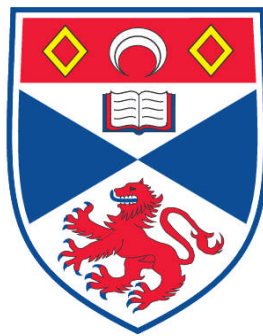# THE BEHAVIOURAL ECOLOGY OF THE TRINIDADIAN GUPPY, *POECILIA RETICULATA*, AS AN INVASIVE SPECIES

Amy E. Deacon

A Thesis Submitted for the Degree of PhD
at the
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The behavioural ecology of the Trinidadian guppy, *Poecilia reticulata*, as an invasive species

A thesis submitted for the degree of Ph.D. at the University of St Andrews

Amy E. Deacon

September 2010

Abstract

This thesis focuses on the guppy, *Poecilia reticulata*, as an invasive species. Its non-native distribution, the biology behind its success and the reasons for its introduction are examined. A worldwide email survey revealed that the guppy is established in at least 73 countries outside of its native range and that mosquito control schemes and the release of unwanted aquarium fish are the two primary routes of introduction. Knowledge gaps were identified; primarily the scarcity of scientific evidence for negative impacts of guppy introductions and similarly for mosquito control efficacy. Replicated mesocosm experiments demonstrated that female guppies are capable of routinely establishing populations, and that these retain behavioural viability over several generations. The first mesocosm study suggested that founders with very different evolutionary histories were equally good at establishing populations. The second mesocosm study suggested that monandrous females were extremely successful at establishing behaviourally viable populations, with no decline in behavioural variation. Two related foraging experiments attempted to examine the effectiveness of guppies as mosquito control agents. The first study found little evidence for the presence of 'prey switching' in guppies, questioning the validity of previous work advocating their introduction to stabilise prey populations. The second study revealed a preference for non-vector mosquito larvae in a two-prey system. However, both mosquito species were consumed equally readily when habitat complexity increased. The presence of conspecifics affected female foraging behaviour. The presence of males reduced the strength of prey preference in the first study, and an interaction between social and habitat factors affected prey preference in the second. Both demonstrate that multi-prey systems have important implications for the efficacy of poeciliids in biological control. Despite severe demographic bottlenecks, their adaptability and ability to rapidly increase in numbers enable guppies to establish and persist when introduced. Such bottlenecks are typical of introduction scenarios, warning that particular caution should be exerted when introducing this species, or other live-bearing fish, to natural water bodies.

Acknowledgements

Declaration

I, Amy Deacon, hereby certify that this thesis, which is approximately 38,000 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.

I was admitted as a research student in September 2006 and as a candidate for the degree of Ph.D. in September 2006; the higher study for which this is a record was carried out in the University of St Andrews between 2006 and 2010.

Date   Signature of candidate

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Ph.D. in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

Date   Signature of supervisor

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Contents

Chapter 1

General Introduction

1.1 Invasive species

Invasive species threaten biodiversity, ecosystem integrity, agriculture, fisheries and public health (Pimentel *et al.*, 2001; Kolar & Lodge, 2001). Alongside habitat destruction, invasions represent one of the most influential components of global change (Vitousek *et al.*, 1997).

Introductions are common worldwide, and consist both of species that have been deliberately placed in a new area and those inadvertently introduced outside of their natural range. Biological control agents and fisheries stocking are examples of deliberate introductions, whilst species arriving by means of ballast water, as unwanted pets or escapees are referred to as 'accidental' or 'by product' introductions (Moyle, 1999). Among species that are introduced to a habitat, it is estimated that only about 10% will persist and become established as self-sustaining populations, and just a fraction of these are expected to become 'invasive species' by spreading further in the habitat (Williamson & Fitter, 1996).

Invasive species are generally considered to have a negative impact on the invaded habitat and ecosystem (Lockwood *et al.*, 2007). Common effects include reduction in biodiversity as a result of interspecific competition, predation, habitat degradation or other modification of environmental conditions (Arim *et al.*, 2006). Such effects can ultimately lead to changes in the productivity and nutrient availability within the habitat, as well as influencing trophic structure and population dynamics such as the abundance and population growth of native species (Parker *et al.*, 1999). Clavero and Garcia-Berthou (2005) found that of 170 extinct animal species on the IUCN red list for which extinction causes could be compiled, 54% included the effects of invasive species. Furthermore, invaders can have negative genetic effects on existing species both by means of hybridisation with closely related native species, or indirectly by altering selection pressures or gene flow within the native community (Parker *et al.*, 1999).

Although the impacts of invasive species have been documented across terrestrial and marine systems, freshwater fish assemblages appear particularly vulnerable to the presence of exotics (Strayer, 2010). Sixty-eight percent of 20th century fish extinctions

in North America are associated with introduced species (Miller *et al.,* 1989) and model predictions suggest that many more potentially harmful species are likely to be introduced (Kolar & Lodge, 2002). In Spain, the acclimatisation of exotic freshwater fishes is one of the most important threats facing native and endemic species (Elvira & Almodóvar, 2001).

This chapter will explore the various life history, genetic, behavioural and ecological characteristics that are thought to affect invasiveness and thus should be considered when attempting to predict and manage invasions. These general characteristics will then be related specifically to the biology of the guppy, *Poecilia reticulata*, which has been widely used in the study of evolutionary ecology (Magurran, 2005), in addition to being an extremely successful invasive species. The worldwide non-native distribution of the guppy will be discussed, including the reasons behind the introductions. One of the common rationales behind guppy, and other species', introductions is biological control. The use of biocontrol and its possible pitfalls will also be explored with specific reference to the guppy.

1.2 Predicting invaders

A comprehensive understanding of the mechanisms behind invasion success is vital for designing effective strategies for controlling invasive species (Suarez *et al.*, 1999; Sakai *et al.*, 2001). Given the number of introductions that either fail to establish or fail to invade a habitat (Williamson & Fitter, 1996*a*), many studies have attempted to develop 'Invasive Species Predictive Schemes' (Whitney & Gabler, 2008). Most of these schemes are based on the idea that it is possible to identify common characteristics of invasive species that explain why certain organisms invade successfully whilst others do not, in the hope that it may be possible to predict future invaders, screen out high risk scenarios and prioritise management efforts (Kolar & Lodge, 2001; Mack *et al.*, 2000; Marchetti *et al.*, 2004*b*).

A wide range of potential 'predictors' have been investigated in relation to invasiveness (Williamson & Fitter, 1996*b*), albeit predominantly in plant and bird taxa (Kolar & Lodge, 2001). These include genetic characteristics (Barrett & Richardson, 1986; Weinig *et al.,* 2007), behavioural plasticity (Sol *et al*., 2002), life

history traits (Rossechi & Crivelli, 2001; Alcaraz *et al*., 2005), taxonomy (Karatayev *et al.*, 2009) and properties of the invaded ecosystem (Moyle & Light, 1996). Such studies indicate that although many of the characteristics that favour invasion success are taxon-specific, it is also possible to highlight similarities in invasion characteristics between groups as diverse as plants and birds (Newsome & Noble, 1986) and plants and fish (Arthington & Mitchell, 1986). This suggests that there are at least some general features that are common to successful invaders across taxa.

Life history traits play an important role in the ability of a species to be a successful invader; of particular importance are reproductive traits and their plasticity in the face of new conditions (Ghalambor *et al.*, 2007). Lodge (1993) summarised the traits that have been proposed by various researchers as common characteristics of invading species, these include: 'r-selected traits (otherwise known as 'fast' life histories, consisting of rapid maturation and production of many small offspring), high dispersal rate, single-parent or vegetative reproduction, high genetic variability, phenotypic plasticity, large native range, eurytopy, polyphagy and human commensalism (see Table 1.1).

Table 1.1: A summary of the traits that are commonly associated with invasion success (as listed in Lodge, 1993), with examples.

Trait	Example taxa	Evidence	Reference
r-selected traits	*Corbicula fluminea* (Asian clams) and *Dreissena polymorpha* (zebra mussels)	These species are highly invasive in North America, and their invasion success is partly attributed to rapid growth, early maturity, short life-spans and high fecundity.	McMahon, 2002
High dispersal rate	*Gambusia* spp. (mosquitofish and relatives)	Invasive species within this genus displayed greater dispersal tendencies than non-invasive congeners.	Rehage & Sih, 2004
Single parent or vegetative reproduction	*Eichhornia crassipes* (water hyacinth)	*E. crassipes* has the most highly developed asexual reproduction strategy within the genus, and is also by far the most invasive.	Barrett & Richardson, 1986
High genetic variability	*Anolis sagrei* (brown anole lizards)	Multiple introductions have elevated genetic variation in introduced populations, leading to widespread invasive success.	Kolbe *et al.*, 2004
Phenotypic plasticity	*Procambarus clarkia* (crayfish)	Invasive species of crayfish were found to use a broader range of predation-risk cues than natives, demonstrating behavioural plasticity.	Hazlett *et al.*, 2003
Large native range	*Bromus tectorum* (Brome grass)	*B. tectorum* has an extremely wide native range including most of Europe and some areas of North Africa and Southwest Asia. It has subsequently invaded Southeast Asia, Australasia and the Americas.	Novak & Mack, 1993
Eurytopy (extent of environmental tolerance)	*Poecilia reticulata* (guppy)	In Brazilian streams, exotic species such as *P. reticulata* were associated with degraded sites with extreme environmental conditions such as siltation, low dissolved oxygen and high ammonia content.	Casatti *et al.*, 2006
Polyphagy	Invasive fish in Californian streams	Alongside piscivores, omnivores were the most successful invaders.	Moyle & Light, 1996
Human commensalism	*Passer domesticus* (house sparrow)	Human commensalism has been identified as an important trait in explaining avian invasion success. For example, *P. domesticus* is associated with human settlements and has been successful in 33/39 introduction attempts.	Sol *et al.*, 2002

Vila-Gispert *et al.* (2005) compared the life history traits of a variety of native and invasive fish species from Californian streams. Using multivariate analyses it was possible to identify some general characteristics that appeared to be associated with the invasive species more often than with the native fish. The strongest associations with the invasive species included longevity, late maturity and high fecundity. As pointed out by Williamson (2006), explaining is not equivalent to predicting, and the authors admit that predicting success of future invasive species may be limited, as

differences in the traits listed above are often small between native and exotic species (Vila-Gispert *et al.*, 2005).

Genetic characteristics of populations can have a huge influence on their invasiveness, and invasions are often described as rapid evolutionary events (Lee, 2002). The most common genetic characteristic examined in relation to invasiveness is level of genetic variation. Invasiveness might be expected to be associated with high levels of genetic variation, as local adaptations enhance the survival of a population in the face of changing conditions, such as are likely to be encountered when establishing and spreading in a new area, and natural selection requires variation on which to act (Gilchrist & Lee, 2007; Sakai *et al.*, 2001; Holt, 2009; Lee *et al.*, 2007). However, this idea is challenged by an apparent genetic paradox (Lee, 2002; Kolbe *et al.*, 2004; Miura, 2007), in that founder effects associated with introduced populations would be expected to cause a decline in genetic variation (Dlugosch & Parker, 2008). Thus, despite depleted genetic variation limiting adaptability, increasing likelihood of inbreeding and rendering populations vulnerable to stochastic extinctions, invasive populations are not only able to persist and adapt to new conditions but are also able to spread and colonise new areas (Kolbe *et al.*, 2004; Lindholm *et al.*, 2005).

Kolbe *et al.* (2004) hypothesised that this success may be explained by the occurrence of repeated introductions from different native populations counterbalancing such founder effects. If multiple introductions represent different samples from the native population then each additional introduction event is likely to add genetic variation to the initial gene pool. In cases where the repeated introductions originate from genetically different populations, the introduced population may contain even greater genetic variation than each individual native population (Lee, 2002; Novak & Mack, 1993; Oliveira *et al.*, 2006). If this is the case, then increased rates of adaptive evolution may be expected in invasive populations. There is some evidence that the phenotypic divergence of the introduced brown anole lizard, *Anolis sagrei*, is consistent with this idea (Kolbe *et al.*, 2004). Furthermore, the 'multiple introductions' hypothesis also accounts for the often unexplained time-lag between the establishment of a self-sustaining population and rapid invasion; it may be that the invasion is only possible once a certain threshold level of genetic variation has been reached (Mack *et al.*, 2000; Kolbe *et al.*, 2004).

However, many successful introductions occur despite considerable reductions in genetic variation (Kolbe *et al.*, 2004; Yonekura *et al.*, 2007; Peacock *et al.*, 2009), and in some cases this appears to confer an invasive advantage. Introduced populations of the Argentine ant, *Linepithema humile*, display reduced genetic variation as a result of a population bottleneck; this has led to reduced intraspecific aggression and subsequently the formation of ant 'supercolonies', encouraging widespread ecological success (Tsutsui *et al.*, 2000).

The importance of evolutionary processes during invasion events is frequently emphasized in the literature, both in terms of how it can help to predict invasive success (Whitney & Gabler, 2008; Miura, 2007; Parker *et al.*, 2003) and what we can learn about evolution by studying introduced populations (Allendorf & Lundquist, 2008; Holt, 2009). Lindholm *et al.*, (2005) argued that the conflicting findings regarding extent of neutral genetic variation and invasiveness is unsurprising given that genes behind quantitative traits that relate directly to invasive success are likely to be more important. In the past the study of phenotypic traits and invasiveness has focused on life history, genetic and ecological traits, largely neglecting the potential importance of behavioural characteristics (Holway & Suarez, 1999).

Behavioural flexibility is thought to be an important component of invasion success for many species (Sol *et al.*, 2002). When invading a new habitat, an organism is likely to face a new suite of habitat characteristics, predators, competitors and food sources (Rehage *et al.*, 2005). Therefore the greater the ability to adapt by both learning and evolving appropriate behaviours, the more individuals will survive, and the greater the likelihood of population persistence and spread. Sol *et al.*, (2002) examined the link between behavioural flexibility and invasion success of avian invaders. Introduced birds with relatively larger brains and with a tendency to generate innovative foraging techniques were, in general, more likely to successfully establish in a new habitat. Similarly, Hazlett *et al.*, (2003) demonstrated that invasive crayfish had a greater affinity to learn a broader range of predation-risk cues than non-invasive relatives, and suggested that this enables them to respond appropriately to novel predators that they might encounter whilst invading new habitats.

Holway & Suarez (1999) pointed out that different specific behaviours are likely to be important at different stages in the invasion process. For example, whilst dispersal ability may be vital during the colonisation stage, competitive interactions may be more important during the subsequent persistence of the population. This is likely to be true of non-behavioural traits too (Marchetti *et al.,* 2004*b*) (Figure 1.1). For example at the immigration stage, human activity is likely to be an extremely important determinant of success, as those species that are used directly in, or indirectly associated with, agriculture, aquaculture, biocontrol or the ornamental trade stand a much higher chance of being introduced to a new habitat in the first place (Marchetti *et al*., 2004*b*; Heger & Trepl, 2003).

Stages of invasion process   Possible traits of importance

1 Immigration   Human commensalism

2 Survival   Environmental tolerances
                    Antipredator defences

3 Establishment   Life history and reproductive traits
                        Habitat and ecosystem characteristics

4 Persistence   Avoidance of inbreeding
                      Competitive ability

5 Spread   Dispersal tendency
                 Phenotypic plasticity

Figure 1.1: Flow chart of the stages of the invasion process and some of the possible traits that might improve success at each stage (compiled using Sakai *et al*., 2001; Williamson, 2006; Heger & Trepl, 2003).

Whether a species is able to invade a habitat is only partly determined by the species characteristics described above. Habitat properties are also critical, and numerous authors have emphasised the importance of physical conditions and existing

assemblages of the habitat in determining the success of invasive populations. Moyle and Light (1996) propose that the most successful aquatic invaders will be those that are adapted to the local hydrological regime of the new habitat.

It seems that certain habitats are more vulnerable to invasion than others (Tobin *et al.*, 2007). Climatic matching, early successional stage, low native diversity, the absence of predators and habitat disturbance have all been proposed as general characteristics of habitats that make them more prone to invasion (Lodge, 1993). Numerous studies point towards human disturbance as a common factor of highly invaded habitats (Rehage *et al.*, 2005; Moyle & Light, 1996). Furthermore, physical conditions can determine the outcome of competitive interactions if the competing species have different ranges of tolerance. Laha and Mattingly (2006) proposed that it may even be possible to promote coexistence between native and invading species by pinpointing particular biological or environmental conditions, such as temperature.

Moyle and Light (1996) argued that although most research tends to focus on the biotic conditions of habitats - such as food availability and the community of species present already, in many cases abiotic habitat conditions are also important (Swincer, 1986). They suggest that if abiotic factors are favourable, then invasion is highly likely, regardless of the other species existing in the habitat. For example, birds are more likely to invade and become established if the invaded habitat is of similar climate to that of their native range (Kolar & Lodge, 2001).

The complexity of the interaction between the various species characteristics and those of the receiving community and habitat remains responsible for our inability to make reliable predictions about specific introduction scenarios (Lodge, 1993). The 'ideal invader' is a hypothetical organism possessing all of the characteristics associated with invasiveness, but in reality, there are many factors that interact to determine whether a particular species is invasive in a specific habitat (Heger & Trepl, 2003). As well as contributing to their likelihood of invasion success in the first place, the behavioural, genetic and life-history characteristics of an invasive species may also play a large role in determining the effects that it has on the invaded ecosystem. This is well illustrated by the case study of invasive brown trout, *Salmo trutta*, in New Zealand. Here, the foraging behaviour of the trout has profound

consequences on community structure and ecosystem functioning. Unlike the native galaxiid fish, brown trout prey upon grazing invertebrates; this leads to a dramatic increase algal primary productivity and ultimately an increased flux of nutrients into the benthic community (Townsend, 2003).

1.3 The guppy as an ideal study species

The guppy is a small, ovoviviparous fish of the family Poeciliidae (Magurran, 2005) and is an ideal species for investigating the characters contributing to invasive success. There is an extensive body of research relating to its evolutionary ecology, as well as to its basic biology, behaviour, life history and ecology (see: Courtenay & Meffe, 1989; Magurran, 2005; Reznick *et al*., 2001). Guppies are ubiquitous in Trinidad (Magurran & Phillip, 2001; Liley & Seghers, 1975), and the island's numerous geographically isolated rivers and streams provide a 'natural laboratory', consisting of many guppy populations with clear behavioural, morphological and life history differences associated with variation in predation regime and other habitat factors (Reznick & Endler, 1982; Magurran *et al.*, 1995). Wild studies are carried out with relative ease in the shallow, clear habitats (Magurran & Seghers, 1991) and likewise in outdoor mesocosms when a semi-naturalistic approach is required (van Oosterhaut *et al.*, 2007). Their small size, hardiness and the ease with which they reproduce means that guppies are also extremely easily maintained in the laboratory for more controlled studies (Magurran & Seghers, 1990*b*).

Their evolutionary history in a temporally and spatially variable environment in Trinidad and northern South America (Chesser *et al.*, 1984; Thibault & Schultz, 1978) is likely to contribute to their survival and spread when introduced to other habitats worldwide. However, within the huge amount of work that has been published on the guppy, only a handful of papers explicitly address the species as an invasive (Lindholm *et al*., 2005; Valero *et al*., 2008; Shoji *et al.*, 2006).

In the guppy we have a unique opportunity to build upon decades of research in the study of an invasive species. As well as contributing to our understanding of the success of the guppy and other poeciliids, this also has the potential to provide valuable insights into invasion ecology more generally. Such insights may ultimately

enable the construction of more effective conservation and management strategies regarding introduced guppy populations as well as other poeciliid and non-poeciliid invasive species.

1.4 The guppy as an invasive species

The guppy has been introduced to every continent with the exception of Antarctica, in many cases deliberately as a mosquito control measure but also inadvertently in the form of unwanted aquaria fish (Froese & Pauly, 2010). Despite being advocated as a biological control agent (Elias *et al*., 1995; Cavalcanti *et al*., 2007), positive impacts are by no means universal. Whilst there is a report of effective mosquito larvae eradication and corresponding malaria control in India (Ghosh *et al*., 2005), of eight other countries in which the introduction impact of guppies was assessed, none reported any positive impacts in terms of the control of malaria epidemics (Courtenay & Meffe, 1989). FishBase lists 45 countries that hold introduced populations worldwide and in the majority of cases the reason for introduction is unknown (Froese & Pauly, 2009). The lack of such documentation, and other basic information such as the number of individuals released and the date of introduction, limit opportunities to study the ecological and evolutionary importance of introductions – to which the guppy would be particularly well-suited, given the body of literature existing based on native and laboratory populations (Carvalho *et al.*, 1996).

Compounding the scarcity of positive reports in terms of malarial control are numerous reports of the negative ecological effects of introduced populations of guppies (see Courtenay & Meffe, 1989; Englund, 1999; Valero *et al*., 2008). Unlike its similarly invasive but more aggressive relatives *Gambusia affinis* and *G. holbrooki*, who predate heavily upon the larvae, eggs and even adults of native species (Courtenay & Meffe, 1989), *P. reticulata* appears to affect native communities by outcompeting native fish for resources such as food and space. For example, invasive guppies have been linked to the declining populations of cyprinodontids in East Africa, and are similarly thought to have played a role in the decline of the Utah sucker, *Catostomas ardens*, at a thermal spring location in Wyoming (Courtenay *et al*., 1988). They are also believed to pose a threat to vulnerable goodeids in Mexico (Magurran, 2005; Valero *et al.*, 2008).

Information on species introductions, including when, why and where they were introduced, is extremely scarce, as is information on the effects of introduced populations on native species and ecosystems (Howarth, 1991). Guppy introductions are no exception, with few published records available and the majority of information held in unpublished reports or retained only anecdotally by individuals working in the field (Carvalho *et al.*, 1996). In Chapter 2, I aim to bring together existing sources of information, together with new reports, to expand on what we know about the distribution, origins and effects of introduced guppies worldwide, whilst identifying remaining gaps in our knowledge.

Key Question 1: **What is the non-native distribution of the guppy?** [Chapter 2]

Key Question 2: **What are the most common modes of introduction for the guppy?** [Chapter 2]

Key Question 3: **What is known about the negative impacts of guppy introductions?** [Chapter 2]

1.5 What makes the guppy a successful invasive species?

A variety of traits may help to explain the success of the guppy as an invasive species, and include characters relating to all of the broad types of trait described in the first half of this chapter: life history, ecological, genetic and behavioural characteristics. Some of these are also summarised in Table 1.2 below:

Table 1.2: A summary of the traits that may influence the invasive success of the guppy, *Poecilia reticulata.*

Trait	Origin*	Evidence	Reference
Ecology & Life History **High dispersal rate**	Wild (Trinidad)	There is considerable gene flow between natural populations	Crispo *et al.*, 2006
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 & Meffe, 1989
		Multiple mating strategies: polyandry often leads to multiple paternities within a brood	Becher & Magurran, 2004
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	Laboratory guppies displayed considerable thermal adaptability	Chung, 2001
	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 & Bryga, 1987; Reznick, 1990
	Wild (Trinidad)	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.	Froese & Pauly, 2009; Ghosh *et al.*, 2005
Genetics **High genetic variability/resistance to loss of genetic variability**	Introduced (Japan)	Evidence for multiple introductions enhancing variation	Shoji *et al.*, 2006
	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

*Key='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

Behavioural traits

Many different behaviours may be critical to the success of an introduced guppy. Not least those relating to predator avoidance, foraging and competition, as a new habitat will almost certainly present the guppy with a new suite of predators, competitors and food sources. Boldness, exploration and dispersal behaviours have also been postulated as behaviours associated with invasive success in poeciliids (Rehage & Sih, 2004).

Behaviours relating to predator avoidance in the guppy include boldness, predator inspection (Magurran *et al*., 1992; Godin & Davis, 1995), predator evasion, schooling (Seghers, 1974), foraging (Krause & Godin, 1996; Dugatkin & Godin, 1992*a*; Godin & Smith, 1988) courtship intensity (Godin, 1995) and predator recognition (Kelley *et al.*, 2003). In Trinidad, different suites of these relate to the specific habitat and predation regime. For example, when coexisting with ambush-hunting predators such as freshwater prawns (*Macrobrachium* spp.), schooling behaviour can cease to be advantageous, and may even increase detectability (Magurran & Seghers, 1990*a*). However, other predators, such as the pike cichlid (*Crenicichla alta*), are 'chasers', and in cases where these are dominant, schooling appears to be a highly advantageous strategy (Seghers, 1974). In some habitats, guppies are required to assess the type of predator (i.e. aerial or aquatic) and relative degree of risk on each encounter, revealing an even greater sophistication to their behavioural plasticity (Templeton & Shriner, 2004). Therefore, although behaviours are likely to be genetically determined to a large extent, the frequency and duration of behaviour appears to be more flexible (Magurran & Seghers, 1990*a*). That strategies can be so divergent between geographically close populations, and behavioural tactics can change dramatically following manipulation of the predation regime, indicates a high degree of behavioural flexibility. This plasticity is likely to play an important role in the invasive success of the guppy.

Sih *et al*. (2004) refer to 'behavioural syndromes' or suites of behaviours that are correlated across a collection of situations. They suggest that behaviours are rarely optimal in individual scenarios, as expression may be advantageous in one situation and disadvantageous in another – for example aggression may be advantageous when

interacting with a competitor, but not necessarily in the context of a predator interaction. The result is limited behavioural flexibility within an individual fish.

The theory behind behavioural syndromes predicts that an 'invasive' species would require sufficient variation in behavioural types between individuals within a population in order to be able to adapt to changing conditions, as each individual alone would have a limited ability to adapt. It is also speculated that the dispersal process that is vital to the success of most invaders would select for bold-aggressive individuals, which in turn would confer a stronger tendency to disrupt existing communities (Sih *et al.*, 2004). Cote *et al.* (2010) found a correlation between sociability and dispersal behaviour in invasive *Gambusia affinis*, with the more asocial individuals dispersing further. Furthermore, when compared with non-invasive close relatives, invasive species of *Gambusia* displayed higher dispersal tendencies (Rehage & Sih, 2004). However, evidence from work with the pumpkinseed sunfish, *Lepomis gibbosus*, suggests that there can be a context-specific aspect to behaviours such as these, rather than existing always as general personality traits, although the existence of behavioural correlations is as yet unexplored in the guppy (Coleman & Wilson, 1998). Nevertheless, bold and shy types can be identified, and foraging success appears to be greater in mixed shoal compositions – suggesting that a mix of behavioural types in an introduced population may enhance success (Dyer *et al.*, 2009).

The ability to learn behaviours during a lifetime has also been demonstrated in the guppy (Kelley & Magurran, 2003), and is yet another advantageous trait for an invasive species. Guppies additionally employ social learning to acquire information concerning novel predators and food sources and to thereby improve their antipredator behaviour and survival (Kelley *et al.*, 2003; Laland & Williams, 1997).

Ecological and life-history traits
McMahon (2002) proposed that invasive aquatic species tend to be characterized by traits including rapid individual and population growth, early maturity, short life-spans, high fecundity, small offspring size and extensive dispersal capacity – all of

which are traits possessed by the highly invasive zebra mussel, *Dreissena polymorpha*, and Asian clam, *Corbicula fluminea*. The guppy also displays many of the general life history and ecological traits associated with invasiveness; it is abundant in its native range, is polyphagous, has short generation times, sperm storage means that a single female is, in theory, capable of colonisation and it has broad physiological tolerances (Constanz, 1989; Dussault & Kramer, 1981; Avise *et al*., 2002; Lodge, 1993). Furthermore, within the natural range of the guppy, certain populations that share their habitat with many piscivorous predators, display even more pronounced 'r-selected' characteristics when compared with those in low predation environments (Reznick & Endler, 1982).

Additionally, like their fellow invasive poeciliids, *Gambusia* spp., and most other members of the family Poeciliidae, guppies have a highly specialised mode of reproduction which appears to suit the requirements of a successful invasive species (Thibault & Schultz, 1978). Arthington and Mitchell (1986) proposed that certain modes of reproduction are suited to invasive species; ovoviviparity and mouth-brooding reduce egg and fry mortality, and thereby produce large numbers of progeny with higher survivorship. It is argued that this leads to widespread dispersal and, ultimately, success as an invader (Arthrington & Mitchell, 1986). Female guppies give birth to between 3 and 30 offspring at roughly monthly intervals throughout the year; there are no specific environmental requirements or seasonal cycles (Magurran, 2005). Furthermore, as they are protected until fully developed there is low prenatal mortality and young are independent of their parents from birth (Courtenay & Meffe, 1989). Chesser *et al*., (1984) suggest that giving birth to live offspring may be an advantage in a rapidly changing environment, as it eliminates the risk of developing young being left behind when populations move between the temporary and permanent aquatic pools and streams that characterise poeciliid habitats.

Ephemeral environments may also be behind the evolution of sperm storage (Chesser *et al*., 1984). Guppies have the ability to store sperm for up to 8 months (Constanz, 1989; Winge, 1937) which allows isolated pools to be colonised even if only female fish remain. This was demonstrated in the case of Kenny's Pond, where a single female guppy successfully founded a persistent population (Carvalho *et al.*, 1996) and could be viewed as an extension or special case of Baker's Rule which suggests that

the ability of an organism to self-propagate is associated with colonisation success as it avoids the need for two compatible organisms to be introduced in close proximity by chance (Baker, 1955).

There is considerable variation in life history tactics among native populations of guppies, part of which is attributable to genetic divergence as a result of isolation and natural selection, and part of which is determined by the environment – referred to as 'phenotypic plasticity'. Studies in Trinidad provide evidence for phenotypic plasticity in several life history traits in the guppy. For example, male and female size at maturity and size of offspring differ according to resource ability (Reznick & Bryga, 1987; Reznick, 1990); it has been demonstrated that females inhabiting 'high' predation sites tend to become sexually receptive at a smaller size and earlier age than those from 'low' predation sites, and that such females also produce larger broods of smaller offspring (Reznick *et al*., 2001; Trexler, 1989; Magurran *et al*., 1995). Theoretical research suggests that this may be an adaptive response to stressful or changing environments (Trexler, 1989). In addition to predation pressure, Crispo *et al*., (2006) emphasised that physical habitat characteristics, such as canopy openness, can also influence life history traits, by influencing primary productivity and food availability. Further evidence of life history plasticity includes the finding that guppies in more isolated populations have a greater capacity to store sperm, another form of flexibility that could be exploited by invading populations (Carvalho *et al.*, 1996).

It is widely accepted that a broad tolerance of environmental conditions is vital to the success of an invasive species spreading into novel and changing habitats (although see McMahon, 2002). Chung (2001) demonstrated considerable thermal adaptability in the guppy. This is likely to reflect an adaptation to the daily temperature fluctuations in their native habitat where the difference between daytime and night temperature is often large. Such adaptability may enhance the ability of the guppy to survive in a huge range of latitudes and climates as introduced populations throughout the world. Chervinski (1984) found that guppies are also extremely adaptable in terms of salinity levels. Findings derived from these laboratory studies are supported by the fact that high abundances of *P. reticulata* are associated with stream degradation in southeastern Brazil, reflecting a broad tolerance to physical and chemical

environmental stressors such as siltation and low dissolved oxygen (Casatti *et al.*, 2006).

In Chapter 3, I will explore how successful single female fish are at establishing new populations and whether fish from wild populations that have tendencies towards more r-selected traits are more successful colonizers.

