam (Magurran et al. 1995, Magurran 2005) and possess reproductive adaptations including multiple mating and sperm storage, which enable a few animals or even a single pregnant female to found viable populations (Deacon et al. 2011, Sievers et al. 2012). As a consequence of these traits, and due to repeated introductions for mosquito control or aquarium release, guppies have established themselves in freshwater habitats in every continent except Antarctica (Deacon et al. 2011).

2.2.4 Aims

Certain characteristics that individuals show in their native environments might be beneficial when they face unknown environments in an invasive context. Invading fish, like the guppy, will be exposed to native heterospecifics with whom they have had no previous opportunities to shoal. Here I tested the hypothesis that guppies will associate with individuals of two other species of poeciliid (of which the females are similar in appearance) even if they have not encountered them before. I measured two aspects of shoaling behaviour: shoaling tendency (willingness to associate with individuals of a given species when this is the only association option) and shoaling preference (inclination to shoal with one species over another). Wild caught Trinidadian guppies were given them the opportunity to associate with the native 'swamp guppy' (*Poecilia picta*) and the exotic 'liberty molly' (*Poecilia sphenops*). All three species occur in rivers in Trinidad, although *P. sphenops* was introduced to the island in the latter part of the 20th century (Kenny 1995). They occupy similar habitats in the rivers and swamps where they coexist, and share similar swimming patterns and social behaviour. Indeed, when collecting in the sites where guppies coexist with *P. picta* (Charlieville) and *P. sphenops* (Maraval) the seine net contained fish from both species. I only collected and used females in this experiment because they have stronger shoaling tendencies than males, which allocate more time to mating activities (Magurran 2005).

2.3 Methods

Experiments were carried out at The University of the West Indies in Trinidad during July 2012. All fish used were collected from the wild (using hand seine nets) in three different locations: (1) Acono – where guppies are the only species of poeciliid fish present; (2) Charlieville – where guppies and the native *P. picta* coexist; and (3) Maraval – where guppies and the invasive *P. sphenops* coexist (Map 2.1, Figure 2.1). All fish were carefully transported to the laboratory in buckets filled with water from the capture location and sorted by species and location. Stock tanks (45 L) contained 20 to 25 fish each and were set up with aged tap water, which was treated with STRESS COAT®. Each tank contained a filter and water pump, rocks and plants. Water temperature was kept at about 24° C and photoperiod was 12L: 12D from 6:00 to 18:00 hrs. Tanks were visually isolated from one another with an opaque sheet. Individuals observed during trials (focal fish) and individuals that composed the shoals (shoal fish) were all females. Focal fish and individuals for the shoals of each species and location were kept in separate stock tanks for two weeks before starting the experiment, to avoid familiarity effects (Griffiths and Magurran 1997a). Fish were fed TetraMin® flakes daily around one hour before, and immediately after, every day observations. After the experiment was completed (28 days), all fish were returned to the location from which they had been collected.

Map 2.1. Localities in the island of Trinidad where experimental fish were collected. In Acono guppies were the only species of poeciliid fish present; in Charlieville there where guppies and the native *P. picta*; and in Maraval there where guppies and the invasive *P. sphenops.*

a) Acono

b) Charlieville

c) Maraval

Figure 2.1. Localities where experimental fish were collected, using hand seine nets. a) Acono, where guppies were the only poeciliid present; b) Charlieville, where guppies coexist with *P. picta*; c) Maraval, where guppies coexist with *P. sphenops* (pictures taken by M. Camacho-Cervantes).

I explored two different aspects of shoaling behaviour in Trinidadian guppies: *tendency* and *preference*. The shoaling *tendency* part of the experiment was designed to test willingness to associate with a shoal (heterospecific or conspecific) when the options were to either join it or remain solitary. The shoaling *preference* aspect was designed to test for the predilection to associate with a shoal from one species over that of a different species. I used a repeated measures approach; each focal fish performed five trials in total. Three trials for *tendency*, to test the willingness of guppies to shoal with either other guppies or *P. picta* or *P. sphenops* respectively; and two trials for *preference*, guppies with *P. picta* and guppies with *P. sphenops*, to examine the preference for conspecifics or heterospecifics (Table 2.1).

Table 2.1. Species used in the trials for the tendency and preference sections of the experiment. Each focal female was tested in five observation trials, three for tendency and two for preference. A total of 14 replicates were carried out.

Trial	Tendency	Preference	Sample size
1	*P. reticulata*		14
2	*P. picta*		14
3	*P. sphenops*		14
4		*P. reticulata – P. picta*	14
5		*P. reticulata – P. sphenops*	14

I decided to use a repeated measures approach because this minimised the number of fish required to be caught from the wild, by allowing higher statistical power at a smaller sample size than other approaches. After these trials the focal fish was not used again. In between trials this focal female was kept in a stand-by tank (20 x 20 x 15 cm) for a period of 25 to 35 minutes during which they never showed signs of stressed behaviour for any longer than 5 minutes. After completion of the trials the focal female was placed in a different stock tank and not reused. Fish for the shoals were randomly selected for each observation from two pools of approximately 30 fish per each species to avoid pseudoreplication (Hurlbert, 1984). Observations were made between 09:00 and 17:00 hours using two identical glass tanks (45x30x30 cm). Each tank contained two transparent plastic bottles (8 cm diameter), perforated so that the water would circulate between the tank and the bottles (Figure 2.2 and 2.3). A group of fish inside the bottle formed a shoal; the focal fish could see and smell the fish inside the bottle, but not interact physically with them.

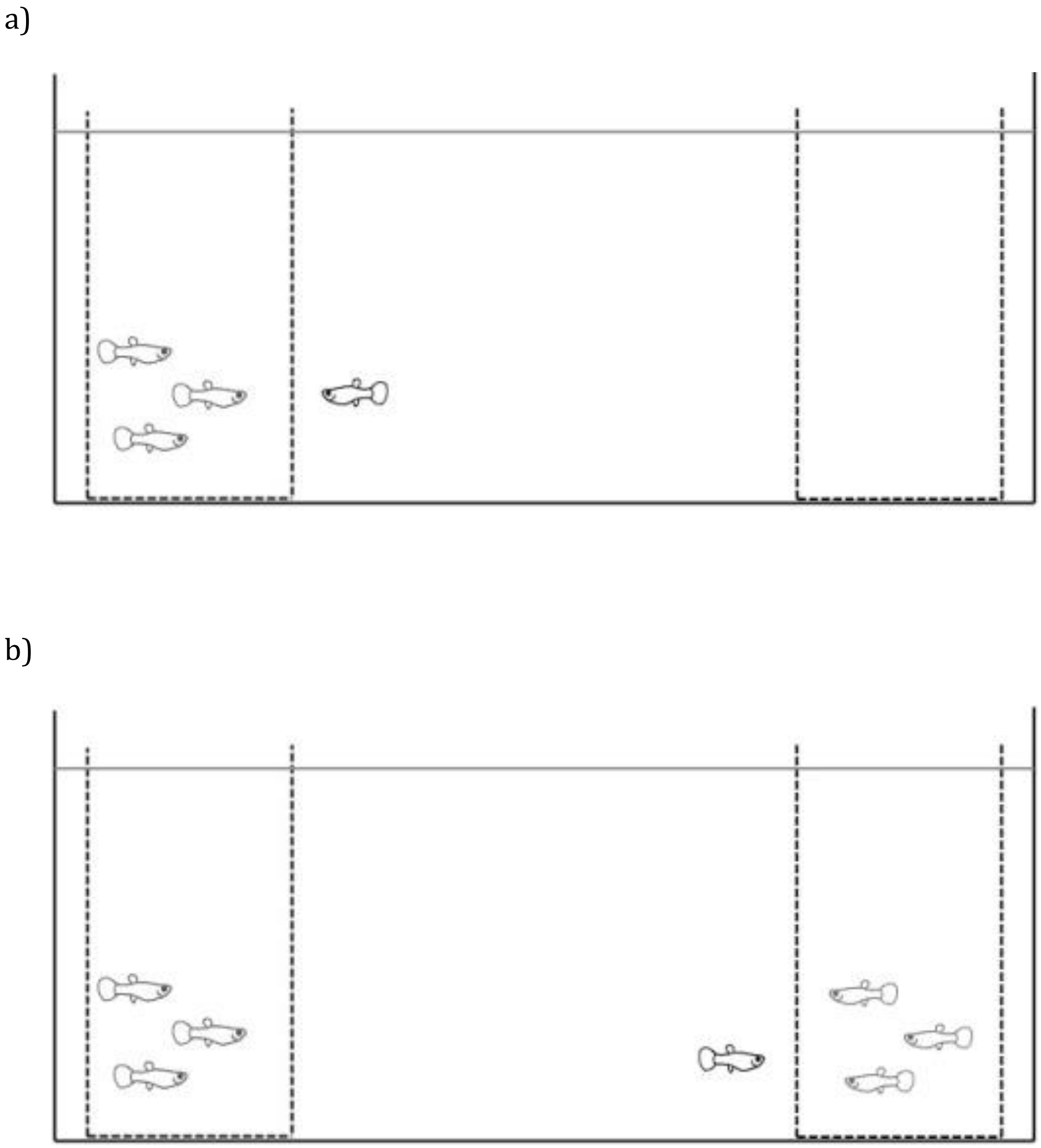

Figure 2.2. Diagram of the tank set up. For the shoaling tendency trials (a), one of the bottles remained empty and for the shoaling preference part (b) each bottle contained a shoal. Time spent shoaling was recorded whenever the fish was within one body length of the bottle containing a shoal.

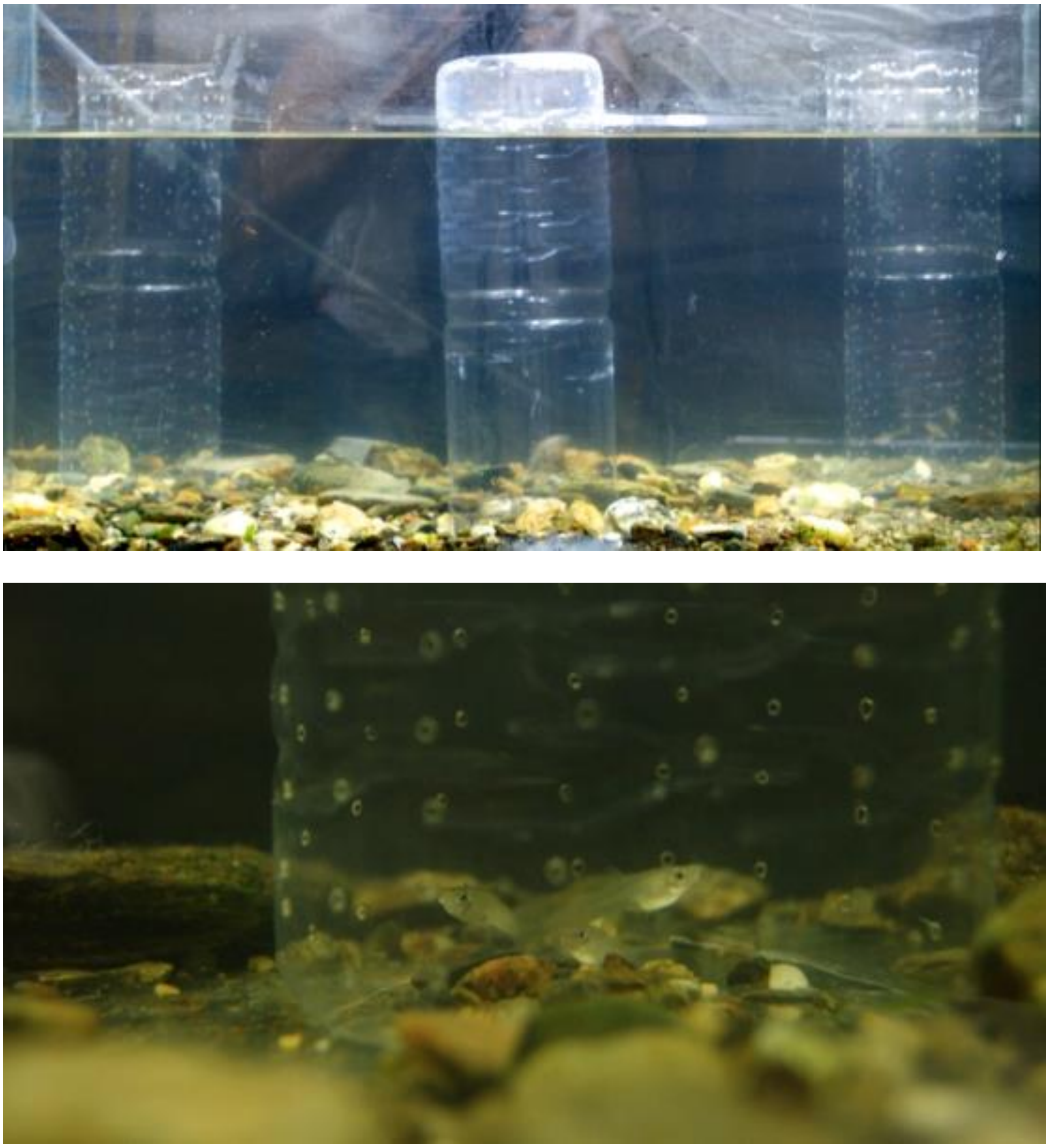

Figure 2.3. Observation tank and bottle containing a guppy shoal while acclimatizing (pictures taken by M. Camacho-Cervantes).

During the trials for shoaling *tendency*, one bottle contained a group of fish and the other remained empty to ensure fish were associating with the shoal contained in the bottle rather than with the bottle itself. For the *preference* trials, both bottles contained shoals. All shoals were composed of three size-matched females and left to acclimatize in the bottles for 10 minutes. The focal fish was introduced to a third bottle and allowed to settle down for at least 10 minutes and no longer than 20 minutes before being released by gently lifting and removing this bottle from the tank. Each observation lasted 10 minutes, during which association was recorded as the amount of time the focal fish spent within one body length of the bottle containing a shoal. The order in which trials were presented to each focal was randomized and the bottle side of the shoals was changed in a random order to avoid side-biased results. Fourteen Trinidadian guppies from each of the three localities, total of 42 individuals, were tested for *preference* and *tendency*. Each fish performed all the trials for both sections. All focal and shoal individuals were photographed as shown in Figure 2.4 and using the ImageJ software I determined their size (Schindelin et al. 2012). Individuals ranged from 1.7 to 3.4 cm total body length. However, fish were size-matched within each observation (maximum difference in size 0.97 cm) and size did not explain any significant proportion of the variation in association time ($R^2 < 0.08$).

Figure 2.4. Example of a focal photograph to measure its size. Using the ImageJ software (Schindelin et al. 2012), I measured the longitude in pixels of a line covering 1 cm of the ruler and then the number of pixels in a line drawn from the mouth to the beginning of the caudal fin to find the fish longitude in centimetres (picture by M. Camacho-Cervantes).

2.3.1. Data Analysis

To analyse the data I used SPSS® statistical software. All variables were expressed as proportions and Arcsine transformed for their distributions to approach normality (Sokal and Rohlf 1981). To test the *tendency* of focal individuals to join a shoal, I compared the observed duration of the focal female's time (in s) in the preference zone against the time she would be expected to be in this zone (30s) if she were swimming randomly in the tank with a one-way t-test. The expected time was calculated using the proportion of the tank volume represented by the association section, and calculating this same proportion for the total trial duration. To test for differences in tendency between populations, species or the interaction between them, I performed a General Linear Model (GLM) repeated measures test with the Arcsine of the proportion of time spent with each shoal species as the within-subjects factor, and the origin population of the focals as the between-subjects factor.

For the *preference* analysis, I used a GLM repeated measures design to test for the difference in the preference for conspecifics over heterospecifics. For this each shoal species was treated as the within-subjects factor and the origin of the focal as the between-subjects factor. To examine the *preference* within the two species presented as a shoaling option, I used one-way t-tests to test if the difference between the times spent with either shoal was significantly different from zero, as a null difference in the times would mean no preference for either of the shoals.

2.4 Results

2.4.1 Shoaling Tendency

Guppies from the three populations spent more time in the proximity of the shoal in the bottle than would be expected if they were swimming randomly, regardless of the species of fish inside the bottle (one-way *t*-test, $t_{13} > 5.51$, $p < 0.001$, Figure 2.5). Tendency to shoal, measured as the time focal fish spent associating with any given shoal, was affected by the species of the shoal (GLM, $F_{2, 78} = 5.25$, $p = 0.007$) but not by the origin of the focal fish (GLM, $F_{2, 39} = 1.19$, $p = 0.312$); the interaction between focal populations and shoal species was not significant (GLM, $F_{4, 78}=1.41$, $P=0.239$; Figure 2.5). Post hoc analysis revealed that tendency to shoal with *P. reticulata* was higher than to shoal with *P. picta* (paired *t*-test, $t_{41} = 3.54$, $p = 0.001$) and there was no difference between the tendency to shoal with *P. reticulata* and *P. sphenops* (paired *t*-test, $t_{41} = 1.34$, $p = 0.187$) nor between *P. picta* and *P. sphenops* (paired *t*-test, $t_{41} = -1.76$, $p = 0.086$).