Key Question 4: **Do single female guppies routinely establish viable populations?** Chapter 3

Key Question 5: **Are guppies from wild populations that tend to have more r-selected traits more likely to establish new populations?** Chapter 3

Genetic traits

The population genetics of invasive guppies have been studied in introduced populations in Australia (Lindholm *et al.*, 2005) and Japan (Shoji *et al.*, 2006) as well as in experimentally introduced populations in Trinidad (Carvalho *et al.*, 1996).

The apparent genetic paradox in invasive species, of the expected low genetic diversity in introduced populations due to founder effects, alongside the association between high genetic diversity and 'invasiveness', may be overcome by a variety of scenarios (Frankham, 2005). In the guppy, explanations include polyandry, the ability for females to store sperm for long periods of time and repeated introductions, all of which have the potential to increase genetic diversity and reduce inbreeding (Cornell & Tregenza, 2007; Winge, 1937; Lindholm *et al.*, 2005).

Polyandry is common in wild populations (Haskins *et al.*, 1961; Herdman *et al.*, 2004). Not only do female guppies mate with multiple males, but there may also be multiple paternities within a single brood (Becher & Magurran, 2004). This may be particularly important after a demographic bottleneck in an introduction scenario, as multiple paternity within each brood means a greater proportion of the founder gene pool is likely to be maintained in the next generation. As well as minimising the chance of inbreeding (Cornell & Tregenza, 2007), this might also increase the between-individual variation in the next generation, thus increasing the chances of the population adapting to new and changing conditions (Lee *et al.*, 2007).

Carvalho *et al.* (1996) examined the genetic consequences of past experimental introductions of the guppy in Trinidad. Reductions in heterozygosity in introduced populations ranged from slight to marked, and there was no significant reduction in mean number of alleles - with the exception of one introduction that had been initiated with a single founding female. These findings may be attributed to life history traits of the guppy, such as their short generation times and non-seasonal reproduction, which allow populations to increase rapidly. In this scenario, rapid initial increase in size can minimise the loss of genetic variation despite a small founding population (Magurran *et al.*, 1995; Nei *et al*., 1975). Although these studies were conducted in the native range of the guppy, it is likely that such a mechanism would also be highly advantageous when establishing populations in new locations.

Shoji *et al*. (2006) found high genetic variation within and among populations of introduced guppies in Japan. This was attributed to repeated introductions, which phylogenetic analysis revealed to be from a wide range of source populations within South America. Such findings are consistent with the idea that high genetic variation increases invasiveness by enhancing adaptability and decreasing the effects of inbreeding (Sakai *et al.*, 2001). In this case the high levels of genetic variation appear to have facilitated rapid adaptive evolution and divergence in the invasive populations (Shoji *et al.*, 2006).

Given the colonisation success and high heritability of morphological traits in introduced Australian guppy populations, alongside historical documentation indicating multiple introductions in the country, Lindholm *et al.* (2005) predicted high levels of neutral genetic diversity in these populations. Instead, strong evidence of recent population bottlenecks was revealed, with both reduced allelic diversity and heterozygosity relative to Trinidadian populations. This is likely to be a product of founder effects and population bottlenecks associated with introductions, but may also be partly attributed to the fact that introduced guppy populations are usually derived from captive breeding lineages, either from the aquaria trade or from laboratory stocks and, as such, tend to have lower genetic variation in the first place (Sakai *et al*., 2001). Conversely, certain populations displayed high levels of additive genetic variance despite having little neutral genetic variation. Lindholm and colleagues speculate that

this may be a result of the bottleneck process itself releasing additive genetic variation as rare alleles become more common through genetic drift (Lindholm *et al.*, 2005), which is consistent with the suggestion that molecular genetic variation is not necessarily correlated with heritability or additive genetic variation (Reed & Frankham, 2001).

Indeed, both high and low levels of neutral diversity can characterise invasive populations not only in different taxa, but also in different populations of the same species. It is reasonable to speculate that the extent of genetic variation underlying 'invasive' life history traits is likely to be more important than measures of general, neutral genetic diversity (Lindholm *et al.*, 2005). For example the genetic diversity behind rapid growth and salinity and temperature tolerance is far more ecologically relevant to invading populations.

Chapter 4 will test the hypotheses that populations founded by polyandrous females have greater establishment success, persistence and within-population behavioural variation when compared with those founded by monandrous females.

Key Question 6: **Are polyandrous females more successful colonisers?** Chapter 4

Key Question 7: **Do populations founded by polyandrous females display more variation in terms of male colouration, courtship and newborn behaviour?** Chapter 4

Social factors

Human commensalism is often linked to invasive success, primarily as it increases the frequency of introduction events (Duggan *et al.*, 2006; Marchetti *et al.*, 2004*b*; Ricciardi & Rasmussan, 1998). In his influential book 'The ecology of invasions by animals and plants', Charles Elton listed three main motivations behind the introduction of freshwater fish; releases for food or sport purposes, releases intended for malaria control, and releases of unwanted aquaria species (Elton, 1958). The latter two are both highly relevant to the spread of the guppy.

Guppies and other poeciliids make good pets for many of the same reasons that they are used so widely in the study of evolutionary ecology: their size, readiness to breed,

hardiness and conspicuous sexually selected colouration. Rixon *et al.*, (2005) reported that 95% of pet shops around the Great Lakes in the USA offered guppies for sale. However, popularity as a pet also carries the risk of being accidentally or deliberately released outside of fish tanks in the home, garden or pet shop by irresponsible or ill-informed individuals (Liang *et al.*, 2006; Keller & Lodge, 2007). Indeed, Duggan *et al.*, (2006) found that 'popularity' as an aquarium species was an important determinant of invasive success among North American ornamental fish. They also found that successful poeciliid introductions were overrepresented even given their high popularity – despite likely being underestimated due to taxonomic difficulties (Duggan *et al.*, 2006). Furthermore, their small size and prolific breeding means that in some localities guppies are reared en masse in outdoor ponds as live food for larger ornamental species.

Aside from the pet trade, guppies have been used for over a century in the biological control of mosquitoes (Lindholm *et al.* 2005; Chandra *et al.* 2008). This involves large numbers being introduced at once, most commonly into artificial water bodies such as troughs and wells (Ghosh *et al.*, 2005). This carries the risk, especially in the tropics, of floods or monsoons facilitating the spread of guppies from these enclosed containers to other natural water bodies where they are likely to come into contact with native species with potentially negative consequences for the invaded ecosystem. (Simberloff & Stiling, 1996).

While information regarding the negative impacts of guppies when introduced remains scarce, it is all the more important to restrict introductions to situations where the potential benefits are likely to justify any possible risk to native fauna. Cost-benefit analyses have been recommended as a means of minimizing the negative effects of biocontrol introductions (Simberloff & Stiling, 1996) but the effectiveness of these is dependent on access to information regarding the positive effects of the introduced species, as well as information on likely negative impacts – both of which are severely lacking in the case of *Poecilia reticulata*. Chapters 5 and 6 of this thesis aim to contribute to our understanding of the former. If foraging studies suggest that guppies are indeed capable of effectively regulating prey populations, then this would indicate that, at least in some circumstances, their introduction may be justified.

1.6 Foraging behaviour of introduced guppies

Despite the widespread introduction of guppies for mosquito control, little is known about their foraging behaviour in this context. Feeding studies largely consist of stomach content analyses (Dussault & Kramer, 1981), the effects of predation on foraging behaviour (Dugatkin & Godin, 1992; Fraser & Gilliam, 1987; Krause & Godin, 1996) and very simple foraging laboratory experiments using single fish (Murdoch *et al.* 1975). Such studies tell us very little about the efficacy of guppies in a biocontrol role, and those studies that do begin to explore these applications (e.g. Murdoch *et al.*, 1975) lack ecological validity. It is often assumed that generalist predators will always regulate prey populations (Symondson *et al.*, 2002; Murdoch & Oaten, 1975).

Prey switching is one phenomenon that has been studied in the guppy (Murdoch *et al.*, 1975). It was found that guppies feed as predicted by optimal foraging theory when faced with two prey types in differing relative abundances, consuming disproportionately more of the most abundant prey type. In some mosquito control scenarios, such as that in Southern India, malarial vector mosquitoes coexist with harmless species (Ghosh *et al.*, 2005). This means that the behavioural response to more than one prey type may be critical to determining the efficacy of the guppy as a biocontrol agent (Manna *et al.*, 2008).

Guppies are highly social fish, and furthermore are commonly introduced to structural habitats, as well as potentially those with more than one choice of prey species. From a biological control perspective it is important to know if and how foraging is affected by these factors, as it may have implications for the efficacy of guppies for biological control.

Furthermore, it seems likely that the factors mentioned above may interfere with the basic predictions and complicate the picture. Chapters 5 and 6 will explore the effects of some of the aforementioned factors, such as social context and habitat structure, on foraging behaviour in the guppy, and relate them back to the suitability of guppies as mosquito control agents.

Key Question 8: **How effective are guppies as mosquito control agents?** Chapters 5&6

1.7 Aims

The aims of this thesis are:

a) To reveal a more complete picture of the current worldwide distribution of the guppy. ^{Chapter 2}

b) To collate information regarding the origins of, rationale behind and effects of introductions and, in doing so, identify key gaps in our knowledge. ^{Chapter 2}

c) To establish whether, once introduced, single pregnant female guppies routinely establish viable populations. ^{Chapter 3}

d) To establish whether evolutionary history in terms of predation regimes can affect colonisation success. ^{Chapter 3}

e) To establish whether polyandry is an important factor in determining colonisation success and population viability. ^{Chapter 4}

f) To explore the validity of the introductions of guppies for mosquito control by:

  a. Establishing whether guppies display prey-switching behaviour in line with optimal foraging theory. ^{Chapter 5}

  b. Establishing whether guppies display a preference for or against malaria vector mosquito larvae in a two prey system and whether this is affected by habitat complexity.^{Chapter 6}

  c. Investigating whether the presence of male or female conspecifics affects the foraging behaviour of female guppies. ^{Chapters 5 & 6}

Chapter 2

Worldwide survey of invasive guppies: distribution, origins and impacts

Abstract

The guppy, *Poecilia reticulata*, is native to north-eastern South America, yet is now found on every continent with the exception of Antarctica. The aim of this chapter was to document the non-native range of the guppy, and the origins of the introductions that have led to their current distribution. An email survey sent to 523 fish biologists worldwide indicated that this species is considerably more widespread than previously thought and that it is present in at least 73 countries. Mosquito control schemes and the release of unwanted aquarium fish are the two primary routes of introduction; both appear to contribute equally to the spread of guppies worldwide. The results also allowed clear identification of important gaps in our knowledge of invasive poeciliid species. These include their effects on native species and ecosystems, and information about when and where guppies were introduced as well as the introduction sources. Finally, despite mosquito control contributing to around 60% of introductions, there is a huge gap in our knowledge of the efficacy of the guppy in this role.

2.1 Introduction

The vast majority of research on the life history (Reznick & Endler, 1982; Reznick & Bryga, 1996), behaviour (Magurran *et al*. 1995, Kodric-Brown & Nicoletto 2005, Croft *et al*. 2004; Magurran & Seghers, 1991, 1994*c*; Dugatkin & Godin, 1992.) and evolution (van Oosterhout *et al*. 2003; Carvalho *et al*. 1996; Gordon *et al*. 2009) of the guppy has focused on the river systems of Trinidad (see also: Magurran, 2005). Recently a few studies have looked at the guppy in non-native habitats, largely focusing on the molecular evolution and population genetics of feral populations (Lindholm *et al*. 2005; Shoji *et al*. 2006; Khoo *et al*. 2002). Meanwhile, very little is known about how and why this species now has a range that stretches across the tropics and beyond.

The native range of the guppy is limited to Trinidad and Tobago and the mainland South American countries of Venezuela, Surinam and Guyana (Magurran, 2005). The online database 'FishBase' (www.fishbase.org), lists 45 countries that support introduced guppy populations (Froese & Pauly, 2009) and that in the majority of cases the origins and rationale behind these introductions is unknown. In 67% of cases the date of introduction is listed as unknown, while the place of origin is unknown in 71% of cases. Carvalho *et al.* (1996) point out that the lack of such documentation and other basic information, such as the number of individuals released and the date of introduction, limit opportunities to study the ecological and evolutionary effects of introductions in such a well known species (Gordon *et al*., 2009; Carvalho *et al*., 1996). Lack of data on introductions also impedes assessment of biological control efficacy (Howarth, 1991).

The guppy is considered to be an invasive species by the Global Invasive Species Database (GISD, 2009). This implies that the species tends to have a negative impact on the invaded habitat (Lockwood *et al.*, 2007). Alongside habitat destruction, invasions represent one of the most influential components of global change (Arim *et al.*, 2006). Invasive species are universally recognized as major threats to biodiversity, ecosystem integrity, agriculture, fisheries and public health (Pimentel *et al*., 2001). Means by which invaders can reduce biodiversity include interspecific competition, predation, habitat degradation or other modification of environmental conditions (Arim *et al*., 2006). Such

effects can ultimately lead to changes in the productivity and nutrient availability within the habitat, as well as influencing trophic structure and population dynamics such as the abundance and population growth of native species (Parker *et al*., 1999). Furthermore, invaders can have negative genetic effects on the existing species both through hybridisation with closely related natives, or indirectly by altering selection pressures or gene flow within the native community (Parker *et al*., 1999).

The impacts of exotic fish introductions tend to include the displacement of, or predation on, native species, the spread of disease and habitat alteration such as changes in ecosystem nutrient cycling (Arthington, 1991; Liang *et al*., 2006). FishBase reports negative effects from 16% of the countries in which the guppy is established (Casal, 2006), yet little is known about the extent and variety of impacts that it is responsible for worldwide.

Given the very limited amount of published literature available, it seems likely that the majority of information regarding the locations, origins and effect of guppy introductions lies in the hands of governments, national organisations, and in some cases also with locally based researchers.

The aim of this chapter is to construct a more complete distribution map for the guppy, to collate available information on the origins and rationale behind the introductions and to identify remaining gaps in our knowledge of the guppy as an introduced species. It is also hoped that the questionnaire responses will provide some information about the potential impacts of introduced guppies on native ecosystems.

2.2 Methods

An email questionnaire was sent to relevant scientists working in universities, governmental organisations and non-governmental organisations worldwide. Recipients were selected primarily by conducting internet searches for key words and phrases such as "freshwater fish research" and the name of the country in question. Some were also found by searching the online scientific literature for similar key words and contacting authors. Others were suggestions made by existing contacts. Contact details and responses were compiled on a spreadsheet, ensuring that the same researcher was not inadvertently contacted more than once, and allowing the tracking of correspondence so that reminders could be sent out where appropriate (usually at least a month after initial email). Electronic and paper copies of completed questionnaires were filed. A map displaying the locations of respondents was updated regularly, so that geographical gaps could be identified and areas with poor response rates specifically targeted.

The questionnaire consisted of five simple questions (see Appendix I) to maximise the number of respondents.

Negative reports include only instances where researchers were confident that they have not come across the species when they would have expected to during their work or the work of others had it be present. Where the respondent was unsure or 'unaware of presence' this was not included as a negative data point. Existing positive reports compiled by FishBase (www.fishbase.org) (Froese & Pauly, 2009) were also included. Other databases such as that compiled by the United States Geological Survey (http://nas.er.usgs.gov), the Global Invasive Species Database (http://www.issg.org/database), the Fisheries and Agriculture Organisation of the United Nations invasive species database (http://www.fao.org/fishery/dias/en) and the South African Biodiversity Information Facility (http://www.sabif.za) were consulted in conjunction with the questionnaire responses to help build up a more comprehensive picture of the worldwide distribution of the guppy.

The questionnaire provided data on:

a) the presence, absence or unknown status of *Poecilia reticulata* and *Gambusia* spp. in a region

b) the year of first introduction, where known

c) the purpose behind the introductions, where known

d) reported negative effects of the introductions

e) more qualitative details concerning the distribution and origins of the introductions

The information on *Gambusia* is summarised in Appendix VI. The rest of this chapter will focus on the responses relating to guppies.

The GIS software Manifold (version 8) was used to visually display the distribution data.

Patterns in the data for year of first introduction per country were investigated as were the relative contributions of the different purposes behind the introductions.

2.3 Results

Emails were sent to 587 fish biologists, wardens and ecologists worldwide between June 2007 and October 2009. Of these, 11% were not delivered, with the highest level of failure in Africa, South America and Asia. Of the 523 emails that were sent successfully, the response rate was 34%. Response rate varied considerably between continents; the lowest levels of response were from Asia (22%), South America (23%) and Africa (27%), and the highest from North America (70%), Australasia (53%), Central America and the Caribbean (52%) and Europe (50%).

Table 2.1: Email distribution statistics for each continent.

Continent	# sent	# failed	# successfully sent (# sent minus # failed)	# replied	% replied
Africa	186	28	158	42	27%
Asia	140	17	123	27	22%
Australasia	86	42	80	42	53%
Central America & Caribbean	36	1	35	18	52%
Europe	62	2	60	30	50%
North America	10	0	10	7	70%
South America	67	10	57	13	23%
Total	587	64 (11%)	523 (89%)	179	34%

2.3.1 Distribution

The native distribution of the guppy is compared below with the non-native distribution, as constructed from a combination of questionnaire responses and existing database information (Figure 2.1 a, b). This species is now found in at least 73 countries outside of its native range. Seven of these countries have populations in water bodies where the temperature is elevated due to geothermal or industrially-created heat. In the remainder, guppies are thought to be established in natural rivers, streams and ponds.

a)
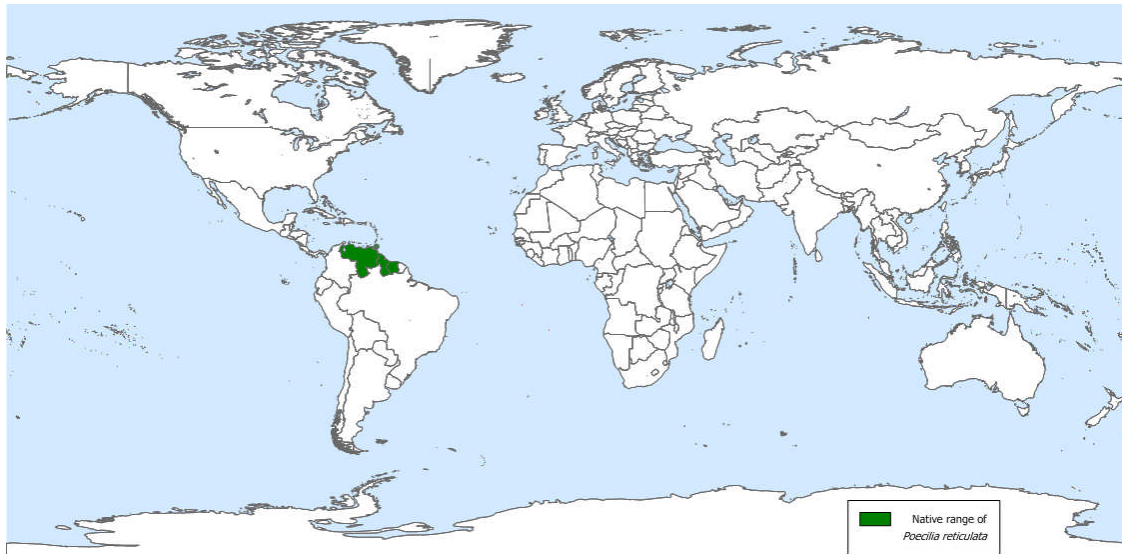
b)
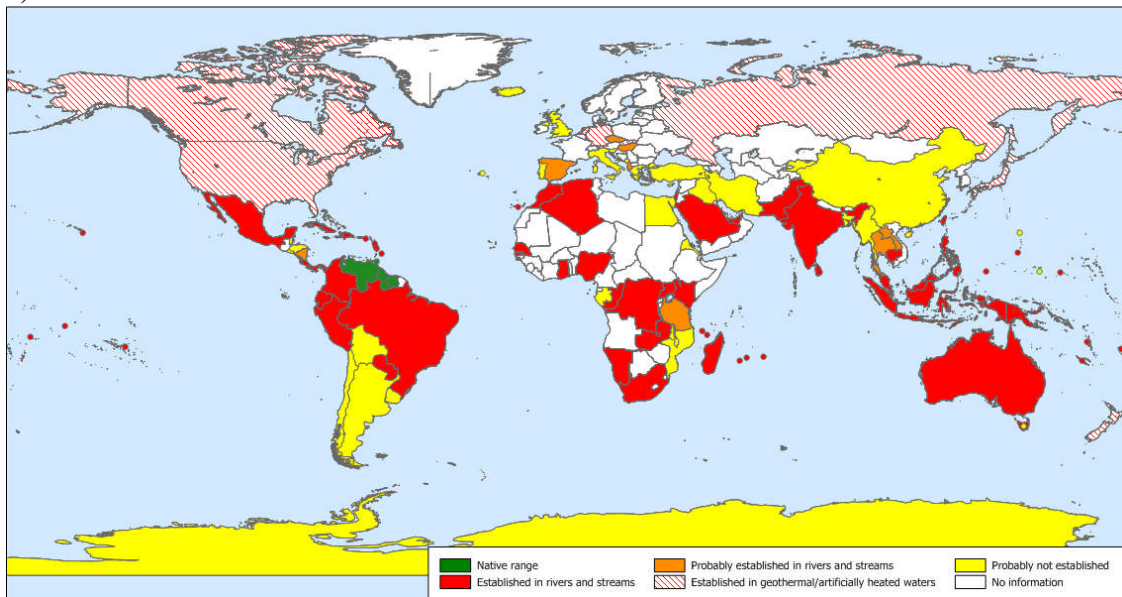
Figure 2.1. a) The native distribution of the guppy compared with **b)** their distribution worldwide as a result of introductions. Includes records from online databases and published literature, in combination with questionnaire responses. Coloured dots have been used where necessary to represent data for small islands.

2.3.2 Origins

The responses to the questionnaire suggest that in 29% of cases the rationale for introduction of guppies is unknown. Of those countries with established populations for which we do have information regarding their origin (N=53), approximately 42% can be attributed to introductions for mosquito control alone. A further 39% can be attributed to accidental release of aquaria fish, and in around 18% of cases, a combination of both mosquito control and aquaria releases are thought to be responsible for the presence of guppies.
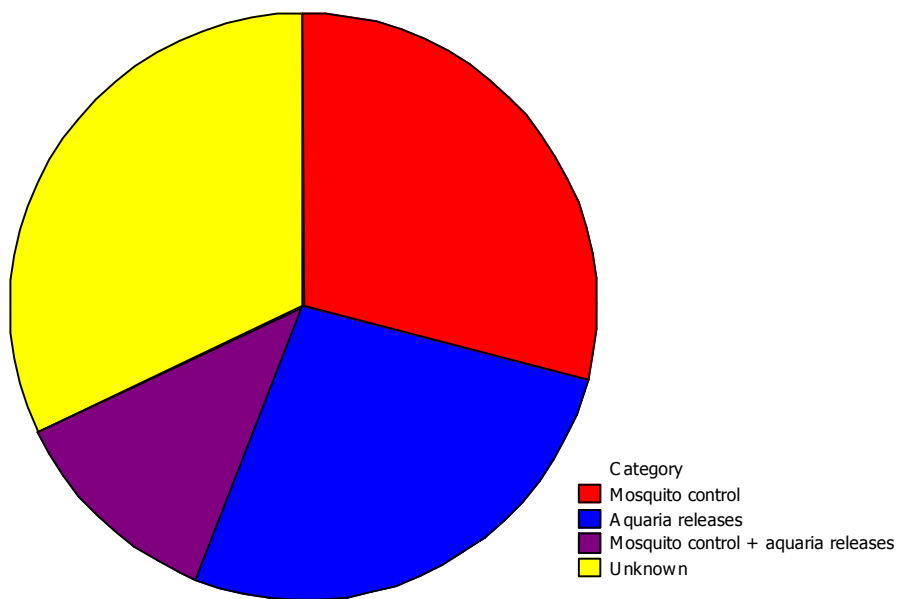
Figure 2.2: Reasons for introduction of the guppy. Information sourced for 73 countries from both questionnaire responses and existing sources.

Introduction for mosquito control tends to be most common in those countries situated close to the equator (Figure 2.3).
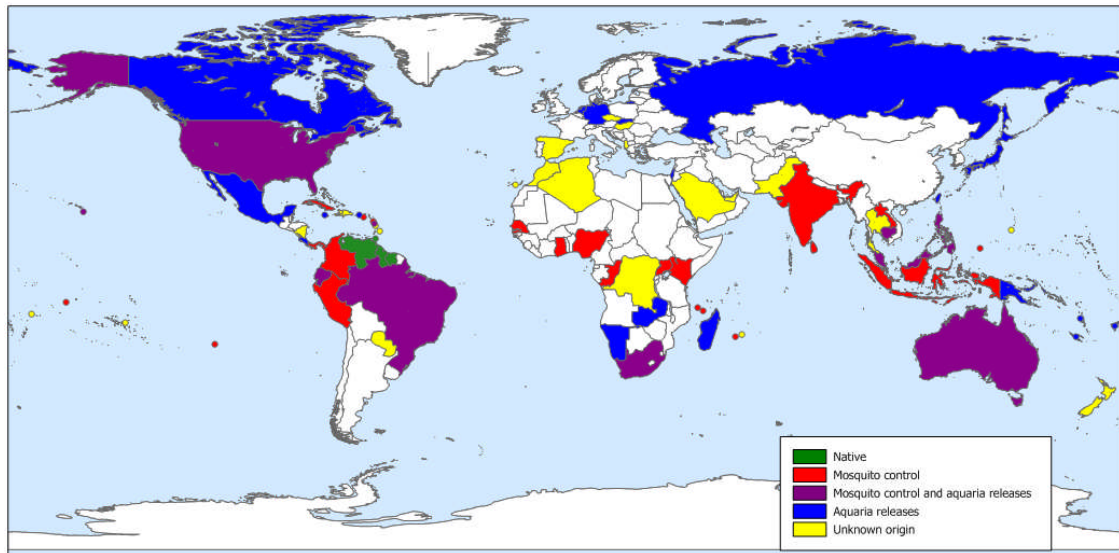
Figure 2.3: Reported reasons for introductions of *P. reticulata* worldwide. Coloured dots have been used where necessary to represent data for small islands.

Information on the date of first introduction was available for a total of 36 out of the 73 countries where guppies have been reported. This was combined with the reported origin of the earliest known introduction - whether it had been for mosquito control, aquarium release, a combination of both or whether the reason is unknown (Figure 2.4).
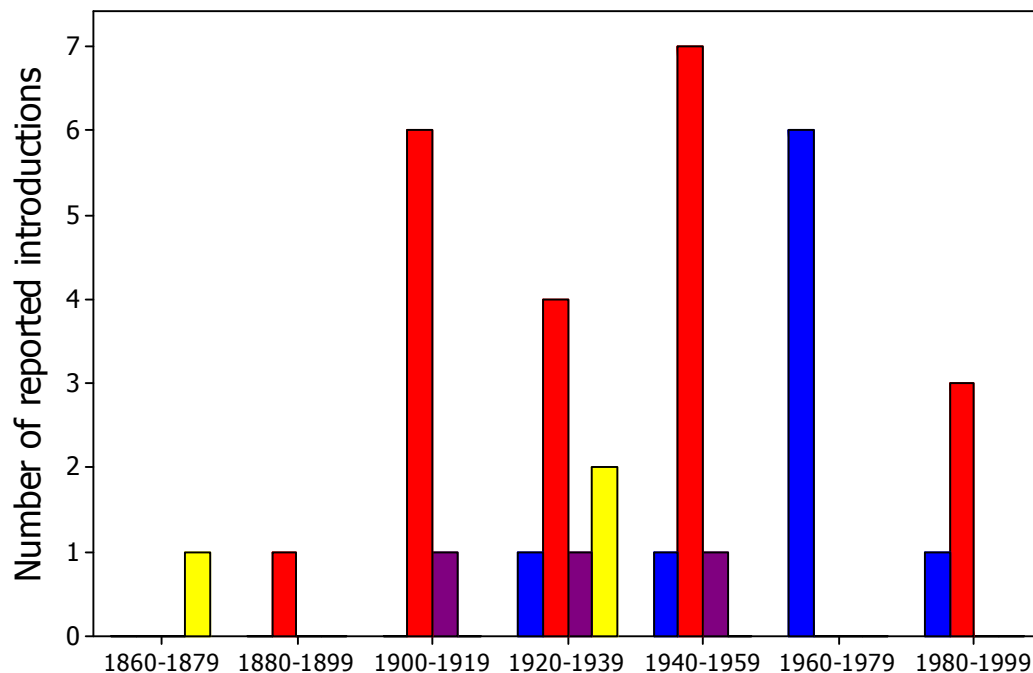
Figure 2.4: Patterns in purpose of introductions over time. Including only those for which an approximate date of introduction is known, and for each country including only the suspected purpose of the earliest known introduction. Colours correspond with key on Figure 2.3 (red=mosquito control; blue=aquaria releases; purple=mosquito control and aquarium releases; yellow=unknown).

Of those where the approximate date is known, 58% of first introductions were for the purpose of mosquito control, 25% resulted from aquaria releases and the remainder were either unknown or from a combination of sources. Between 1900 and 1960, 68% of introductions were for mosquito control alone and just 8% were from aquaria releases. Of the countries for which a date of introduction is available, 50% had an introduction of guppies by 1941. Between 1900 and 1985 the rate of introductions appears to have been reasonably constant (Figure 2.5).
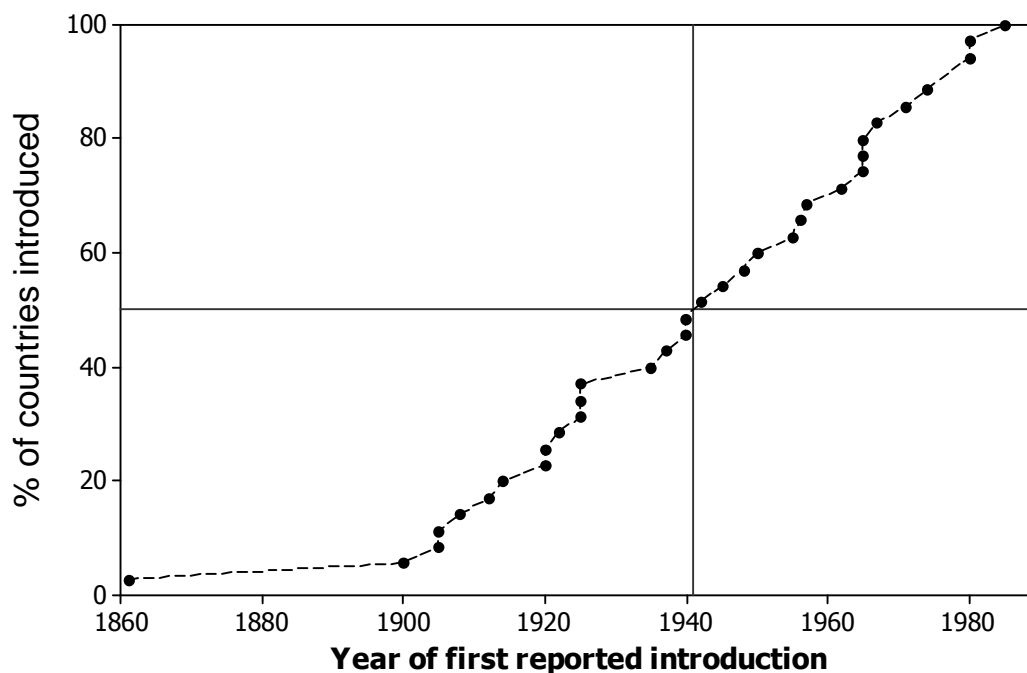
Figure 2.5: Cumulative percentage of those countries for which the date of first introduction is known, that were subject to guppy introductions over time. Gridlines indicate date by which 50% of these countries had been subject to their first guppy introduction.

The data point at 1861 represents the first reported presence of the guppy in Barbados by De Filippi (De Filippi, 1861).

2.3.3. Continent-by-Continent summary

(See Appendix III for information in full. Citations with the format '# number' refer to an individual email response, all of which can be looked up in Appendix V, Table 1. See Appendix II for photographs of guppies from various introduced populations).

Africa

Emails were sent to potential contacts in 49 African countries, and information received in relation to 21 of these. Guppies are established in the wild in at least 17 African countries. Four report their likely absence, and no information was available concerning the remainder. Africa had the highest rate of non-deliverable email addresses – 15% - which reflects the difficulty in finding up-to-date contact details of fish biologists from

this continent (Table 2.1). Both mosquito control and released aquarium fish have contributed to the African distribution of the guppy. Most of the mosquito-control related introductions were conducted, initially at least, during the colonial period – for example in Kenya (Welcomme, 1981), Uganda (Lever, 1996) and Nigeria (#28). However, more recent introductions are also reported, such as that in the Democratic Republic of Congo where they were introduced to channels around the capital, Brazzaville, as part of a malaria control workshop held in 1985. Despite persisting for 25 years, in this instance they appear to have had little impact on mosquito populations (#109).

Asia

Contacts from 26 Asian countries were emailed, and information relating to 18 of these received in return. Positive reports were associated with fourteen countries.

The aquarium trade is particularly popular in South-East Asia (Khoo *et al*., 2002; Liang *et al*., 2006) and responses suggest that repeated aquarium releases, in some cases alongside mosquito-control schemes, are likely to be blamed for the widespread distribution of the guppy in this part of the world. Indian and Sri Lankan populations originated from mosquito control efforts in colonial times (#86) while in the more northerly Asian countries of Russia and Japan, populations originated from released pets and are restricted to geothermally or artificially heated water bodies (#11; #106; #107).

Australasia

A total of 17 countries within Australasia (including the islands of the South Pacific – see Appendix III) were contacted. Information was obtained relating to 15 of these, 13 of which included positive reports of guppies.

In Australia guppies were first introduced as early as 1910, as part of a colonial office mosquito control effort. Since then they have been introduced as part of more mosquito control schemes as well as by way of aquaria releases (#38; #56; Lindholm *et al*., 2005). However, they currently appear to be restricted to the Northern states, probably due to the climate in the south of the country being less suitable (#40). A mixture of mosquito

control and aquaria releases is also accountable for the distribution of guppies throughout the Australasian and South Pacific islands (#63; #1; #2; #26; #70; Lever, 1996).

Central America and Caribbean

Sixteen Caribbean and Central American contacts were emailed, and information was obtained in relation to all of these. The introduction of guppies to Barbados in 1861 represents the earliest reported introduction of guppies worldwide (#44). Most Caribbean and Central American populations originate from released aquaria fish (#101; #66; Lever, 1996), with the exceptions of Cuba and Panama, where they were originally introduced for mosquito control (Lever, 1996; #83).

Europe

Contacts from 21 European countries were emailed, and responses received in relation to sixteen. Of these, only three countries have verified reports of established, self-sustaining guppy populations: Spain (both on the mainland and in the Canary Islands), the Netherlands and Slovakia (Elvira *et al*. 2001; #79; #92; #72). However, there are less certain reports from a number of others.

The low winter temperatures experienced in many European countries is likely to blame for the ephemeral nature of many populations - leading to unverified or contradictory reports from different sources as to the status of guppies in countries such as Albania, the Czech Republic, Germany and the Netherlands (Crivelli, 1995; Holcík, 1991; #119; #92; #72; #87).

The majority of European introductions have been inadvertent aquaria releases. The climate is generally not warm enough for guppies to overwinter, and most self sustaining populations are associated with artificially or geologically elevated water temperatures, for example in thermal ponds, or streams near to power stations. For this reason, even those populations that can be said to be established and self sustaining tend to be localised, with little potential for spread.

North America

Emails were sent to contacts from all three North American countries; Canada, the USA and Mexico, and information received relating to each of them. There was a very high, 70%, response rate to emails sent to North American contacts (Table 2.1).

In the USA and Mexico guppies have been released primarily as unwanted aquaria fish (Lever, 1996), but populations in California and Hawaii probably originate from a combination of mosquito control and aquaria releases. A total of 15 states within the USA are thought to support self-sustaining guppy populations (Nico, 2009). In Alberta, Canada, there is an aquarium-fish initiated population which exists near some hot springs (#46).

South America

Emails were sent to contacts in twelve South American countries, and replies received in relation to eight of these. This is not including the three South American countries that comprise the native range of the guppy. Towards the southern end of the continent, temperatures are too low for year-round survival under natural conditions, and the guppy is reported as absent from these countries. Both mosquito control and aquarium releases are likely to have contributed to the non-native South American distribution of the guppy (Welcomme, 1988; #23; #100; #35).

2.3.4 Impacts

Responses that mentioned negative effects were received in reference to sixteen countries, whilst in seventeen countries guppy introductions were associated with neutral effects by at least one respondent. Although the question was designed to extract information about negative effects, responses mentioning the positive effects of the guppy introductions were also received in reference to four countries.
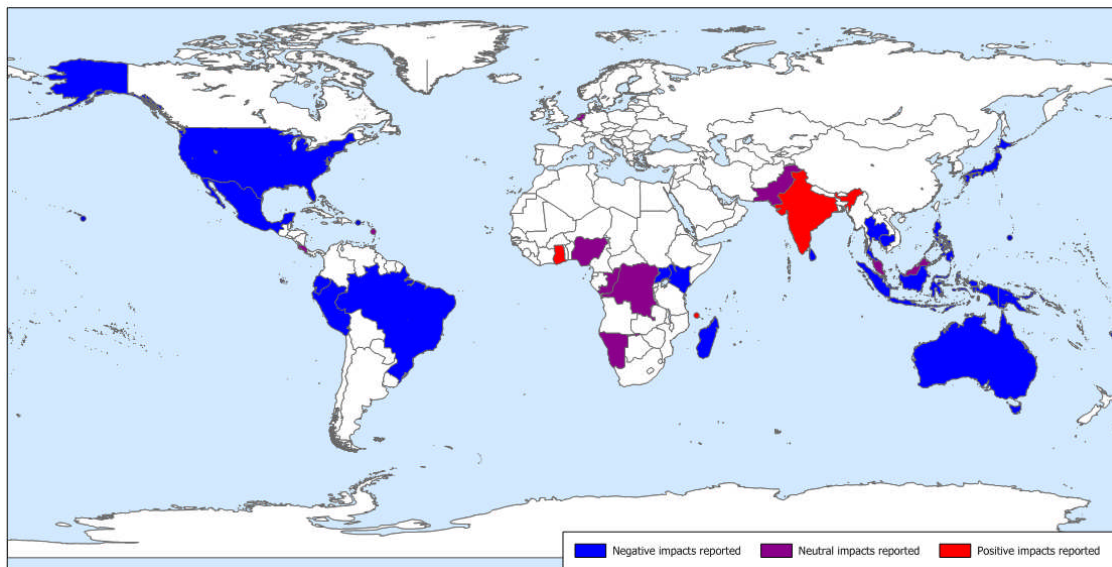
Figure 2.6: Reported impacts of the guppy worldwide. Negative and neutral impacts refer to the reported effects of guppies on native species and ecosystems or aquaculture practices, and positive impacts refer to cases where there appear to be considerable mosquito control benefits. Coloured dots have been used where necessary to represent data for small islands.

The reports of negative impacts of guppies include their implication in the decline of certain native species, both through the spread of disease and competition for resources (Table 2.2.). They are also associated with more general ecosystem level effects and in a few cases are reported to interfere with aquaculture processes.

Table 2.2: Negative and positive reports of the effects of guppies worldwide.

	Negative effects	Positive effects	Reference
Kenya	Decline in native cyprinodontid species		Welcomme, 1981; #46
Uganda	Decline in native cyprinodontid species		Welcomme, 1981; #46
Madagascar	Decline in native fish *Pachyanchax sakaramyi*		#54
Ghana		Believed to be effective at mosquito control	#14
Comoros		Effective in cisterns and basins, but not in wild.	Sabatinelli *et al.* 1990 & 1991.
Sri Lanka	Decline of native *Davario malabaricus* and native aquatic invertebrates.		#86; #91
Hong Kong	Decline of the native minnow, *Aphyocypris lini*.		Man and Hodgkiss, 1981.
Japan	Probably predate upon or compete with native *Oryzias latipes*		#107
India		Reduced Malaria in state of Karnataka.	Ghosh *et al.* 2005
Thailand	Seem to compete directly with native species: *Aplocheilus panchax* and *Oryzias danceana*		#118
Philippines	A pest in milkfish, *Chanos chanos*, ponds.	Some mosquito-control benefits	#70; #26; Juliano *et al.*, 1989.
Papua New Guinea	Exclusion of native species from some streams.		Allen, 1991 (as cited in Lever, 1996)
Australia	Regarded as a pest		Lever, 1996; Arthington,1989
Fiji	Associated with low native species abundance. Natives better at mosquito control.		#1
Puerto Rico	Possibly affects native invertebrate and fish communities		#95
USA: Hawaii	Decline in native shrimp, amphipod and Odonata species and spread of disease to native fish. Density of poeciliids positively correlated with nitrogen levels.		Englund, 1999; Font & Tate, 1994; Eldredge, 2000; #117
USA: Nevada	Decline of native goodeid, *Crenichthys baileyi*		Deacon *et al.*, 1964; Courtenay & Deacon, 1982; Courtenay & Deacon, 1983.
USA: Wyoming	Decline of Utah sucker, *Catostomas ardens*		Courtenay *et al.*, 1988
Mexico	Decline in native goodeid species through competition and desert topminnows through spread of disease		Valero *et al.* 2008; Leberg & Vrijenhoek, 1994.
Peru	Displace native ichthyfauna and cause food poisoning.		#35

Native species

In Kenya and Uganda, guppies have been blamed for outcompeting native cyprinodontids for food and space, leading to declines in some species. (Lever, 1996; Welcomme 1981). In Madagascar, alongside *Gambusia* spp., guppies are blamed for extirpating endemic fish *Pachyanchax sakaramyi* from the low gradient slopes of the Massif d'Ambre. However, in general *Gambusia* seem to pose much more of a problem here (#54).

In Sri Lanka, it has been observed that rather than controlling mosquito populations, guppies actually tend to selectively feed on non-mosquito larvae prey, and have simultaneously invaded the natural habitat of indigenous fish *Davario malabaricus* (#86).

In Hong Kong, guppies have been implicated in the decline of the native minnow, *Aphyocypris lini* (Man & Hodgkiss, 1981) and in Mexico they appear to be associated with the extirpation or decline of some native fish populations or assemblages (#76), posing a particular threat to threatened species of goodeids (Valero *et al*., 2008). Leberg and Vrijenberg (1994) raised concerns about introduced guppies acting as a reservoir for gyrodactylid parasites which may threaten native poeciliids.

In several parts of the USA the guppy is thought to have had a damaging effect on endemic autochthonous fishes (Courtenay & Meffe, 1989). In Nevada in particular it is blamed for adversely affecting the white river springfish, *Crenichthys baileyi*, a native cyprinodontid (Courtenay & Deacon, 1983). They have also been associated with the decline of the Utah sucker, *Catostomas ardens*, in a spring in Wyoming and are considered a potential threat to this and other species due to the exotic trematodes that they can carry (Courtenay *et al*., 1988).

Helminth, tapeworm and nematode parasites carried by guppies and other introduced poeciliids are blamed for having a negative impact on native fish in Hawaii (Eldredge, 2000). Englund (1999) implicated guppies in the decline of native Zygoptera in Hawaii; distributions of guppies and damselflies were often mutually exclusive, possibly as a

result of fish predating upon insects. Native atyid shrimps and amphipods also tend disappear from ponds and streams after the introduction of guppies (Brock & Kam, 1997).

In the outskirts of Bangkok in Thailand, guppies and *Gambusia* appear to compete directly with native species *Aplocheilus panchax* and *Oryzias danceana* (#118). Similarly, in habitats where guppies and *Gambusia* are found in Australia endemics tend to be rarer, and it is suggested that this may be due to feeding interactions between the two overlapping invaders and the endemic species (Arthington, 1989). As a result both are considered to be 'undesirable' here, although there have been few specific studies on their impact (Arthington, 1989). The guppy is also regarded as a pest in Papua New Guinea, where its rapid breeding has been linked with the exclusion of native species from some streams (Lever, 1996).

Ecosystems

In Brazil, the presence of guppies is regarded as a good predictor of low native fish abundance and diversity (#23). Similarly in Peru, they appear to displace native ichthyofauna, and are even reported to have poisoned one family who used them for food (#35).

In most cases where poeciliids are found in Fiji they are associated with a decline of water quality and reduced number of native species. This is likely to be due to poor land use practice and poor water quality, creating a competitive advantage for the invaders and resulting in declining numbers of native species. Indeed, native forest cover is strongly negatively correlated with presence of introduced poeciliids and cichlids. Many feel that there is little justification for the introduction of these species here as there are many, better, native biocontrol species that could be used (#1), although others found that in streams and rivers where invasive species were present, at least one or two native species coexisted. Such habitats were often muddy or silty, which might suggest that the invasive poeciliids tend to thrive in already-degraded habitats (#2).

Environmentalists in Sri Lanka claim that guppies affect native aquatic invertebrates, but there does not seem to be any scientific evidence to support this (#91). Raghavan *et al.* (2008) speculate that there is great potential for the species to become a pest to native fish fauna in the Western Ghats in India, as self-sustaining populations are already established in this system. This is of particular concern as the region is considered to be one of the world's 'biodiversity hotspots'.

In Hawaii, Richard MacKenzie (#117) has found that invasive poeciliids (guppies, mollies and mosquitofish) excrete more nitrogen than native species, which together with high densities and their omnivorous habitats, results in a positive correlation between numbers of poeciliids and total nitrogen levels.

Another common opinion is that the effects of guppies may be minimal (#68). In Kenya's Lake Baringo, stable isotope analysis suggests that there is little scope for competition with native species due to only a marginal overlap in preferred food sources (#10). Guppies are not considered a particular threat in Nigeria either, as here they tend to thrive in degraded, polluted habitats where native fish cannot survive anyway (#28). However, this is purely anecdotal and no studies of their impact have been conducted (#51). In many parts of Malaysia, guppies are considered to occupy empty niches, or to be established in areas without native species; there are no records of negative effects (#55). The same is generally true in Hong Kong, where *Gambusia* pose a much bigger problem (#57) and in Costa Rica, where guppies are often found in polluted ditches that are uninhabitable by native fishes. Another example is Laos, where they tend to be introduced to areas that support already depleted numbers of native fish species due to having been sprayed with DDT (Kottelat, 2001).

In a few cases, guppies are found to interfere with aquaculture practices, for example they are considered a pest in milkfish ponds in the Philippines (#70) and in shrimp ponds in Thailand (#118).

2.4 Discussion and conclusions

The worldwide distribution of the guppy is considerably more extensive than previously described in the literature or in any single online database. Despite the native range of this species being confined to approximately a 10° latitudinal range just north of the equator in Trinidad and Tobago and the north-eastern coastal margins of Venezuela, Guyana and Surinam, its introduced range spans every continent with the exception of Antarctica, as well as numerous oceanic islands.

The populations reported at the most extreme latitudes such as in Canada, Russia and parts of northern Europe are established exclusively in water bodies where the temperature is elevated due to geothermal or industrially-created heat. Although self-sustaining, these populations do not have any invasive potential as they will always be limited by temperature. Nevertheless, their success in such habitats demonstrates a remarkable opportunism, where they have colonised narrow bands of habitat where environmental conditions allow their survival. McDowall (2004) also points out that simply because some introduced fish might be limited to geothermal habitats, this does not necessarily mean that they do not have adverse impacts in such places.

Climate change is an important consideration regarding the future of the distribution of aquatic invasive species worldwide (Rahel & Olden, 2008; Walther *et al*., 2009). Increasing temperatures are predicted to impact on invasive species in a number of ways; Hellmann *et al.* (2008) predict that the lower temperature limits of some invaders will be reduced, allowing colonisation at higher latitudes and elevations. It is also likely that their establishment, spread and success in general will increase because invasive species tend to already have traits that make them better at adapting to a changing environment - such as broad environmental tolerances, short generation times and high rates of dispersal (Hellmann *et al*., 2008). The guppy certainly possesses many of the physiological (Chervinski, 1984; Chung, 2001; Casatti *et al*., 2006), behavioural (Magurran *et al*., 1992; Kelley et al., 2003) and life-history (Reznick and Bryga, 1987; Reznick, 1990; Carvalho *et al*., 1996) characters that are associated with extreme adaptability and it is clear that its current range is at least partly dictated by temperature constraints. Inevitable escapees

and releases from the pet trade mean that the guppy is frequently being introduced to places that are outside of its environmental tolerance range, but as water temperatures rise, an increasing number of these introductions may result in the establishment of self-sustaining populations (McDowall, 2004).

It is likely that in many cases these two powerful components of global change, climate change and biological invasions, may act synergistically (Rahel & Olden, 2008), a possibility that has already begun to be investigated in relation to another invasive poeciliid: *Gambusia holbrooki* (Benejam *et al.*, 2009).

Human commensalism has frequently been associated with the success of invasive species (Sol *et al.*, 2002). In the case of the guppy, its use by humans both as a popular pet and as a biocontrol agent has allowed it to be transported throughout the world, constantly providing opportunities for invading new habitats. A total of 115 species of invasive freshwater fish are already established worldwide as a result of the global aquarium trade, which continues to grow by 14% annually (Padilla & Williams, 2004). Fish introduced by this route tend to be healthy adults, who have often been already inadvertently selected for traits such as hardiness by the domestication process (Padilla & Williams, 2004; Duggan *et al.*, 2006). Furthermore, Liang *et al.*, (2006) suggest that certain families of fish, including the Poeciliidae, have more survival potential, once escaped from captivity or discarded, than others. Indeed, of 18 species of poeciliid listed in FishBase as 'established' or 'probably established' outside of their native range, 14 are popular aquaria species (Froese & Pauly, 2009).

This survey allowed the identification of important gaps in our understanding of the distribution and impact not only of introduced guppies, but gaps that are likely to exist in our knowledge of many introduced species. The most immediate knowledge gap is that of when and where introduced guppies have come from originally. Despite the fact that most introductions have occurred within the last 100 years, it is incredibly difficult to source information about the origins of introduction events. This is partly because such information is stored in governmental and other unpublished reports, rather than in the

public domain (Casal, 2006; Howarth, 1991). The email survey method proved successful in that some such information has been passed on anecdotally within the country concerned, but it remains extremely patchy. Another approach is to use genetic analyses to trace introduced guppies back to the various populations in their native range (e.g. Lindholm *et al.*, 2005).

A further knowledge gap is the impact of introduced species on native species and ecosystems, which was identified as an important under-studied area in invasion biology in general by Crooks (2002). This survey brought together many reports of the destructive impact of guppies on a range of native species and ecosystems worldwide. The majority of these are anecdotal, and many specifically mention the scarcity of studies examining potential effects of invasive species such as guppies (Gurevitch & Padilla, 2004; Howarth, 1991). Fears of the effect of guppies on native species coupled with the fact that it is commonplace to introduce guppies for mosquito control and as unwanted pets, suggests an urgent need for properly controlled studies in order to fill this gap in our knowledge.

As with many threats to biodiversity, the problem of invasive guppies is largely restricted to the regions that are least well equipped in terms of scientific and financial resources and therefore where very little is done in terms of impact assessment. At the same time, the control of malaria and other mosquito-borne disease continues to be of utmost importance in many of these countries, and larvivorous poeciliids such as guppies are frequently seen as a cheap and easy potential solution (Dash, 2009).

It is essential that more is done to inform sensible decision making in such scenarios, both in terms of the potential threat to biodiversity posed by guppies, and their potential to reduce mosquito-borne diseases. In Hawaii, Richard MacKenzie and colleagues are taking an experimental approach to examining the ecosystem level impacts of introduced guppies, by setting up a series of flumes to represent streams with and without guppies (R. Mackenzie, pers. comm. (#117a)). Findings from studies such as this will provide a

valuable contribution to our currently limited understanding of the potential impact of introduced guppies.

Studies that allow ecosystems to be assessed pre- and post- guppy introduction would be essential, and it would be important to design an experiment in which the guppy-mediated effects can be separated from other independent effects that may be correlated on a temporal scale with introductions, such as habitat degradation or the introduction of other exotic species, or even acting synergistically with other factors (Didham *et al*., 2007). A common response in the survey was that presence of guppies seemed to be correlated with low native fish abundance and diversity. However, determining whether the guppies have *caused* the low diversity, or whether they are simply better at colonising habitats which are already depauperate of native fish is extremely difficult without studies such as that described above (and in more detail in Box 2.1). This is especially challenging given that many respondents emphasised that guppies often tend to colonise habitats that are uninhabitable by most native fish – for example in degraded, polluted or disturbed habitats.

Box 2.1: Proposed experiments for closing the knowledge gap regarding the impact of guppies and other poeciliids on existing habitats, communities and species.

Hypothesis:
Introduced guppies have an adverse effect on biodiversity:

       a) they reduce species richness and diversity

       b) they reduce abundances of particular species

       c) they reduce ecosystem health

Two main approaches are possible here:

1) The first is to conduct an entirely naturalistic experiment, where guppies are introduced to natural, pristine aquatic habitats. This would best represent a real introduction scenario, yet would likely be much more difficult to control for the many environmental variables that might affect the success and impact of introduced guppies. There are also ethical issues associated with deliberate introductions into pristine habitats, and this should only be conducted in a region where guppies have already been introduced to other areas and it seems highly likely that they will eventually find their way to these localities anyway.

An ideal scenario would involve a series of at least 40 pristine, naturally occurring ponds or streams which are lacking any introduced species. These should all be comprehensively surveyed for species richness, diversity and ecosystem health using standardised methods, and then a small founding population of guppies introduced to half of the localities. All sites should be monitored, using identical survey methods as in the initial survey, every 3-6 months for at least 2 years and temporal patterns in biodiversity compared between the guppy sites and non-guppy sites.

2) The second approach would involve a collection of mesocosm tanks.

A more convenient and perhaps realistic alternative would be to construct the same number of large mesocosms instead. These would represent natural communities as closely as possible, containing fish, invertebrates, plants and algae, and would be established for at least a year before the beginning of the experiments. The pre- and post-guppy introduction surveys can then be conducted as described for the first approach.

A combination of these two methods would provide a great insight into the effects that guppies might have on new habitats, and would provide some evidence for whether the success of guppies is indeed the cause of declines in richness, diversity, the decline in the abundances of certain species and the general health of the invaded ecosystem.

The effect of habitat degradation

An additional variable of interest would be whether the habitat being invaded was already disturbed. This could be investigated as part of the same study by doubling the number of sites/mesocosms and adding 'disturbed/pristine' as an additional factor:

	Pristine	Disturbed
Guppies	20	20
No guppies	20	20

'Disturbed' habitats could be created by means of physical disturbance, species removal or pollution. This design would allow the detection of any synergistic effects of disturbance and guppy presence acting on the invaded communities. Here the additional dependent variable to be measured would be the success of the introduced guppy population (population size) between the disturbed and pristine conditions, and the hypothesis that guppies are more successful at colonising already-disturbed habitats could be tested.

Another important knowledge gap here is the scarcity of evidence of the benefits of guppies as biocontrol agents. Sixty percent of established feral populations are known to have originated purely or partly from introductions for the purpose of mosquito control, yet there is very little evidence to confirm their efficacy in this role. Sabatinelli *et al.* (1991) suggested that guppies were highly effective in the Comoros islands because most water bodies harbouring mosquito larvae were self-contained manmade structures, such as troughs and wells. They suggested that guppies would be far less successful in areas where mosquitoes were concentrated in natural habitats. The most convincing study of the success of guppies as mosquito control agents is that by Ghosh *et al.* (2005) in Karnataka, India, where the focus was also on villages with water-filled troughs and wells. It seems likely that the precise circumstances of the ecology of the mosquito and habitat in a specific area would be critical in determining the suitability of guppies as a tool in the fight against mosquito-borne diseases and that this should be taken into account when considering introducing guppies.

The findings of this chapter confirm that mosquito control measures and aquaria releases are the two main sources of introduced guppies, and therefore it is of concern that the same findings also indicate that two main gaps in our knowledge are the efficacy of guppies in mosquito control and their impact on native ecosystems when introduced. An interesting continuation of this work would be to perform follow up surveys every five or ten years. This would reveal whether the range of the guppy is expanding – particularly at the extremes of their current range where small increases in temperature as a result of climate change might make the difference between temporary or seasonal populations and those that are persistent and invasive.

Chapter 3

Colonisation success in an invasive species: single female guppies routinely establish viable populations

Abstract

The Trinidadian guppy is a successful invasive species, and is now established in more than 70 countries outside of its native range. It has been proposed that specialised reproductive traits contribute to this success but this has never been investigated experimentally. Here I demonstrate the remarkable ability of single pregnant female guppies to routinely establish viable populations. In 86% of cases these populations persisted for the two years of the experiment. Establishment success was independent of founder origin, as was population size, population structure and condition factor. There was no reduction in behavioural performance of mesocosm newborns in terms of schooling, evasion, time in cover, activity or reaction behaviour, although predator inspections were less frequent and more cautious in mesocosm fish. Differences in certain behaviours between upstream and downstream populations were evident; some of these persisted in the mesocosm populations (differences in newborn reaction behaviour and evasion ability) while others did not (differences in male courtship vigour). These results demonstrate that introductions consisting of a single individual can lead to thriving populations and suggest that particular caution should be exerted when introducing this species, or other live-bearing fish, to natural water bodies.

3.1 Introduction

It is widely recognised that invasive species represent a serious threat to biodiversity (Vitousek *et al.*, 1997; Crooks & Soule, 1999). Freshwater fish assemblages have been shown to be particularly vulnerable to the impacts of invasive species (Miller *et al.*, 1989). Fish introductions occur for a number of reasons including ad hoc releases of pet fish and deliberate introductions designed, however misguidedly, to improve human wellbeing (Elton, 1958).

The guppy, *Poecilia reticulata*, is one example of a species whose range has expanded as a result of human intervention. In addition to being a popular aquarium species (Rixon *et al*., 2005; Chapman *et al*., 1997), it has also been employed in the biological control of malarial mosquitoes (Courtenay & Meffe, 1989). The guppy's native range is Trinidad and Tobago, and the coastal zone of NE South America yet it is now established in over 70 countries worldwide (see Chapter 2). Although there is some support for the view that guppies can reduce the incidence of malaria (Ghosh *et al.,* 2005) there is growing evidence that the species has a detrimental impact on invaded communities in many regions (for example: Courtenay & Deacon, 1983; Courtenay *et al.,* 1988; Valero *et al*., 2008). As a consequence the guppy is now listed in the Global Invasive Species Database (www.issg.org/database/species).

One explanation for the extraordinary invasive capacity of the guppy is the ability of a single female to give birth to multiple broods of live offspring (Courtenay & Meffe, 1989). A female can store sperm for over eight months, and broods may be fathered by several males (Winge, 1937; Becher & Magurran, 2004). To date there is one documented case where a female successfully founded a population (Carvalho *et al*., 1996). Guppies and other poeciliids naturally occur in ephemeral or isolated habitats where females have limited chances of encountering a mating partner (Magurran, 2005). Sperm storage, combined with ovoviviparity (live birth), is advantageous in such circumstances (Chesser *et al.*, 1984) but may also preadapt these fish for invasive success. Thus, while single females clearly have the potential to establish viable populations it is not known whether this is a routine event.

There are a number of studies that have examined the effect of demographic bottlenecks on the genetics of poeciliid populations, both in the context of experimental manipulations (Leberg, 1992; Spencer *et al.*, 2000), as well as in relation to native (Carvalho *et al.*, 1996; Barson *et al.*, 2009) and feral (Lindholm *et al.*, 2005; Shoji *et al.*, 2006; Grapputo *et al.*, 2006) populations. When very small founder numbers (<10) are involved, bottlenecks are almost always detectable using molecular markers, which reveal reductions in allele frequency and heterozygosity (Spencer *et al.*, 2000; Leberg, 1992; Carvalho *et al.*, 1996). Studies of introduced populations produce mixed findings; in some cases bottlenecks were revealed (Lindholm *et al.*, 2005; Grapputo *et al.*, 2006), and in other cases there was little evidence of founder effects (Shoji *et al.*, 2006). Introduced populations of poeciliids, particularly those originating from unwanted pets, or from fish placed in a water tank to control mosquito larvae, are likely to have descended from very few founding individuals. Consequently, demographic bottlenecks may be a common occurrence in the evolutionary history of non-native poeciliid populations.

Thus far, most studies have used molecular approaches to detect changes in neutral genetic variation proceeding bottleneck events; here I examine their effects on phenotypic traits. In the context of introduced populations, phenotypic differences are likely to be more important to invasive success than the extent of neutral genetic variation (Lindholm *et al.*, 2005).

An additional issue is the extent to which evolutionary history influences founding success. Guppies exhibit marked geographical variation in behavioural and life history characteristics, primarily linked to variation in predation regime (Magurran *et al.*, 1995). Fish that have historically coexisted with many large predators tend to have more pronounced predator evasion behaviours, such as a stronger tendency to shoal (Seghers, 1974) and improved escape ability (O'Steen *et al.,* 2002). Low predation localities tend to be upstream of waterfalls, where large predatory species have failed to colonise, whilst high predation sites tend to be further downstream. Life history strategies also differ

markedly: 'High predation', downstream guppies tend to be characterised by what have been termed by ecologists as 'r-selected' traits, (sometimes referred to as 'faster' life-histories) which include a tendency to mature more quickly and give birth to more, smaller offspring per brood. Upstream guppies that have evolved under 'low predation' regimes often tend towards a more 'K-selected' strategy (with 'slower' life histories), giving birth to fewer, larger young (Pianka, 1970; Reznick & Endler, 1982; Reznick, 1982). Together these observations lead us to predict that single pregnant females derived from downstream localities where they experience high predation risk will be more likely to found a viable population than those originating upstream, from low risk sites.

Differences in predation regime also explain some of the variation in male sexual behaviour that has been documented between populations. Individual guppies can adjust their courtship strategy according to perceived risk of predation (Magurran & Seghers, 1990*a*; Godin, 1995). When perceived risk is higher, males tend to perform fewer sigmoid courtship displays and engage in more 'sneaky' mating instead. Furthermore, it has been demonstrated that populations from high predation populations are better able to adjust their behaviour in such scenarios than those from low predation populations (Magurran & Seghers, 1990*a*). Recently it has also been found that male courtship vigour can act as an indicator of inbreeding in guppies, with reduced courtship intensity and following behaviour after just one generation of full sibling mating (Mariette *et al.*, 2006). Although environmental factors are thought to be of greatest importance, there is also an inherited component and observable correlations between variation in courtship behaviour and the predators that are characteristic of the habitat (Rodd & Sokolowski, 1995; Endler, 1987; Farr, 1975).