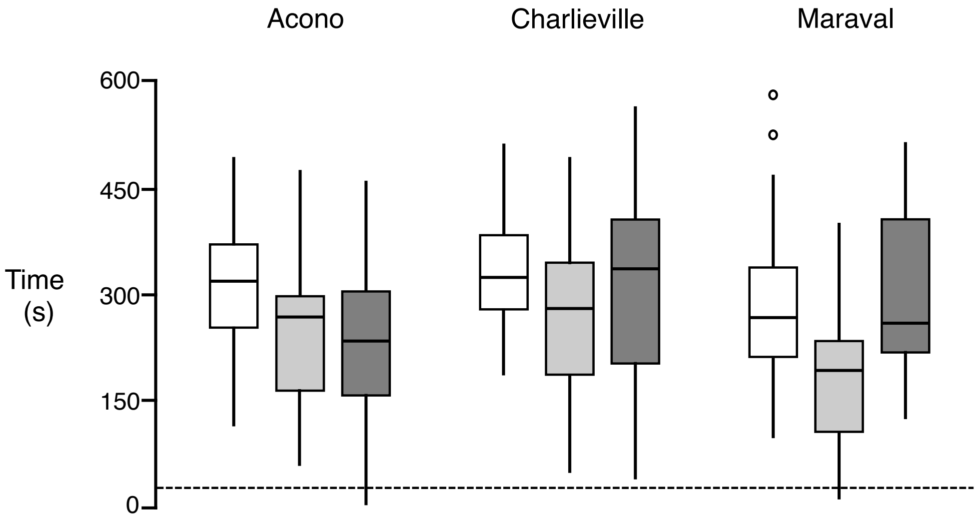

Figure 2.5. Shoaling tendency. White bars represent the time (out of a possible maximum of 600 seconds) focals from Acono (where guppies were the only poeciliid present), Charlieville (where guppies coexist with *P. picta*) or Maraval (where guppies coexist with *P. sphenops*) spent with *P. reticulata* shoals, light grey is for *P. picta* shoals and dark grey for *P. sphenops* shoals. Dotted line shows the expected time of association if randomly swimming (30 s). Horizontal lines in the bars represent the median, boxes indicate interquartile ranges and vertical lines show the range excluding outliers (circles).

2.4.2 Shoaling Preference

The preference and tendency tests confirmed Trinidadian guppies readily shoal with heterospecifics. There were no significant differences between localities in terms of the extent of their preference for conspecifics (GLM, $F_{2, 39} = 1.49$, $p = 0.237$) nor between species (GLM, $F_{1, 39} = 0.26$, $p = 0.724$) and there was no interaction (GLM, $F_{2, 39} = 1.41$, $p = 0.255$, Figure 2.6).

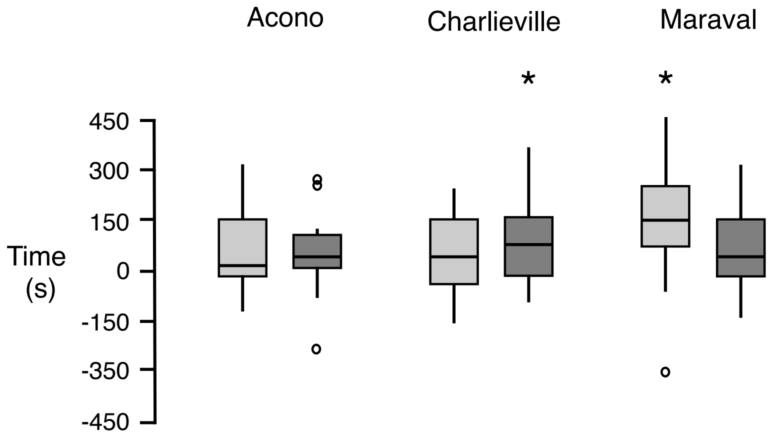

Figure 2.6. Preferences between conspecifics and heterospecifics. The vertical axis shows the difference in time spent with one over another shoal. Positive numbers show preference for conspecifics and negative numbers show preference for heterospecifics. Light grey bars represent the difference in time between *P. reticulata* and *P. picta*, grey bars represent the difference between *P. reticulata* and *P. sphenops*. Asterisks indicate significant differences from zero. Guppies from Acono had no preference between conspecifics and heterospecifics (one-way *t*-test, $t_{13} > 1.51$, $p > 0.13$); guppies from Charlieville (where they coexist with *P. picta*) had no preference between *P.picta* and conspecifics (one-way *t*-test, $t_{13} = 1.586$, $p = 0.137$) but preferred conspecifics over *P. sphenops* (one-way *t*-test, $t_{13} = 2.62$, $p = 0.021$), and focals from Maraval (where they coexist with *P. sphenops*) showed no preference between *P. sphenops* and conspecifics (one-way *t*-test, $t_{13} = 2.11$, $p = 0.054$) but preferred conspecifics over *P. picta* (one-way *t*-test, $t_{13} = 2.88$, $p = 0.013$).

2.5 Discussion

I have shown that wild-caught Trinidadian female guppies have a strong *tendency* to associate with other poeciliid females and, often, no *preference* for conspecifics over heterospecifics. I conclude that female guppies readily shoal with morphologically similar fish regardless of the species. This builds on Warburton and Lees' (1996) results, which showed that guppies are willing to associate with swordtails (*Xiphophorus helleri*) when reared with them. In a similar way, Schlupp and Ryan (1996) demonstrated that *Poecilia latipinna* and *Poecilia formosa* preferred a larger heterospecific shoal than a smaller conspecific one. In the case of gynogenetic *P. formosa* this outcome is expected as they need heterospecific males in order to reproduce, but for *P. latipinna* it suggests that the non-reproductive benefits of associating with heterospecifics must outweigh the costs. Although associations with heterospecifics could be beneficial for guppies, they may have a cost for the other species. For example, Valero et al. (2008) argued that heterospecific associations between guppies and the goodeid *Skiffia bilineata* lead to a decrease in the goodeid's fitness due to the harassment of *S. bilineata* females by guppy males.

Invasions typically begin with the introduction of small numbers of individuals. In fact, mesocosms experiments have shown that guppies can establish viable populations with just one pregnant female (Deacon et al., 2011). However, since guppies are social and form large shoals in their natural range (Croft et al., 2006), it remains unclear how they deal with the need for large numbers of conspecifics in order to improve food location and effective defence against predators (Stephens & Sutherland, 1999).

The disadvantage of being part of a small population is known as the Allee effect and has been reported in some fish species (Stephens & Sutherland, 1999; Drake & Kramer, 2011). Based on my findings that guppies readily associate with heterospecifics, I hypothesise that associating with heterospecifics allows guppies to be part of larger groups, mitigating the Allee effect. Behaviour may play a crucial role in enabling the invaders to form a viable population as individuals could choose to increase their group numbers by associating with others (Holway & Suarez, 1999; Stephens & Sutherland, 1999). In the two locations were guppies coexisted with other species (Acono and Maraval), guppies shoal with heterospecifics (pers. obs.) and the seine net collected mixed species groups of fish, which suggests that the daily activity patterns of these species are similar. Here, I examined the shoaling behaviour of female guppies when given the opportunity to associate with groups of similar species. Females have a higher shoaling tendency than males and devote more time to anti-predator responses (Magurran, 2005). Males move between these female shoals in the pursuit of mating opportunities (Griffiths & Magurran, 1998; Croft et al., 2003). Wild shoals of guppies are not haphazard associations but rather form social networks (Edenbrow et al., 2011) and in the case of females, actively choose shoaling partners (Croft et al., 2004). It would be interesting in future work to ask how social interactions between heterospecific females shape social interactions, and how they influence network structure. Moreover, to demonstrate that invading fish benefit from heterospecific shoaling, the next step will be to find evidence of information exchange (Couzin, 2009) about food and predators, and whether the advantages of group living, such as more effective predator avoidance, are shared with all group members.

It is increasingly clear that behaviour plays a role in the colonization of invasive species and can influence the probability that an invasion succeeds (Holway & Suarez, 1999), yet there have been relatively few investigations of the mechanisms involved. This study is a first step towards the identification of heterospecific shoaling as an invasive success trait. Further research should be carried out to demonstrate the survival benefits of heterospecific association in an invasive species. Information on the interactions of exotic species with native species will help us to understand which species are likely to become established when introduced (Alcaraz et al., 2005) and may also be important in identifying those assemblages most vulnerable to invasion. In the next chapter, I will go further into guppies willingness to associate with heterospecific by testing if the tendency they showed in Trinidad to shoal with other species remains in their invasive context.

Chapter 3

High sociability as a key trait for invasion success: a case study in the Mexican Central Plateau

3.1 Abstract

From all the species that arrive to a novel environment, very few manage to form a viable population. The guppy, a very successful invader, is a highly social species that performs some of its vital tasks (*e.g.*, foraging, avoiding predators) in groups. It is known guppies are willing to associate with native species, but it is still uncertain if native species associate with them as well, thus facilitating successful establishment. I found that guppies readily associate with native heterospecifics. At the same time, native heterospecifics were also inclined to associate with the invasive guppies. My results suggest that guppies might have a greater chance of successfully invading an area when arriving in environments were native species cooperate with them to enhance their chances of surviving by shoaling with them.

3.2 Introduction

3.2.1 Facilitation between species

Invasions typically begin with few individuals colonizing a novel environment; this is one of the crucial stages of invasion (Mack et al. 2000). During this stage, individuals are vulnerable to the disadvantages of being part of small groups; known as Allee effects, which decrease their establishment success, see 1.4 Allee effects section in the Introduction (Courchamp et al. 2008, Tobin et al. 2011). The term facilitation is used when interactions between individuals have a net positive effect, it can occur within species and between species (Bertness and Callaway 1994). During invasion, facilitation could be a key promoting establishing success (Sheley and James 2014).

Invaders might have a weak defence against generalist local predators, be poor competitors against natives or lack mutualists to perform vital tasks (Alpert 2006). Thus, they could require time to adapt to the novel community and local abiotic conditions; invaders might survive for only a few generations or form a viable population with a limited range of distribution (Andow et al. 1999, Sakai et al. 2001). On the other hand, some invaders might be better in novel areas due to the lack of enemies, some might be in need of other individuals to effectively forage or escape predation (Simberloff and Von Holle 1999).

Once a species has succeeded in reaching a new site, they are likely to be destroyed quickly by a multitude of physical or biotic agents of the novel environment (Andow et al. 1999). For instance, native species might resist invasion by parasites or predate the introduced ones (Levine 2000). Sometimes, even after invaders survived to have descendants these may only live for a few generations before going locally extinct (Mack et al. 2000). In some cases, the environment where invasive species arrive has particular characteristics that might enhance invaders chances of successfully form a viable population (Mack et al. 2000, Simberloff 2006). For example, in Great Britain, the invasive zebra mussel (*Dreissena polymorpha*) has been found to provide a substantial food source for the American signal crayfish (*Pacifastacus leniusculus*) without suffering declines in its populations, enhancing signal crayfish's establishment success (zu Ermgassen and Aldridge 2011). Most of the animal studies on facilitation have been made between invasive species, identified as the key interaction necessary for invasion meltdown – the acceleration of species invasions through interspecies interactions – by Simberloff (1999, 2006).

3.2.2 Native species under risk

The Trinidadian guppy (*Poecilia reticulata*) is a very successful invasive species native to Trinidad, Guyana, Venezuela and Surinam (Magurran et al. 1995). They possess behavioural and phenotypic traits that have enabled them to invade over 70 countries around the globe (Deacon et al. 2011). Guppies have physiological traits that enhance their chances of succeeding as an invader, for example they are able to form a viable population with just a pregnant female (Magurran 2005). However, being a highly social species, this represents a challenge for them in aspects such as finding food and protecting themselves from predators (Magurran 2005, Croft et al. 2009a), see also 2.1.3 The social guppy section in the Introduction of Chapter 2. Guppies are known to associate with other species, potentially to overcome small group disadvantages (Warburton and Lees 1996, Camacho-Cervantes et al. 2014).

In México, guppies can be found in many locations (Gesundheit and Macias Garcia 2007), including the Lerma-Santiago River system, the main basin of the Mexican High Plateau and a watershed noted for its high levels of endemicity. Endemics include Goodeinae, a clade consisting of *ca.* 45 species of small livebearing fish (Froese and Pauly 2013), 17 of which are included in the IUCN Red List of Threatened Species (IUCN 2013); see also (De La Vega-Salazar et al. 2003, Dominguez-Dominguez et al. 2008). The Goodeinae are mostly omnivorous freshwater topminnows that inhabit shallow ponds, lakes and rivers. They are the focus of this study because many species are morphologically similar to guppies, feed on the same resources and occupy the same habitat. See Map 1.1 and 1.7 Vulnerable Mexican fish communities section in the Introduction for more details. Guppies have been reported to harass goodeidae females (Valero et al. 2008) and in some cases population declines have been directly attributed to guppy invasions (Magurran 2009).

3.2.3 Aims

Mixed species associations occur in many taxa (Sazima et al. 2007, Farine and Milburn 2013) when they are beneficial to the participants (Ward et al. 2002). Even very phylogenetically distant species (*e.g.* monkeys and birds) are able to locate food faster when foraging together (Boinski and Scott 1988). This is a way in which invasive species could gain benefits and potentially overcome Allee effects (Chapter 5). However, it is uncertain if guppies are the only ones inclined to join heterospecific shoals (Camacho-Cervantes et al. 2014) and native species avoid them, or if native species are as well willing to associate with heterospecifics, in this case the invasive guppy and another native topminnow. Here I tested the hypothesis that Mexican topminnows (*Skiffia bilineata* and *Poeciliopsis infans*), like guppies, associate with heterospecific individuals; and this could help invasive guppies to avoid Allee effects by increasing the group size. Willingness of natives to interact with invaders could be one of the environmental characteristics of a place that increase its risk of invasion. In this Chapter I aim to explore the social interactions of invasive guppies with native Mexican topminnows, particularly if the tendency guppies showed to associate with heterospecifics (Chapter 2) remains when they are invaders and if natives are as well willing to associate with them.

3.3 Methods

Experiments were carried out at the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH) in Morelia, México, during the months of March and April 2012. Experimental fish were collected from the wild (using hand seine nets) in three different locations: *Poecilia reticulata* were collected in Maravatío, Michoacán; *Skiffia bilineata* were originally from Álvaro Obregón in Michoacán; and *Poeciliopsis infans* from La Mintzita, Michoacán (Map 3.1 and Figure 3.1). Species were collected in different locations to ensure that none of them had previous contact with the other species. All fish were carefully transported to the laboratory in breathable plastic bags filled with water from the sites and kept in separate tanks. Stock tanks (50 L) were set up with aged tap water treated with STRESS COAT® and contained 25 to 30 fish each. All tanks contained a filter and water pump, some rocks and plants and were visually isolated from each other. Water temperature was kept at about 24° C and photoperiod was 12L: 12D from 7 am to 7 pm. Fish were fed daily at least one hour before observations and at the end of it with commercial flake food. Each focal fish was used only once, after the completion of the experiment (37 days) all fish were returned to the location from which they were collected. Only females were used in this experiment to exclude mating behaviour, as males and females allocate time in a different way being females the ones that devote more time to shoaling. Focal fish and individuals for the shoals from each species and location were kept separately in different stock tanks at least two weeks before starting observations to avoid familiarity effects (Griffiths and Magurran 1997a).

Map 3.1. Localities in the Mexican Central Plateau where experimental fish were collected. *P. reticulata* were collected in Maravatío, *S. bilineata* in Álvaro Obregón, and *P. infans* in La Mintzita.

a) Maravatío

b) Álvaro Obregón

c) La Mintzita

Figure 3.1. Localities where experimental fish were collected, using hand seine nets. a) Maravatío, where only guppies were present; b) Álvaro Obregón, where only *S. bilineata* were present; c) La Mintzita, where only *P. infans* were present (picture a) and c) by Adrián Ortega and b) by M. Camacho-Cervantes).

Observations were made between 1000 and 1700 h using a medium size glass tank (50 cm x 35 cm x 35 cm) that contained two bottles (diameter 8 cm) – during observations only one bottle contained a shoal and the side of it was rotated after each observation (Figure 3.2 and 2.3 in previous chapter). The bottles were perforated to allow chemical cues to travel freely in the tank. Fifteen focal fish of each species were tested using a repeated measures approach. Each focal was presented haphazardly with three shoals, one of each species (*P. reticulata*, *S. bilineata* and *P. infans*). Outside the trials, focals were kept in an individual tank (20 L) to keep track of their identity. Observations lasted 10 minutes, during which association was recorded whenever the focal fish was within one body length of the bottle containing the shoal. All fish were measured (see Figure 2.4 in Chapter 2) to explore the relationship between body size and association time.