This study has the following aims. The first is to ask whether single pregnant female guppies routinely establish viable populations. The primary measure of viability is a self-sustaining population that persists for at least a year. In addition I compared the performance of the newborn fish in the newly founded populations with those from wild caught controls to produce an additional measure of viability. Performance here is a composite measure based on schooling behaviour, evasion ability, time spent in cover,

activity and reaction distance. Predator inspection behaviour of newborns was also examined. The focus on newborn fish is important as poeciliids can be highly cannibalistic (Rose, 1959; Loekle *et al.*, 1982; Smith & Reay, 1991), and populations will not establish if juveniles are unable to escape predatory attacks from older conspecifics. The second aim was to test the prediction that successful populations are established at a reduced rate when females originate from low risk, upstream, localities. Finally, I also investigated whether differences exist between the high and low risk evolutionary histories in terms of newborn performance and courtship vigour and whether these differences persist in the mesocosm populations.

I tested these ideas using a replicated mesocosm setup at the University of the West Indies (UWI), Trinidad & Tobago.

3.2 Methods

3.2.1. Mesocosm set-up

Thirty-two plastic mesocosms (100 x 40 x 30 cm deep), were placed on the roof of the Department of Life Sciences (Figure 3.1). These were filled with dechlorinated water to a depth of 20 cm. Well-rinsed river gravel and vegetation (water hyacinth, *Eichhornia crassipes* and Canadian pondweed, *Elodea canadensis*) provided cover. Tanks were covered with wire mesh to prevent aerial predation and to stop fish from jumping. A single wild-caught female was introduced to each mesocosm. Half of these were from the Upper Tunapuna (upstream, 'low predation') river, the remainder from the Lower Tacarigua (downstream, 'high predation') river. Guppy origin was alternated along the line of mesocosms. There was no significant difference in size between females from the two localities (t=0.35; df=28; p=0.732); in both cases mean total length was 35 mm (±0.7 SE). Wild guppy females of this size are almost invariably pregnant and have reserves of stored sperm (Winge, 1937; Becher & Magurran, 2004). The mesocosms relied on natural productivity; no food supplements were added for the duration of the experiment. Tanks were topped up with water when necessary. The experiment ran for 2 years from April 2007 to May 2009.

Figure 3.1: Mesocosms on the rooftop at the University of the West Indies.

Tanks were monitored daily for the first few weeks, and at this stage newborns could be easily counted. This enabled the size of first brood to be estimated for each tank.

3.2.2. Population assessment

All mesocosms were censused at 12 and 24 months. Each fish was caught, measured (total length) to the nearest millimetre using a ruler and weighed on an electronic balance in a petri dish. A fine paint brush was used to gently manipulate the fish where necessary. Number of adult males, adult females and juveniles (including newborns) were recorded. Male fish were distinguished by coloured markings and/or by the presence of a gonopodium. Fish were recorded as adult females if they were 16 mm or more in length and lacking any signs of male secondary sexual characteristics. Fish <12 mm were not weighed.

Condition factor (**K**) was calculated for weighed fish in every mesocosm with a population of ≥4, using the following equation (Williams, 2000):

$$K = (100,000 * weight(g))/(totallength^3)\,1$$

The mean **K** for each mesocosm was calculated.

3.2.3. Newborn performance

Females measuring >16 mm were considered likely to be sexually mature (Reznick & Endler, 1982). These were isolated in individual containers and checked for offspring several times each day. This system generated newborn fish for the performance tests. Remaining fish were released back into their respective mesocosms immediately following the census. Wild-caught females from the original upstream and downstream sites were isolated in the same manner as the mesocosm females. Containers were labelled according to an arbitrary code with a corresponding key to enable blind testing. Females were removed, measured and returned to their mesocosms as soon as a brood had been produced. Schooling, evasion ability, time in cover, activity, reaction distance and predator inspection were assessed in this order in newborn fish using the following methods:

Schooling

Pairs of newborns were transferred to a circular white tray (30 cm in diameter; water 2 cm deep) (Figure 3.2). They were allowed to settle for 5 min, and the length of time the focal fish spent schooling was recorded for the proceeding 5 min. *Schooling* was defined as the time it spent swimming within 3.5 body lengths of its companion (see Evans & Magurran, 2000). All pairs tested were siblings.

Evasion ability

A small circular net (3 cm in diameter) was used to catch each newborn from the tray at the end of the schooling behaviour trial (Figure 3.2). The net was placed in the water at the opposite side of the tray to the fish, and the time taken to catch it was recorded as a measure of *evasion ability* (see Evans & Magurran, 2000).

Figure 3.2: Tray used in schooling and evasion ability trials. Net used to assess 'evasion ability'.

Time in cover and activity

The fish was released into a shallow white tub (21 x 15 x 8 cm deep) containing water to a depth of 2.5 cm. The base of the tub was divided into quarters, two of which were covered in a shallow sprinkling of gravel and two of which were left bare (Figure 3.3). After every two trials, the gravel/bare sections were reversed to control for any spatial differences. Fish were allowed to explore the arena for 5 min during which two measures were taken: the time spent on gravel-covered areas (*time in cover*) and the number of times that the fish crossed from a gravel-covered to a gravel-free section or vice versa (*activity*).

Figure 3.3: Arena for assessment of *time in cover* and *activity*.

Reaction distance

Each fish was then transferred to an identical but gravel-free container containing water to a depth of 1.5 cm. Graph paper was attached to the base to aid the estimation of

distance. A dark-coloured pencil-end was introduced 12 cm away from the fish and moved towards it at a constant speed of 2.5 mm/sec. The distance at which the fish reacted to the object, in the form of an obvious directional movement, was measured as *reaction distance*.

Predator inspection

This behaviour was only assessed in newborns at 24 months post introduction. Pairs of newborns were transferred to an aquarium (45 x 22 x 23 cm) filled with water to a depth of 5 cm, containing a free-swimming adult female guppy. Three laboratory stock females were used alternately for this purpose throughout the experimental period, they measured between 4 – 5 cm and were taken from wild caught mixed population stock tanks. Adult guppies are known to cannibalise young (Smith & Reay, 1991) and as such are a good means of initiating inspection behaviour in newborns. Graph paper was attached to the glass base to aid the estimation of distance within the arena. Each pair of newborns was released at the opposite end of the arena to the adult and allowed to roam for 10 min. Inspection behaviour was conspicuous, and consisted of a directional approach towards the female, followed by a sideways glide and then retreat. The number of inspections conducted (*frequency*), the mean approach distance of all inspections (*mean distance*), the closest approach (*closest*) and the number of inspections conducted alone rather than as a pair (to calculate *% alone*) during a trial were all recorded. Approach distances were estimated to the nearest cm.

Altogether 82 broods were tested for evasion ability, reaction distance, time in cover and activity; 58 of these were also tested for schooling and 26 tested for predator inspection behaviour. Fewer trials were conducted for schooling because they required pairs of fish, as did predator inspection, for which even fewer trials were conducted as this was only performed in year two.

3.2.4. Courtship vigour

The aim here was to establish if there are population differences in courtship vigour between the two wild populations, and whether these differences persist in the mesocosm

populations. 20 wild upstream, 20 wild downstream, 32 mesocosm upstream and 32 mesocosm downstream males were tested. The mesocosm males were taken from populations that had been established for 24 months. The wild males were collected from either the Lower Tacarigua (downstream) or Upper Tunapuna (upstream) localities and groups were kept in separate aquaria in the laboratory. Each fish was tested only once, and trials were alternated between the four conditions.

Two tanks were set up (30 x 20 x 20 cm) with gravel and dechlorinated water. Four wild female and two wild male fish were introduced to each tank; two females and one of the males were from an upstream locality and the remainder from a downstream locality. These fish were allowed to settle overnight. New companion fish were used for each new day of the experiment. As far as possible I ensured that wild fish were unfamiliar with their companions before the trial, as they were always taken from different holding tanks.

At the start of each trial, the focal male was gently introduced into a startbox, which consisted of a polystyrene cup weighed down with gravel in the base, and with a small window cut in the side (2 x 2 cm). The trial began when the fish exited the cup and lasted 10 minutes. The time that the focal male spent following females was recorded, as were the number of sigmoid displays and the number of gonopodial thrusts. *Following* was defined as swimming within two body lengths of the female and in the same direction, *sigmoid displays* were recorded when the male arched his body into an S-shape, raised his dorsal fin and quivered while within sight of the female, *gonopodial thrusts* were recorded when the male approached the female from behind and attempted or succeeded in making contact with the female by cocking his gonopodium forward at least 90˚ while positioned within one body length of her. Copulations and post-copulatory jerks were also recorded but occurred so infrequently that they were not included in any analyses.

3.2.5. Statistical analyses
All statistical tests were performed using SPSS v.17.0.0. Differences in mesocosm population size, size of first brood, population structure, condition factor, male length and

size of newborns between conditions (upstream and downstream) were compared using t-tests or Mann-Whitney U-tests.

The mean value per brood for all newborn performance data (schooling, evasion, time in cover, activity and reaction, inspection frequency, mean inspection distance, closest inspection and % inspections conducted alone) was calculated and used in all further analyses. A MANOVA was used to examine for the effect of evolutionary history (upstream and downstream) and origin of mother (wild or mesocosm). As an integrated measure of performance a principal components analysis was performed using the following behaviours: *evasion ability*, *time in cover*, *activity* and *reaction distance*. Resulting PC1 and PC2 values were analysed using a two-way ANOVA. Data for *Time in cover* were transformed (squared) in order to meet the assumptions of an approximately normal distribution and homogeneity of variance.

The predator inspection behaviours were analysed in the same way; calculation of means followed by a MANOVA, principal component analysis and ANOVA of PC1 and PC2 values. *Mean distance* and *closest* data were transformed (natural logarithm) in order to meet assumptions.

Differences between the two wild populations and the two mesocosm conditions in terms of time spent following, sigmoid displays and gonopodial thrusts were examined using a two-way ANOVA or Mann-Whitney U test. The sequential Bonferroni correction was applied to correct for multiple testing (Rice, 1989).

In all tests, data were transformed where necessary to meet the assumptions of a normal distribution and homogeneity of variance. When this was not possible, an equivalent non-parametric test was used.

3.3 Results

3.3.1 Establishment success

Two fish from the initial thirty tanks died within the first week of the experiment, and were promptly replaced. Overall, 91% of mesocosm populations persisted at the end of year one; 86% at the end of year two (Table 3.1).

Table 3.1: Statistics for mesocosm populations one and two years after they were founded by single female guppies from downstream or upstream localities.

	# tanks started	# excluded (*Rivulus*)	Actual sample size	# Extinctions	# Survived	% Success	Mean pop size	Median pop size
12 months								
Downstream	17	1	16	2	14	87.5%	21.00	17.5
Upstream	18	2	16	1	15	93.75%	18.58	14
Total	**35**	**3**	**32**	**3**	**29**	**90.6%**	**19.88**	**16.5**
24 months								
Downstream	17	1	16	3	13	81.25%	22.15	17
Upstream	15	2	13	1	12	92.3%	14.25	12
Total	**32**	**3**	**29**	**4**	**25**	**86.21%**	**18.36**	**15**

Rivulus hartii was discovered in three of the tanks, presumably these were in the original vegetation which was taken from the University pond and remained in spite of thorough rinsing. These mesocosms were excluded from the study.

There was no significant difference in population size between those founded by females from the two different localities after 12 months (t=0.258; df=25; p=0.798) or 24 months (t=0.878; df=25; p=0.388) (Figure 3.4).
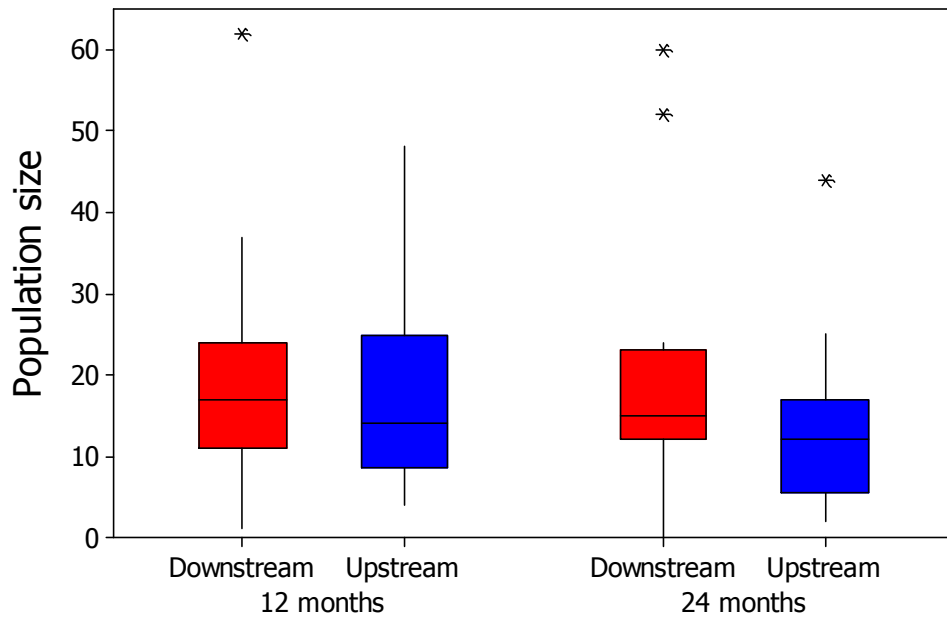
Figure 3.4: Population size of mesocosms founded by females originally from downstream and upstream localities after 12 months and after 24 months. Medians, interquartile ranges and outliers are shown. N=16 for all conditions except 'upstream, 24 months', where N=13.

3.3.2 Life history and population structure

Brood size

Newborns were recorded in all tanks by eight weeks post-introduction. There was a significant difference in first brood size between the two conditions, with females originating from downstream populations having larger broods (t=2.27; df=28; p=0.031). Females from downstream populations had a mean brood size of 13 (+1.66SE) whilst those from upstream populations had a mean of 8.6 (+0.99SE) (Figure 3.5).
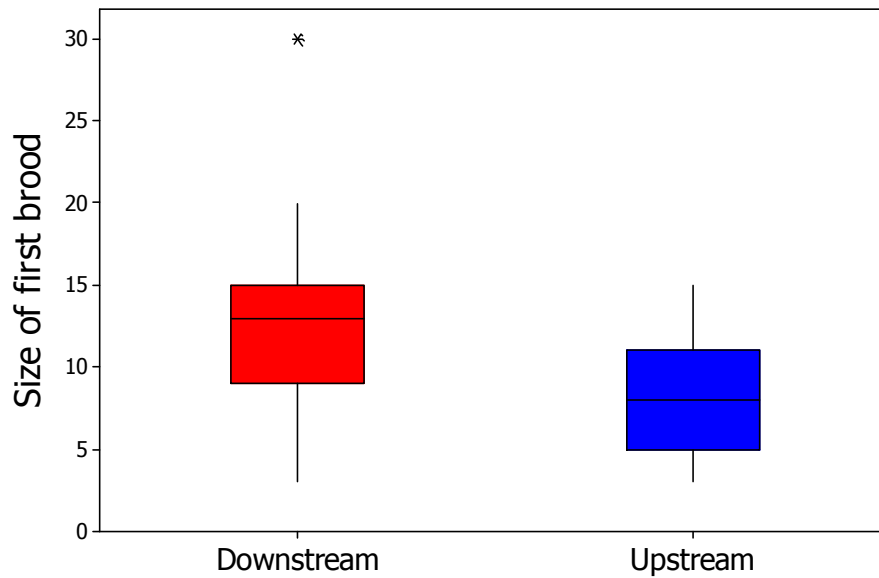
Figure 3.5: Size of first brood born to females from the downstream (N=15) and upstream (N=15) localities. Medians, interquartile ranges and outliers are shown.

Population age-structure

Population age-structure varied with the evolutionary history of the female founder (Figure 3.6). The proportion of juveniles was significantly greater in mesocosms founded by downstream fish ($F_{1,48}$=9.07; p=0.004), but did not differ significantly between one and two years post-introduction ($F_{1,48}$=0.025; p=0.874). There was not a significant interaction between evolutionary history and year ($F_{1,47}$=0.500; p=0.483).
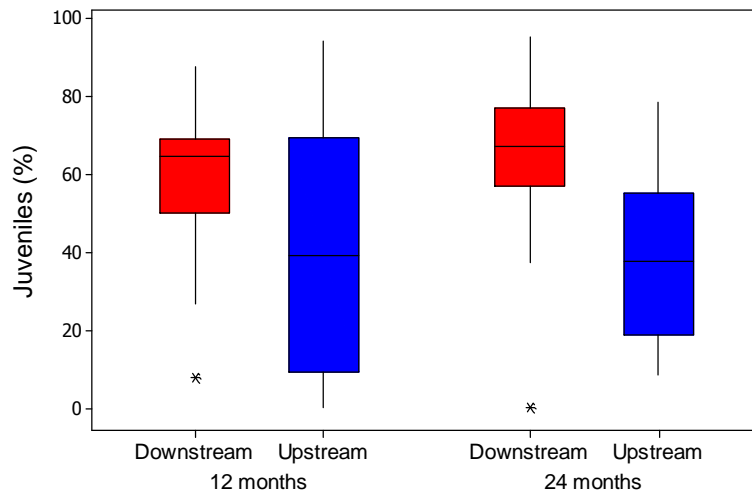
Figure 3.6: Percentage of juveniles (as opposed to adults) in the mesocosm populations founded by downstream and upstream fish both 12 and 24 months post-introduction. Medians, interquartile ranges and outliers are shown.

Condition factor

There was no difference in condition factor, K, in fish from mesocosms founded by downstream or upstream fish, either at 12 months (t=0.711; df=24; p=0.484) or 24 months (t=-0.509; df=23; p=0.616) (Figure 3.7).
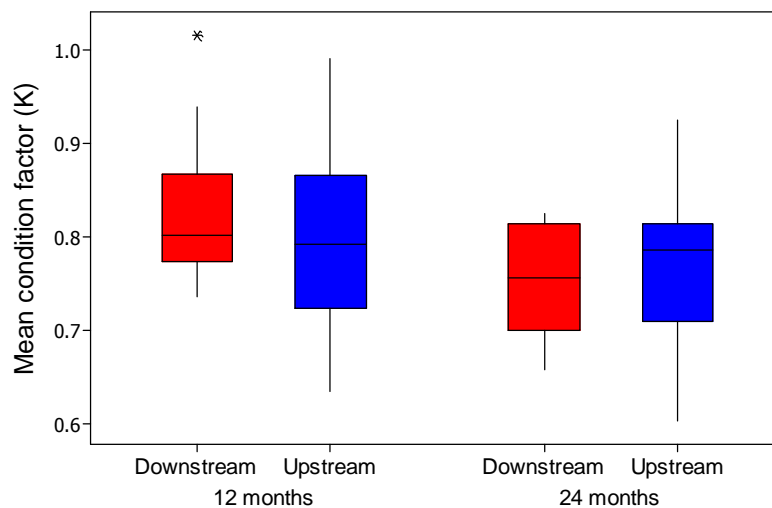
Figure 3.7: Mean condition factor (K) per mesocosm for adult fish in tanks founded by either downstream or upstream females, and after both 12 and 24 months post-introduction of founder. Medians, interquartile ranges and outliers are shown.

Male length

Twelve months after the mesocosms were established, adult males from the upstream-founded mesocosms were significantly larger than those from the downstream-founded tanks (U=427; N1=34; N2=46; p<0.001). At 24 months post-establishment, this difference was no longer evident (t=-0.782; df=77; p=0.437) (Figure 3.8).
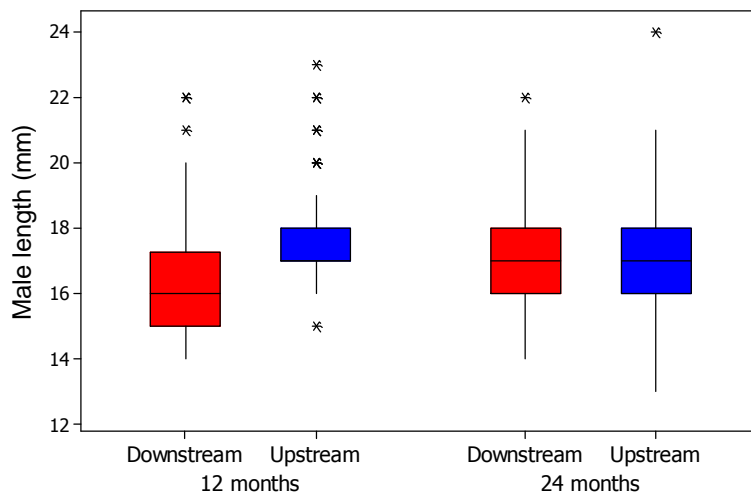
Figure 3.8: Male total length (mm) for mesocosm populations founded by downstream and upstream females, 12 months and 24 months post-establishment. Medians, interquartile ranges and outliers are shown.

Size of newborns

There was a significant interaction between the effects of evolutionary history (downstream or upstream) and origin of mother (wild or mesocosm) ($F_{1,76}$=17.40; p<0.001) on newborn length; the effect of evolutionary history was evident only between the two wild populations (Figure 3.9).
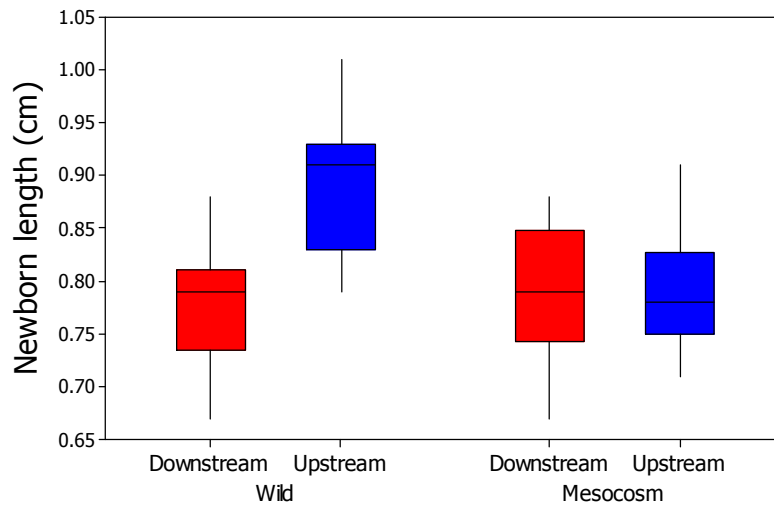
Figure 3.9: Mean length of newborns per brood for each condition; broods born to wild fish from a downstream or upstream locality and broods born to females from mesocosms that were originally established by fish from either a downstream or upstream locality. Medians, interquartile ranges and outliers are shown.

3.3.3 Newborn performance

There was no evidence for any improvement in experimenter catching ability when escape times were plotted against time (Pearson's correlation=0.056; p=0.449) (Figure 3.10).
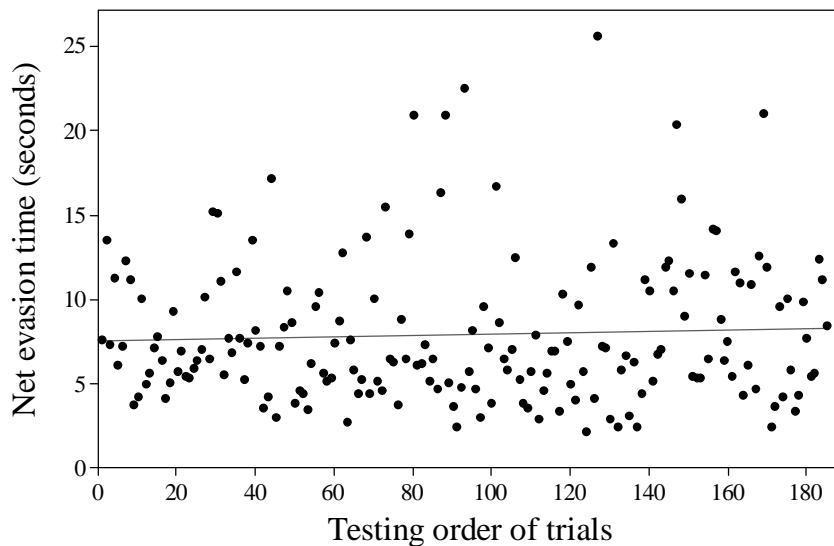
Figure 3.10: Net evasion time and test order of trials.

There was no significant difference between the behavioural performance of offspring born to mesocosm or wild fish ($F_{5,47}=1.642$; $p=0.168$). There was a significant effect of evolutionary history ($F_{5,47}=4.020$; $p=0.004$), with offspring born to descendents of upstream fish displaying greater evasion ability and reaction distance (Table 3.2). There was no interaction between the two factors ($F_{5,46}=1.127$; $p=0.360$) (Figure 3.11).

a)

b)

c)

d)

e)
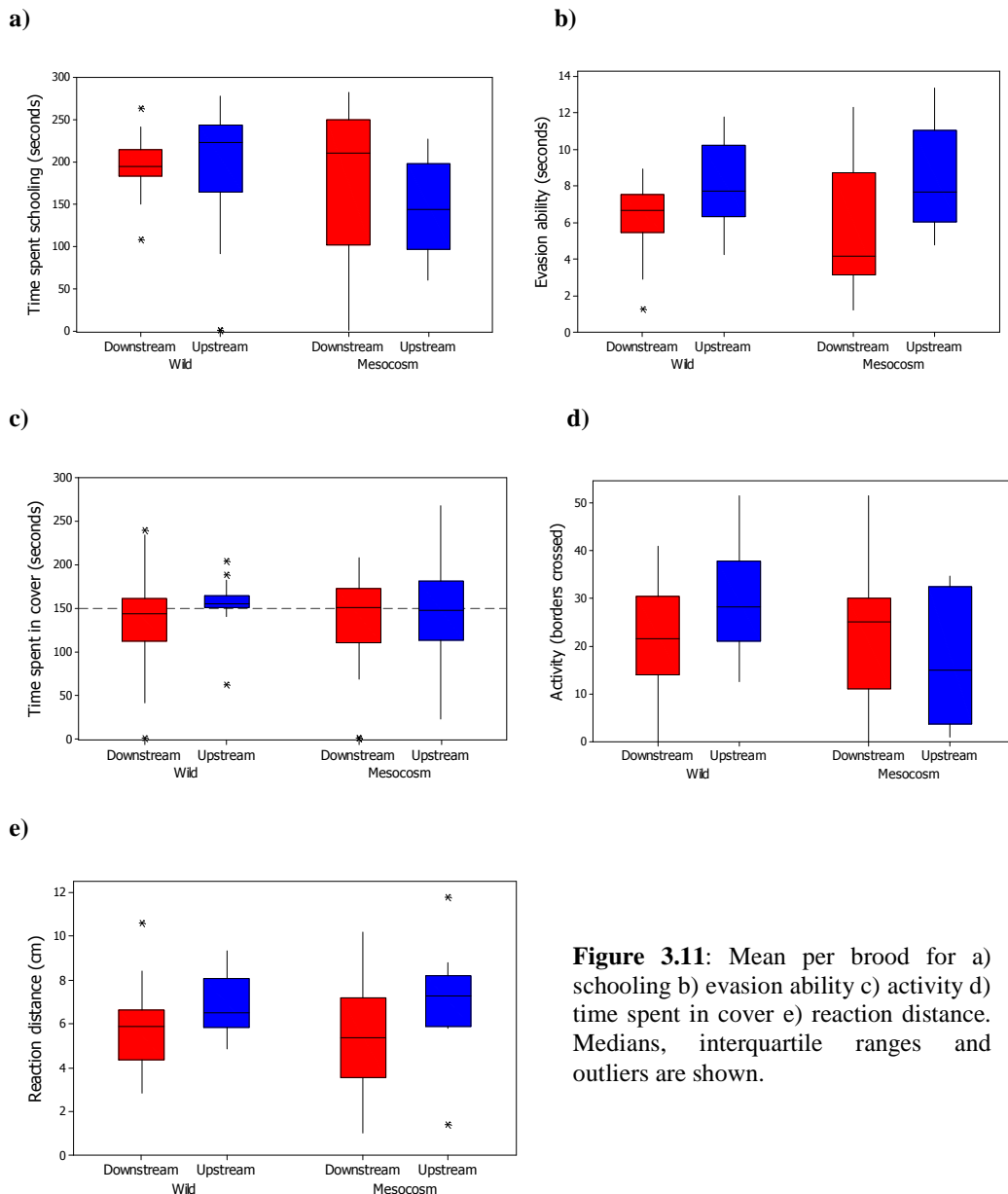
Figure 3.11: Mean per brood for a) schooling b) evasion ability c) activity d) time spent in cover e) reaction distance. Medians, interquartile ranges and outliers are shown.

Table 3.2: Results of the MANOVA analysis of behavioural performance, with origin of mother and evolutionary history as fixed factors.

Multivariate tests	Wilks' λ	Df		F	p
Origin of mother (wild or mesocosm)	0.851	5,47		1.642	0.168
Evolutionary history (downstream or upstream)	0.700	5,47		4.020	0.004**
Between-subjects tests	SS	Df	MS	F	p
Origin of mother (wild or mesocosm)					
Schooling	14325.56	1	14325.56	3.221	0.079
Evasion	7.39	1	7.39	1.257	0.269
Cover	268900000	1	268900000	2.209	0.143
Activity	54.16	1	54.16	0.423	0.518
Reaction	1.72	1	1.72	0.556	0.459
Evolutionary history (downstream or upstream)					
Schooling	1356.46	1	1356.46	0.305	0.583
Evasion	37.43	1	37.43	6.319	0.015*
Cover	4434357.37	1	4434357.37	0.036	0.849
Activity	294.99	1	294.99	2.304	0.135
Reaction	22.01	1	22.01	7.134	0.010**
Error terms					
Schooling	226834.88	51	4447.743		
Evasion	302.11	51	5.924		
Cover	6209000000	51	121700000		
Activity	6530.42	51	128.047		
Reaction	157.319	51	3.085		
Total					
Schooling	2058770.12	54			
Evasion	2950.66	54			
Cover	33340000000	54			
Activity	40478.05	54			
Reaction	2.359.75	54			

* significant at the 5% level; **significant at the 1% level.

The first principal component (PC1) explained 34% of the variation, and PC2 explained a further 26%. Higher values of PC1 were positively associated with activity (variable weighting: 0.828) and reaction distance (0.805), whilst higher values of PC2 were positively associated with time in cover (0.774) and evasion ability (0.651) (Figure 3.12).
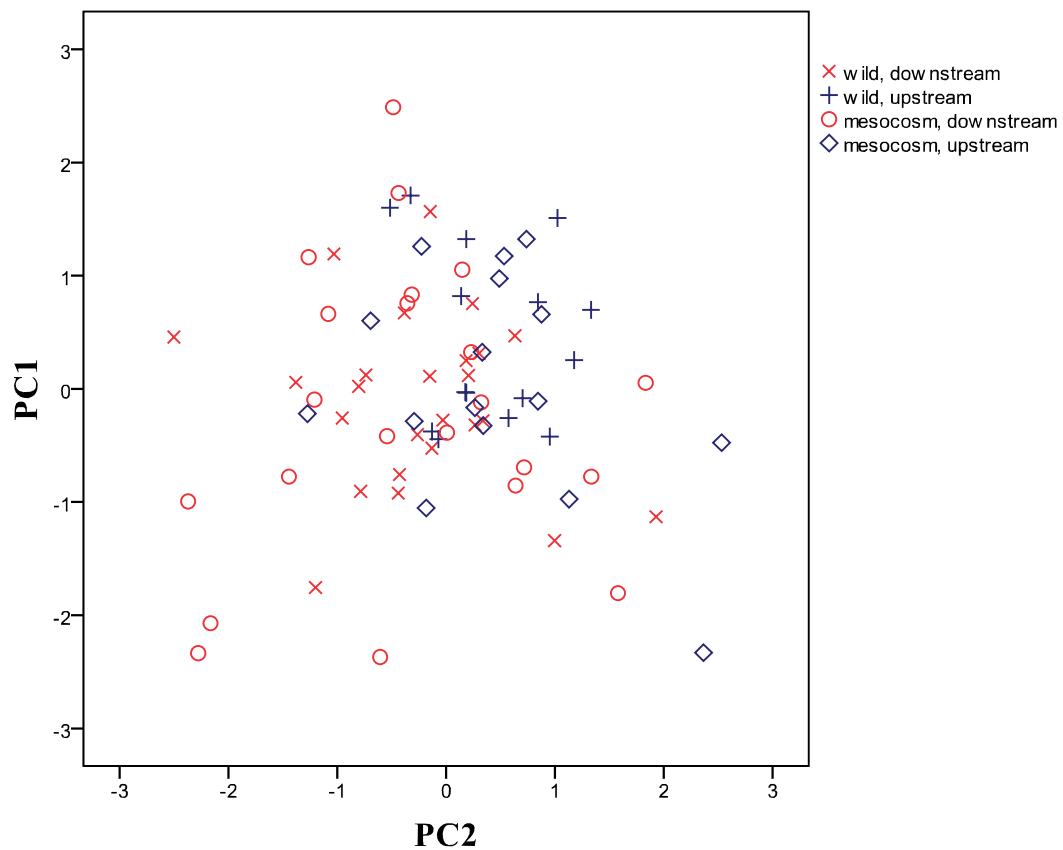
Figure 3.12: Newborn antipredator behaviours, plotted according to the first two principal components.

There was no significant difference in PC1 scores between mesocosm and wild populations ($F_{1,75}$=1.125; p=0.292) or between high and low predation origins ($F_{1,75}$=3.187; p=0.078). PC2 scores also did not differ significantly between mesocosm and wild populations ($F_{1,75}$=0.025; p=0.876) but, like the MANOVA, indicated a significant effect of original locality ($F_{1,75}$=11.904; p=0.001). There was no significant interaction in either case (PC1: $F_{1,74}$=0.569; p=0.453; PC2: $F_{1,74}$=0.152; p=0.689), and this term was removed from both final models.

Predator inspection

There was a significant difference in predator inspection behaviour between wild and mesocosm fish ($F_{4,22}$=4.920; p=0.005), but no difference between downstream and

upstream evolutionary histories ($F_{4,22}$=0.720; p=0.588) (Table 3.3; Figure 3.13). There was no significant interaction ($F_{4,22}$=1.429; p=0.258).
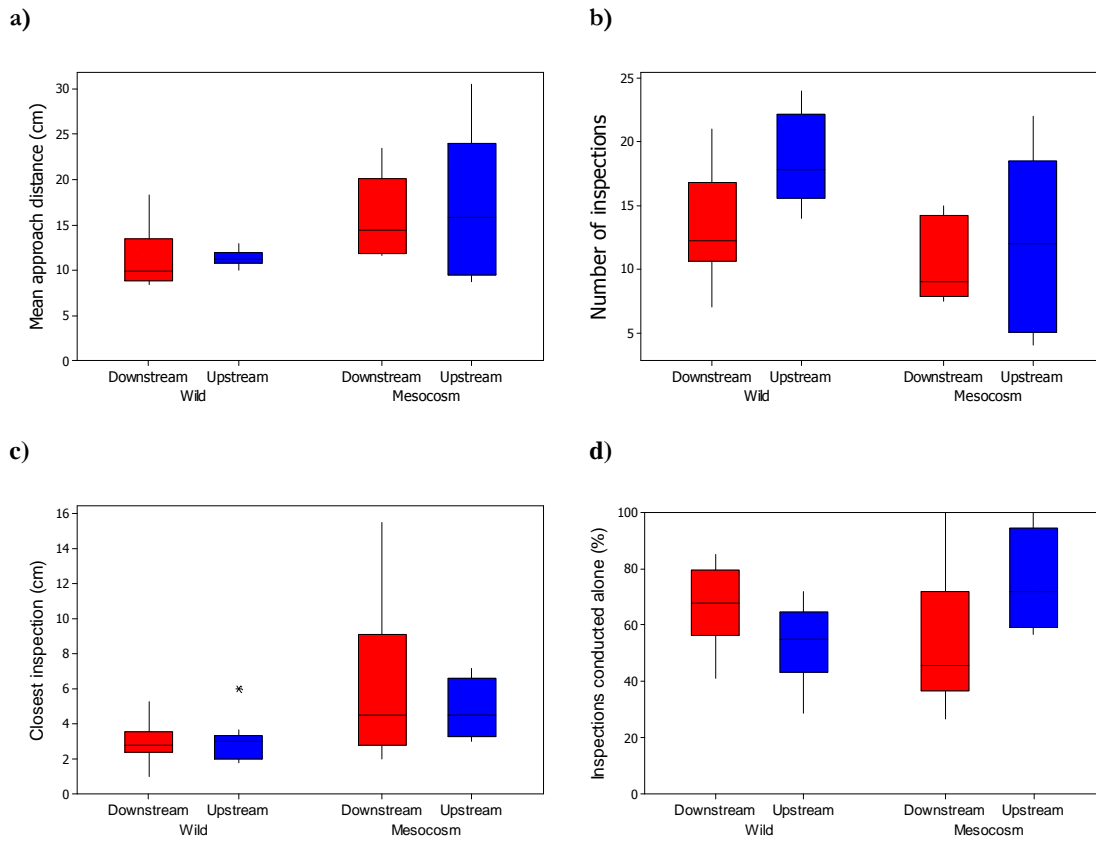
Figure 3.13: a) Mean number of inspections conducted by newborn guppies directed at a large adult female guppy within a ten minute period; b) the closest inspection within that time period; c) the mean approach distance of all inspections (cm); d) the percentage of inspections that were conducted alone as opposed to in a pair. Medians, interquartile ranges and outliers are shown.

Table 3.3: Results of MANOVA for predator inspection behaviours.

Multivariate tests	Wilks' λ	Df	F	p
Origin of mother (wild or mesocosm)	0.528	4, 22	4.920	0.005**
Evolutionary history (downstream or upstream)	0.884	4, 22	0.720	0.588
Interaction	1.429	4, 22	1.429	0.258
Between-subjects tests	**SS**	**Df**	**MS**	**F**	**P**
Origin of mother (wild or mesocosm)					
Frequency	90.270	1	90.270	4.129	0.053
Closest	1.796	1	1.796	7.473	0.011*
Mean distance	0.366	1	0.366	5.911	0.023*
% alone	122.728	1	122.728	0.398	0.534
Evolutionary history (downstream or upstream)					
Frequency	56.512	1	56.512	2.585	0.120
Closest	0.0000523	1	0.0000523	0.000	0.988
Mean distance	0.000	1	0.000	0.007	0.936
% alone	125.827	1	125.827	0.408	0.529
Interaction term					
Frequency	0.704	1	0.704	0.032	0.859
Closest	0.012	1	0.012	0.050	0.825
Mean distance	0.122	1	0.122	1.964	0.173
% alone	1914.626	1	1914.626	6.208	0.020*
Error terms					
Frequency	546.615	25	21.865		
Closest	6.009	25	0.240		
Mean distance	1.549	25	0.062		
% alone	7710.861	25	308.434		
Total					
Frequency	6447.734	29			
Closest	49.068	29			
Mean distance	183.049	29			
% alone	117066.918	29			

* significant at the 5% level;** significant at the 1% level.

Newborns born to mesocosm fish inspected the adult female from a significantly greater distance than wild fish, and marginally significantly less frequently (Table 3.3). There was a significant interaction between the effects of origin of mother and evolutionary history in terms of the % of inspections conducted alone. Upstream, mesocosm newborns conducted the greatest percentage of inspections alone (Figure 3.13).
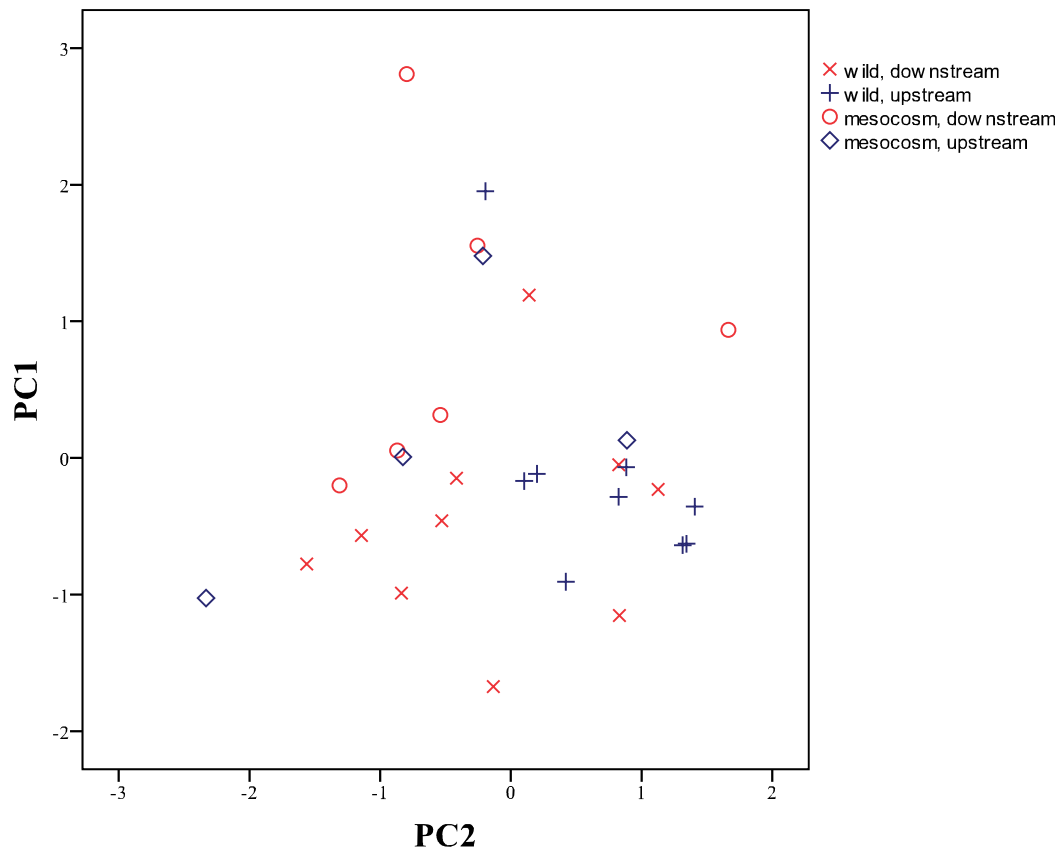
Figure 3.14: Predator inspection measures, plotted according to the first two principal components.

PC1 explained 50% of the variation, and PC2 explained a further 29%. Higher values of PC1 were positively associated with inspection distance (0.880), closest inspection (0.791) and negatively with % of inspections conducted alone (-0.675). Higher values of PC2 were positively associated with inspection frequency (0.813) (Figure 3.14).

As with the MANOVA, there was a significant difference in PC1 scores between mesocosm and wild populations ($F_{1,26}$=6.460; p=0.017). There was no significant effect of evolutionary history ($F_{1,26}$=0.005; p=0.946), and no significant interaction between the two factors ($F_{1,25}$=2.423; p=0.132). There was no significant difference in PC2 scores according to the origin of the mother ($F_{1,26}$=3.252; p=0.083) or according to evolutionary history ($F_{1,26}$=1.903; p=0.180). Again, there was no significant interaction between these factors ($F_{1,25}$=2.458; p=0.129).

3.3.4 Courtship vigour

There was no significant effect of evolutionary history on time males spent following females after a Bonferroni correction was applied ($F_{1,101}=5.014$; p=0.027; Bonferroni correction: $\alpha=0.0167$) nor on the number of sigmoid displays (U=1115; N1=52; N2=52; p=0.105) or thrusts ($F_{1,101}=0.395$; p=0.531) performed.

a)

b)

c)
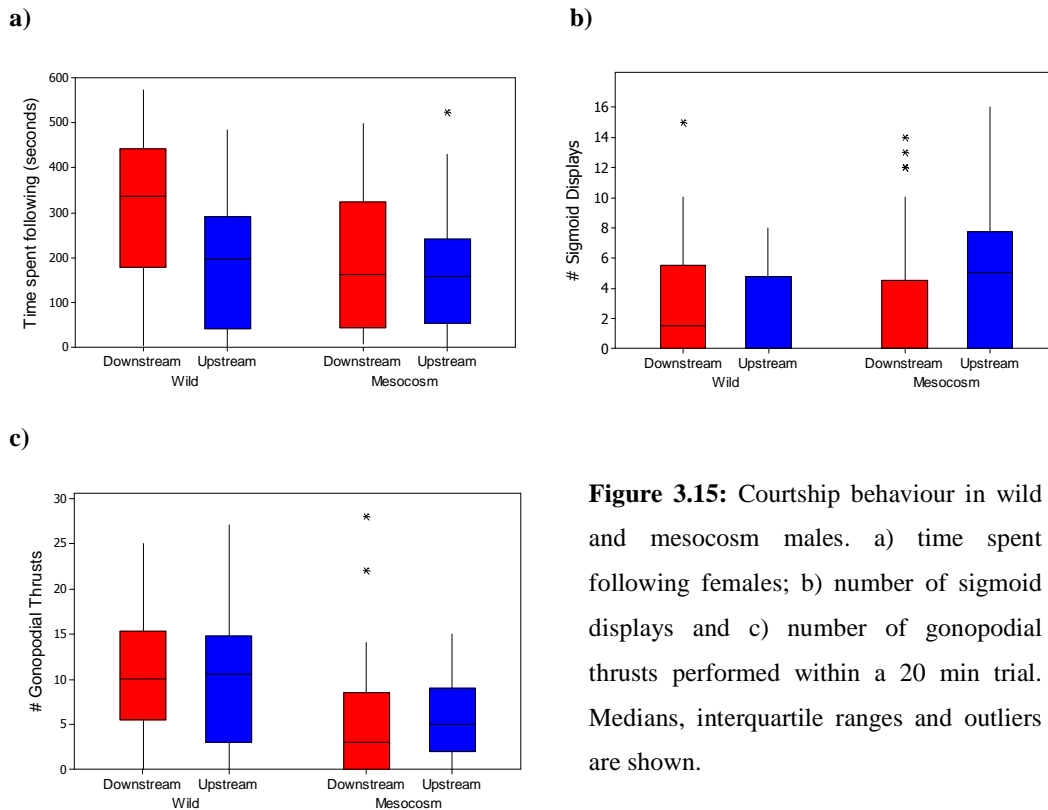
Figure 3.15: Courtship behaviour in wild and mesocosm males. a) time spent following females; b) number of sigmoid displays and c) number of gonopodial thrusts performed within a 20 min trial. Medians, interquartile ranges and outliers are shown.

Wild males performed significantly more thrusts ($F_{1,101}=13.117$; p<0.001; $\alpha=0.0167$) than mesocosm males. Time spent following females did not differ significantly between wild and mesocosm males once the Bonferroni correction was applied ($F_{1,101}=4.254$; p=0.042; $\alpha=0.025$) and number of sigmoid displays was not significantly different between wild and mesocosm fish (U=1106.5; N1:40; N2:64; p=0.223) (Figure 3.15; Table 3.4).

Table 3.4: Summary of outcomes of courtship analysis.

	Effect of evolutionary history	Effect of origin of mother
Following	p=0.027[a*]	p=0.042[a**]
Thrusts	p=0.531[a]	**p<0.001[a*]**
Sigmoids	p=0.105[b**]	p=0.223[b]

[a]two-way ANOVA; [b]Mann-Whitney U test; Bonferroni correction critical values: *α=0.0167; **α=0.025.
p-values in **bold case** indicate those still significant at the 5% level after the sequential Bonferroni correction for multiple tests has been applied.

3.3.5 Results summary

The size of the first brood was larger for downstream females and one year after introduction of the female males tended to be larger in upstream-founded mesocosms. Two years after the mesocosms were founded, this difference had disappeared (Table 3.5). The only remaining difference after two years was a significant juvenile-skewed age structure in downstream-founded populations when compared with upstream-founded populations.

Table 3.5: Summary of mesocosm findings

	12 months post-intro	24 months post-intro
Establishment probability	No difference	No difference
Population size	No difference	No difference
Size of first brood	Larger for downstream	N/A
Size of males	Larger for upstream	No difference
Condition factor	No difference	No difference
Population age structure	More juvenile-heavy in downstream	More juvenile-heavy in downstream

Evasion and reaction behaviours were more pronounced in newborns born to females descending from upstream populations. Predator inspections were more frequent and closer in newborns born to wild females. Other behaviours did not differ with either origin of mother or evolutionary history. Wild males spent more time following females and performed more gonopodial thrusts than mesocosm males. Downstream males also spent more time following females than upstream males did (Table 3.6).

Table 3.6: Summary of behavioural findings.

	Origin of mother (wild, mesocosm)	Evolutionary history (downstream, upstream)
Newborn size	Interaction: wild, upstream newborns are larger.	
Newborn performance	No differences	Evasion & reaction better in those born to upstream fish
Newborn inspections	More frequent and closer in those born to wild fish	No differences
Male courtship	Wild males performed more gonopodial thrusts.	No significant differences

3.4 Discussion

3.4.1. Establishment success

This study demonstrates that single female guppies are capable of routinely establishing new populations. In the majority of cases populations persisted for the two years of the experiment, suggesting the viability of such populations.

3.4.2. Life history and population structure

Populations founded by females from the downstream locality were just as successful as those founded by females from the upstream locality. Size of first brood was greater for females from the upstream, low-predation, 'K-selected' wild population, as would be expected from previous studies of wild fish (Reznick & Endler, 1982; Reznick & Bryga, 1987; Reznick, 1990, Reznick *et al.*, 2001). However, this initial difference in life history strategy does not appear to have a bearing on the probability of establishment or population size after one or two years.

The evolutionary history of the founders did not affect the mean condition factor of fish in mesocosm populations at either 12 or 24 months post-introduction.

Mesocosm population age-structure differed according to evolutionary history, with those populations founded by downstream females containing a significantly greater proportion of juveniles. This may be a consequence of the 'fast' life history traits that are characteristic of downstream populations, such as larger brood sizes (Reznick & Endler, 1982). In a resource-limited environment, this may lead to a more juvenile-dominated population even in the absence of predation pressure. Identifiable males from the downstream-founded mesocosms were significantly smaller than those from upstream-founded mesocosms, suggesting that descendents of downstream fish mature faster and/or at a smaller size (Reznick & Endler, 1982). However, differences in male size disappeared by 24 months post-introduction.

Population differences in the size of newborns are well documented; they are associated with various factors including differences in predation regime (Reznick & Endler, 1982; Reznick, 1982). The wild populations in this study support the general findings of previous work, with upstream females giving birth to significantly longer young than downstream females. However, this difference does not persist in the mesocosm populations, where newborns of both evolutionary histories tend to be the same size as downstream wild newborns.

It seems that the effect of evolutionary history on the population and life history characters in mesocosm populations is relatively small, and any differences that do exist 12 months post-establishment, soon disappear. With the exception of population age-structure, by 24 months post-establishment there were no differences in any of the parameters measured between mesocosm populations founded by wild downstream or upstream fish.

3.4.3. Newborn performance

I found no significant differences in performance between the offspring of wild and mesocosm fish for most of the behavioural measures, which suggests that behavioural viability is maintained in the mesocosm populations. The persistence of antipredator behaviours in the absence of predation pressure has been found previously in highly

inbred ornamental guppy strains (Bleakley *et al*., 2006) as well as in wild-derived guppies reared in isolation (Paxton, 1996).

Original location had a significant effect on both evasion ability and reaction distance, with newborns born to fish originally descended from upstream locations being better at evading a net and reacting at a shorter distance from an approaching object. This is also reflected in the PC2 analysis, where there was a significant effect of evolutionary history. While behavioural differences between populations of guppies in Trinidad, especially between those from upstream and downstream localities, are unsurprising and well documented in the literature (Seghers, 1974; Templeton & Shriner, 2004; Magurran *et al.*, 1995; Magurran *et al.*, 1992; Magurran & Seghers, 1991), here the direction of the pattern is the opposite of what might be expected if I had tested adult guppies from the same localities. In all instances where there was a difference, it was those newborns born to, or descending from, fish from upstream locations - typically free from large predatory fish - that were in fact better at evading a net and reacting to an approaching stimulus.

Past studies have also emphasised that predation pressure on adult and juvenile guppies can differ markedly in the same location (Magurran & Seghers, 1990*b*). In this case, while the upstream locality in this current study supports fewer predators of adult guppies, the abundance of smaller species such as *Rivulus hartii*, and possibly higher levels of cannibalism due to higher densities and larger adults (Reznick & Endler, 1982), may lead to a greater selective pressure on newborn anti-predator behaviour here than in the 'high predation' location population further downstream.

Predator inspection appears to show the opposite pattern to the other behavioural measures. Here there was a significant effect of origin of mother, with closer and more frequent inspections conducted by those born to wild fish, while there was no evidence suggesting differences between upstream and downstream populations.

3.4.4. Courtship vigour

Direct comparisons between wild and mesocosm male courtship behaviours from this part of the study should be made with caution, as the experimental design does not provide a true control as it did with the newborn performance experiment. Here, it is not possible to separate the effects of genetics and experience, although in fact there were no significant differences between the two localities after the correction for multiple tests.

Wild fish performed almost twice the number of gonopodial thrusts during a 20 minute trial than the mesocosm males. This does not necessarily imply an effect of inbreeding; studies suggest that population differences tend to take the form of differences in risk sensitivity rather than in absolute behavioural differences (Magurran & Seghers, 1990*a*). As the mesocosm fish have never been exposed to a predator of adult fish within their lifetimes, whilst the wild fish probably have been, it makes sense that any differences in courtship behaviour would be more apparent in the wild fish, regardless of any potential inbreeding effects. Frequency of sigmoid displays did not differ with evolutionary history or immediate origin.

The relative effects of immediate origin or evolutionary origin appear to be different for different behaviours. Even within antipredator behaviours, it seems that differences in evasion and reaction behaviours are determined by evolutionary history (upstream or downstream), whilst for predator inspection behaviours whether they were born to wild or mesocosm parents proved to be more important and indicates some reduction in predator inspection behaviour in mesocosm newborns.

3.4.5. General discussion in the context of introduced species

Most species will fail to survive, reproduce or establish a self-sustaining population when introduced to a new habitat (Williamson & Fitter, 1996). As indicated by its extensive worldwide distribution, the guppy appears to be particularly good at overcoming the usual barriers to establishment. Many of the traits that are associated with invasive success (Lodge, 1993) can be seen in the guppy, including phenotypic plasticity (Reznick & Bryga, 1987; Reznick, 1990; Carvalho *et al.*, 1996), polyphagy (Dussault & Kramer,

1981), eurytopy (Chervinski, 1984; Chung, 2001; Casatti *et al.*, 2006), and a specialised reproductive system (Courtenay & Meffe, 1989; Thibault & Schultz, 1978). The remarkable establishment success demonstrated in this study, independent of evolutionary history, emphasises the critical importance of the latter.

Arthington & Mitchell (1986) identified three fundamental reproductive strategies among successful Australian introduced fish: oviparity, ovoviviparity and mouth-brooding, and suggest that they all share an end result of producing large numbers of progeny. Single-parent or vegetative reproductive strategies are commonly associated with invasives in a wide range of taxa (Baker, 1955; Lodge, 1993). For example, the water hyacinth *Eichhornia crassipes* has the most highly developed asexual reproduction strategy within its genus, and is also by far the most invasive (Barrett & Richardson, 1986). Taylor & Hastings (2005) suggested that this is partly because such strategies minimize Allee effects in small introduced populations, increasing their invasive potential. This is supported by the documented success of other introduced livebearing fish; the family Poeciliidae contributes to 11% of the fish species listed on the Global Invasive Species Database (http://www.issg.org/database/welcome/).

Although this study did not examine the likely role of species interactions in determining colonisation success (Lodge, 1993), it does reveal that female guppies have the potential to routinely establish viable populations on a short temporal scale, and demonstrates that even very small numbers, such as those introduced as unwanted pets, are capable of colonising a new environment. Furthermore, in many guppy introduction scenarios, particularly those associated with mosquito control, species interactions may not be a concern in the initial stages of establishment. For example, in villages in India and in the Comoros, the primary habitats of malarial mosquito larvae (*Anopheles* sp.) are manmade wells and troughs (Ghosh *et al.*, 2005; Sabatinelli *et al.*, 1991) – in some cases not dissimilar to the mesocosm tanks used here. Guppies are introduced to these containers to reduce the incidence of mosquito-borne disease, and their initial increase in numbers will depend largely on juveniles successfully evading cannibalistic attacks by older individuals (Rose, 1959; Smith & Reay, 1991). It may be many months or years before a

monsoon or flood enables them to escape to more natural environments where they will encounter competitors or predators, thus it is of great interest that these severe bottleneck events do not necessarily reduce colonisation ability or the behavioural viability of resulting populations. Such resistance greatly increases the likelihood of their successful spread in localities where they have been introduced for the purpose of mosquito control.

A highly specialised reproductive system, coupled with a remarkable adaptability (Kelley *et al.*, 2003; Magurran *et al.*, 1995) is likely to have led to the phenomenal success of the guppy outside of its native range. Moreover, these results show how introductions consisting of only a few animals, or even a single individual, can lead to thriving populations of invasive species. This strongly suggests that particular caution should be exerted when considering introducing the guppy, or other poeciliids, to both natural and manmade water bodies.

Chapter 4

Polyandry and colonisation success in the guppy

Abstract

It has been proposed that by increasing genetic and phenotypic variation, polyandry may help founding populations minimise the effects of demographic bottlenecks. Invasive species may benefit from such a mechanism, as founding populations can be small. The guppy is a successful invasive species in which females mate multiply and store sperm for several months. Chapter 3 demonstrated that single female guppies are capable of establishing populations, but it is not known whether multiple mating contributed to this success. Here, I tested the hypothesis that female guppies that had mated with four males were more likely to establish viable populations than those that had mated with a single male. Treatments were monitored for one year. Establishment success was high for both conditions (88%). There were however no differences in a range of measures including population size, population structure, behavioural performance of newborns and courtship vigour of male descendents. Moreover, there were no differences in behavioural variability. These findings suggest that the invasive success of guppies is not dependent on polyandry.

4.1 Introduction

There is a growing body of literature discussing the possible evolutionary benefits of polyandry (Jennions & Petrie, 2000; Hosken & Blanckenhorn, 1999; Simmons, 2001; Cornell & Tregenza, 2007), several of which may apply in particular to invasive species (Figure 4.1). Potentially the most important is that mating with several males increases the overall genetic and phenotypic diversity of offspring (Becher & Magurran, 2004). Producing a brood with multiple paternity can help to minimise loss of genetic diversity during the severe demographic bottlenecks often experienced by small founding populations during and soon after their introduction, by ensuring that a larger proportion of the gene pool is retained and that effective population size of the next generation is maximised (Frankham, 1995; Pearse & Anderson, 2009). Cornell & Tregenza (2007) suggested that polyandry also acts to minimise inbreeding risk in very small populations because it creates an F1 of half siblings rather than full siblings, and this can dramatically reduce the extent of inbreeding in the second generation. Furthermore, there is evidence that in some species pre-copulatory mate choice can bias paternity in favour of the 'most different' or the 'most compatible' males (Tregenza & Wedell, 2002; Eakley & Houde, 2004).
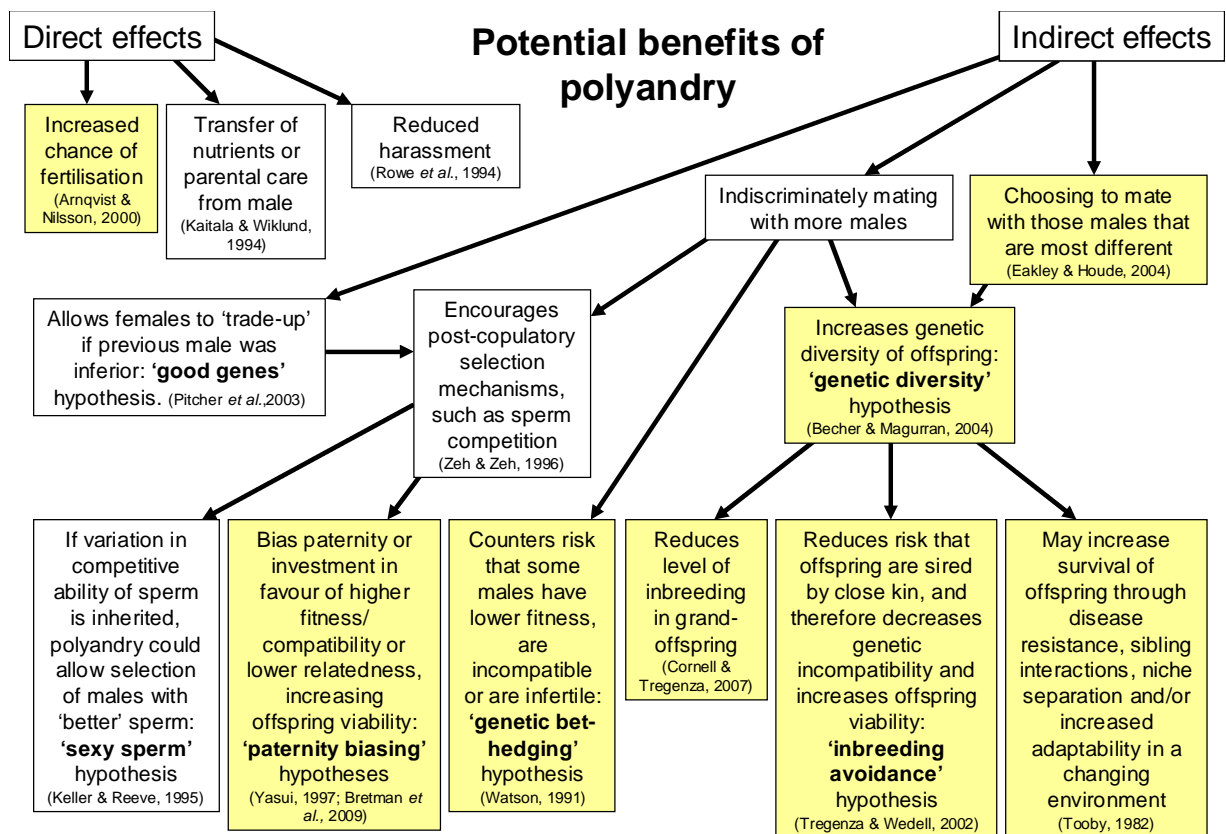
Figure 4.1: Multiple (and non-mutually exclusive) potential benefits of polyandry. Those that may have particular relevance to introduction scenarios and invasive success are shaded in yellow.