Size of the focal individual relative to that of their shoal mates did not vary with the species of the focal or the shoal (ANOVA, $F_{2,126} < 1.63$, $p > 0.2$), nor did the relative size explain the tendency of fish to associate with *P. reticulata* ($r^2 = 0.004$, $p = 0.28$), *S. bilineata* ($r^2 = 0.001$, $p = 0.34$) or *P. infans* ($r^2 = 0.010$, $p = 0.54$).

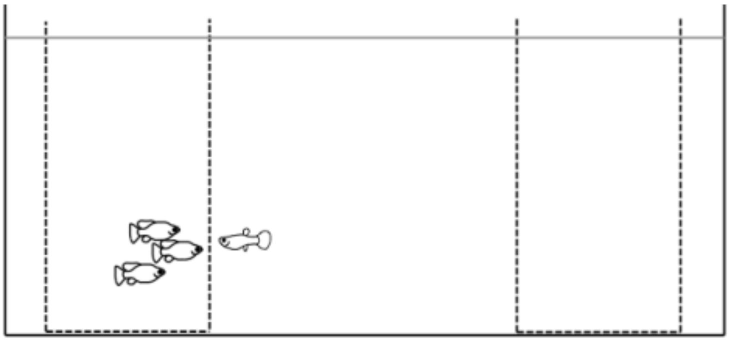

Figure 3.2. Diagram of the tank set up. One of the bottles remained empty and the bottle containing a shoal was changed each observation. Time spent shoaling was recorded whenever the fish was within one body length of the bottle containing a shoal.

3.3.1 Data analysis

To test the tendency of focal individuals to join a shoal, I compared the observed duration of the focal female's time (in s) in the preference zone against the time she would be expected to be in this zone (23s) if she were swimming randomly in the tank with a one-way t-test. The expected time was calculated using the proportion of the tank volume represented by the association section, and calculating this same proportion for the total trial duration. I performed a linear mixed effects model (lme) to evaluate shoaling tendency differences and interactions between focal species and shoal species. *A posteriori* Tukey HSD test was carried out. An ANOVA test was carried out for each focal species set of observations to evaluate differences between shoal species. All analysis were carried out with the statistical software R (R-Core-Team 2013).

3.4 Results

Fish of all species spent more time in the proximity of the shoal in the bottle than would be expected if they were swimming randomly, regardless of the species of fish inside the bottle (one-way *t*-test, $t_{14} > 3.256$ $p < 0.005$, Figure 3.3). Tendency to associate with other species was different between *P. reticulata*, *S. bilineata* and *P. infans* (lme, $F_{2,82} = 22.69$, $p < 0.001$; Figure 3.3). Post-hoc Tukey HSD test showed *P. reticulata* and *S. bilineata* are alike, and both are different from *P. infans*. The difference between shoal species was not significant (lme, $F_{2,82} = 2.38$, $p = 0.098$; Figure 3.3); but there was an interaction between focal species and shoal species (lme, $F_{4,82} = 4.01$, $p = 0.005$; Figure 3.3). *P. reticulata* showed significant differences in the tendency to associate with the given shoal species (lme, $F_{2,11} = 4.94$, $p = 0.029$; Figure 3.3). They had a higher tendency to associate with other guppies and the same tendency when the shoal partners where *S. biliniata* or *P. infans*. *S. bilineata* and *P. infans* showed no differences in the tendency to associate with the three shoal species (lme, $F_{2,11} < 3.52$, $p > 0.07$; Figure 3.3).

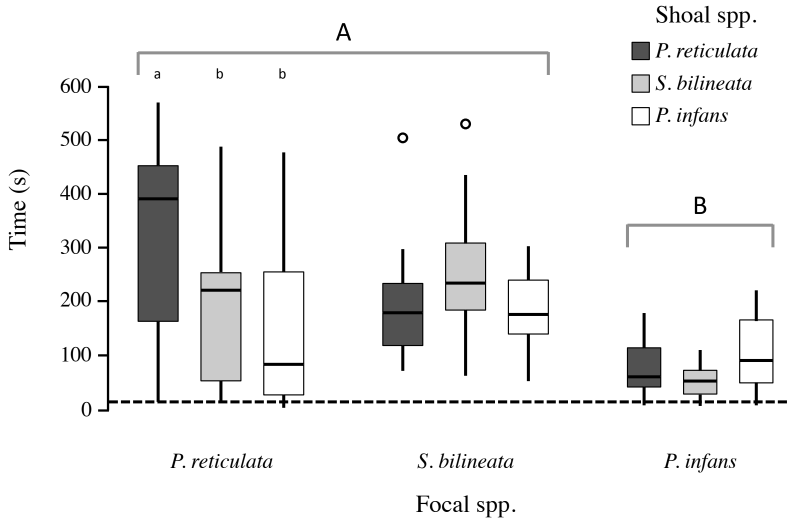

Figure 3.3. Time (max = 600 s) the focal fish was associated with the given shoal. Horizontal lines in the bars represent the median, boxes indicate interquartile ranges and vertical lines show the range excluding outliers (circles). Uppercase letters represent significant differences between focal species and lowercase letters significant differences within *P. reticulata* focals to shoal with the given species. *S. bilineata* and *P. infans* focals showed no significant differences time associating with the given shoal species.

3.5 Discussion

Associations with morphologically similar heterospecifics that share the same habitat increase the size of groups and this is one way to avoid Allee effects. Results of this chapter show that invasive guppies are willing to associate with heterospecifics, just as they are in their native environment (see Chapter 3). Guppies in Trinidad readily shoaled with native poeciliids indicating that this is a pre-existing trait that can be exploited during invasion (Camacho-Cervantes et al.

2014). Then, my results corroborate this and showed that invasive guppies are also willing to shoal with other native topminnows and not only that, but native topminnows are as well inclined to form heterospecific shoals with invasive guppies.

Many studies on native-exotic interactions focus on the resistance of natives to being colonized by exotic species. Contrary to my results, a study carried out in Florida, U.S.A., showed that the resistance to be invaded by native species is reducing the success of introduced fishes; the eastern mosquito fish (*Gambusia holbrooki*) attacked and killed non-native poeciliids (*Xiphophorus variatus* and *Xiphophorus hellerii*) causing a negative effect on invader's populations (Thompson et al. 2012). In Thompson et al. experiment the density of the eastern mosquito fish was positively related with the negative effects on the non-native poeciliids. Results of this chapter, on the other hand, suggest that the native goodeinae are associating with the invasive guppy, by joining heterospecific groups both species are more likely to avoid Allee effects due to the higher availability of individuals to associate with. In the case of guppies, the later could enhance their establishment success when invading.

In the same fashion, some studies suggest that empty niches enhance invasion rates (Davis et al. 2000, Fridley et al. 2007). For instance, Elton (1958) suggested that more diverse communities are more resistant to invasion through competitive processes. Nevertheless, my results found native species were willing to associate with the invasive guppies. This finding is in accordance with Simberloff and Von Holle's (1999) research on interspecific facilitation between invaders leading to an accelerating increase in the number of introduced species and their impact, except that my results point to a potential facilitation from a native, and not another invasive, species.

My study suggests that social species, like the invasive guppy, can overcome the problems of low numbers during early stages of invasion by associating with groups of individuals regardless of the species and that some native species might as well facilitate invasion by not discriminating between other native and invasive shoal partners. Native Mexican topminnows might be providing guppies with the advantages of being part of a bigger shoal. In some cases, invasion success depends on finding a time or place where invaders can coexist and even outcompete resident species (Shea and Chesson 2002). By being willing to associate with guppies during the critical initial stages of invasion, goodeinaes might be enhancing guppies' chances of successfully establish themselves.

Chapter 4

Boldness and exploratory behaviour with heterospecifics in complex and simple habitats

4.1 Abstract

Individual behaviour is strongly influenced by the environment. Animals associate to protect themselves from predators, as well as gaining other benefits such as foraging efficiency or mating opportunities. When accompanied by others, fish have been shown to increase their boldness. Bold individuals tend to expand their range further than shy ones, as an invader, this could be a desirable trait when trying to establish in new environments or expand its range. In this chapter I explore the willingness of guppies to leave a refuge when accompanied by another guppy or a fish from a different species in a simple or complex environment. My hypothesis is that individuals are bolder when accompanied by an individual of the same species, thus guppies will be more eager to leave the refugee when the couple is conformed by two guppies than when it is one guppy and one goodeinae. In the same direction, I explore guppies' tendency to associate with their partner while exploring. I hypothesised guppies would have a higher tendency to associate with individuals of the same species. Guppies are more willing to leave the refuge when the environment is more complex and that their tendency to associate with a given partner is higher when the environment is simpler. The species of the partner had no effect in boldness or association tendency. These results reinforce the idea that guppies' ability to associate and gain benefits from heterospecifics as much as they do from conspecifics. Usually, invaders arrive to a novel habitat in small groups, which is a disadvantage for social species like the guppy. In the cases were native species are potential shoal-mates, invaders might associate with them to overcome the disadvantages of being part of a small group and thus enhance its chances of success.

4.2 Introduction

Personality has been shown to have an effect on several traits of ecological importance such as dominance, natal dispersal, territory quality, survival, recruitment and physiological responses to social stress (Reale et al. 2007, Quinn et al. 2012). An individual's behaviour is influenced by its environment, which is partially related to the presence or absence of conspecifics and or heterospecifics (Ward 2012). Complex habitats provide enough structure for fish to cover themselves, thus, when in this environments fish might be less willing to associate (Hamilton 1971). Temperament traits, such as boldness, appear to affect the ways an individual interacts with its environment, whether in its reactions with predators, food sources, and habitat (Reale et al. 2007). Shoaling with other fish is a way of sheltering from predators and gaining other benefits, such as being more efficient when foraging (Magurran and Nowak 1991, Bleakley et al. 2007).

4.2.1 Refuge use and exploring behaviour

Animals do not necessarily behave optimally when facing the fundamental problem of choices between foraging and risk avoidance (Dammhahn and Almeling 2012). The structural complexity of the environment can influence social interactions (Edenbrow et al. 2011). As Refuge use could result in lost feeding opportunities, an animal continuously must decide whether to stay in the refuge or to emerge into open habitat; this decision may depend on its energetic state and vulnerability to predation (Sih 1992, Godin and Dugatkin 1996, Dowling and Godin 2002). Habitat complexity may also influence the frequency and outcome of behavioural interactions between individuals. For example, Hibler and Houde (2006) demonstrated that the structural complexity of the environment plays an important role in sexual interactions of guppies.

It has been demonstrated that fish in larger groups are more willing to engage in exploratory behaviour and swim more actively than those on their own or in smaller groups (Krause and Ruxton 2002, Ward 2012). For example, (Orpwood et al. 2008) compared the shoaling behaviour of European minnows (*Phoxinus phoxinus*) between simple and complex habitats in the presence of a predator. Minnows formed larger shoals in simple habitats when exposed to predators, supporting the hypothesis that individuals shoal as a sheltering mechanism (Hamilton 1971).

4.2.2 Boldness

The foraging cost of refuge use is the difference between the expected rate of energy gain in the open habitat and that in the refuge (Sih 1992). It has been suggested that bolder or proactive individuals prioritize the reduction of starvation risk, while reactive animals do the opposite (Biro and Stamps 2008). For many prey species, hiding in a refuge prevents detection by predators, but at the same time there are associated costs of staying in the refuge such as loss of feeding and mating opportunities (Sih 1992). Individuals that are part of populations that face strong predation pressure tend to be bolder (Harris et al. 2010). Being bold may be very beneficial in terms of reproduction and acquiring food resources, while at the same time the risk of encountering predators and aggressive conspecifics may increase (Wilson et al. 1994).

4.2.3 Aims

In structurally simpler habitats fish form larger shoals to reduce their chances of being caught by a predator, locate food faster and/or find mating partners (Orpwood et al. 2008). In the case of guppies, males and females prefer to associate with bolder individuals (Brosnan et al. 2003). As well, being proactive has shown to confer bold guppies with mating advantages (Godin and Dugatkin

1996). In this chapter I assessed guppies exploratory behaviour, I hypothesised that they will exit a refuge and explore an unknown environment when accompanied by a conspecific and by an heterospecific. I specifically tested for the willingness of guppies to explore simple and complex environments and, while doing so, their tendency to associate with a given shoaling partner, either another guppy or a heterospecific individual (*Ameca splendens*). This species was selected because guppies are likely to encounter them when invading the Mexican Central Plateau. Moreover, both species share ecological requirements and have a similar phenotype, see section 1.7 Vulnerable Mexican fish communities in the Introduction. I hypothesised that in simpler habitats fish would be less eager to leave the refuge and keener to remain together while exploring. In the presence of conspecifics, individuals are more likely to express a given behaviour, or express it a greater rate (Ward 2012). I expected guppies to show a higher inclination to leave the refuge and be more social with other guppies than with *Ameca splendens*.

4.3 Methods

Experiments were carried out at the University of St Andrews, during October and November 2013. Guppies and goodeinae used in this experiments were descendants from wild individuals collected in their native habitats, Trinidad and México respectively. Fish were kept in stock tanks (45 L) that contained a maximum of 25 fish. Water in these tanks was continuously aerated and filtered. Tanks were furnished with gravel, rocks and plastic plants. Water temperature was kept at about 23°C (ranging from 20 to 26 °C) and the photoperiod was 12L: 12D from 800 to 2000 h Experimental fish of each species were kept in separate stock tanks for at least two weeks prior to the start of the experiment to avoid familiarity effects (Griffiths and Magurran 1997a). Fish were fed once a day with Tetramin® flakes. I used only female guppies as they allocate more time to social behaviour than males (Sievers et al. 2012) and juvenile goodeinae as they resemble female guppies in colour, size and behaviour. To ensure results were not biased by fish size, all focals and shoaling partners of both species were size matched (ANOVA, $F_{1,72} = 2.39$, $p = 0.12$).

For the purposes of this experiment I placed a bottle with a window opening (5 x 6 cm) on the side at the bottom inside an observation tank (40 x 30 x 30 cm) (Figure 4.1). Focal and shoaling partner were gently released inside the bottle (time zero) and were given up to 15 minutes to abandon the refuge (exit the bottle through the window) and explore either a complex (plants in the tank) or a simple (no plants in the tank) environment (Figure 4.2). Observations lasted 8 minutes after both fish had exited the bottle and I recorded the distance between the two fish every 20 seconds. I made 38 observations using a complex environment and 38 using a simple environment. Half of each had a guppy as a shoaling partner and the other half a goodeidae (19 replicates for each treatment). Each focal was used only once and therefore all observations were independent from each other.

Figure 4.1. Picture of the window opening on the bottle (by M. Camacho-Cervantes).

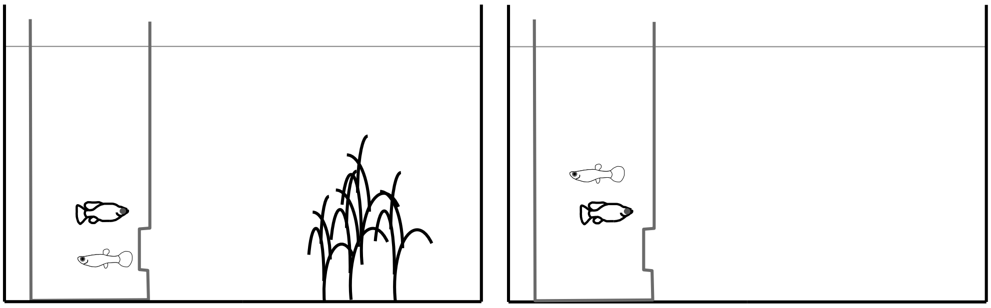

Figure 4.2. Diagram of the tank set up. Tank with plants as complex environment and tank without plants as simple environment. Refuge (bottle) had a window (5 x 6 cm) on the side at the bottom.

4.3.1 Data analysis

To compare the willingness of heterospecific or conspecific couples to explore the given environment (simple or complex) I performed proportion difference binomial tests. Next, I examined differences in the species of the first fish to abandon the refuge when in the different environments, as well using binomial tests, and I tested the effect of habitat and partner species on the difference between the focal and the partner time to abandon the refuge using an ANOVA. Effect of habitat and species partner on the time focal took to abandon the refuge was examined using an ANOVA. On exploring behaviour, I used an ANOVA to test for differences in the times fish were found within one body length from each other when the partner was another guppy or a goodeidae in a simple or complex environment. All analysis were performed using the R statistical software (R-Core-Team 2013).

4.4 Results

4.4.1 Boldness

Fish had a higher inclination to abandon the refuge and explore the habitat when this was more complex regardless of the species of the partner and there was no difference in the species of the first fish to abandon the refuge in either of the two habitats (Table 4.1). Time difference between the focal and the partner exiting the bottle was not affected by the species of the partner (ANOVA, $F_{1,45} = 0.27$, $p = 0.6$), nor by the complexity of the habitat (ANOVA, $F_{1,45} = 0.73$, $p = 0.4$). Habitat complexity had an effect on the time it took the focal to abandon the refuge, fish in more complex habitat exited the bottle faster (ANOVA, $F_{1,48} = 5.23$, $p = 0.027$, Figure 4.3) but there was no effect of the partner species (ANOVA, $F_{1,48} = 0.65$, $p = 0.422$, Figure 4.3).