In addition to maintaining offspring (and population) viability by reducing the risk of inbreeding depression (Stockley *et al*., 1993; Cornell & Tregenza, 2007), maintaining phenotypic variation within a brood may be especially beneficial in an introduction situation where individuals are faced with a novel biotic and abiotic environment. The more variation that exists in the population, the more likely that the population can adapt by the process of natural selection, in order to survive, persist and invade (Lee *et al.*, 2007). It has even been proposed that individuals could be selected for 'invasibility' (Lee, 2002).

Polyandry also encourages post-copulatory selection mechanisms such as sperm competition or differential investment, ensuring that the paternity of offspring is biased in favour of the most compatible males (Zeh & Zeh, 1996). Often the most compatible

males are those that are least closely related, and recent evidence suggests that females may manipulate sperm storage in favour of unrelated males in order to minimise inbreeding (Bretman *et al.*, 2009).

The guppy, *Poecilia reticulata*, is an invasive species that is now established in more than 70 countries outside its native range of north-eastern South America (Chapter 2). There are many aspects of the biology of this species that equip it to be a successful invasive species (Lodge, 1993), including the birth of live, free-swimming young (Courtenay & Meffe, 1989), the ability of females to store sperm for up to 8 months (Winge, 1937), flexible anti-predator behaviour (Kelley & Magurran, 2003), a wide tolerance to environmental conditions (Chervinski, 1984; Chung, 2001; Casatti *et al.*, 2006) and generalist, omnivorous feeding habits (Dussault & Kramer, 1981). An additional trait that may also contribute to the invasive success of this species is polyandry.

In the wild, guppies tend to mate multiply (Haskins *et al.*, 1961; Herdman *et al.*, 2004), Not only do female guppies mate with multiple males, but there may also be multiple paternities within a single brood (Becher & Magurran, 2004). Up to 64% of females in certain populations give birth to broods with multiple paternity (Kelly *et al.,* 1999), and Becher & Magurran (2004) found that a single brood may be sired by as many as 5 males.

Laboratory studies have detected multiple benefits of polyandry in the guppy; Evans and Magurran (2000) found that those that were allowed to mate with multiple males had shorter gestation times, larger broods and offspring displaying better antipredator behavioural defences than those who had been mated with a single male. All of these characteristics have the potential to increase the success of colonisation of a new habitat, by encouraging rapid population growth and improved survival in the face of new conditions.

This study investigated whether number of mates increased colonisation success of single females and behavioural viability, as an indicator of the role that multiple mating might play in the invasive success of the guppy. I tested the following hypotheses:

- Populations founded by females who mated with one male should have a lower establishment probability, longer gestation periods, smaller brood sizes and a smaller total population size after 12 months when compared with those founded by females who mated with multiple males.

- After 12 months, newborns and males from colonies founded by 'monogamous' females should show signs of inbreeding, in the form of reduced behavioural performance and reduced variation in behavioural performance.

4.2. Methods

4.2.1. Mesocosm set up

In the tropical fish laboratory of the University of St Andrews, 40 tanks (45 x 30 x 25 cm were set up with gravel, plastic plants, large pebbles and Java moss, and filled with dechlorinated water. Healthy virgin females measuring between 25 and 28 mm were selected, measured and each placed in individual tanks. All fish used were descendents of fish taken from the lower portion of the Tacarigua River in Trinidad, from stocks that had been bred in the laboratory for a number of years. Fish were paired according to precise size and one of each pair allocated to condition 1, and the other to condition 2. Fish were left to settle for 72 hours.

Males were selected from the stock tanks according to size; all measured between 20 and 23 mm. On the first day, a single male was randomly allocated to each mesocosm tank and gently introduced. The male-female pair was observed for the proceeding thirty minutes. The number of apparently successful copulations was recorded, including both those due to 'sneaky' gonopodial thrusts and those immediately following a sigmoid display.

After 24 hours, the male was removed from the tank. In half of the tanks (condition 1: 'single'), the same male was replaced immediately, whilst in the other tanks, a new male was introduced (condition 2: 'multiple'). This procedure was repeated for four days, resulting in half of the females having been exposed to a different male each day for four days, and the other half having only encountered one during the same period.

All of the females in the 'multiple' condition were observed to mate with at least 2 males of the four that were presented during the 30 minute observation periods. The mean number of males mated with was 3 (+0.2SE) and 70% of females mated with 3 or 4 of the males. This does not necessarily mean that sperm was transferred or that every male's sperm contributed to the female's offspring, but did confirm that the females in the multiple mating condition all had sexual contact with more than one male. It seems likely, given the full 24 hour period, that females will have had some sexual contact with all 4 males.

At the end of the four days, all males were removed and females (and descendent populations) were fed daily on a diet of flake food for the following 12 months. Water was topped-up when necessary.

4.2.2. Population size and structure

Mesocosm tanks were checked daily for offspring in the early stages of the experiment in order to assess length of first gestation period. After 12 months, a full census was conducted of each mesocosm. This involved catching, counting, measuring and weighing each fish, as well as sexing adult fish. Numbers of adults and juveniles were recorded.

4.2.3. Male colouration

During the census, all adult males were photographed using a Nikon CoolPix digital camera. Orange and black colour patterns of each male were scored according to position on the fish (Figure 4.2). A basic body plan of a male guppy was divided into 12 sections and each patch of orange or black colour was recorded according to which of these sections the majority of the patch fell into. This resulted in 24 possible spot-types.
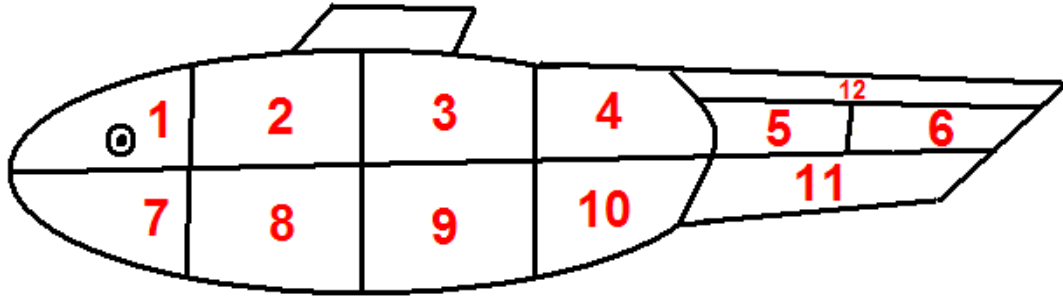
Figure 4.2: Schematic diagram of a male guppy, showing sections that were used to classify black and orange markings on each mesocosm male. The section that the majority of a marking fell into was recorded.

A mean index of complementarity **C** was then calculated for each mesocosm (Colwell & Coddington, 1995). This involved creating a matrix for each mesocosm in which each fish was compared with every other fish in the mesocosm and for each comparison the number of spot-types unique to either fish was divided by the total number of spot-types possessed by both fish combined (Equation 4.1; Appendix VII).

Equation 4.1:

$$C_{jk} = U_{jk} / S_{jk}$$

S_{jk}= total number of spot-types possessed by fish j and k combined

U_{jk}= number of spot-types unique to either fish j or k.

The mean 'C_{jk}' for all possible comparisons within each mesocosm was calculated and presented as the complementarity score (**C**).

An ANCOVA was used to compare differences in within-mesocosm complementarity between conditions, with number of males as a covariate. The hypothesis tested was that multiply mated mesocosms would possess lower between-fish similarity, reflected in higher complementarity scores.

4.2.4. Newborn performance

Adult females (>18mm) were removed from the mesocosms and each placed in separate small glass tanks filled with dechlorinated water where they were fed twice daily on dry flake food. Care was taken not to use the founder female who, where present, was conspicuously larger in size. Tanks were labelled according to an arbitrary code with a corresponding key to enable 'blind' testing.

At least once a day the tanks were checked for babies. On finding a brood, the mother was carefully removed, photographed for later measurement, and kept in a separate container until the tests were over and she could be returned to her original mesocosm. The following tests were then conducted in the order that they are described below:

Schooling

Pairs of newborns were transferred to a circular pale tray (40 cm in diameter; water 2 cm deep). They were allowed to settle for 5 minutes, and the length of time the focal fish spent schooling was recorded for the proceeding 5 minutes. '*Schooling*' was defined as the time it spent swimming within 3.5 body lengths of its companion (see Evans & Magurran, 2000).

Evasion ability

A small net (with a circular rim of 3 cm in diameter, as depicted in Chapter 3, Figure 3.2) was used to catch each newborn individually from the tray at the end of the schooling behaviour trial. The net was placed in the water at the opposite side of the tray to the fish, and the time taken to catch was recorded using a stopwatch as *evasion ability*. No improvement in experimenter catching ability was apparent when escape times ranked according to time and date were plotted.

Time in cover and activity

On being caught, the net containing the fish was placed in the centre of a shallow container (21 x 15 x 8cm, with water 2.5cm deep) and the fish gently released. The bottom of the tub was divided into four equal sections, two of which were covered in a shallow sprinkling of gravel and two of which were left bare white. After every two trials, the gravel/bare sections were reversed to control for any effect of unforeseen spatial differences, such as minor differences in lighting. Fish were allowed to explore the arena for five minutes; the time spent on gravel-covered areas (*time in cover*) and the number of times that the newborn crossed from a gravel-covered to a gravel-free section or vice versa were recorded (*activity*).

Reaction distance

At the end of the 5 minutes, the fish was gently caught from the exploration arena and placed in another container (same dimensions as before, with a water depth of 1.5cm), this time with graph paper showing through the translucent base. Once the fish was settled (sometimes immediately, sometimes after a few minutes), a pencil end was introduced 12cm away from the fish. This was moved towards the fish's head at a constant rate (2.5 mm/sec). The distance at which the fish reacted to the object – usually in the form of an obvious directional movement – was measured as *reaction distance*.

Inspection of a novel object

Newborns were gently transferred two at a time to the inspection arena. This was the same tray as used earlier for schooling, but filled to a deeper level with water (3 cm). A coloured, plastic fish-shaped object (Figure 4.3) was placed in a position at the back edge of the tray. The two babies were released at the opposite end of the arena and allowed to roam for 10 minutes. During this time, the number of inspections (*frequency*), proximity of approach during each inspection (*mean distance*) and group size at approach (to calculate *% alone*) were all recorded. Inspection behaviour was conspicuous, and consisted of a directional approach towards the object, followed by a sideways glide and then retreat. The methods here differed from those in the previous chapter, in that an

object was used to induce inspection behaviours rather than an adult guppy. This was because adult guppies from the St Andrews laboratory stock are more relaxed in the experimental arena, resulting in a higher risk of cannibalism during the tests.

Figure 4.3: Novel object used in inspection trials.

Measurement of newborns

Newborns were placed in a Petri dish filled with water, placed on top of a piece of graph paper and a small piece of paper labelled according to their container code. When six fish had been accumulated (or fewer in smaller broods), they were photographed for later measurement using Image J software (version 1.37) (Figure 4.4).
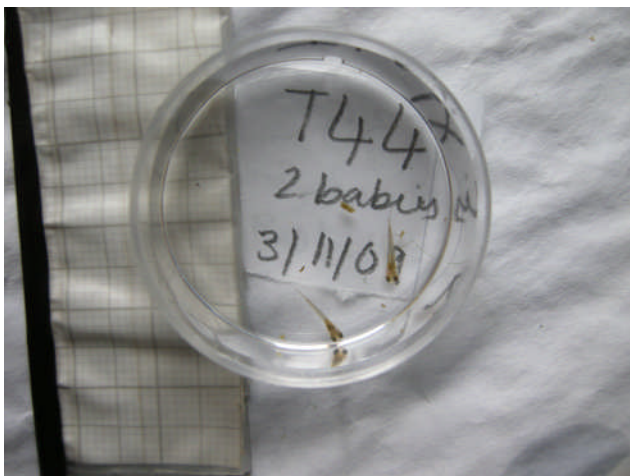
Figure 4.4 Example photograph of newborns, used in conjunction with Image J software to measure the length of each fish.

Altogether 54 broods were tested for evasion ability, reaction distance, time in cover and activity; 45 of these were also tested for schooling and 39 tested for inspection behaviour. Fewer trials were conducted for schooling because they required pairs of fish, as did novel object inspection, for which even fewer trials were conducted. Only one brood from each female was used, with 10 mesocosms from the monandrous condition and 9 from the polyandrous condition contributing females who gave birth during the experimental period.

4.2.5. Courtship behaviour

To test for differences in courtship vigour between the two conditions, observation tanks were set up (30 x 20 x 20 cm) with three male and four female companion fish from stock tanks. All were unfamiliar individuals. The companion fish were allowed to settle overnight before a set of trials.

Between three and six males from each mesocosm were tested. Trials were alternated between males from the monandrous and polyandrous conditions.

The focal male was gently introduced into the tank and observed for 10 minutes. An hour after the initial 10 minutes, the male was observed for an additional 10 minutes. This was in case males were more or less likely to court once settled for longer in the tank, but as there was no such effect, these periods were combined to form one 20 minute data period.

The time that the focal male spent following females was recorded, as were the number of sigmoid displays and the number of gonopodial thrusts. '*Following*' was defined as swimming within two body lengths of the female and in the same direction, '*sigmoid displays*' were recorded when the male arched his body into an S-shape, raised his dorsal fin and quivered while within sight of the female, '*gonopodial thrusts*' were recorded when the male approached the female from behind and attempted or succeeded in making contact with the female by cocking his gonopodium forward at least 90˚ while positioned

within one body length of her. Copulations and post-copulatory jerks were also recorded but occurred so infrequently that they were not included in any analyses.

At the end of each trial, the male was captured and returned to its mesocosm of origin.

4.2.6. Statistical methods

All statistical tests were performed using SPSS v.17.0.0. Differences in mesocosm population size, gestation time, size of first brood, population structure and size of newborns between conditions were compared using t-tests or Mann-Whitney U-tests.

The mean per brood for performance (schooling, evasion ability, time in cover, activity, reaction distance, frequency of inspection, mean inspection distance and % conducted alone) was calculated. A MANOVA was used to examine for the effect of condition (mesocosms founded by single or multiple mated females). As an integrated measure of performance, a principal components analysis was conducted using all the behaviours listed above. Resulting PC1 values were analysed using a two-way ANOVA.

Courtship behaviour (time spent following, sigmoid displays and gonopodial thrusts) were examined using t-tests or Mann-Whitney U tests.

Data were transformed where necessary to meet the assumptions of a normal distribution and homogeneity of variance. When this was not possible, an equivalent non-parametric test was used instead.

To explore the extent of newborn variation within mesocosms and whether this is affected by condition, the coefficient of variation (CV) for each mesocosm was calculated for evasion ability, activity, time in cover, reaction distance and length, using Minitab 14.0. A minimum of 6 and a maximum of 10 newborns were included in the CV calculations; therefore only those mesocosms from which at least 6 newborns had been tested were included. Where possible, the ten were selected to include newborns from 3 different broods from the same mesocosm. A MANOVA was used to examine whether

the CV differed significantly between mesocosms founded by singly or multiply mated females. Similarly, the CV was calculated for the courtship behaviours of males within each mesocosm for all mesocosms where at least 4 fish were tested, and compared using a MANOVA.

4.3. Results

4.3.1. Establishment success

Of the 40 mesocosm tanks set up, 35 (87.5%) had successfully established populations that still persisted one year after initial introduction. Of the five extinctions, three were from the single male condition and two from the multiple male condition. In all cases, the extinction was the result of a failure of the female to establish a population at all; no offspring were recorded in any of these five tanks at any point.

4.3.2. Life history and population structure

There was no significant difference in gestation time between singly and multiply mated founding females (t=-0.844; df=18; p=0.410) (Figure 4.5a). There was also no significant difference in size of first brood between the two conditions (t=0.50; df=33; p=0.961), which in both cases consisted of a mean of 7 (+0.8 SE) offspring (Figure 4.5b).

a) b)
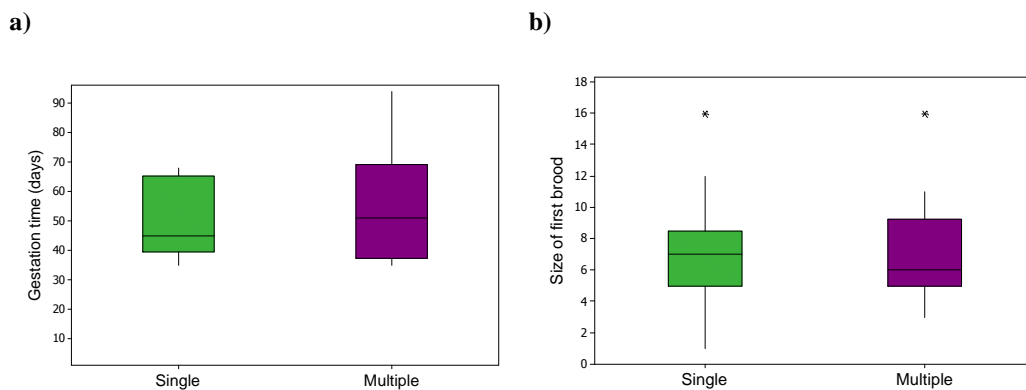
Figure 4.5: a) Gestation time and b) size of first brood in mesocosms established by females that had been allowed to mate with one or multiple males. Medians, interquartile ranges and outliers are shown.

One year after establishment, there was no significant difference in population size between those founded by singly or multiply mated females (t=0.504; df=33; p=0.618) (Figure 4.6).
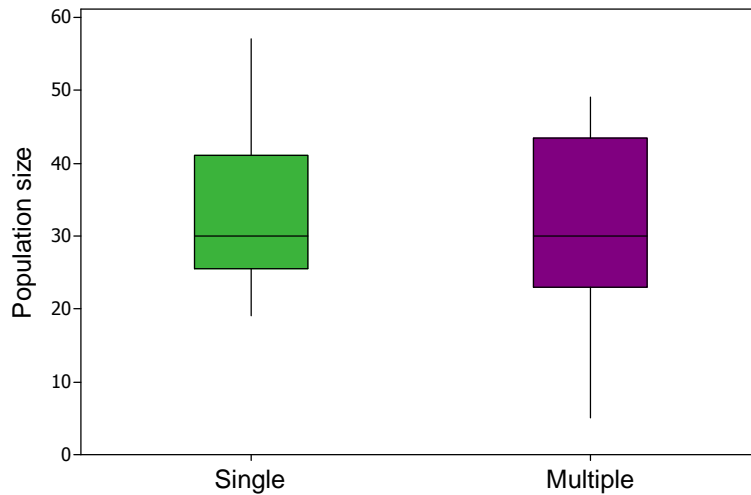
Figure 4.6: Population size in mesocosms one year after establishment. Medians and interquartile ranges are shown.

Tank populations were composed of a mean of 47% (±2SE) juveniles. There was no significant difference in the percentage of juveniles in tanks founded by monandrous or polyandrous females (t=0.301; df=33; p=0.765) (Figure 4.7).
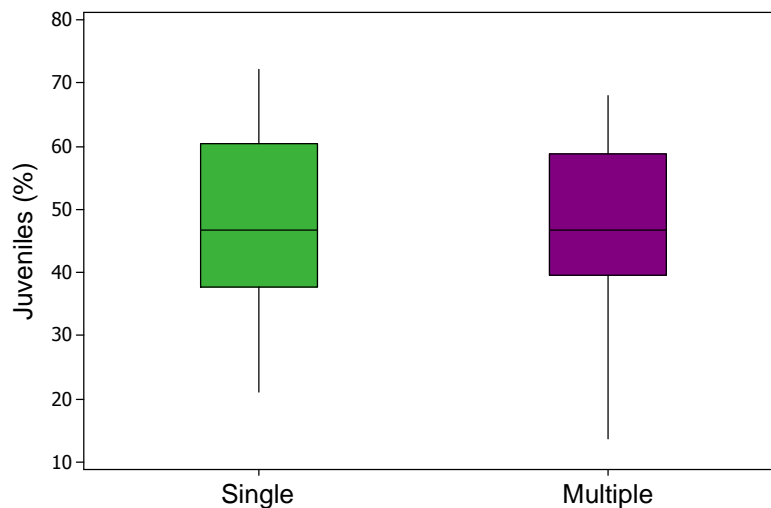
Figure 4.7: Proportion of juveniles present in mesocosm populations founded by singly or multiply mated females. Medians and interquartile ranges are shown.

Size of newborns

There was no difference in mean size of newborns per brood (t=-1.08; df=50 p=0.285) (Figure 4.9).
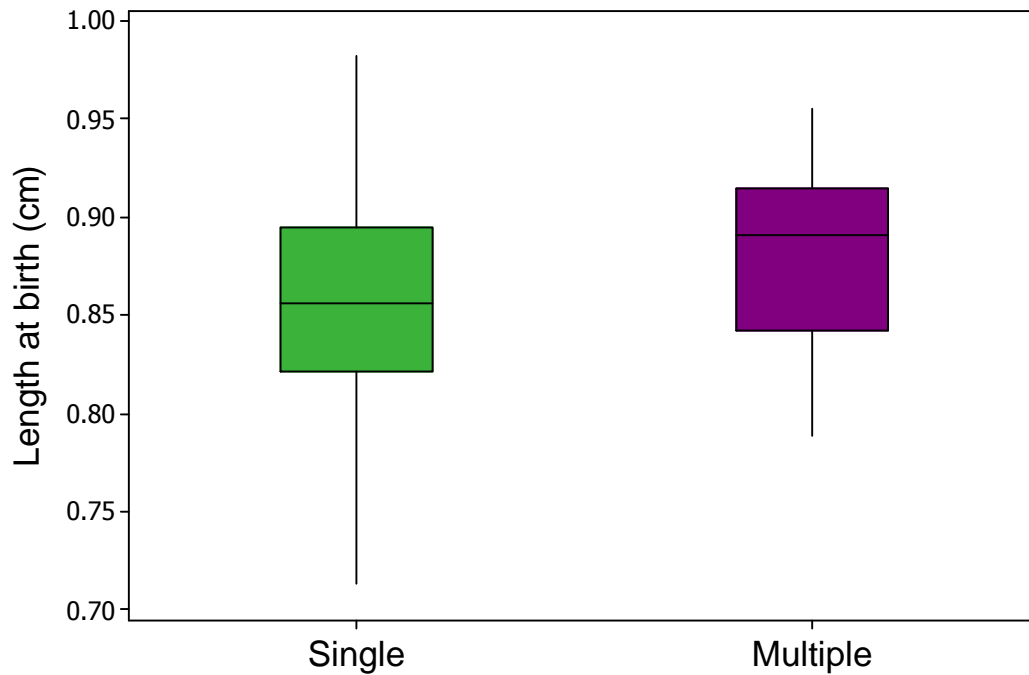
Figure 4.9: Size of newborns (mean per brood) born to females from the mesocosms founded by singly or multiply mated females. Medians and interquartile ranges are shown.

4.3.3 Male colouration diversity

Mesocosm populations in which the female founder had the opportunity to mate multiply showed significantly greater between-fish complementarity in colour patterns ($F_{1,30}=6.432$; p=0.017) (Figure 4.8).
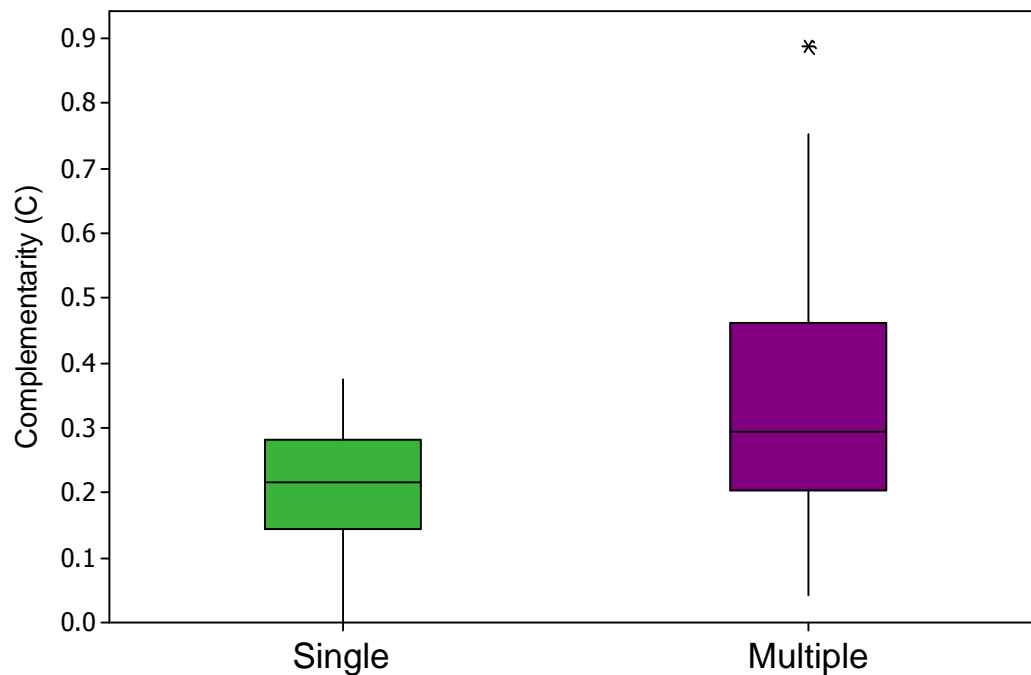
Figure 4.8: Mean complementarity scores per mesocosm. Lower values of '*C*' represent greater within-mesocosm similarities between fish in terms of their black and orange colour patterns. Medians, interquartile ranges and outliers are shown.

4.3.4. Newborn performance

There was no difference in the behavioural performance of offspring born to fish from mesocosms founded by singly or multiply mated females in 6 out of the 7 of the behaviours tested (Figure 4.10; Table 4.1). However, offspring from mesocosms founded by singly-mated females were significantly more active than those from mesocosms founded by multiply mated females (see Table 4.1).
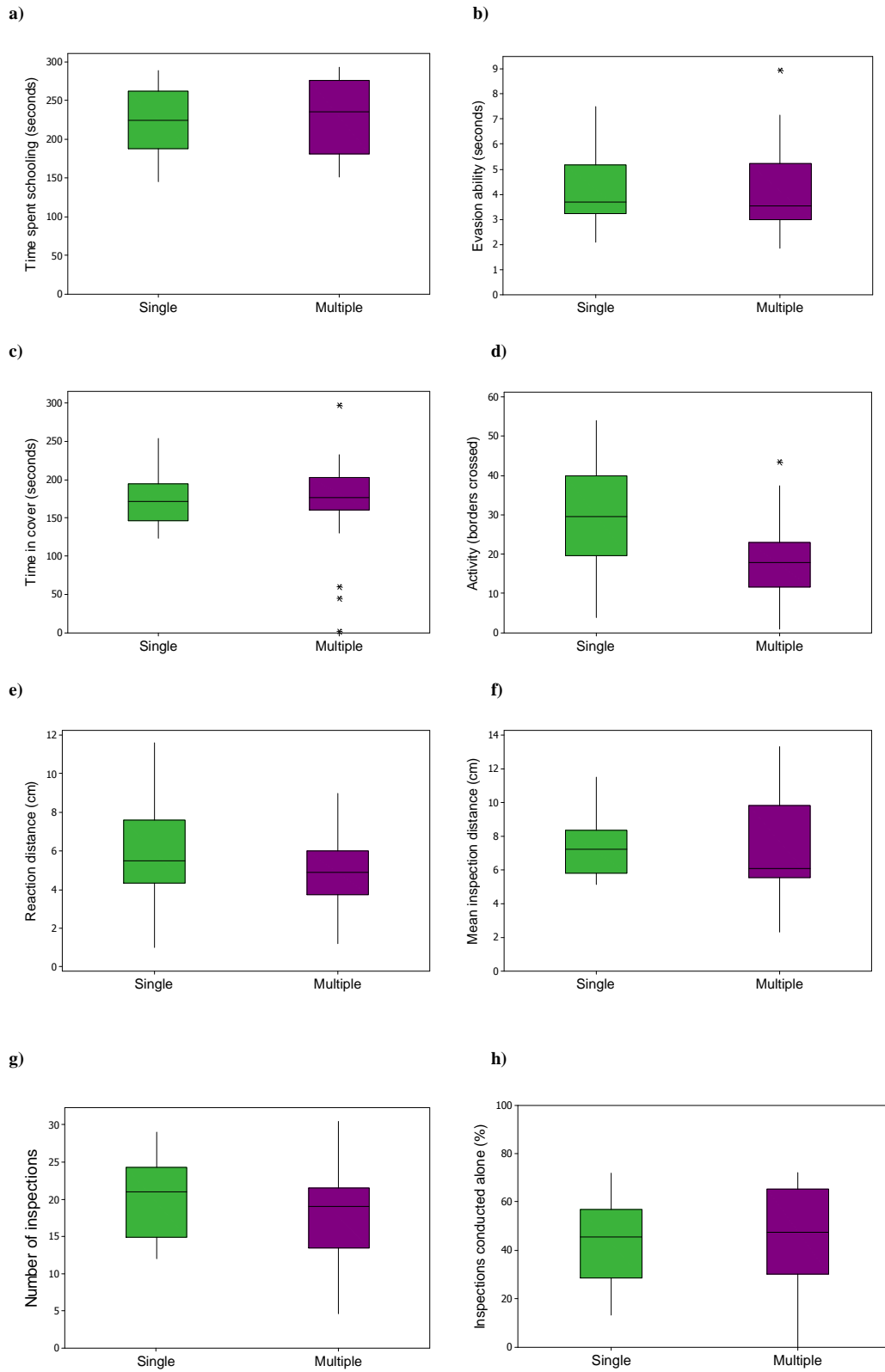
Figure 4.10: Behaviours quantified in newborns from mesocosm populations founded by either singly mated (single) or multiply mated (multiple) females. Medians, interquartile ranges and outliers are shown.

Table 4.1: MANOVA analysis of newborn behavioural performance with condition (single or multiply mated female founded populations).