Table 4.1. The number of trials when both fish exited the bottle and the species that exited first. Binomial tests for difference in proportions.

Habitat		Simple	Complex	
Times both fish exited the bottle (maximum =19)				Z
P. reticulata		9	16	-2.39*
P. reticulata - A. splendens		8	16	-2.69**
	Z	0.32	0	
Species of the first fish to exit the refuge (maximum in brackets)				Z
P. reticulata		5 (9)	8 (16)	0.27
A. splendens		6 (8)	9 (16)	0.89
	Z	-0.83	0.35	

Signif. codes: 0.001 ‘**’ 0.01 ‘*’

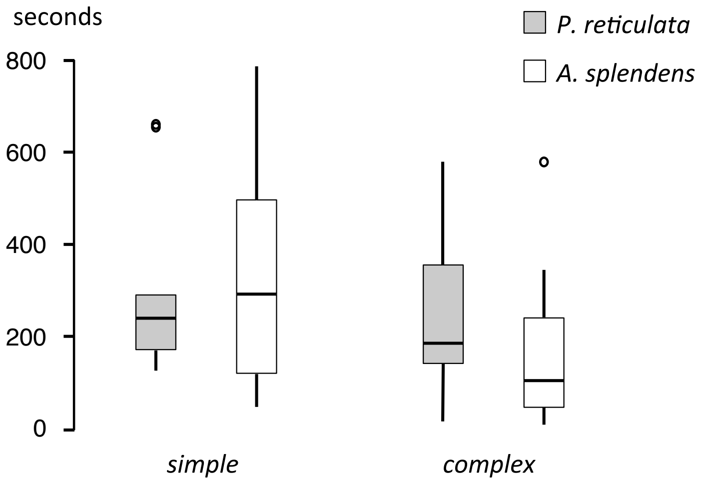

Figure 4.3. Time it took the focal individual to abandon the refuge. Horizontal lines in the bars represent the median, boxes indicate interquartile ranges and vertical lines show the range excluding outliers (circles).

4.4.2 Exploring behaviour

There was a significant effect of habitat complexity on the times fish were found within one body length from each other after both abandoned the refuge, fish were more willing to associate in the simpler habitat (ANOVA, $F_{1,45} = 50.43$, $p < 0.001$, Figure 4.4), species of the partner did not have any effect (ANOVA, $F_{1,45} = 0.63$, $p = 0.431$, Figure 4.4).

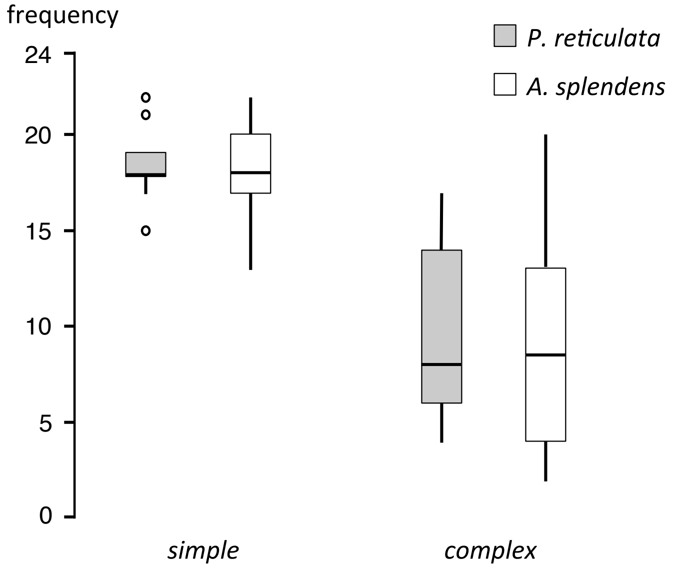

Figure 4.4. Times fish were found within one body length from each other after exiting the bottle (max = 24). Horizontal lines in the bars represent the median, boxes indicate interquartile ranges and vertical lines show the range excluding outliers (circles).

4.5 Discussion

Guppies in my experiment were bolder when in more complex habitats and more willing to associate with the given partner in the simpler habitat. There was no difference in behaviour when the shoaling partner was another guppy or a heterospecific. Moreover, there was no difference in the species of the first fish to engage in exploring behaviour, nor in the difference in time between the first and the second fish to exit the refuge. These results are consistent with the ideas around common interests presented by Leimar and Hammerstein (2010), individuals may decide to remain together when the interaction is beneficial for both. In their study both individuals would benefit equally from exiting the refuge and remaining together when exploring a simple habitat, even if they are not the same species.

Predation risk and the presence of other fish determine exploratory behaviour (Ward 2012). Fish might be more willing to explore when accompanied by other individuals; for example, Magurran and Pitcher (1983) showed that larger groups of minnows (*Phoxinus phoxinus*) and goldfish (*Carassius auratus*) made more visits to an exposed and potentially risky food patch and spent more time there than smaller groups did. Complex habitats are used as refuges from predators (Werner et al. 1983), with shoaling tendency and shoal sizes increasing in simpler habitats (Orpwood et al. 2008). In previous experiments, guppies have been found to be bolder when associating with bold (Brown and Laland 2003) and familiar individuals (Bhat and Magurran 2006). My results show that the species of the partner had no effect in the guppies´ willingness to leave the refuge, but the environment did.

Being bold represents benefits, such as locating food and finding mating partners, therefore being able to gain these benefits is an advantage for survival (Godin and Davis 1995). My results show that complex environments encouraged guppies to engage in exploring behaviour faster than simpler ones, regardless of the species of the partner. In a similar direction, it has been shown that guppies increased boldness of *Rivulus hartii* to explore zones of high predation, equivalent to the effect of conspecifics, exhibiting exploratory behaviour to reach new habitats favourable for growth and reproduction (Fraser et al. 2011). Since behaviour under predation risk is one of the key determinants for fitness (Sih et al. 2004), it is likely to be an ecologically relevant personality trait (Dammhahn and Almeling 2012).

Bold individuals have higher reproductive investment but with the handicap of a reduction in survival (Smith and Blumstein 2010).

Fast explorers that are also bold are assumed to pay a predation cost associated with it (Wilson et al. 1994). However, it has been suggested that proactive individuals may be able to compensate for their higher risk of predation by enhancing antipredator behaviours (Jones and Godin 2010) and thus, do not necessarily have to pay a predation cost (Pascual and Senar 2014). My results suggest that sociability in guppies increases when the predation risk seems higher – simple environments – regardless of the species of the partner, conferring guppies with a potential advantage when colonising novel environments, where availability of conspecifics to associate with might be low. The results of this chapter are consistent with the obtained in Chapters 2 and 3, guppies interact with heterospecifics that they might encounter when invading the Mexican Central Plateau, which provides them with the possibility of forming bigger shoals.

Chapter 5

Transmission of information between native and invasive fish species

5.1 Abstract

Foraging in groups confers individuals with advantages, such as being more efficient locating food and get protection from predators. Information on food availability can be transferred between animals when socialising. Usually, this transmission is between individuals of the same species. However, in some cases animals can learn from a different species. I asked whether guppies are able to acquire information through visual cues from heterospecific individuals as well as they do from conspecifics. My results demonstrate that they are able to use this information. Being able to exploit information in this way could be a trait that enhances invasion success, particularly because when arriving to novel environments invaders might have few conspecifics to associate with and these might not be as knowledgeable as natives.

5.2 Introduction

Animals that are part of a group are more efficient foraging, avoiding predator attacks and finding mating partners (Krause and Ruxton 2002). In most cases, individuals associate with others of their species and even genetically related as it enhances the benefits obtained by doing so (Griffiths and Magurran 1997a, Croft et al. 2003, Mathot and Giraldeau 2010). When individuals are related to their associating partners, not only they gain individual benefits, also, they maximize their inclusive fitness (Okasha et al. 2014). However, the benefits of grouping extend to heterospecific associations as well as conspecific ones (Frank 1994, Barakat et al. 2009). For example, some species of birds join mixed species flocks to engage in foraging activities or antipredator behaviour (Sridhar and Shanker 2014).

5.2.1 Foraging in groups

Individuals increase their foraging efficiency when being part of a group (Magurran and Nowak 1991, Srinivasan and Quader 2012, Angulo et al. 2013, Camacho-Cervantes et al. 2014). When foraging in groups, apart from being more efficient in finding food, animals are able to react better in case of a predator attack and increase their chances of survival, which enables them to forage longer (Toth et al. 2014). However, when foraging in groups, individuals must share resources and even decide between staying at a foraging site or follow the group if they decide to move in order not to lose other grouping benefits (Kazahari 2014).

5.2.2 Social learning

Social interactions allow animals to acquire information about their environments rapidly and efficiently (Laland and Williams 1997), which may be critical in their survival (Galef and Laland 2005). Information that individuals acquire by observing or interacting with others, usually conspecifics, is considered to be social learning (Heyes 1994). Acquisition of information on nutritious and safe food to eat or avoidance of unpalatable food are some of the advantages of foraging with others (Galef and Laland 2005). Social learning about food within species is well known to occur in different taxa, such as bats (Clarin et al. 2014), birds (Belmaker et al. 2012), or fish (Brown and Laland 2003). But transmission of information can also occur between species (Lefebvre et al. 1997, Seppanen et al. 2007, Clarin et al. 2014). For example, fathead minnows (*Pimephales promelas*) responded to heterospecific chemical alarm cues and decreased their probability to be attacked when encountering a predator (Chivers et al. 2002).

5.2.3 Aims

Associating with heterospecifics could be particularly useful in situations when there are not many conspecifics around, in which case a heterospecific partner could potentially be an option. There have been many studies on the conspecific interactions of guppies, and it is known that guppies obtain information about how or where to locate food sources by shoaling with informed conspecifics (Laland and Williams 1997, Swaney et al. 2001). However, during invasion, guppies might not find informed conspecifics but will encounter native fish already familiar with the local habitat. To identify the mechanisms through which guppies could derive foraging benefits when associating with goodeinaes, I assessed if guppies can acquire information from other fish species through visual cues on food availability. The experiment investigated if individual guppies changed their behaviour when watching groups of fish that smelled food. I used guppies and a species of goodeinae (*Ameca splendens*) native from México. *Ameca splendens* is a species that resembles guppies during its juvenile stages and guppies are likely to encounter it in México because they share the same ecological requirements, see 1.7 Vulnerable Mexican fish communities section in the Introduction. I hypothesised, after proving that guppies interact with heterospecifics (see Chapter 2, 3 and 4), that derive from these interactions the possibility of learn from them.

5.3 Methods

This experiment explored the ability of guppies to acquire information from heterospecifics by observing goodeinaes' swimming pattern when they had information on food availability and guppies did not. Fish used in this experiment were descendants from wild individuals, however they had spent all their life kept in aquarium tanks. Through all their life, these fish had been fed with commercial flakes, which tend to float. Thus, their food searching activity is mostly on the upper part of the tank. Indeed, pilot experiments showed that when food scent was added to the water, fish responded by changing their swimming pattern and spending more time in the upper half of the tank. I tested the responsiveness of a previously selected guppy (focal) to the change in swimming behaviour of a group of fish (informant shoal) after they received a chemical food cue.

Guppies and goodeinaes used in the experiment were descendants from wild individuals collected in their native habitats, Trinidad and México respectively. Fish were kept in stock tanks (45 L) that contained a maximum of 25 fish. Water in these tanks was continuously aerated and filtered and the tanks were furnished with gravel, rocks and plastic plants. Water temperature was kept at about 23 °C (ranging from 20 to 26 °C) and the photoperiod was 12L: 12D from 800 to 2000 h. All observations were conducted at the University of St Andrews, Scotland, UK.

Experimental fish of each species were kept in separate stock tanks for at least three weeks prior to the start of the experiment to avoid familiarity effects (Griffiths and Magurran 1997a). Fish were fed once a day with Tetramin® flakes a minimum of 12 hours before the observations to ensure food searching behaviour. I used females as they allocate more time to associating behaviour than males (Sievers et al. 2012); female guppies also resemble juvenile goodeinae in colour, size and behaviour. All fish used in this experiment were size matched. However I photographed and measured all fish to ensure there were no relation between size and behaviour (see Figure 2.4 in Chapter 2)

For the purpose of this experiment I placed the focal guppy on a 15 L tank and the informants in an identical tank located right next to it, so both could see each other but there were no exchange of chemical cues (Figure 5.1). I formed the informant shoals with a random selection of three individuals from the stock tanks to avoid pseudoreplication (Hurlbert 1984). In order to give the informants a signal without focals noticing, I prepared a colourless food cue. I prepared the signal cue adding 5 g of Tetramin® flakes to 100 ml of clear water and removing the flakes sediment after five minutes, which left a clear food scented water that provided informants with information that the focal did not have.

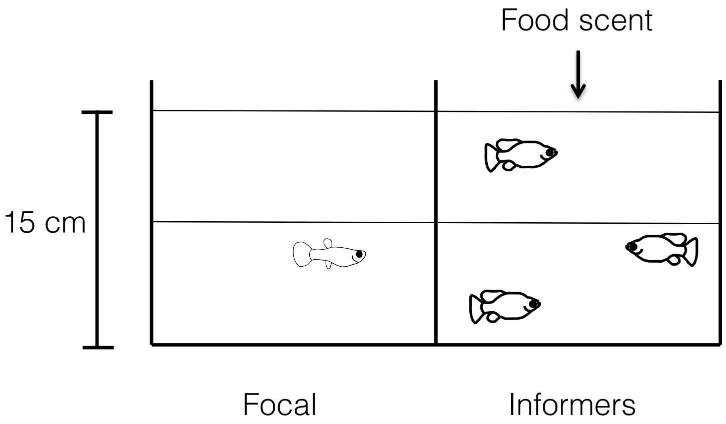

Figure 5.1. Diagram of the tank set up. Fish were considered up when within 7.5 cm below the water surface or down when within 7.5 cm above the tank bottom.

I observed each focal during 16 minutes between 1000 and 1500 h. The observation tank (filled with water up to15 cm from the bottom) was imaginary divided in two sections of 7.5 cm each. Every 20 seconds I recorded the position of the focal and informer fish as *up* (within the 7.5 cm close to the surface) or *down* (within the 7.5 cm close to the bottom). After the first 8 minutes I injected 8 ml of scented water (food cue) in the informants tank to assess the change on fish vertical swimming pattern.

After every observation, water in both tanks was discarded and replaced to make conditions in both tanks equal every time. Each focal fish was used only once in each of the two treatments. In between trials, focal fish were kept in a stand-by tank (15 L) for a period of 30 to 40 minutes. Fish for the shoals were haphazardly selected for each observation from a pool of 23 fish per each species to avoid pseudoreplication (Hurlbert 1984). The order in which trials were presented to each focal and the side of the tanks were randomized.

To exclude the possibility of the food scent traveling by air or guppies being able to smell the food from their tank rather than from the informants, I carried out a control in which there were no fish in the informants' tank; these observations were recorded in the same way as the experimental trials injecting scented water to the empty informants' tank. Additionally, I carried out a fourth treatment with conspecifics as informants and injecting only clean water in the tank to control for fish switching swimming patterns for other reasons than food cues. And finally, to make sure fish were engaging in searching behaviour and not only intending to associate with the informants shoal, I recorded along with being up or down in the tank if they were associated or not to the informants shoal. I counted association when the focal was within one body length from the wall of the tank adjacent to the informants as is the closest they could be from the informants shoal.

5.3.1 Data analysis

I used a repeated measures approach in which 21 focal fish performed two trials: one with three goodeinaes and the other with three guppies as informants. An ANOVA and linear regression were performed to ensure all fish were same size and there were no relation between size and behaviour, respectively. Relative size of fish was calculated subtracting the size of the focal from the average size of the shoal. A linear mixed effect (lme) model was performed to analyse the effect of adding food scent to the informants' tank in association patterns of the focal.

A lme model including the within-group error (species of the shoal and controls) was performed to test for the change in focals' behaviour when injecting scented water in the informers' tank and the difference between treatments. Finally I carried out a Tukey post hoc analysis to explore the differences between trials. To analyse the data I used the R statistical software (R-Core-Team 2013).

5.4 Results

Size of the focal individual relative to that of the informants did not vary when the shoal was composed by guppies or *A. splendens* (ANOVA, $F_{1,42}= 0.47$ $p = 0.83$), nor did relative size explain the focal fish behaviour (with guppies $r^2 = 0.017$, $p = 0.43$; or with *A. splendens* $r^2 = 0.051$, $p = 0.86$). Focals were found more often associated with the informants after adding the food to the informants tank except when the informants were injected with non-scented water (lme, $F_{5,79}= 40.91$, $p = 0.001$, Tukey post hoc). This implies that guppies were more often associated to the informants after these changed their behaviour when receiving information. Therefore, I discarded the possibility that focals following the informants at all times and change their swimming pattern as a result.

There was a significant difference in the times the focals were found in the upper part of the tank before and after the introduction of the food scent (lme, $F_{7,176}= 79.52$ $p < 0.001$). Post hoc Tukey HSD analysis revealed there were no significant differences between having *P. reticulata* and *A. splendens* as informants and there was a significant difference between experiment trials and control trials (Figure 5.2).