Multivariate tests	Wilks' λ	Df		F	p
Condition (single or multiple)	0.698	8, 28		1.517	0.196
Between-subjects tests	**SS**	**Df**	**MS**	**F**	**p**
Condition (single or multiple)					
schooling	67.707	1	67.707	0.035	0.853
evasion ability	2.753	1	2.753	1.445	0.237
time in cover	9.884	1	9.884	0.005	0.947
activity	1106.139	1	1106.139	7.598	0.009**
reaction distance	2.865	1	2.865	0.728	0.399
mean inspection distance	0.088	1	0.088	0.018	0.895
inspection frequency	33.141	1	33.141	1.082	0.305
% inspections alone	235.361	1	235.361	0.836	0.367
Error terms					
schooling	68223.616	35	1949.246		
evasion ability	66.674	35	1.905		
time in cover	75868.571	35	2167.673		
activity	5095.301	35	145.580		
reaction distance	137.706	35	3.934		
mean inspection distance	174.754	35	4.993		
inspection frequency	1071.942	35	20.627		
% inspections alone	9850.451	35	281.441		
Total					
schooling	2033826.300	37			
evasion ability	811.253	37			
time in cover	1287814.099	37			
activity	29106.983	37			
reaction distance	1182.704	37			
mean inspection distance	2137.528	37			
inspection frequency	14585.868	37			
% inspections alone	2137.528	37			

**significant at the 1% level.

Principal component analysis (PCA)

A PCA extracted four principal components. PC1 explained 21% of the variance and was negatively correlated with activity (-0.831) whilst positively correlated with evasion ability (0.775). PC2 explained a further 20% and was positively correlated with schooling (0.754) and time in cover (0.537), whilst negatively associated with % of inspections conducted alone (-0.611). PC3 explained another 16% and was positively associated with reaction distance (0.712) and negatively associated with inspection frequency (-0.605). PC4 explained 15% of the total variance and was positively associated with time in cover (0.634).
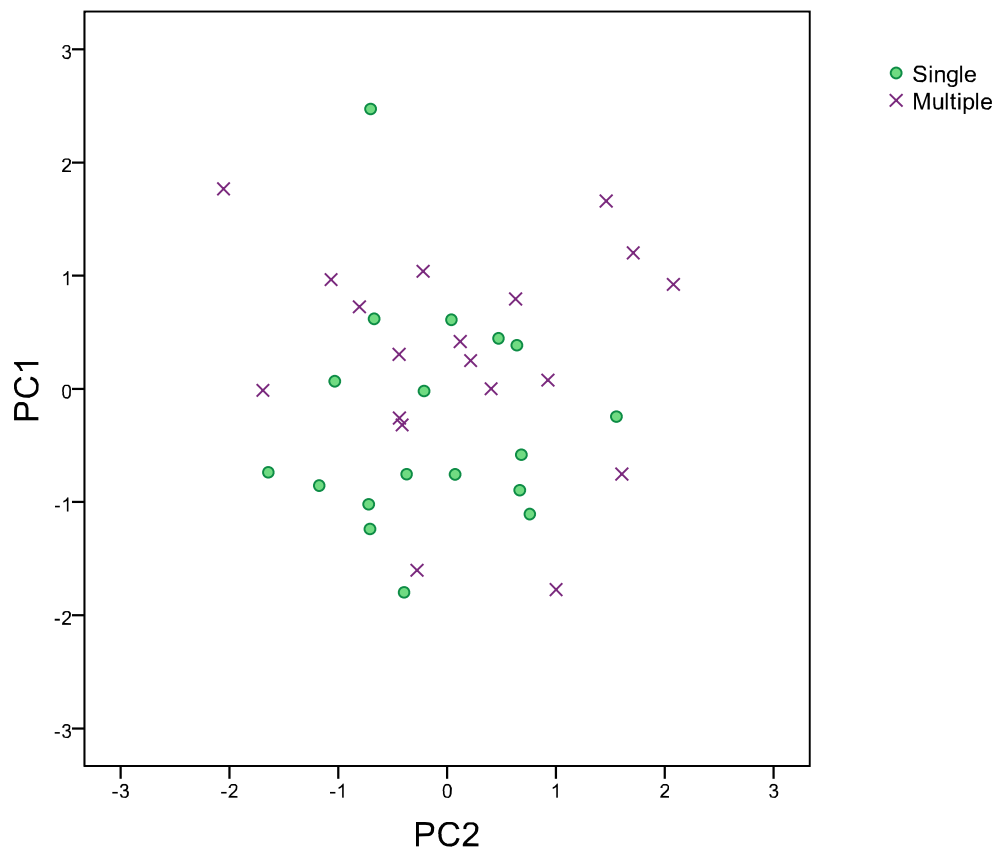
Figure 4.11: Plot of the first two behavioural performance data principal components.

There was a marginally significant difference in PC1 values between the two conditions, (t=-1.835; df=35; p=0.075) which is reflected in the spread of data along the y-axis of the multivariate plot (Figure 4.11).

4.3.5. Courtship vigour

A MANOVA revealed no significant differences in courtship vigour between the two conditions ($F_{3,30}$=1.884; p=0.154) (Figure 4.12; Table 4.2).

a)

Time spent following (secs)

Single   Multiple

b)

Number of sigmoid displays

Single   Multiple

c)

Number of thrusts

Single   Multiple
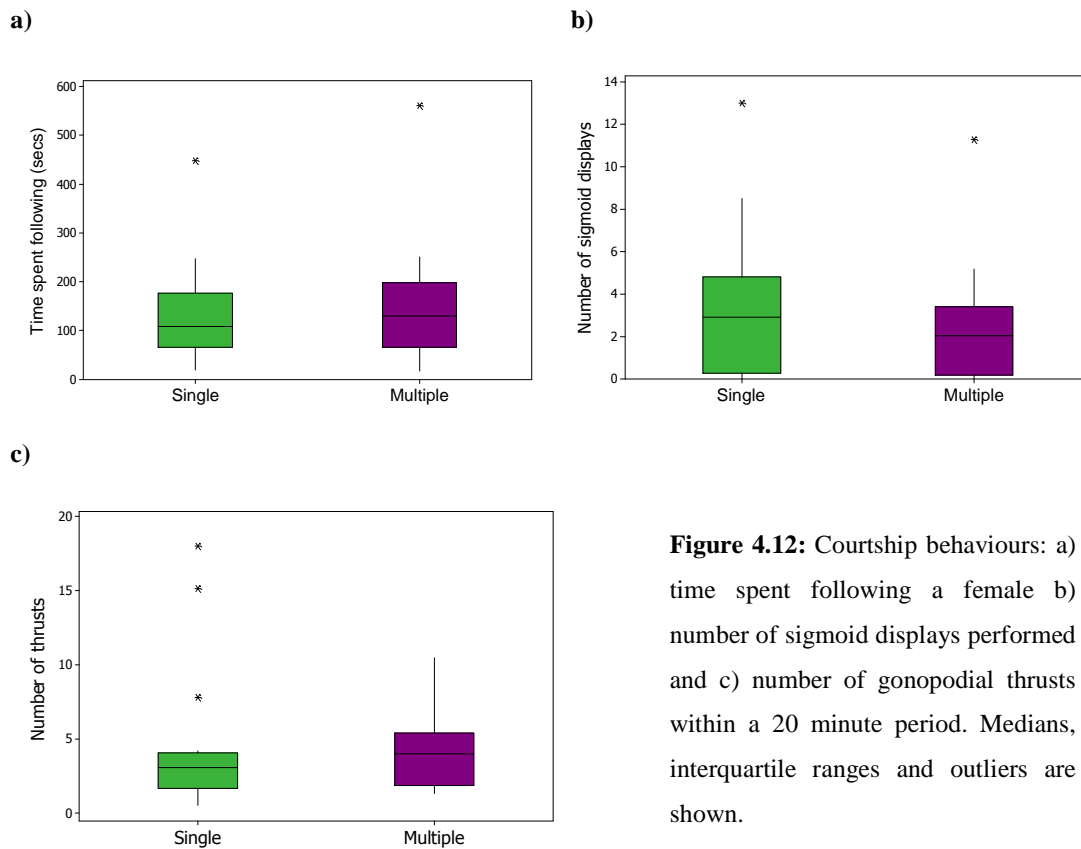
Figure 4.12: Courtship behaviours: a) time spent following a female b) number of sigmoid displays performed and c) number of gonopodial thrusts within a 20 minute period. Medians, interquartile ranges and outliers are shown.

Table 4.2: MANOVA analysis of courtship vigour, with condition (founded by single or multiply mated females).

Multivariate tests		Wilks' λ	Df		F	p
Condition (single or multiple)		0.841	3, 30		1.884	0.154
Between-subjects tests		SS	Df	MS	F	p
Condition (single or multiple)						
	following	2636.374	1	2636.374	0.200	0.658
	sigmoids	6.989	1	6.989	0.686	0.414
	thrusts	1.682	1	1.682	0.111	0.741
Error terms						
	following	422216.710	32	13194.272		
	sigmoids	326.173	32	10.193		
	thrusts	484.699	32	15.147		
Total						
	following	1128691.983	34			
	sigmoids	612.232	34			
	thrusts	1090.770	34			

4.3.6. Behavioural variation

A MANOVA revealed no significant difference in the per mesocosm coefficient of variation between the two conditions for any of the newborn antipredator behaviours (Wilks' λ=0.814; $F_{4,7}$=0.399; p=0.804), newborn lengths (t=1.369; df=9; p=0.204) or courtship behaviours (Wilks' λ=0.989; $F_{3,17}$=0.064; p=0.978) (Figure 4.13; Appendix VIII).

a)
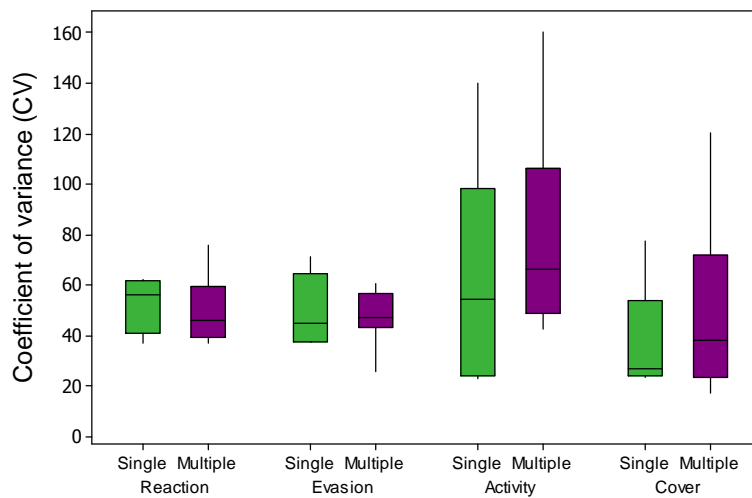
b)
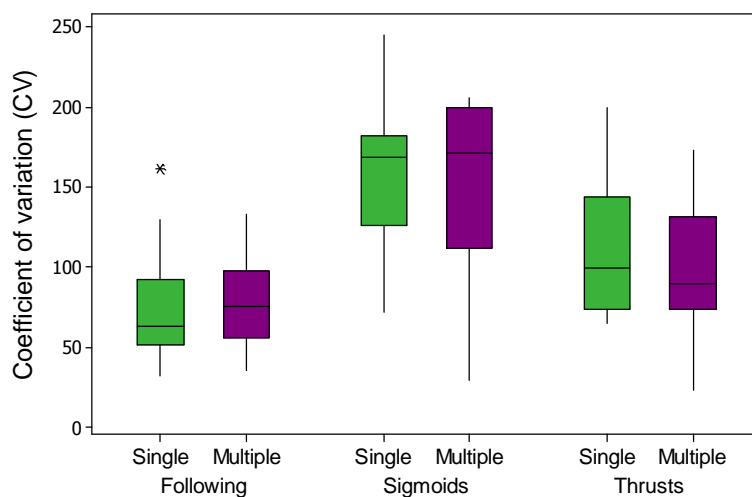
Figure 4.13: Coefficient of variation values for each of a) the newborn behaviours: evasion ability, activity, time in cover and reaction distance, and b) the three measures of courtship vigour; time spent following females, number of sigmoid displays and number of gonopodial thrusts within each trial.

4.3.7. Results summary

Table 4.3: Results summary

	Populations founded by singly or multiply mated females
Establishment probability	No difference
Size of first brood	No difference
Gestation time	No difference
Population size	No difference
Population structure	No difference
Male colour pattern diversity	More variation in multiply mated tanks
Newborn performance	Higher activity levels in single-mated newborns; no difference in any other behaviours.
Variance in newborn performance	No difference
Male courtship vigour	No difference
Variance in courtship vigour	No difference

4.4. Discussion

At 88%, establishment success was equally high in mesocosms founded by singly and multiply mated females. There was no difference in gestation time or size of the first brood between the two conditions, or in population size after 12 months. The age-structure of the mesocosm populations did not differ significantly between conditions and there was no difference in size of newborns.

Overall, there was no significant difference in behavioural performance of newborn fish between the two conditions. The exception was activity levels, which were significantly greater in newborns of descendents of the singly-mated fish. Bleakley *et al*. (2006) found that extremely inbred ornamental strains of guppies also retained antipredator behavioural responses.

Previous studies have indicated that between 1 to 3 generations of full-sibling inbreeding can be enough to detect reductions in male courtship vigour in the guppy (Mariette *et al.,* 2006; van Oosterhaut *et al*., 2003), specifically in terms of frequency of sigmoid displays and time spent following females. Similar reductions in courtship vigour have also been documented in other species of poeciliid (Ala-Honkola *et al*., 2009). I tested males in mesocosm populations that had been established for 12 months (1-3 generations) and

found that descendents of singly-mated and multiply-mated females courted with equal vigour.

Colour pattern complementarity analysis suggests that the tanks founded by multiply mated females did indeed contain more genetic heterogeneity than the single-mated tanks. This difference was not detectable in the within-mesocosm behavioural variation, which did not differ significantly between the two conditions. I might have expected that monandry would lead to reduced variation in behavioural characteristics, particularly in newborn antipredator behaviours as these have been demonstrated to have a strong inherited component (Magurran & Seghers, 1990*b*; Seghers, 1974; O'Steen *et al.,* 2002 Seghers & Magurran, 1995). This has been postulated as one of the potential advantages of a polyandrous strategy in an introduction situation (Lee, 2002), as greater within brood variation in novel or changing environments would maximise the variation on which natural selection could act. However, this study found no evidence to support such a hypothesis. Instead it seems that females are equally good at colonising, persisting and producing viable offspring regardless of whether they have had the opportunity to be polyandrous.

This study demonstrates that, at least in the initial establishment phase of the invasion process, a polyandrous strategy is not necessary for successful colonisation and maintaining behavioural viability. Indeed, Lindholm *et al*. (2005) found no evidence to support the idea that high neutral genetic diversity leads to invasive success in introduced guppies in Australia; genetic analysis revealed severe bottleneck events and low diversity even in highly successful invasive populations. They propose that rapid growth and wide environmental tolerances, and selection for the genes that underlie such life history traits are more important to invasive success (Lindholm *et al*., 2005).

The ability to produce many offspring in a short time and therefore rapidly increase the size of small founding populations is often the key to minimising the loss of genetic variation in an introduced population (Magurran *et al*., 1995; Nei *et al*., 1975) and this may be more important than the strategy of multiple mating itself. Indeed, Nei *et al*.,

(1975) mathematically modelled colonisation events and found that the amount of heterozygosity lost during a population bottleneck is likely to be more dependent on the rate of population growth immediately afterwards than the size of the bottleneck itself, a finding supported empirically by a mesocosm study of *Gambusia holbrooki* (Leberg, 1992). The ability of the guppy to give birth to broods of up to 30 precocious young once a month throughout the year (Alkins-Koo, 2000; Reznick *et al.*, 2001) and the short generation time of just 2 – 3 months from birth to sexual maturity (Magurran, 2005) all encourage a rapid rate of population growth. However, this does not necessarily apply to allelic diversity which is vulnerable to a reduction during a severe bottleneck regardless of the rate of population growth (Nei *et al.*, 1975). Ideas about how these questions could be pursued are outlined in Box 4.2.

Many studies suggest that propagule pressure is the most critical factor in determining invasive success (Kolar & Lodge, 2001; Allendorf & Lundquist, 2003). In the case of the guppy this is potentially high both due to rapid population growth immediately following introduction, and also in many situations due to multiple introductions (Lindholm *et al.* 2005). The latter may also dramatically increase the genetic and phenotypic variation in the population (Kolbe *et al.*, 2004).

In conclusion, polyandry does not appear to be the key to colonisation success in the guppy, as even when founders are restricted to one male and one female, resulting populations thrive. Instead it seems likely that rapid initial population growth is critical as it may both help minimize loss of genetic diversity as well as increasing propagule pressure early on in the invasion process. Further work is required to distinguish between the relative contributions of these two potentially important benefits in this and other invasive species.

Box 4.2: Proposed methods for testing the hypothesis that rapid population growth soon after introduction increases establishment success and viability of an introduced population.

There are two main approaches available: theoretical (e.g. Nei *et al.*, 1975) and experimental (e.g. Leberg, 1992).

Theoretical: This would involve mathematical modelling of hypothetical scenarios. Population growth rate could be varied, to account for differences in reproductive output and survival. Number of founders could also be varied between 2 and 5. It would then be possible to model how these two factors affect heterozygosity after a set number of generations. This could be carried out by expanding on the equations laid out in papers such as Nei *et al.* (1975) or perhaps by using or adapting a population viability analysis computer model such as Vortex (Lacy *et al.*, 1993).

Empirical: Mesocosms founded by singly and multiply mated female guppies could be set up in the same manner as for the study described in this chapter. However, half of the tanks in each condition could have their population growth rate limited during the first 6 months by the systematic removal of sexually immature juveniles. After 6 months, the population limitation procedure is stopped and the tanks left to their own devices for a further 12 months. At the end of this time period, establishment success, behavioural viability and genetic diversity can be assessed in each condition, and the relative contributions of rapid initial population expansion and polyandry can be examined.

Chapter 5

The effect of conspecifics on foraging behaviour in the guppy: prey switching?

Abstract

Prey switching describes when a predator feeds disproportionately on the most abundant prey in a two-prey system, switching when the alternate prey becomes more abundant. This behaviour can be viewed as an application of optimal foraging theory, enabling the most efficient exploitation of food resources. The concept has been applied to biological control scenarios and it has been suggested that prey-switching may lead to effective stabilisation of mosquito larvae populations by generalist predators such as the guppy. Classic ecological studies have demonstrated prey switching in the guppy, but such studies have been laboratory based and severely lacking in ecological validity. In particular, they fail to consider the potential effect of the presence of conspecifics on foraging behaviour in this highly social species. I addressed this by testing the hypothesis that the presence of conspecifics has an effect on foraging choices in female guppies. The relative abundance of two prey types, *Daphnia* and *Tubifex*, available to each fish within trials was shifted over an 8 day period, and the number of each prey type consumed was recorded. Focal fish were either foraging alone, in the presence of two males or in the presence of two female conspecifics. Number of prey items consumed by the focal fish was unaffected by the presence of conspecifics, and there was a strong preference for *Tubifex* in all trials. I found little evidence for prey switching in any of the social conditions. However, the results indicate that the presence of male guppies affects the behaviour of focal females, by reducing the strength of their preference for the preferred prey. Interestingly, these effects were dependent upon which prey type was presented in greatest abundance initially, suggesting the presence of search image formation in foraging guppies.

5.1 Introduction

The guppy, *Poecilia reticulata*, has been introduced to water bodies in many tropical countries as a biological control agent of mosquitoes (Chapter 2), often in keeping with government policy (Dash, 2009). Despite this widespread practice, the success of such introductions is largely undocumented and the few reports that are available present conflicting results (Ghosh *et al*., 2005; Seng *et al*., 2008; Courtenay & Meffe, 1989).

The introduction of guppies for mosquito control is based on the assumption that they will consume sufficient numbers of water-borne larvae to substantially reduce mosquito populations, and ultimately reduce the incidence of malaria in humans. However, there is surprisingly little literature concerning guppy feeding behaviour, and even less relating specifically to their use in biological control. One of the few studies with relevance to biological control was conducted by Murdoch *et al*. (1975). Murdoch and colleagues presented evidence for the existence of 'prey-switching' in guppies; a behaviour defined as consuming more than is proportional of the most abundant prey in a multi-prey system –switching to an alternative prey type if this becomes most abundant (Murdoch, 1969) (Figure 5.1). Switching behaviour can be viewed as an application of optimal foraging theory, and as such is a means by which an organism might most efficiently exploit the food resources in a habitat (Ringler, 1985). By employing switching behaviour, guppies may be more likely to control invertebrate prey populations and thus be deemed a suitable biocontrol agent (Murdoch & Oaten, 1975).
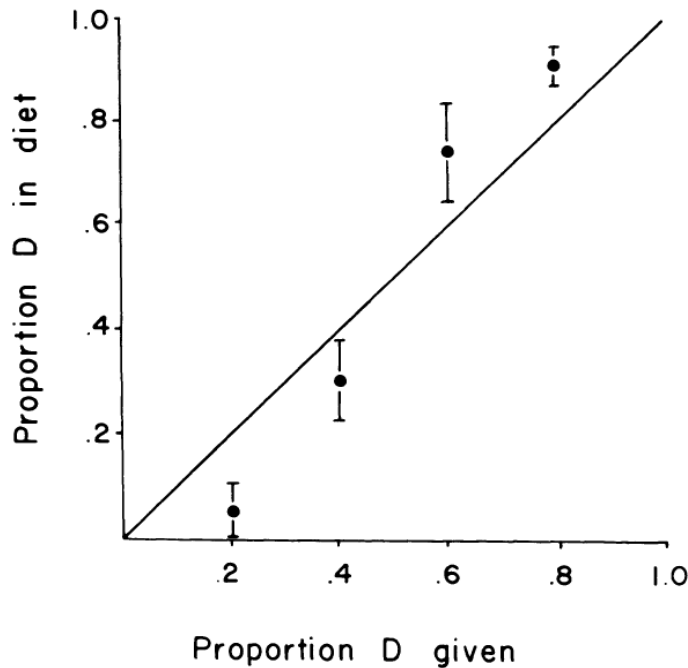
Figure 5.1: A classic case of prey switching. Assuming no prey preference in the predator, the line shows expected consumption if individuals were consuming prey 'D' in proportion with availability. In the case of switching, when one prey type is rare, foragers consume less than would be predicted, and when that prey type becomes the more abundant, they tend to consume more than would be predicted. Taken from Murdoch *et al.*, (1975).

However, the ecological validity of findings from simple laboratory experiments is questionable, particularly given the social nature of guppy populations; shoaling behaviour can encourage intraspecific competition (Bertram, 1978), and persistent sneaky mating and courtship behaviours displayed by male guppies have been shown to be costly to harassed females by reducing their foraging time and consequently their fecundity (Magurran & Seghers, 1994 *a, b*). Recently, Darden & Croft (2008) demonstrated that female habitat preference is affected by the presence of males, with females preferring shallow, safer areas when in an all-female environment and only venturing into deeper, high-predation risk areas when the shallower habitat is occupied by males. This suggests that the presence of conspecifics could also have an important bearing on foraging behaviours such as prey-switching, particularly when two prey patches are spatially distinct.

It is important to improve our knowledge of guppy foraging behaviour given that this species is now established in at least 70 countries outside its native range of Trinidad and north-eastern South America. In approximately 60% of these countries, the presence of guppies is wholly or partly due to introduction for mosquito control (Chapter 2), yet there is growing evidence that introduced guppies have a negative impact on native species and invaded ecosystems (Chapter 2 of this thesis; Courtenay & Meffe, 1989; Valero *et al.*, 2008). Until more is known about the foraging behaviour of the guppy, we cannot evaluate their efficacy as mosquito control agents and therefore cannot justify their introduction to natural habitats for this purpose (Simberloff & Stiling, 1996).

This study aims to investigate whether previous findings relating to guppy foraging behaviour can be applied to more naturalistic scenarios such as that of a mosquito control introduction; firstly by testing for the existence of 'switching' behaviour in female guppies, and secondly by exploring whether their foraging behaviour is affected by the presence of male or female conspecifics.

I tested the following hypotheses:

1) Female guppies should display switching behaviour when presented with two prey types in shifting relative abundances.

2) The presence of conspecifics should alter the foraging behaviour of female guppies.

3) The effect of male conspecifics on foraging behaviour of females should differ from that of females.

5.2 Methods

5.2.1. Experimental methods

This study took place in the tropical aquarium at the University of St Andrews during August and September 2008. Twelve tanks (45 x 23 x 23 cm) were fitted with a filter, gravel and a black plastic lid and filled to a depth of 20 cm with dechlorinated water. Two holes were cut in the lid, one on either side of the front of the tank. Lengths of transparent plastic tubing (8 cm long with an internal diameter of 12 mm), were positioned in these holes so that part of the end of the tube was submerged. Funnels were fitted to the tops of the tubes (see Figure 5.2 for basic tank set up).

A focal female guppy was introduced into each tank. A third of the tanks were allocated to each of three conditions: 1) a single focal female, 2) a focal female with two male guppies, and 3) a focal female with two smaller females. Laboratory stock fish were used, all of which were descendents of fish from the lower section of the Tacarigua River in Northern Trinidad. Focal females were healthy adults measuring between 25-30 mm (total length). Fish were introduced a few days before the start of the experiment and during this time were fed food granules via both feeding tubes so as to encourage an association between the exit of the tube and food.

The two prey types used were *Daphnia* and *Tubifex* worms. These were selected because they are similar in size, available in large numbers from aquatic suppliers and both readily consumed by guppies.

The fish were presented with two different live prey types at ratios that shifted over an eight day period. As in Murdoch *et al.* (1975), four different ratios were used; 4:1, 3:2, 2:3, 1:4 and the fish were fed at each specified ratio for two days. Half of the guppies were presented with a ratio shifting from abundant *Daphnia* prey to abundant *Tubifex* prey over an eight day period, and half subject to a shift in the opposite direction (Figure 5.2). A total of approximately ten prey items were in the water column around the two feeding tubes at any one time, present in the correct proportions and maintained by

adding more of each prey type as it was consumed or drifted away to other regions of the tank. Prey of both types were pre-counted and loaded into many small syringes for easy replenishment of prey patches during each trial. The number and type of each prey item consumed was recorded using a hands-free digital voice recorder (Olympus VN1100PC), so that the experimenter could continue to replenish prey whilst recording data.
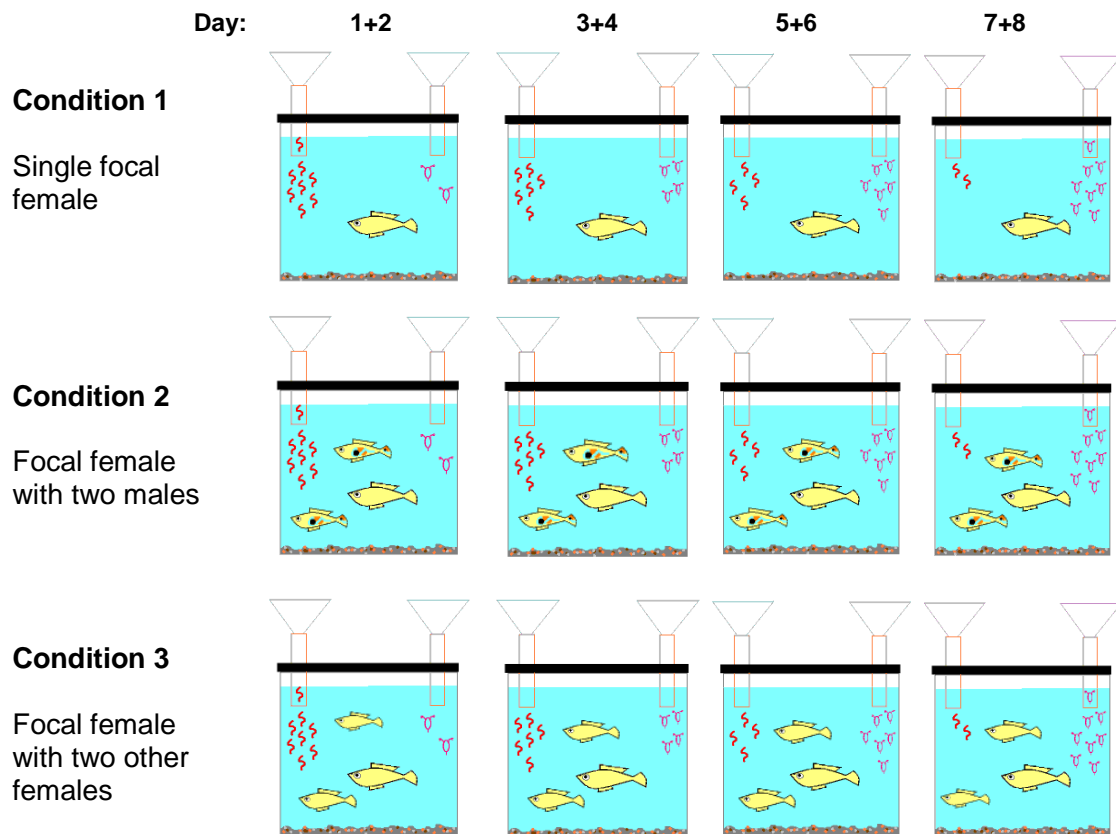
Figure 5.2: Experimental set up showing shift in prey abundances over each set of 8 day trials. Half of the fish in each set of trials experienced the shift from 4:1 to 1:4 *Tubifex*:*Daphnia*, and the other half experienced the shift in the opposite direction.

A repeated measures design was employed: at the end of the first 8 day period, the experiment was repeated, but with each focal female now experiencing a different social condition, and then again, so that by the end of the experiment each female had been tested under all three social conditions (Figure 5.3).
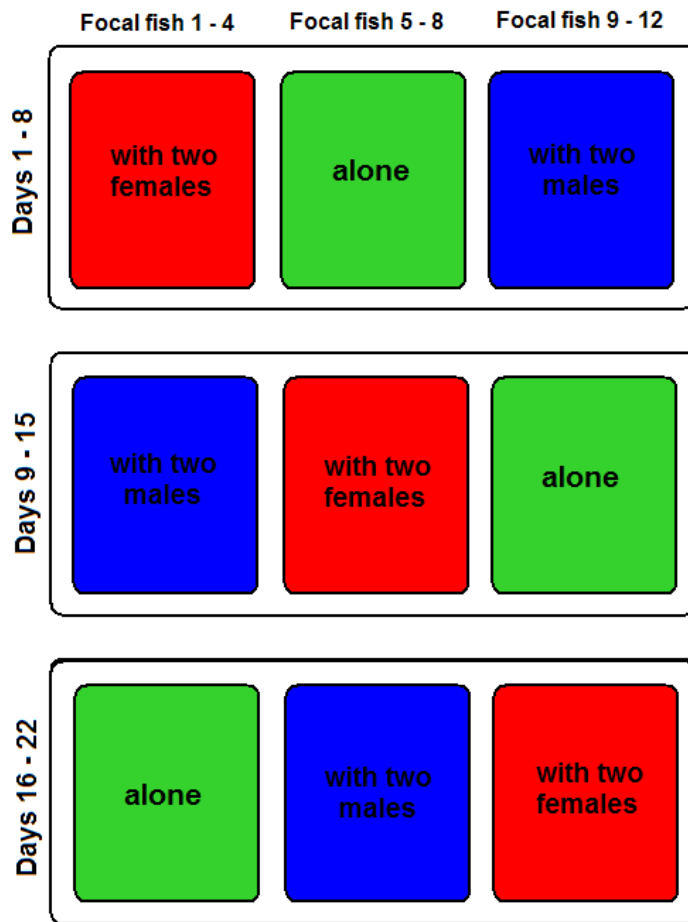
Figure 5.3: Experimental design. All focal females experienced each of the three social conditions.

5.2.2. Statistical methods

Proportion of Daphnia consumed

A repeated measures ANOVA was used to examine the effect of social condition and prey ratio on P_D (*Daphnia* eaten/total prey eaten=proportion of *Daphnia* consumed in a trial). P_D was calculated using the mean number of prey for each pair of days for each fish in each social condition (i.e. days 1+2, 3+4, 5+6, 7+8). Analyses used SPSS version 17.0.

Preference parameter

In order to establish the strength of prey preference in the focal guppies and to compare preference between prey ratios, social conditions and individual fish, a preference parameter was calculated as follows (based on the methods of Murdoch *et al.*, 1975):

I used N_D to denote the number of *Daphnia* consumed, and N_T to denote the number of *Tubifex* consumed. H_D and H_T denote the proportion of *Daphnia* and *Tubifex* presented, respectively. The total N_D, N_T, H_D and H_T were calculated for any given set of trials (e.g. subset by condition, individual fish or ratio).

The preference parameter c_D was calculated by applying the following equation to the totals:

$$c_D = (N_D / N_T)/(H_D / H_T)$$
                                                                   (Equation 5.1).