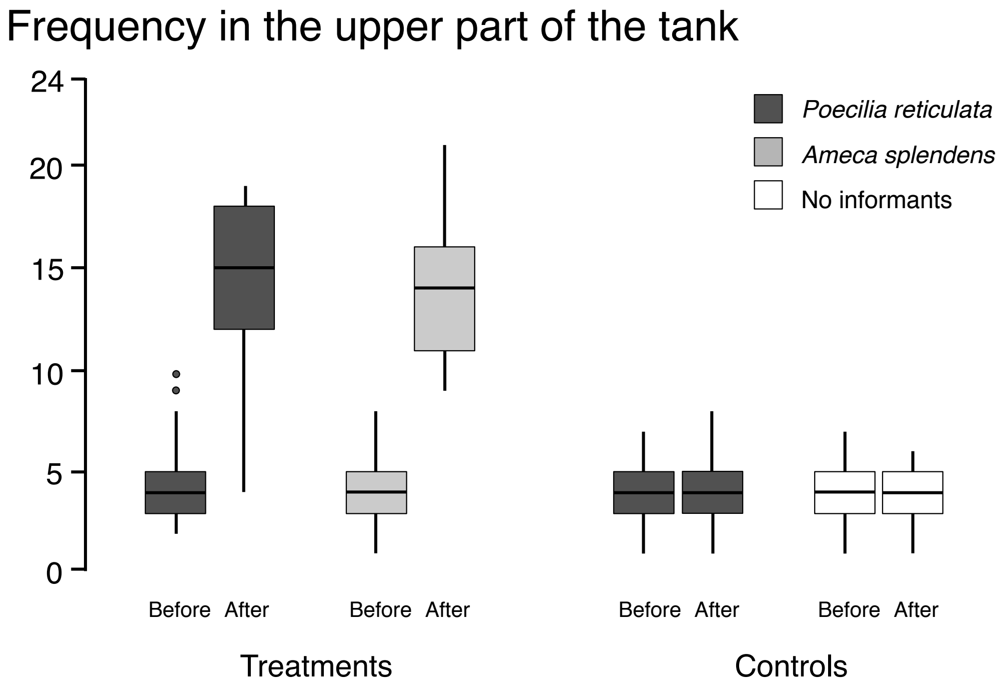

Figure 5.2. Times the focal guppy was found in the upper part of the tank (max=24) before and after the food scent was introduced in the informants' tank. In the case of the *P. reticulata* control treatment, only unscented water was injected in the tank. A set of 21 fish performed the two treatment trials and a different set of 21 fish performed the two control trials. Horizontal lines in the bars represent the median, boxes indicate interquartile ranges and vertical lines show the range excluding outliers (circles).

5.5 Discussion

My results show that guppies are able to gain the same information about feeding opportunities from heterospecifics as from conspecifics. When a group of fish knows that there is food around, guppies watching the group, but not accessing the information, initiate food-searching behaviour. This change in behaviour is the same regardless of the species of the informants. This could be particularly relevant for invasive species that typically occur in small numbers during the initial stages of invasion (Mack et al. 2000). Being able to efficiently find food can be easily linked to survival and fitness or ecological success. In the case of guppies, it is known that under risk of predation they reduce effort on foraging activities. Therefore, the more efficient they are when foraging the more time they are able to allocate to other vital tasks, such as avoiding predators or mating (Magurran and Nowak 1991, Magurran and Seghers 1994b). This chapter provides evidence of a direct benefit from associating with heterospecifics, a behaviour that has already been described in guppies (Warburton and Lees 1996, Camacho-Cervantes et al. 2014).

Attraction to a particular location because of the presence or success of other species has been demonstrated experimentally in the field for many taxa (Monkkonen and Forsman 2002, Seppanen and Forsman 2007, Seppanen et al. 2007). Fitness in species that are very sociable, depends on group size and group dynamics (Cote et al. 2012). Fish find food sources by sampling and observing other fish (Warburton 2003) and sometimes copying them (Laland 2008). Acquiring knowledge from other species may have a significant effect on local adaptation and thus on spread and success of populations (Seppanen et al. 2007).

Information on the environment enables an individual to adapt to changing circumstances and modify its behaviour to suit these circumstances (Girvan and Braithwaite 1998). Shoaling plays a role in transmission of information in fish (Laland and Williams 1997). A study carried out by Cote et al. (2011) suggests that individuals with a higher sociability rate are more successful as invaders. In species that gain fitness benefits from being social, foraging information may be transmitted between individuals by processes as simple as following (Laland and Williams 1997). In my study fish were more willing to associate with the informants after these got information on food availability.

In novel environments, invaders might encounter that food sources could be different, therefore those that can obtain information from locals could potentially increase their chances of survival. When colonizing a new habitat the chances of finding conspecific shoaling mates are uncertain, thus being able to acquire information from heterospecific individuals could be a useful trait to succeed. Efficient foraging requires information on which patches to forage and how long to spend at each patch, this information can be obtained directly, by sampling or indirectly by attending social cues produced intentionally or inadvertently by other individuals (Kendal et al. 2005).

Guppies have been found to be willing to associate with heterospecific individuals when these are familiar to them (Warburton and Lees 1996) and even when they have not encounter the species before (Camacho-Cervantes et al. 2014). Here I proved that guppies are able to read information from a species of goodeinae *A. splendens*. Being able to acquire information from non-familiar heterospecific individuals could increase invasion success by decreasing guppies' investment in food searching activities.

Identifying traits and conditions that facilitate establishment of invasive species, such as species that are beneficial for invaders, promotes better assessment of invasion risk and conservation planning.

Chapter 6

Exotic invaders gain foraging benefits by shoaling with native fish

6.1 Abstract

Freshwater habitats are under increasing threat due to invasions of exotic fish. These invasions typically begin with the introduction of small numbers of individuals unfamiliar with the new habitat. One way in which the invaders might overcome this disadvantage is by associating with native taxa occupying a similar ecological niche. I used Trinidadian guppies (*Poecilia reticulata*) to test the prediction that exotic shoaling fish can associate with heterospecifics, and that they improve their foraging efficiency by doing so. Guppies have invaded the Mexican High Plateau and are implicated in the declines of many native Goodeinae species. I show that heterospecific associations between guppies and goodeinaes can deliver the same foraging benefits as conspecific shoals, and that variation in foraging gains is linked to differences in association tendency. These results uncover a mechanism enabling founding individuals to survive during the most vulnerable phase of an invasion and help explain why guppies have established viable populations in many parts of Mexico as well in every continent except Antarctica.

A version of this chapter was published in November 2014 in the scientific journal *Royal Society Open Science*. Additional material and discussion is included in this thesis chapter.

M. Camacho-Cervantes, C. Macías-Garcia, A. F. Ojanguren, A. E. Magurran (2014) Exotic invaders gain foraging benefits by shoaling with native fish. *Royal Society Open Science*. DOI:10.1098/rsos.140101

6.2 Introduction

In fish, as in other taxa, social behaviour can enhance survival (Krause and Ruxton 2002). Apart from for mating, fish associate with other individuals in contexts such as hibernation, sleeping and foraging (Bleakley et al. 2007), thus gaining benefits including being able to avoid predators more efficiently (Magurran and Nowak 1991), increased foraging efficiency (Day et al. 2001) and reductions in the energetic costs of movement (Krause and Ruxton 2002). However, animal associations are not limited to single species groups. Heterospecific aggregations occur regularly in nature when they are beneficial to the participants (Ward et al. 2002). Examples include fish (Sazima et al. 2007, Camacho-Cervantes et al. 2014), birds (Farine and Milburn 2013) and even members of very distant taxa (e.g. monkeys and birds; (Boinski and Scott 1988).

6.2.1 Colonization of novel environments

Invasive species are a major agent of global change (Mack et al. 2000, Lockwood et al. 2006). They modify the environment at multiple ecological levels, lead to community disassembly and alter species interactions across a range of spatial and temporal scales (Sanders et al. 2003, Lockwood et al. 2006, Ehrenfeld 2010). Most introductions of non-native species are the direct or indirect result of human activities (Sakai et al. 2001). The arrival and establishment – colonization– of an invasive species are the two first, and probably most crucial, stages of invasion (Lockwood et al. 2006). Although many species are translocated from their native range, the majority do not manage to establish viable populations (Mack et al. 2000, Lockwood et al. 2006). After arrival to a new habitat, individuals face predators, competitors and food sources that are unknown for them, the establishment success of these individuals depends on its adaptive capacity to the novel environmental conditions (Sax et al. 2007). Indeed, in order to succeed, some species modify their behaviour and develop phenotypes that allow them to cope with environmental novelty (Escoriza et al. 2014).

6.2.2 Allee effects on the invasion context

Invasions typically begin with the introduction of just a few individuals (Mack et al. 2000), and behaviour may play a crucial role in enabling such individuals to survive until they can reproduce (Holway and Suarez 1999, Stephens and Sutherland 1999). Allee effects are the disadvantages linked to membership of a small population (Stephens and Sutherland 1999, Courchamp et al. 2008, Tobin et al. 2011). During the first stages of invasion, many invaders, specially the most social species, are subject to Allee effects when locating mates, avoiding predators or when foraging (Taylor and Hastings 2005, Tobin et al. 2011). Moreover, invasion success is affected by the invasibility of the habitat (Lonsdale 1999), which can interact and even intensify Allee effects (Tobin et al. 2011). Colonising individuals subject to these, are more likely to have longer lag times, spread slower and even not being able to form viable populations (Taylor and Hastings 2005).

6.2.3 Aims

Guppies are known to be willing to associate with native Mexican goodeinaes (see also Chapter 3) and other poecilids (Warburton and Lees 1996). However, it is still uncertain if this association will provide them with direct benefits. In this chapter. I tested the hypothesis that small shoals of invading guppies gain foraging benefits by increasing shoal size by associating with goodeinaes. I expected that fish would locate food faster and increase the time spent foraging when in larger groups (Pitcher et al. 1982). I predicted that foraging advantages would also apply when the additional shoal members were heterospecific rather than conspecific fish. To assess whether these effects can be generalized across species I repeated the experiments with four goodainae species (*Skiffia bilineata*, *Zoogoneticus tequila*, *Xenotoca eiseni* and *Girardinichthys viviparous;* Figure 6.1) that are morphologically similar to guppies (Valero et al. 2008)). In addition, I asked whether the differences in the foraging advantages that accrue when individuals belong to a larger shoal can be linked to the guppy's tendency to associate with a given species.

6.3 Methods

Experiments were carried out at the main campus of the National Autonomous University of México (UNAM) in México City from July to September 2013. Goodeinae were originally collected from the wild (using hand seine nets and traps) under the permit SGPA/DGVS/09253 provided by the Mexican Ministry for the Environment (SEMARNAT) and used to establish populations in outdoor ponds at UNAM. Guppy (*P. reticulata)* individuals were collected from a population established in the wild in Ahuisculco, Jalisco, where no other species used in this experiment occur. In the case of the goodainaes, *Z. tequila* were originally from Teuchitlán in Jalisco; *G. viviparus* originated in Texcoco, México; *S. bilineata* were originally from Álvaro Obregón in Michoacán and *X. eiseni* from San Sebastián in Jalisco (Map 6.1). All fish were carefully transported to the laboratory in plastic bags half filled with water and half filled with air. Stock tanks (45L) contained 15 to 20 fish each and were set up with aged tap water, which was treated with STRESS COAT®. Each tank contained a filter, water pump and plants. Photoperiod was 12L: 12D from 0700 to 1900 h. Water daily temperature ranged between 19 and 22 °C. Tanks were visually isolated from one another with an opaque sheet. I used only female fish in the experiment as they devote more time to shoaling and foraging than males (Sievers et al. 2012). Individuals in a given trial were kept separate for several weeks prior to observations to avoid familiarity effects (Griffiths and Magurran 1997a). Fish were fed with commercial flake food

(SeraVipan®) daily at the end of each day. After the experiment was completed (70 days), all fish remained in stock tanks in the laboratory.

Map 6.1. Localities in the Mexican Central Plateau were experimental fish were collected. *P. reticulata* individuals were from Ahuisculco, *Z. tequila* were from Teuchitlán, *G. viviparus* from Texcoco, *S. bilineata* from Álvaro Obregón and *X. eiseni* from San Sebastián.

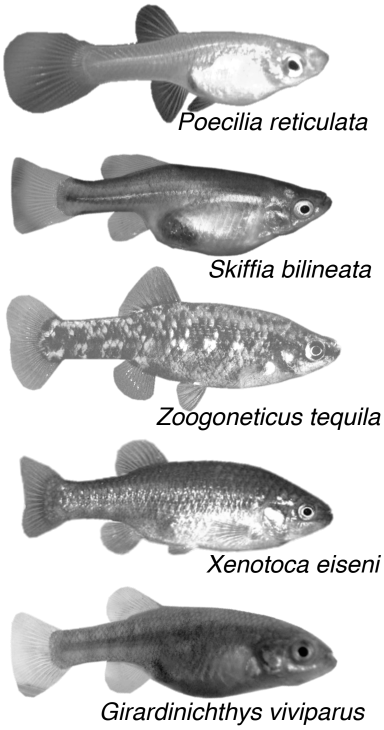

Figure 6.1. Species used in these experiment, all individuals are adult females (photo composition by M. Camacho-Cervantes).

This study was divided into two phases: in the first I measured foraging behaviour in the presence of mixed or single species shoals (*foraging benefits test*). I then evaluated whether guppies would shoal with goodeinaes (*heterospecific association test*). Focal fish were used only once and returned to stock tanks after each trial. Fish used to form the shoals were haphazardly selected from three tanks holding ~15 fish of one species each to avoid pseudoreplication (Hurlbert 1984). Observations were made between 10:00 and 16:00 h using two identical glass tanks (45x25x30 cm) each with a gravel bottom.

6.3.1 Foraging efficiency

In the *foraging* trials, pelleted fish food (Pleco Sticks®) was placed at the bottom of a randomly-selected corner of the tank at the beginning of the day. Shoals were assembled with a female guppy from the focal tanks and haphazardly selected individuals from the shoal tanks to produce the desired composition for a given trial (Table 6.1), then gently introduced to the observation tank. Shoals typically consisted of three guppy females and three females of one goodeinae species (Table 6.1). I also included two conspecific shoal sizes (of three or six guppies) to assess whether a change in food finding linked to an increase in a single species group size is matched when 50% of the conspecific individuals are replaced by heterospecifics. The shoal was observed for 10 minutes and the time and species of the first fish to locate the food was recorded. I then recorded the time spent foraging by the focal female during the rest of the trial. Each of the six treatments was replicated twenty-two times. Replicates for all treatments were performed in a random order.

6.3.2 Heterospecific association

For the *heterospecific association* trials (Table 6.1) all shoals consisted of six fish (in one treatment these were all guppies, in the others the shoal consisted of three guppies and three goodeinaes of the same species). Shoals were assembled as before and then gently placed in a bottomless bottle inside the observation tank to acclimatise for 10 min; the bottle was then carefully lifted and removed. The focal female was then followed for eight minutes. Every 15 seconds I recorded the species and distance, in body lengths, to the closest heterospecific and conspecific fish. Each of the five treatments was replicated 15 times in a random order.

Table 6.1. Composition of shoal in the trials to test *foraging benefit* (22 replicates) and *heterospecific association* (15 replicates). All fish used were females and focal individuals are included in the number of guppies contained in each trial.

Heterospecific sp.		Guppy females	Total fish
Foraging benefits			
	-	3	3
	-	6	6
S. bilineata	3	3	6
Z. tequila	3	3	6
X. eiseni	3	3	6
G. viviparous	3	3	6
Heterospecific association			
	-	6	6
S. bilineata	3	3	6
Z. tequila	3	3	6
X. eiseni	3	3	6
G. vivipaurs	3	3	6

6.3.3 Data analysis

Size of the focal individual relative to that of their shoal mates did not vary across treatments (ANOVA, $F_{5,126}$= 0.72 p = 0.61), nor did relative size explain the focal fish behaviour (latency to find food: r^2 = 0.007, p = 0.16; percentage of time spent foraging: r^2 = 0.001, p = 0.86). Fish size was therefore not included in the analyses.

To evaluate the foraging benefits obtained by guppies in shoals of different compositions I first asked (using χ^2 tests) whether one species in the two species trials consistently found the food first. Next I examined the time taken by the focal female to begin foraging. These data were log transformed to approximate normality. An ANOVA, followed by Tukey HSD post-hoc tests, was then used to assess the differences amongst treatments.

I examined association patterns using analyses of variance (ANOVA). In the first test I asked whether the number of occasions in a trial (out of a maximum of 32) on which the focal female was shoaling with a conspecific, (defined as the focal female being within one body length, or less, of another guppy, varied between treatments. In the second test I asked whether the extent to which focal females shoaled with heterospecifics, defined using the one body length criterion as before, depended on the species of goodeinae involved. post-hocTukey tests were used when treatment effects were significant. All analyses were performed using R statistical software (R-Core-Team 2013).

6.4 Results

6.4.1 Foraging efficiency

The time taken for the focal fish to find food varied across treatments ($F_{5,75} = 20.39$, $p < 0.001$; Figure 6.2). Post-hoc tests revealed that when guppies were in a single species shoal of six, the focal female found food more quickly than when there were three guppies in the tank. This advantage also occurred in three out of the four cases when the shoal was composed of both guppies and goodeinaes (i.e. in the presence of *S. bilineata*, *Z. tequila* or *X. eiseni* but not when the additional fish were *G. viviparus).* Focal individuals also increased the proportion of time they spent foraging when the shoal increased from three to six in all treatments, except -again- in the case of *G. viviparus*, where the focal female behaviour was indistinguishable from that exhibited in a shoal of three guppies ($F_{5,75} = 26.65$, $p < 0.001$; Figure 6.3). With the exception of the trials with *G. viviparus,* the heterospecific shoal members located the hidden food more quickly, or as quickly as shoal with only guppies did (Table 6.2).

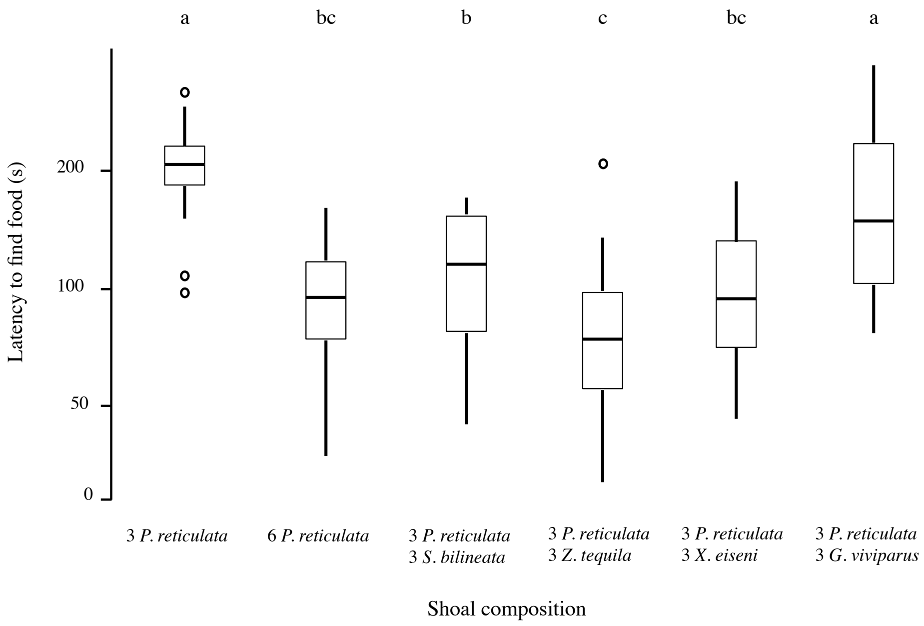

Figure 6.2. Time (max = 600 s, in a log scale) the focal fish took to find the food for each shoal composition. Horizontal lines in the bars represent the median, boxes indicate interquartile ranges and vertical lines show the range excluding outliers (circles). Letters represent the results of a Tukey HSD post-hoc test.

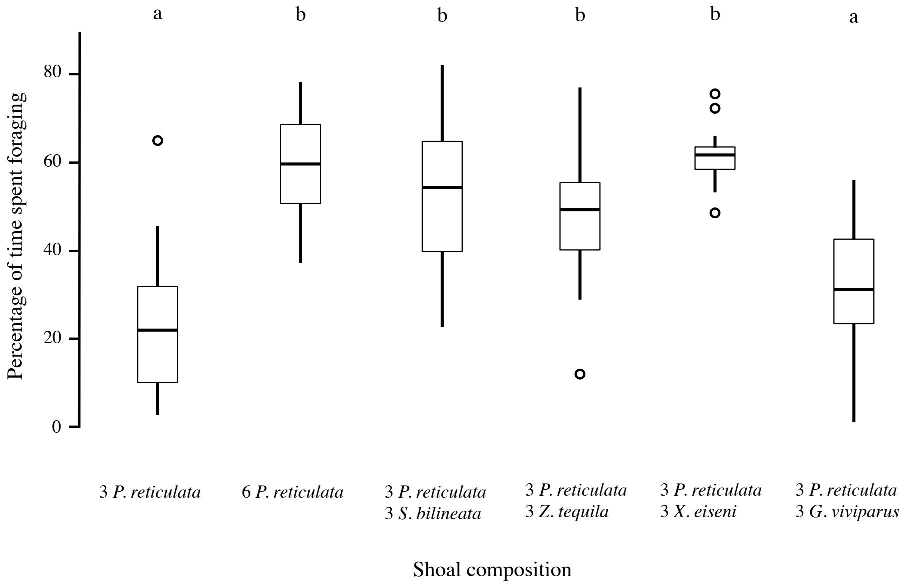

Figure 6.3. Percentage of the time after finding food that the focal spent eating for each shoal composition. Horizontal lines in the bars represent the median, boxes indicate interquartile ranges and vertical lines show the range excluding outliers (circles). Letters represent the results of a Tukey HSD post-hoc test.

Table 6.2. Species of the first fish to locate the food in the 22 replicates of the trials to evaluate *foraging benefits* p-values from χ^2 tests.

Tretment	Conspecific	Heterospecific	p-value
3 *P. reticulata* 3 S. *bilineata*	6	16	0.033
3 *P. reticulata* 3 *Z. tequila*	1	21	< 0.001
3 *P. reticulata* 3 *X. eiseni*	9	13	0.393
3 *P. reticulata* 3 *G. viviparus*	17	5	0.010

6.4.2 Heterospecific association

The number of times the closest guppy was found within one body length of the focal female was not significantly different in all treatments ($F_{4,75} = 2.25$, $p = 0.071$). However, the extent to which the focal females shoaled with heterospecifics varied between treatments ($F_{3,60} = 23.49$, $p < 0.001$, Figure 6.4). Post-hoc tests revealed that guppies were less likely to associate with *G. viviparous* than with any of the other three species of goodeinaes, but equally likely to associate with them as with conspecifics.

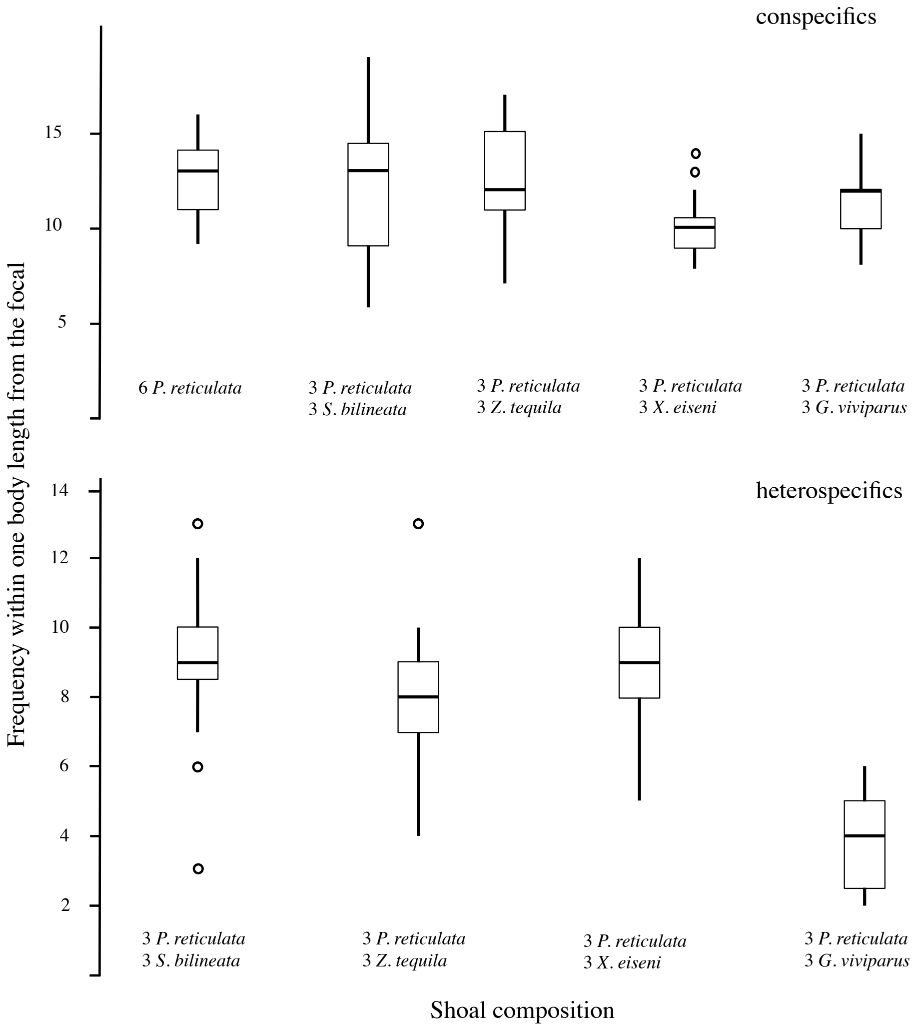

Figure 6.4. Times (max = 32) focal fish were found within one body length or less from the closest conspecific and heterospecific. Horizontal lines in the bars represent the median, boxes indicate interquartile ranges and vertical lines show the range excluding outliers (circles).

6.5 Discussion

My results demonstrate that guppies – regarded as one of the world's most invasive freshwater fish – are able to find food equally fast and spend more time foraging, when shoaling with native heterospecifics as they would by belonging to a conspecific shoal of the same size. Being part of a large shoal of conspecifics enhances foraging success of the individuals that constitute it (Pitcher et al. 1982, Krause and Ruxton 2002). Guppies are amongst the species in which it has been shown that social interactions can result in foraging benefits (Laland and Williams 1997, Day et al. 2001, Reader et al. 2003). Individuals lacking information about the local environment can, if joining a group, learn from other more knowledgeable conspecifics (Suboski and Templeton 1989). Indeed, foraging information may be transmitted by processes as simple as the tendency to follow other fish (Laland and Williams 1997). Here I have shown that these benefits extend across, as well as within, species.

In the trials of this experiment, goodeinaes were often the first to find the food, with guppies subsequently locating it. Being able to follow other individuals and/or join groups to find food more efficiently would annul one major disadvantage that locally scarce invading fish have to face (Tobin et al. 2011). Yet there are advantages of belonging to a larger group other than faster location of hidden food. A major benefit of these associations is the increased vigilance associated with 'many eyes' (Magurran and Higham 1988). It is believed that there is a positive relation between being a successful forager and avoiding predators (Cresswell et al. 2003). Larger flocks or shoals are better at detecting approaching predators and taking advantage of the dilution effect, but, crucially, the individual members devote less time to scanning for potential threats (Metcalfe 1989, Cresswell et al. 2003). This effect, which leave more time to, for instance, feeding, occurs even in the absence of an evident predation risk, and helps reduce the individual fitness cost of predation (Lind and Cresswell 2005). I showed that focal females not only found food faster in the larger shoals (whether the additional shoal members were conspecifics or heterospecifics) but devoted more time to foraging. Indeed, the link between the tendency to associate with a given goodeinae species and the foraging advantages that accrue when it is present, directly imply shoaling behaviour as a cause of the foraging gains. In short, my results substantially extend earlier research on single species shoals by showing that the foraging advantages of increased shoal size apply when the additional conspecifics are replaced by heterospecifics.

The natural habitat of most of the goodeinae species used in this study would already have been invaded to a lesser or greater extent by guppies. It is therefore likely that invading guppies in México have already been able to exploit the foraging and other benefits of heterospecific shoaling. However, a further important finding of this research is that not all native species that might be encountered will deliver the same foraging gains. Indeed, in this study associations with *G. viviparus* brought no foraging gains. This outcome highlights the context dependent nature of invasions (Arim et al. 2006), and highlights the need to give more attention to exotic native interactions when assessing invasion risk. Indeed, some species might be more helpful to guppies than others, and thus be under higher risk of invasion.

Chapter 7

General discussion

The invasion success of a species depends on its ability to cope with the novel conditions it will encounter outside its native range, these include unknown food sources, predators and competitors (Sax et al. 2007). Guppies are well known for being social and gaining many benefits from doing so. In Chapter 2, I tested if their shoaling tendency remains when their only option is to shoal with heterospecific individuals. Guppies prefer to associate with other guppies; nevertheless, they will shoal with heterospecifics rather than remain alone. Chapter 2's experiments were carried out in Trinidad, where guppies are native, using another native and an invasive to Trinidad species. I hypothesised that if guppies were willing to associate in Trinidad, where they are native and thus adapted to the environment, they would also be willing to do so in their invasive context.

In México, as in Trinidad, guppies showed a tendency to associate with heterospecifics. When their options were to remain alone or associate with heterospecifics, guppies joined Mexican topminnows shoals. This could be an example of a trait species exhibit in their native environment that is also useful in an invasive context. Gould (1991), coined the term 'exaptation' to name species' traits that were selected for a given environment (adaptation) and can be used in another one where they have never been. This type of trait enhances survival and ultimately establishment of species when relocated to a non-native area with fairly similar environmental conditions (Cote et al. 2008). I tested the tendency of Mexican topminnows to associate with guppies (Chapter 3), and albeit while less social, goodeidae are still willing to shoal with heterospecifics. It is unknown whether goodeidae benefit in anyway from shoaling with guppies, but these results support the idea of guppies being able to join their shoals.

Mexican topminnows' tendency to associate with heterospecifics provides guppies with opportunities to mitigate Allee effects. However, Mexican topminnows might suffer from these interactions. Valero et al.'s (2008) carried out a series of experiments where she found that guppies harass Mexican *S. bilineata* females. Still, I found in all the experiments with *S. bilineata* (Chapter 3 and 6), other goodeidae and guppies that they are willing to interact with each other. It is possible that goodeids, as well as guppies, gain benefits when increasing their shoal size, even if the shoaling partners are invasive guppies. This has never been tested before.

Despite the fact that individuals from different species are not genetically related, heterospecific interactions sometimes can provide individuals with similar advantages to those provided by kin, because these interactions ultimately enhance the fitness and reproductive success of individuals (Wyatt et al. 2013). Results of Chapter 4 are consistent with these ideas; I found that pairs formed by a guppy and a goodeidae tend to spend more time in close proximity to each other when exploring simple novel environments than when exploring complex ones and this behaviour was the same when two guppies formed the pair. Thus, when there are few of any conspecific available to associate with, but there are fairly similar heterospecifics, guppies are still able to enjoy the benefits of being in a group.

As explained in the Introduction, many biological invaders are subject to Allee effects –disadvantages of being part of a small population. The ways in which they manage to avoid these costs (*e.g.* higher investment in reproduction) is of interest in attempts to prevent and manage biological invasions and ultimately biodiversity loss (Tobin et al. 2011). The number of individuals that survive in transit from their native environment to the novel one is believed to be a good indicator of invasion success, the more individuals trying to establish in the novel environment the more likely they are to succeed (Lockwood et al. 2006). According to Holway and Suarez (1999), behaviour is a key component in invasion success, as it influences competitive ability and patterns of spatial spread. In the case of guppies, tendency to associate with other species opens an opportunity for invaders to increase their shoal size when few conspecifics are available to shoal and thus avoid Allee effects.

Understanding the traits common in successful invader species is one of the major ways of preventing invasions (Chapple et al. 2012). These traits help managers to locate key areas to implement plans to prevent and manage biological invasions (Guisan et al. 2013). While Poeciliids, including the guppy, possess many of the traits associated with successful invaders (Lodge 1993, Kolar and Lodge 2001, Magurran 2005, Deacon et al. 2011) such as phenotypic plasticity (Carvalho et al. 1996, Auer 2010), ovoviviparity (Magurran 2005) and a flexible life history (Rodd and Reznick 1997) the likelihood that founders will establish a viable population may depend on many local factors including the traits of the species that already occur there. There is no consensus regarding which species or community attributes promote invader success or explain spread dynamics (Arim et al. 2006, Garcia-Berthou 2007). In Chapter 3, I found that a tendency to associate with heterospecifics is present in guppies as well as in Mexican topminnows, which opens an opportunity for guppies to avoid the disadvantages of being part of a small population. Thus, places where species that help or might help guppies to overcome the first stages of invasion and form a viable population are at a higher risk of invasion. After finding that guppies benefit from increasing the shoal size with heterospecifics as much as with conspecifics, the second section of Chapter 6 corroborated not only that the amount of benefit was related to the willingness of guppies to associate with the different species (Figures 6.2 and 6.4), but that Mexican topminnows are willing to associate with guppies. This second section was not designed to test specifically the willingness of goodeinae to associate with guppies, nevertheless the experimental design allowed fish to swim freely in the tank and heterospecific associations were seen frequently.

The prevention and eradication of invasive species requires considerable effort, and one of the first steps is to determine which factors regulate the different invasion phases (Sakai et al. 2001). Chapter 3 assessed guppies' boldness to explore novel environments. Boldness may be a key trait during the establishment, lag period and spread phases of invasion (Sakai et al. 2001, Cote et al. 2011). I found that guppies prefer to explore complex habitats and that their decision to leave the shelter was unaffected by whether their partner fish was a conspecific or a heterospecific. This is consistent with the findings of Chapter 4, where I found guppies are able to acquire information about food availability equally from heterospecifics and conspecifics. In Chapter 4 I measured the swimming patterns of guppies in accordance to goodeinaes, as it is possible to acquire knowledge from processes as simple as following (Laland and Williams 1997). Guppies changing their behaviour in accordance with goodeinaes might explain why they were able to locate food faster when shoaling with the species they had a higher tendency to associate with (Chapter 6).

Ecological overlap of species niches, such as similar or equal food sources and problems, leads to learning processes across species. For example, keeping track of the foraging choices of other species with the same food requirements can lead individuals to information as valuable as the one gathered from individuals of its same species (Avargues-Weber et al. 2013). As an invader, acquiring information via trial-and-error strategies from the unknown environment can be costly and even fatal (Wright et al. 2010). As noted in the Introduction, the availability of conspecifics during the first stages of invasion is uncertain; moreover, these conspecifics might not have either knowledge on suitable food sources or sheltering areas.

Chapter 5 explored the ability of guppies to acquire information from goodeinaes when the goodeinaes had information on food availability that guppies did not. Mönkkönen and Forsman (2002) found that migratory birds prefer to associate with residents to acquire information on food availability and potential breeding grounds. Consistent with this, I found that guppies were able to coordinate searching behaviour with the knowledgeable individuals regardless of their species. Moreover, results of Chapter 6 show that not only do they gain information from other species but also that this enables them to be more efficient foragers. Heterospecific interactions, like the ones I found among Mexican topminnows and invasive guppies, lead individuals to a faster and safer way, compared to trial-and-error, of acquiring information about their surroundings (Danielson 1991).

Among freshwater fish invasions, establishment success is the most studied phase and it seems to be multi-factorial and dependent on the context. For example in the U.S.A., 87 species of fish are known to have been introduced to California, and among these the main predictors of establishment success are physiological tolerance, smaller size of native range and -somewhat circularly- prior invasion success (Garcia-Berthou 2007). The number of species that successfully establish themselves outside their native range is increasing, as is the number of these that cause economic and ecological damage (Holway and Suarez 1999, Simberloff et al. 2013).

My results suggest that plastic social behaviour can help invading species to overcome initial numerical disadvantages and become effective ecological competitors. This, together with direct negative effects on local species (e.g. introduction of novel parasites and sexual disruption), may facilitate the establishment of viable populations and the eventual replacement of native species.

Population Viability Analysis (PVA) is a tool to estimate a population's growth or decline in a given amount of time, it takes into account parameters relating species life history characteristics and other population statistics, such as mortality rate or inbreeding coefficients (Morris and Doak 2002). PVA is mostly applied to model the extinction probabilities of endangered or reintroduced species. However, they can also be used as a tool to manage invasive populations. A PVA carried out for by Brassil (2001) for a metapopulation revealed that the Allee effect threshold has an important impact on the expected time of extinction (Brassil 2001).Understanding how social behaviour, in response to heterospecifics and well as conspecifics, can mitigate Allee effects, will improve the predictive power of PVA. Invaders' responses to other individuals being present or not have a great influence in their survival. Individuals that invaders encounter in the novel places where they arrive might represent predation, competition or an aid to protect from predators and take better decisions on where to shelter and/or forage (Reale et al. 2007). The results of this thesis demonstrate that heterospecific group dynamics could help invaders to avoid Allee effects when colonizing novel environments. Fogarty *et al.* (2011) developed a simulation model that integrated life-history theory, animal personalities, network theory, and spatial ecology knowledge to explain variation in animal invasion success. This allowed them to predict spread for given characteristics of invaders.

Assessments like the one done in this thesis, contribute to improved models and estimates.

The goodeidae is an important group of endemic freshwater fish in México; invasive species colonizing its habitat, along with habitat destruction, is one of the main causes of the severe reduction of its populations (De la Vega-Salazar and Macias-Garcia 2005). At the moment, there is no strategy focused on the protection of the Goodeidae family; however, they are included in CONABIO's (National Mexican Commission for Biodiversity) plans to protect Mexican biodiversity. CONABIO intends to gather scientific knowledge on biodiversity issues and make it available to the public. Their plans set out to preserve areas as a whole through the CONANP (National Mexican Commission of Protected Natural Areas), areas with high indexes of biodiversity are always the priority (CONABIO 2014). At the same time, some universities (including UNAM and UMSNH) have *ex situ* conservation programs to keep as many species as possible in aquaria. These *ex situ* programs have helped avoid extinction of some species while also making it possible to further research the species. For example, the goodeidae species *Ameca splendends* (used in experiments of Chapters 4 and 5), has few, and small, populations in the wild. However, there are populations of them in the Aquaculture Laboratory at the School of Biology (UMSNH), Animal Behaviour Laboratory at the Ecology Institute (UNAM) and the St Andrews Aquarium. These *ex situ* programs are a short-term partial solution and much more needs to be done to preserve endemic Mexican topminnow species in the long term. Suggested plans include habitat restoration, including extirpation of invasive species, and reintroduction of population in the best-preserved areas (Dominguez-Dominguez and Pérez Ponde de León 2007). Results of this thesis are the beginning of the study of native species that aid guppies to successfully colonize new habitats.

Further assessment on the species that are of most aid to invaders and their presence or absence in freshwater bodies could help to better quantify invasion risk of rivers and lakes containing highly endemic and/or endangered populations of fish.

7.1 Future research

Identifying future invaders and taking effective steps to prevent their dispersal and establishment constitutes an enormous challenge to both conservation and international commerce (Mack et al. 2000). This study reveals that sociability is one of the key predictors of species establishment in novel localities. It highlights the need to pay attention to behavioural traits when assessing the invasion risk associated with releases or escapes of exotic species.

Many of the studies done in biological invasions are focused on describing the characteristics of species that enable them to be successful when invading; indeed, predicting invaders has been a long-standing goal of ecologists (Kolar and Lodge 2001). Results of my thesis show that not only certain characteristics of invaders but also the interactions they might have with natives have an effect on the likelihood of establishment success. I demonstrated that natives provide advantages to invaders. However, benefits are not the same from all species. Thus, further research needs to be done to define which species are more helpful to guppies and explore which sites composition might be under higher invasion threat.

Identifying the species composition of freshwater bodies containing highly endemic species (such as the goodeidae) and which of these species are of any benefit to invaders will help focus conservation efforts in the most needed places to manage and prevent invasive species from establishing. When invasive populations are already established there are aspects that might be helpful to eradicate them, such as the Allee effect threshold of the invasive populations. Indeed, in the ponds where guppies have been identified to compete with native goodeids researchers are selectively removing invaders using electrofishing techniques (Macias-Garcia, pers. com. 2014). In this thesis I assessed ways in which guppies might overcome Allee effect, however, it is still uncertain where is the Allee effect threshold for invasive guppies populations. It is known that a single pregnant guppy female is able to form a viable population (Deacon et al. 2011, Deacon et al. 2014). Nevertheless, Deacon et al. (2011 and 2014) tested guppies' ability to form a viable population from a single individual alone, without species that could compete with them for resources or even predate their juveniles.

A different and more realistic approach to assess guppies' Allee effect threshold when invading would be to do a similar mesocosm experiment in a guppies' invasive context. In the Mexican case, it would be worth including native species, especially the ones expected to aid guppies, like *Z. tequila* or *S. bilineata*, and the ones expected to aid them less or none, like *G. viviparous* (see Chapter 6), to test if my findings on the benefits guppies get from natives remains in the long term. The hypothesis would be that some species aid guppies more than others and the ones adding the most are under higher risk of being outcompeted by guppies. To test this hypothesis, it would be necessary to collect data on the species composition and abundance in the mescosoms through the longest possible period of time. Ultimately, I expect species of more aid to guppies would help them to form a viable population and then guppies' population would grow to displace natives. Species that facilitate invaders are, potentially, under higher risk of being outcompeted after colonization. Studies like the ones carried out in this thesis and the proposed in this section could help to identify them. Sociability is a key predictor for species ability to invade. I highlight the need to consider behaviour when assessing invasion risk of exotic species and deciding which areas should be prioritized for biological conservation.

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Appendix

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Association tendency and preference for heterospecifics in an invasive species

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Accepted 9 December 2013; published online 17 January 2014
DOI:10.1163/1568539X-00003169

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Cite this article: Camacho-Cervantes M, Macías Garcia C, Ojanguren AF, Magurran AE. 2014 Exotic invaders gain foraging benefits by shoaling with native fish. *R. Soc. open sci.* **1**: 140101.
http://dx.doi.org/10.1098/rsos.140101

Received: 11 June 2014
Accepted: 20 October 2014

Subject Areas:
behaviour/ecology/environmental science

Keywords:
interspecific interactions, invasion success, guppies, goodeids, sociability, Allee effects

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rsos.140101 or via http://rsos.royalsocietypublishing.org.

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Exotic invaders gain foraging benefits by shoaling with native fish

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1. Summary

Freshwater habitats are under increasing threat due to invasions of exotic fish. These invasions typically begin with the introduction of small numbers of individuals unfamiliar with the new habitat. One way in which the invaders might overcome this disadvantage is by associating with native taxa occupying a similar ecological niche. Here we used guppies (*Poecilia reticulata*) from a feral population in Mexico to test the prediction that exotic shoaling fish can associate with heterospecifics, and that they improve their foraging efficiency by doing so. Guppies have invaded the Mexican High Plateau and are implicated in the declines of many native topminnow (Goodeinae) species. We show that heterospecific associations between guppies and topminnows can deliver the same foraging benefits as conspecific shoals, and that variation in foraging gains is linked to differences in association tendency. These results uncover a mechanism enabling founding individuals to survive during the most vulnerable phase of an invasion and help explain why guppies have established viable populations in many parts of Mexico as well in every continent except Antarctica.

2. Introduction

Invasive species, a major agent of global change [1,2], modify the environment at multiple ecological levels, lead to community disassembly and alter species interactions across a range of spatial and temporal scales [2–4]. These changes result in biodiversity loss and wildlife homogenization [5] and are considered some of the greatest threats to ecosystem services [6,7].

Although many species are translocated from their native range, most do not establish viable populations [1,2]. Invasions typically begin with the introduction of just a few individuals [1], and behaviour may play a crucial role in enabling such

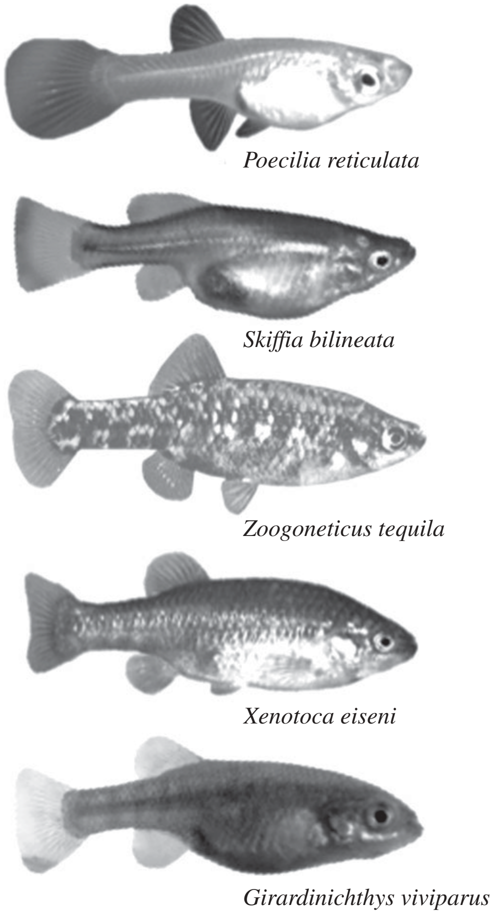

Figure 1. Species used in these experiments, all individuals are adult females (photo composition by the authors).

individuals to compensate for Allee effects—the disadvantages linked to membership of a small population [8,9]—and to survive until they can reproduce [10,11].

In fish, as in other taxa, social behaviour can enhance survival [12]. Apart from for mating, fish associate with other individuals in contexts such as hibernation, sleeping and foraging [13], thus gaining benefits including protection from predators [14], increased foraging efficiency [15] and reductions in the energetic costs of movement [12]. However, animal associations are not limited to single species groups. Mixed-species (heterospecific) aggregations, i.e. two or more species associating in time and space [16], occur regularly in nature; examples include fish [17,18], birds [19] and even members of very distant taxa (e.g. monkeys and birds [20]). Heterospecific aggregations occur when they are beneficial to the participants [21]. For example, fathead minnows (*Pimephales promelas*) can learn to recognize heterospecific alarm cues, and this decreases their probability of being attacked and captured during predator encounters [22].

Freshwater ecosystems are among the most altered and invaded in the world [23]. Like islands, they are vulnerable due to their geographical isolation and high rates of endemicity [24]. Common routes of fish invasion include introductions of biological control agents [25], releases designed to provide food and sport or discards of aquarium fish and bait buckets [26]. Although in some cases freshwater fish invasions may have a positive outcome for the local fish communities and on human economy [27], in others their effects are catastrophic [28]. Freshwater invaders are responsible for effects that range from local extinctions to alterations in nutrient and energy fluxes [29].

The guppy (*Poecilia reticulata*) is native to Trinidad, Guyana, Venezuela and Surinam [30,31]. It is a remarkably opportunistic species with reproductive adaptations that enable a few individuals or even a single pregnant female to found a viable population [31]. Guppies possess many of the physiological, behavioural and life-history characters that are associated with extreme adaptability [31]—traits associated with increased invasion success [32]. During the past century, guppies have been released into environments outside their native range to control mosquitoes and reduce malaria, and also accidentally as a consequence of escapes from home aquaria. There are now established populations in at least 72 different countries across the globe [33]. This includes Mexico [34], where they are found in many localities including the Lerma-Santiago River system, the main basin of the Mexican High Plateau and a watershed noted for its high levels of endemicity. Endemics include Goodeinae, a clade consisting of *ca* 45 species of small livebearing fish [35], 17 of which are included in the IUCN Red List of Threatened Species [36] (see also [37,38]). The Goodeinae are mostly omnivorous freshwater topminnows that inhabit shallow ponds, lakes and rivers. They are the focus of this study because many species are morphologically similar to guppies, feed on the same resources and occupy the same habitat. In some cases, population declines have been directly attributed to guppy invasions [39].

We tested the hypothesis that small shoals of invading guppies gain foraging benefits by associating with topminnows. We quantified foraging benefits associated with an increase in conspecific shoal size. We expected that fish would locate food faster and increase the time spent foraging when associating with others [40]. We predicted that foraging advantages would also apply when the additional shoal members were heterospecific rather than conspecific fish. To assess whether these effects can be generalized across species we repeated the experiments with four topminnow species (*Skiffia bilineata*, *Zoogoneticus tequila*, *Xenotoca eiseni* and *Girardinichthys viviparous*; figure 1) that are morphologically similar to guppies [41]. In addition, we asked whether the differences in the foraging advantages that accrue when individuals belong to a larger shoal could be linked to the guppy's tendency to associate with a given species.

3. Material and methods

Experiments were carried out at the main campus of the National Autonomous University of México (UNAM) in México City from July to September 2013. Guppy (*P. reticulata)* individuals were collected from a population established in the wild in Ahuisculco, Jalisco, where no other species used in this experiment occur. In the case of the topminnows, *Z. tequila* were originally from Teuchitlán in Jalisco; *G. viviparus* originated in Texcoco, México; *S. bilineata* were originally from Álvaro Obregón in Michoacán and *X. eiseni* from San Sebastián in Jalisco. All fish were collected from either the wild or outdoor ponds within a two-week period, and carefully transported in plastic bags half filled with water and half filled with air to the laboratory, where they remained for roughly the same amount of time (*ca* 12 days) before trials. Stock tanks (45 l) contained 15–20 fish each and were set up with aged tap water, which was treated with Stress Coat. Each tank contained a filter, water pump and plants. Photoperiod was 12 L : 12 D from 7.00 to 19.00 h. Water daily temperature ranged between 19°C and 22°C. Tanks were visually isolated from one another with an opaque sheet. We used only female fish in the experiment as they devote more time to shoaling and foraging than males [42]. Individuals in a given trial were kept separate for several weeks prior to observations to avoid familiarity effects [43]. Fish were fed with commercial flake food (SeraVipan) daily at the end of each day. After the experiment was completed (70 days), all fish remained in stock tanks in the laboratory. In the wild, species used in this study have similar foraging patterns and forage from similar sources: plants, detritus and smaller animals [35]. Nevertheless, *Z. tequila* is, among the species used in these experiments, the most likely to feed at the bottom [44].

Our study was divided into two parts: in the first we measured foraging behaviour in the presence of mixed or single species shoals (*foraging benefits test*). We then evaluated whether guppies would shoal with topminnows (*heterospecific association test*). In the two parts, we selected a guppy prior to the start of each observation (focal) and recorded its behaviour; they were easily distinguished from the rest of the fish due to minor individual differences, such as eye size or fin scars. Focals were used only once and returned to stock tanks after each trial. Fish used to form the shoals were haphazardly selected from three tanks holding approximately 15 fish of one species each to avoid pseudoreplication [45]. Observations were made between 10.00 and 16.00 h using two identical glass tanks (45 × 25 × 30 cm) each with a gravel bottom.

In the *foraging* trials, pelleted fish food (Pleco Sticks) was placed at the bottom of a randomly selected corner of the tank at the beginning of the day. Shoals were assembled with a female guppy from the focal tanks and haphazardly selected individuals from the shoal tanks to produce the desired composition for a given trial, then gently introduced to the observation tank. Shoals typically consisted of three guppy females and three females of one Goodeinae species. We also included two conspecific shoal sizes (of three or six guppies) to assess whether a change in food finding linked to an increase in a single species group size is matched when 50% of the conspecific individuals are replaced by heterospecifics. The shoal was observed for 10 min to determine both the time (seconds) and species of the first fish to locate the food. We recorded the time (seconds) it took the first fish to locate the food and also the time (seconds) it took the focal guppy to do it. We then recorded the time spent foraging by the focal guppy female during the rest of the trial. As some individuals had more time left than others, data for this variable were analysed using the proportion of time spent foraging from the available time (time spent foraging divided by the remaining time after the food was located). Each of the six treatments was replicated 22 times. Replicates for all treatments were performed in a random order.

For the *heterospecific association* trials, all shoals consisted of six fish (in one treatment these were all guppies, in the others the shoal consisted of three guppies and three Goodeinae of the same species). Shoals were assembled as before and then gently placed in a bottomless bottle inside the observation tank to acclimatize for 10 min; the bottle was then carefully lifted and removed. The focal female was then followed for 8 min. Every 15 s we recorded the species and distance (spot sampling), in body lengths, to the closest heterospecific and conspecific fish. Each of the five treatments was replicated 15 times in a random order.

Standard length of the fish used in these experiments ranged from 17.2 to 35.1 mm. However, the shoals and focals were size assorted trying to minimize differences in size that could influence behaviour. Average (±s.d.) difference between the standard length (SL) of the focal and the average SL of the shoal fish (i.e. relative size of the focal fish) was -0.6 ± 1.5 mm (ranging from -4.1 to 3.2 mm) and was not significantly different across treatments of shoal composition (ANOVA, $F_{5,126} = 0.72$, $p = 0.61$). However, all analyses were performed including difference in size as a covariate. Since neither difference in size ($F < 0.783$, $p > 0.39$) nor the interaction between difference in size and treatment ($F < 0.465$, $p > 0.5$) had a significant effect, we concluded that size did not play a role in foraging or association patterns in this experiment. Therefore, for the benefit of clarity, the Results section only presents the analyses with treatment as the main factor.

In the Foraging benefits section, in order to evaluate the foraging benefits obtained by guppies in shoals of different compositions we first asked (using χ^2-tests) whether one species in the two species trials consistently found the food first. Next we examined the time taken by the focal female to begin foraging. These data were log transformed to approximate normality. An ANOVA, followed by Tukey HSD post-hoc tests was then used to assess the differences among treatments.

In the Heterospecific association section, we examined association patterns using ANOVA. In the first test, we asked whether the number of occasions in a trial (out of a maximum of 32) on which the focal female was shoaling with a conspecific, defined as the focal female being within one body length of another guppy, varied between treatments. In the second test, we asked whether the extent to which focal females shoaled with heterospecifics, defined using the one body length criterion as before, depended on the species of topminnow involved. Post-hoc Tukey tests were used when treatment effects were significant. All analyses were performed using R statistical software [46].

4. Results

4.1. Foraging benefits

The time taken for the focal fish to find food varied across treatments ($F_{5,75} = 20.39$, $p < 0.001$; figure 2). Post-hoc tests revealed that when guppies were in a single species shoal of six, the focal female found food more quickly than when there were three guppies in the tank. This advantage also occurred in three out of the four cases when the shoal was composed of both guppies and topminnows (i.e. in the presence of *S. bilineata*, *Z. tequila* or *X. eiseni* but not when the additional fish were *G. viviparus*). Focal individuals also increased the proportion of time they spent foraging when the shoal increased from three to six in all treatments, except—again—in the case of *G. viviparus*, where the focal female behaviour was indistinguishable from that exhibited in a shoal of three guppies ($F_{5,75} = 26.65$, $p < 0.001$; figure 3). With the exception of the trials with *G. viviparus*, the heterospecific shoal members located the hidden food more quickly, or as quickly as shoal with only guppies did (table 1).

4.2. Heterospecific association

The number of times the closest guppy was found within one body length of the focal female was not significantly different in all treatments ($F_{4,75} = 2.25$, $p = 0.071$). However, the extent to which the focal

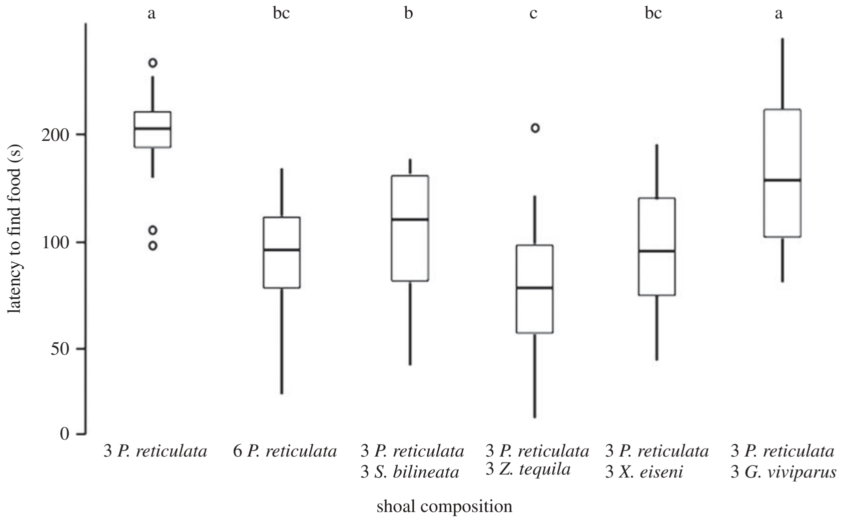

Figure 2. Time (maximum = 600 s, in a log scale) the guppy focal fish took to find the food for each shoal composition. Horizontal lines in the bars represent the median, boxes indicate interquartile ranges and vertical lines show the range excluding outliers (circles). Letters represent the results of a Tukey HSD post-hoc test.

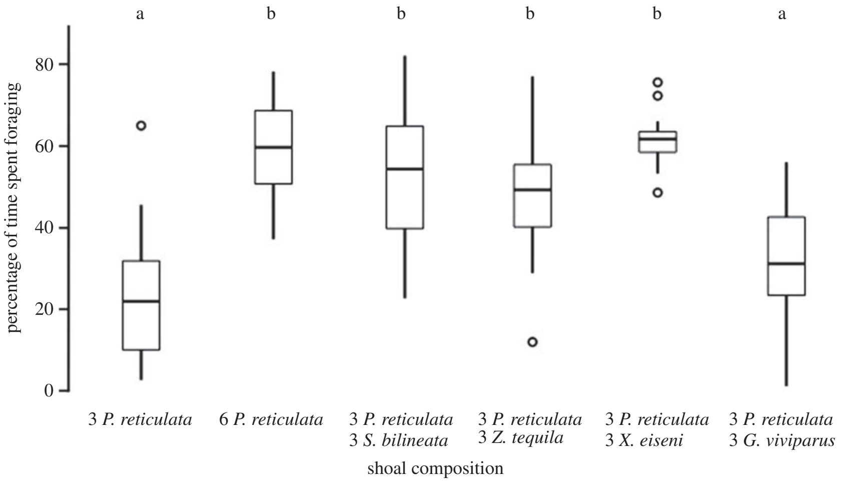

Figure 3. Percentage of the time after finding food that the focal spent eating for each shoal composition. Horizontal lines in the bars represent the median, boxes indicate interquartile ranges and vertical lines show the range excluding outliers (circles). Letters represent the results of a Tukey HSD post-hoc test.

Table 1. Species of the first fish to locate the food in the 22 replicates of the trials to evaluate *foraging benefits*; *p*-values from χ^2-tests. All treatments included three individuals of each species.

treatment	guppies first	heterospecific first	*p*-value
P. reticulata *S. bilineata*	6	16	0.033
P. reticulata *Z. tequila*	1	21	<0.001
P. reticulata *X. eiseni*	9	13	0.393
P. reticulata *G. viviparus*	17	5	0.010

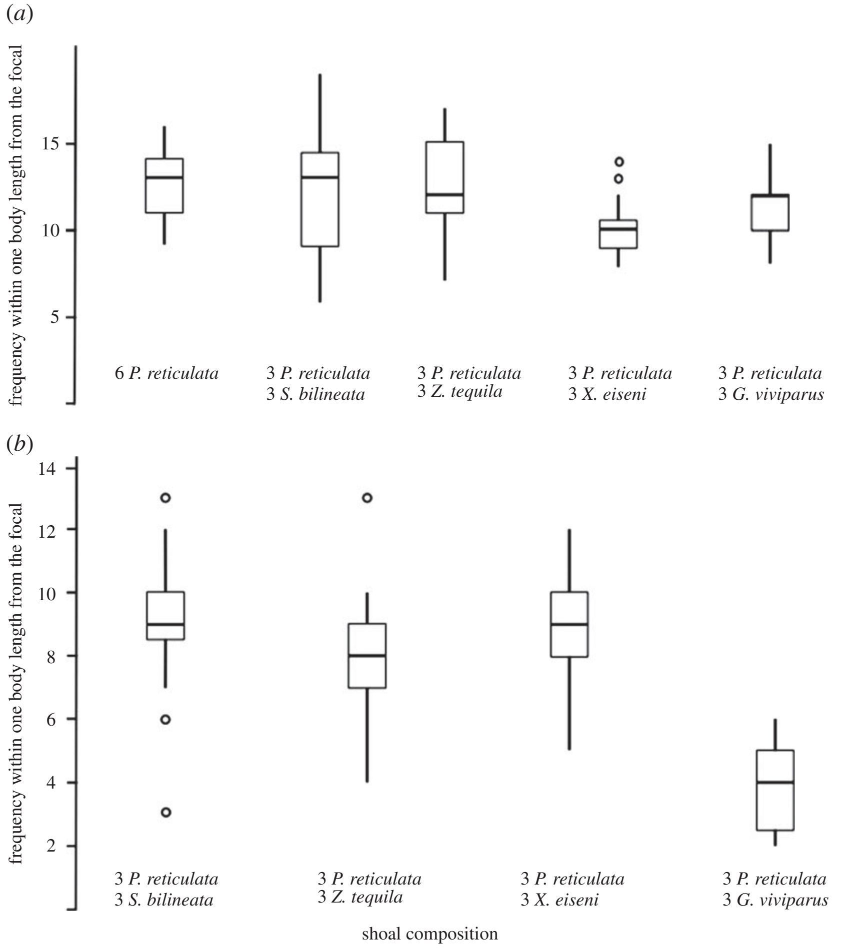

Figure 4. Times (maximum = 32) focal fish were found within one body length or less from the (*a*) closest conspecific and (*b*) heterospecific. Horizontal lines in the bars represent the median, boxes indicate interquartile ranges and vertical lines show the range excluding outliers (circles).

females shoaled with heterospecifics varied between treatments ($F_{3,60} = 23.49$, $p < 0.001$; figure 4). Post-hoc tests revealed that guppies were less likely to associate with *G. viviparous* than with any of the other three species of Goodeinae, but equally likely to associate with the latter three species as with conspecifics.

5. Discussion

Our data demonstrate that guppies—regarded as one of the world's most invasive freshwater fish—gain the same benefits, in terms of finding hidden food sources, when shoaling with native heterospecifics as they would by belonging to a conspecific shoal of the same size. Being part of a large shoal of conspecifics enhances foraging success of the individuals that constitute it [40]. Guppies are among the species in which it has been shown that social interactions can result in foraging benefits [15,47,48]. Individuals lacking information about the local environment can, if joining a group, learn from other more knowledgeable conspecifics [49]. Indeed, foraging information may be transmitted by processes as simple as the tendency to follow other fish [47]. Here we have shown that these benefits extend across, as well as within, species.

In our trials, topminnows were often the first to find the food, with guppies subsequently locating it. We refer to Goodeinae fish as topminnows because they, as the guppies, regularly forage at the water surface, yet they also forage at the bottom, and it has been reported that *Zoogoneticus* spp. are more likely to forage from the substrate than other Goodeid genera [44]. Thus, it is possible that our protocol made topminnows more likely to find the food pellets than the guppies. If so, the fact that female guppies were better able to find and consume pellets at the bottom when shoaling with topminnows is evidence that their behaviour is flexible enough to allow them to benefit from shoaling with native species. It must be noted, however, that guppy females are also likely to forage at the bottom under some circumstances [31], which may explain why they were also able to locate food faster and spend more time foraging when in larger shoals of conspecifics.

Being able to follow other individuals to find food more efficiently would annul one major disadvantage that locally scarce invading fish have to face [8]. Yet there are advantages of belonging to a larger group other than faster location of hidden food. A major benefit of these associations is the increased vigilance associated with 'many eyes' [50]. It is believed that there is a positive relationship between being a successful forager and avoiding predators [51]. Larger flocks or shoals are better at detecting approaching predators and taking advantage of the dilution effect, but, crucially, the individual members devote less time to scanning for potential threats [51,52]. This effect, which leaves more time for feeding, occurs even in the absence of an evident predation risk and helps reduce the individual fitness cost of predation [53].

In our investigation, the focal females not only found food faster in the larger shoals (whether the additional shoal members were conspecifics or heterospecifics) but devoted more time to foraging. Indeed, the link between the tendency to associate with a given topminnow species and the foraging advantages that accrue when it is present, directly implies shoaling behaviour as a cause of the foraging gains. In short, our results substantially extend earlier research on single species shoals by showing that the foraging advantages of increased shoal size apply when the additional conspecifics are replaced by heterospecifics. However, as our experimental design included only females, further research should be carried on to explore whether these advantages remain when guppy and Goodeinae males are part of the group. Indeed, it is known that guppy males interact with native Mexican topminnows and even attempt to copulate with them [41].

While Poeciliids, including the guppy, possess many of the traits associated with successful invaders [31,33,54,55] such as phenotypic plasticity [56,57], ovoviviparity [31] and a flexible life history [58] the likelihood that founders will establish a viable population may depend on many local factors including the traits of the species that already occur there. There is no consensus regarding which species or community attributes promote invader success or explain spread dynamics [5,59]. Among freshwater fish invasions, establishment success is the most studied phase and it seems to be multi-factorial and dependent on the context. For example in the USA, 87 species of fish are known to have been introduced to California, and among these the main predictors of establishment success are physiological tolerance, smaller size of native range and—somewhat circularly—prior invasion success [59].

The natural habitat of most of the topminnow species used in this study has already been invaded to a lesser or greater extent—and often intermittently—by guppies. It is therefore likely that invading guppies in Mexico have already been able to exploit the foraging and other benefits of heterospecific shoaling. Indeed, in the site were we collected guppies for this study (Ahuisculco, Jalisco) they were in close association with other species, as inferred from the fact that we found more than one species in our nets. However, a further important finding of our work is that not all native species that might be encountered will deliver the same foraging gains. Indeed, in our study associations with *G. viviparus* brought no foraging gains. This outcome highlights the context-dependent nature of invasions [5].

The number of species that successfully establish themselves outside their native range is increasing, as is the number of these that cause economic and ecological damage [10,60]. Our results suggest that plastic social behaviour could help invading species to overcome initial numerical disadvantages and become successful invaders. This, together with direct negative effects on local species (e.g. introduction of novel parasites and sexual disruption), may facilitate the establishment of viable populations and the eventual replacement of native species. This study reveals that sociability is one of the key predictors of species establishment in novel localities. It highlights the need to pay attention to behavioural traits when assessing the invasion risk associated with releases or escapes of exotic species.

Ethics statement. Topminnows (Goodeinae) were originally collected from the wild (using hand seine nets and traps) under the permit SGPA/DGVS/09253 provided by the Mexican Ministry for the Environment (SEMARNAT) and used to establish populations in outdoor ponds at UNAM.

Data accessibility. Data can be found in the electronic supplementary material.

Acknowledgements. We thank Edgar G. Ávila Luna and members of the Animal Behaviour research group at UNAM for help in the collection and maintenance of experimental fish. Hideyasu Shimadzu, Miguel Barbosa, Alan Reeve and Maria Dornelas provided helpful comments during earlier stages of this research. We thank the University of St Andrews School of Biology Research Committee for funding. M.C.-C. thanks Topo Cortés and Bati Loch for fieldwork assistance. A.E.M. acknowledges the ERC (project BioTIME 250189) and the Royal Society.

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