A value of c_D =1, indicates no directional preference, a value greater than 1 indicates a preference for *Daphnia*, and a value below 1 indicates a preference for *Tubifex*.

Box 5.1: Worked example of prey preference parameter calculations.

For example, if on Day 1, three fish are presented with *Daphnia*:*Tubifex* in a ratio of 4:1:

H_D=0.8

H_T=0.2

Fish one consumes 16 *Daphnia* and 4 *Tubifex* within the trial:

N_D=16

N_T=4

c_D=(16/4)/(0.8/0.2)=1

Confirming no preference for either prey; they are being consumed in proportion with their availability.

Fish two consumes 28 *Daphnia* and 19 *Tubifex*:

N_D=28

N_T=19

c_D=(28/19)/(0.8/0.2)=0.375
Indicating a preference for *Tubifex*.

Finally, fish three consumes 20 *Daphnia* and 3 *Tubifex*:
N_D=20

N_T=3

c_D=(20/3)/(0.8/0.2)=1.67
Indicating a preference for *Daphnia*.

Using the summed totals rather than calculating c for each trial and then finding the mean avoided the problem of zeros – i.e. where a fish consumed one of the prey types exclusively during a trial. However, it did not allow the construction of 95% confidence intervals and subsequent statistical analysis. Mean values and confidence intervals were therefore constructed by bootstrapping the data 1000 times using the 'boot' package in 'R' (version 2.4.0). A general preference parameter was calculated for the entire data set, and separate values of c were calculated for relevant subsets of the data i.e. when the data were aggregated according to individual fish, social condition, direction, prey ratio or a combination of these.

Preference parameters c_D were calculated again, this time when data were aggregated by social condition, direction and prey ratio.

The proportion of Daphnia eaten, P_D, was predicted for each subset of the data using the bootstrapped preference parameters c and the following equation:

$$_{pred}P_D = (c_D * H_D)/((1 - H_D) + (c_D * H_D))$$
(Equation 5.2).

A null model was calculated by applying the same methods to data subset only by direction, not by social condition or prey ratio. This means that deviations from the null model indicate an effect of social condition or prey ratio on foraging behaviour. Data were subset by direction as initial results indicated that it had an effect on foraging behaviour.

Prey switching

Switching behaviour can be detected if c is an increasing function of proportion of prey presented (H_D/H_T). Increasing values of c_D as *Daphnia* becomes the more abundant prey would indicate the presence of switching behaviour (Murdoch, 1969).

Those trials in which no prey of either type was consumed were omitted from all analyses.

5.3 Results

Focal fish consumed significantly more *Tubifex* (mean of 75 ± 5 SE per set of trials) than *Daphnia* (39 ± 3 SE) overall ($F_{1,66}$=33.810; p<0.001) (Figure 5.4). There was no significant difference in the total number of prey items consumed between the three social conditions ($F_{2,66}$=0.486; p=0.617). There was no significant interaction between prey type and social condition ($F_{2,64}$=1.857; p=0.164).
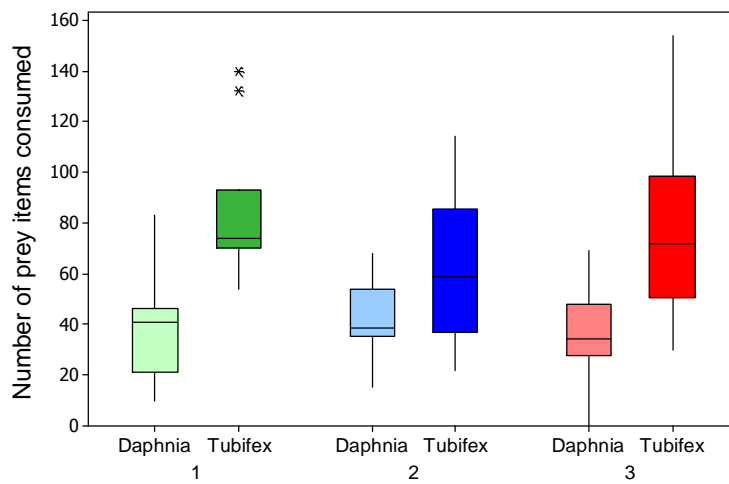
Figure 5.4: Total number of prey of each type (*Daphnia* and *Tubifex*) eaten during each 8 day set of trials for each of the three social conditions: 1 = focal female alone (green); 2 = focal female with two males (blue); 3 = focal female with two other females (red). Medians, interquartile ranges and outliers are shown.
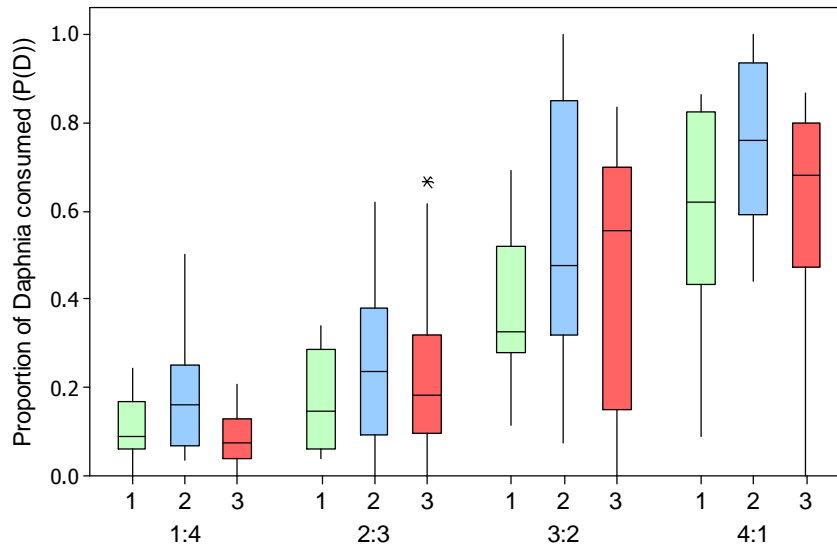
Figure 5.5: Proportion of *Daphnia* consumed (as a proportion of total prey consumed=P_D), at different ratios of prey, showing differences between conditions. 1=single female; 2=with two males; 3=with two females. Medians, interquartile ranges and outliers are shown.

There was a significant effect of both prey ratio ($F_{3,123}$=43.007; p<0.001) and social condition ($F_{2,123}$=5.037; p=0.008) on mean proportion of *Daphnia* consumed (P_D) (Figure 5.5). There was no significant interaction between these factors ($F_{6,117}$=0.498; p=0.809) and no significant effect of individual fish ($F_{11,123}$=1.412; p=0.175). Pairwise analysis of the ratios revealed that the differences were significant in all comparisons with the exception of the two low *Daphnia* ratios (Tukey's pairwise p<0.010 for all comparisons except 1:4 and 2:3 where p=0.202). Pairwise analysis of social condition revealed that condition two differed significantly from both of the other conditions (p<0.050 in both cases), which did not differ significantly from each other (p=0.962).

Prey preference parameter analyses

The overall preference parameter c_D for all data was 0.33 (bootstrapped CIs: upper=0.36; lower=0.29) confirming a general preference for *Tubifex* worms.

All fish have a mean preference parameter of c_D <1, and most have upper 95% CIs lying below 0.5 which is strong evidence for a preference for *Tubifex* in most cases. Despite

substantial variation in the mean estimated values of c_D both within and between individual fish, in almost all cases the CIs overlap (Figure 5.6). The only exception is between fish 9 and 11, where fish 9 appears to have a stronger preference for *Tubifex*.
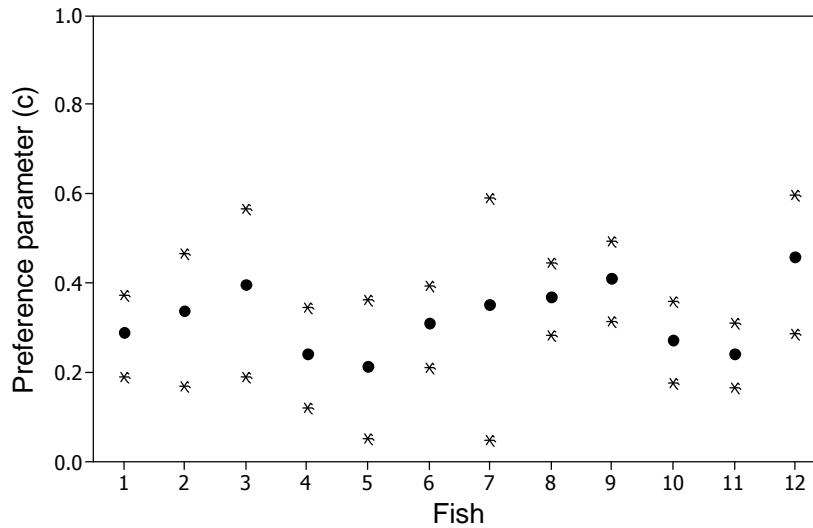
Figure 5.6: Bootstrapped preference parameter estimates for each fish (N=24 for all fish except fish 7 where N=16) with 95% confidence intervals.

In sets of trials where *Daphnia* was presented in highest abundance initially, the focal fish in the condition with two male companions showed a higher preference for *Daphnia* than in other conditions (Figure 5.7). In all cases the overall preference parameter was $c_D<1$, indicating an overall preference for *Tubifex* in all conditions.
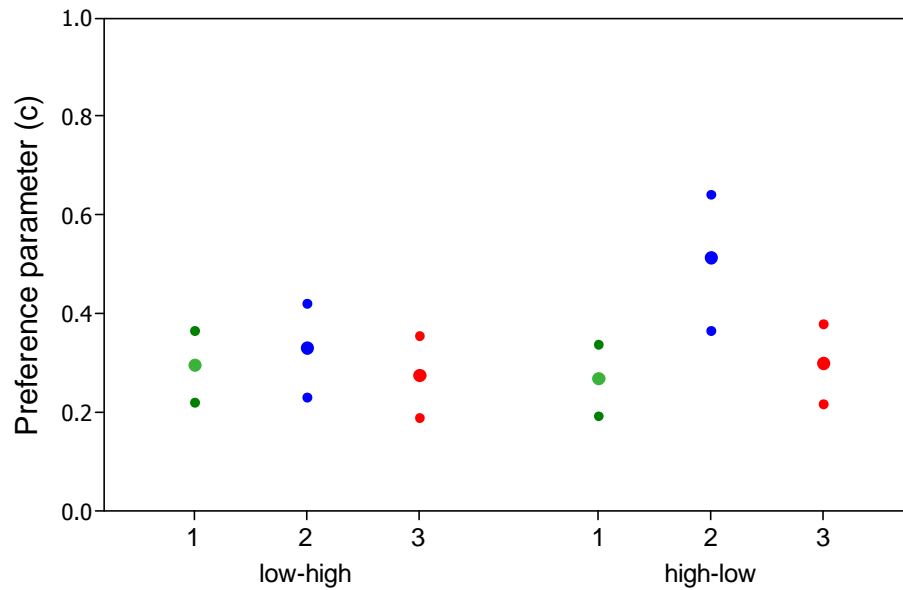
Figure 5.7: Preference parameters and percentile 95% confidence intervals for each condition when calculated for those trials in which *Daphnia* was presented shifting from low to high abundances and from high to low abundances.

When preference parameters are calculated according to social condition, prey ratio and direction, condition 2 trials indicate a higher preference for *Daphnia* when *Daphnia* has been presented as the most abundant prey at the start of the set of trials (Figure 5.8). However, there is a great deal of variation and the only instance where the CIs do not overlap between conditions is at the 2:3 ratio in the high-low condition where it seems the 'two male' condition fish display a stronger preference for *Daphnia* than the single females. The only cases where confidence intervals overlap with *c*>1 (i.e. overall preference is for *Daphnia*, rather than *Tubifex*) are for the females in the two-male condition at high relative abundance of *Daphnia* (4:1 and 3:2).
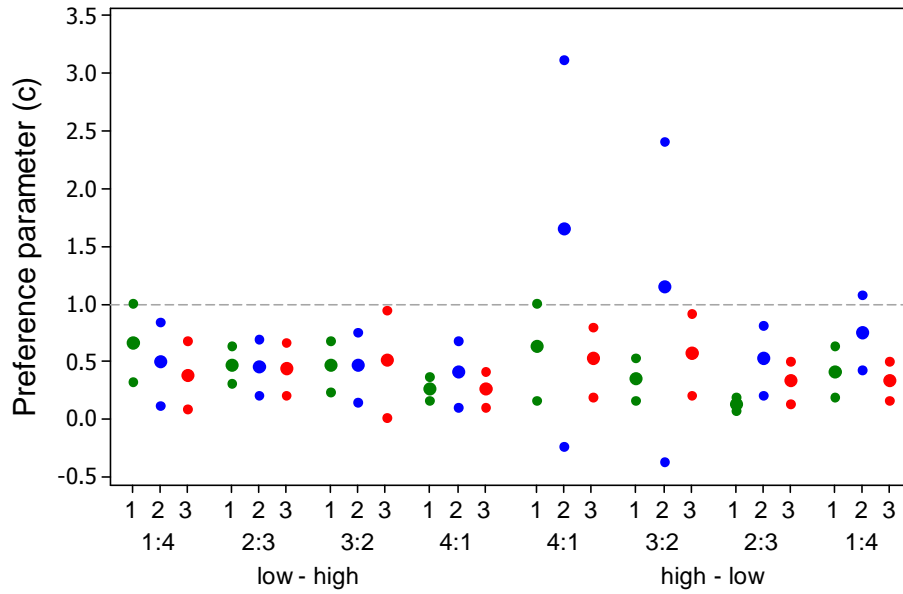
Figure 5.8: Bootstrapped preference parameters with CIs when direction, ratio and condition are all taken into account. The grey dotted line represents equal preference for *Daphnia* and *Tubifex*; values below indicate an overall preference for *Tubifex*, and those above represent an overall preference for *Daphnia*.

Prey switching

Preference parameter c_D was not a function of changing prey ratio (i.e. increasing H_D/H_T). There was an increase in mean c_D for condition two, high-low, but the confidence intervals all overlapped (Figure 5.8).

The graphs below show the model's predictions for proportion eaten ($_{pred}P_D$) in the three conditions and the null model over the four prey ratios and in the two directions, given the preference parameters calculated as above (Figure 5.9).

a)
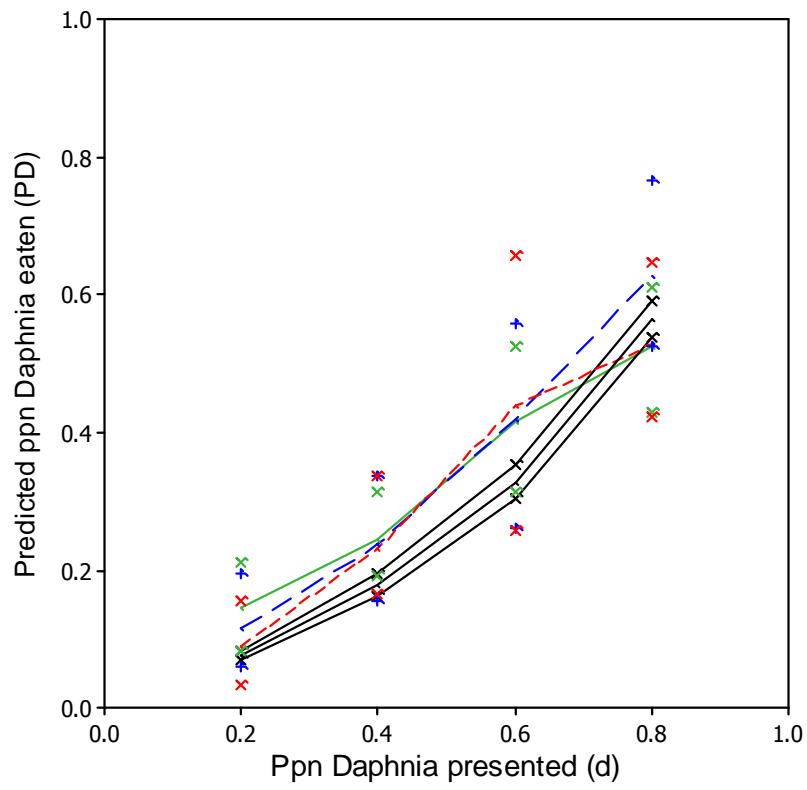
b)
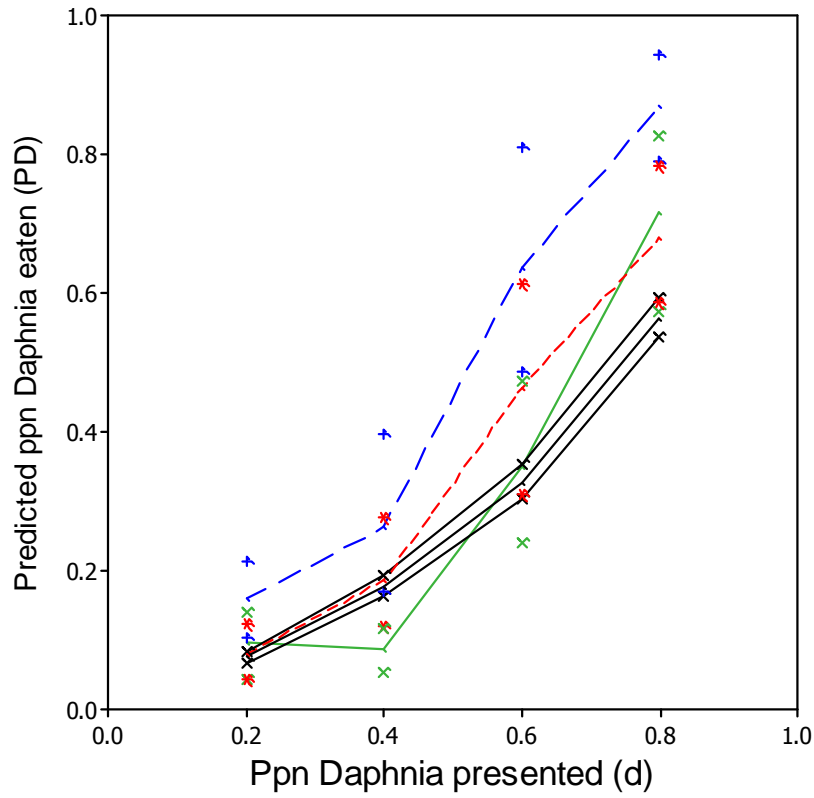
Figure 5.9: Predicted proportions ($_{pred}P_D$) and bootstrapped percentile 95% confidence intervals for *Daphnia* consumed, generated by model in which ***c*** was calculated for each social condition and prey ratio a) when calculated for those trials in which *Daphnia* was presented shifting from low to high abundances and b) from high to low abundances. Colour key: Green = Condition 1, single female; Blue = Condition 2, focal female with two males; Red = Condition 3, focal female with two additional females; Black = null model (the middle line is the mean prediction, and the outer lines represent the 95% CIs).

When trials began with *Daphnia* presented in low abundance, more Daphnia was being consumed by the single fish than was predicted by the null model when H_D=0.2. All other CIs overlapped.

When trials began with *Tubifex* presented in low abundance, the single fish consumed a lower proportion of Daphnia than the other two social conditions and the predictions of the null model, when H_D=0.4. When H_D=0.6, females in the presence of male conspecifics consumed a greater proportion of *Daphnia* than single fish and the null model predictions. Again at H_D=0.8, females with male companions consumed more *Daphnia* than predicted by the null model, and more than is consumed by focals with two female companions.

5.4 Discussion

This study did not reveal convincing evidence for the occurrence of prey switching in the guppy. Foraging behaviour in focal fish did not appear to be affected by the presence of female conspecifics, however I did find evidence that male conspecifics can reduce the strength of female preference for a preferred prey.

Fish displayed a preference for the *Tubifex* prey; the total number of worms eaten throughout all trials was almost double that of *Daphnia* (2630 *Tubifex*, 1377 *Daphnia*). Reddy and Shakuntala (1979) also found that guppies consistently preferred *Tubifex* when given a choice between *Tubifex* and *Culex* sp. mosquito larvae.

a) Did the fish display 'switching' behaviour?

Murdoch and Marks (1973) proposed that the conditions for switching to occur would include a weak and variable prey preference among individuals. Here the focal fish displayed a strong and consistent preference; there was a mean preference parameter value for *Daphnia* of below <0.5 in all cases (when 1 indicates no preference), and all but one pair of CIs overlap between individuals, indicating the consistency of the preference.

It is commonly suggested that if switching is occurring, it may be detected by examining whether c is an increasing function of the proportion of prey presented (prey ratio), because the null model would predict that preference is independent of prey ratio (Murdoch, 1969; Murdoch & Marks, 1973; Murdoch *et al*., 1975). Here, there is no obvious relationship between c and presented prey ratio in any of the social conditions (Figure 5.8). In trials where *Daphnia* was presented at high abundance initially, there were values of c_D that appeared to increase at higher abundances of *Daphnia* when there were two males present. However, there were very large CIs associated with the mean preferences and, as there were only four data points, it was not possible to test for a correlation here. The other two social conditions showed no sign of a positive relationship between preference and prey ratio, with c_D remaining more or less constant as proportion of prey presented (H_D/H_T) changes.

Similarly, switching behaviour was not detected when predictions of proportions of prey eaten were generated using bootstrapping. Here, if there was switching occurring, I might expect the model predictions and associated confidence intervals to fall below the null model predictions at the lower abundances of *Daphnia* (1:4 and 2:3), and to rise above those of the null model at higher abundances of *Daphnia* (3:2 and 4:1). Although this trend is clearly visible at the higher proportions for the fish with two males present, and some evidence for this in the lower proportions for the single fish, none of the conditions alone exhibit both of these features, which would be necessary to be confident of detecting switching behaviour. Particularly as these analyses are restricted to comparisons of confidence intervals, which in some cases will be non-overlapping by chance.

Despite the lack of evidence for switching, certain individual fish in certain sets of trials showed a classic sigmoid switching curve (Appendix IX). However, there was little consistency within condition or even within individual fish. Other studies investigating prey switching in fish since the work of Murdoch and colleagues, have also found it difficult to find such clear patterns, with individual variation often obscuring more general patterns (Ringler, 1985; Colgan & Silbert, 1984).

b) Does the presence of conspecifics affect the foraging behaviour of focal females?

Overall, there was no significant difference in the total number of prey items consumed per trial between fish experiencing the trials alone, or with male or female conspecifics. In the 'two male' condition, preference for *Tubifex* was weaker among the focal females than in the other social conditions. Darden and Croft (2008) found that female guppy spatial distribution within a habitat was affected by the presence of males. Females chose to venture into areas associated with higher predation risk only when males were present in the lower risk areas. A similar phenomenon appears to have occurred here, with females choosing to feed on a patch of non-preferred prey when males were present at the preferred patch. However, this pattern was only evident when the ratio of prey was presented beginning with high *Daphnia* abundance and shifting towards low, and not when the trials shifted from high to low *Tubifex* abundance. It seems that which prey type is initially most abundant is important to the subsequent foraging behaviour.

Possible explanations include the formation of a search image that might predispose an individual to a particular prey type (Tinbergen, 1960; Dawkins, 1971). The use of the term 'search image' has been much debated in the literature as it has been used to describe a very specific as well as a broader phenomenon (Dawkins, 1971; Lawrence & Allen, 1983; Ishii & Shimada, 2010). For the purpose of this discussion, the term is used to refer to a strategy whereby perceptual changes allow a predator to 'learn to see' a recently encountered cryptic prey type (Guilford & Dawkins, 1987).

Daphnia is considerably more cryptic a prey, and therefore predators may require learning through experience at high densities and formation of a search image for effective foraging (Warburton & Thomson, 2006). As those females who were accompanied by males and were initially exposed to high densities of *Tubifex* may not have had the opportunity to learn how to effectively detect and exploit the more cryptic *Daphnia*, they may be less prepared to switch to the alternative prey despite the benefits of escaping the male harassment and competition. There are a variety of evolutionary and optimal foraging advantages of a flexible learned prey selection mechanism for generalist

predators, such as the guppy (Cornell, 1976; Warburton & Thomson, 2006). In particular, it is a mechanism closely associated with switching behaviour, as it allows the learned preferred prey to change as relative prey abundances shift (Cornell, 1976).

Other studies have also found that experience of a prey type or habitat can affect foraging decisions in fish (Werner *et al.*, 1981; Warburton & Thomson, 2006), and that predators may not forage 'optimally' until they have had the opportunity to assess the different types of available prey (Jaeger & Rubin, 1982).

This finding suggests that the phenomenon of switching is likely to be more complex than previously described in Murdoch *et al.* (1975). The presence of conspecifics affects aspects of foraging behaviour that are in turn highly likely to affect the efficacy of prey consumption in more naturalistic situations. Furthermore, these findings also suggest it is important to consider the behavioural mechanisms behind switching, such as the possibility of search image formation or similar, and the implications these may have to the outcomes of such behaviours.

c) Conclusions and implications

The strong preference for one prey type over the other suggests that the vulnerability of mosquito larvae prey to predation by guppies would depend greatly on the availability of multiple alternative prey types in the habitat. Manna *et al*. (2008) found that the presence of chironomid larvae and tubificid worms reduced the efficiency of guppies as biocontrol agents of the mosquito *Culex quinquefasciatus,* and similar findings have been described in the use of *Gambusia affinis* for mosquito control (Blaustein, 1992).

The presence of males appears to affect decision making in foraging females, consistently weakening preference for the preferred prey. Female guppies suffer a substantial cost of sexual harassment both through short term fitness losses (Ojanguren & Magurran, 2007) and reduced feeding opportunities (Magurran & Seghers, 1994*b*). It seems likely that in this case harassment by males is causing spatial segregation of the sexes leading to the

observed shift in prey preference (Darden & Croft, 2008; Croft *et al*., 2006). This may have further implications for their effectiveness in the control of mosquito larvae.

The foraging behaviour of male and female guppies can be quite distinct (Dussault & Kramer, 1981; Magurran & Seghers, 1994 *a, b*), with females tending to consume greater numbers of prey (Elias *et al*., 1995). Therefore, depending on whether the targets of the biological control are the 'preferred' or 'non-preferred' prey in an ecosystem the effectiveness of guppies may be either improved or reduced. If the malarial vectors were the preferred prey, then this might reduce effectiveness, as females may tend to feed at an alternative prey patch to minimise male harassment. Conversely, if the vector species was not the preferred prey, male harassment could increase effectiveness by forcing females to feed more upon this species.

If this study was to be repeated it would be insightful, with the help of a video recorder or second observer, to record the feeding behaviour of the companion as well as that of the focal females – either in terms of prey items consumed or by recording the time they spend at each of the two prey patches. This would confirm whether sexual segregation is behind the change in female prey preference in the presence of males. Given the findings of this study, I would predict that male fish will spend more time at the *Tubifex* patch, and have a correspondingly stronger preference for *Tubifex*, and that females spend more time at the *Daphnia* patch when in the presence of males than in the other two conditions.

A broad diet, such as that of the guppy, can be advantageous in a biological control scenario. It means that the introduced fish are able to persist in the habitat for longer, reducing the need for frequent replenishment of stocks; even when the target prey is at low density, guppy populations can survive by consuming non-target alternatives such as zooplankton and algae. However, if there is a strong preference for a non-target prey, this flexibility might reduce the effectiveness of guppies as biological control agents, and, at the same time, lead to negative impacts on the non-pest prey communities and subsequently further up and down the food chain.

These findings emphasise that we must apply optimality theory to natural situations with caution, as often individuals will have constraints preventing them from behaving 'optimally', even in laboratory situations. The lack of evidence for classic prey switching and the strong preference for one prey over the other in this study warn that the introduction of biological control agents such as guppies will not necessarily tend towards the stabilisation of prey populations in a multi-prey system, as has been suggested by some authors (Murdoch & Oaten, 1975). The relative attractiveness of alternative prey types may have a strong bearing on the success of introductions in terms of disease control (Manna *et al*., 2008). These complexities should be borne in mind when making predictions about their behaviour in naturalistic scenarios, particularly those relating to important issues such as biological control. Chapter 6 will investigate this in a more relevant system to biological control; that of a two-prey system consisting of one potential malarial vector and another non-vector species of mosquito.

Chapter 6

The foraging behaviour of the guppy in relation to its use in the biological control of mosquito larvae
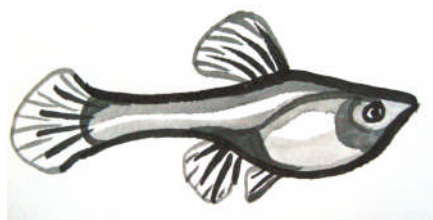
Abstract

The guppy has been introduced widely for mosquito control, yet very little is known about its foraging behaviour or efficacy in this role. This is of particular interest in situations where the fish are faced with a choice of vector and non-vector prey types. Few studies have examined guppy foraging in the presence of alternative prey, or the effects of social and physical aspects of the foraging environment. The aim of this study was to investigate the foraging behaviour of the guppy in a two-prey system under different social and physical conditions. These included foraging alone or alongside male or female conspecifics, and in the presence or absence of cover. Females ate more larvae and at a faster rate than males. Both the length of feeding bout and number of larvae eaten by females were unaffected by the presence of companions or cover. Prey preference in female guppies was affected by both the social and physical environment in which they were foraging. When feeding alone, both male and female guppies consumed more of the non-vector larvae, *Culex*. However, the extent of preference was significantly affected by both the presence of conspecifics and cover, in a non-additive manner. These results suggest that the presence of alternative prey types could have an important bearing on the effectiveness of guppies in biological control, the exact nature of which would be dependent on an interaction between both social and habitat factors.

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6.1. Introduction

The guppy has been widely introduced as a biocontrol agent throughout the tropics. In India it has been used since 1908 for mosquito control purposes (Chandra *et al.* 2008), and this rationale is behind introductions in ~60% of countries that now support non-native guppy populations (Chapter 2). Despite this, surprisingly little is known about its foraging behaviour and efficacy in relation to the control of mosquito larvae.

In the 1980s, Sabatinelli and colleagues introduced guppies to 120 basins and cisterns in a village on Grande Comore in the Western Indian Ocean. Post-introduction, they found a reduction in *Anopheles* breeding sites, bites per person per night and mosquito-borne disease in school children (Sabatinelli *et al.*, 1991). Similarly, Ghosh *et al.* (2005) found that introducing guppies into wells virtually eradicated malaria from villages in Karnataka, India. Seng *et al.*, (2008) also found that guppies reduced the dengue-carrying *Aedes aegypti* larval infestation by 79% in villages where guppies were introduced, compared to control villages. It is of note that all of the aforementioned studies involve situations where manmade containers were the main breeding ground for mosquitoes, rather than natural water bodies (Table 6.1). S. Blanchy (pers. comm.) suggests that this may be crucial in determining the success of guppies as a biocontrol tool.

Table 6.1: Published studies regarding the effectiveness of guppies as mosquito control agents.

Location	Introduced habitats	Positive findings	Negative findings	Species	Reference
Hardwar, India	Drain		Guppy inefficient as mosquito control agent	*Culex quinquefasciatus*	Dua *et al.*, 2007
Karnataka, India	Wells and troughs	Virtual eradication of malaria.		*Anopheles* sp.	Ghosh *et al.*, 2005
French Polynesia	Ponds, wells, water tanks, drums and covered wells		No reduction in biting rate	*Aedes aegypti*	Lardeux, 1992
Comoros, Indian Ocean	Ablution basins and cisterns	Reduction in larval density, biting rate and malaria infection		*Anopheles gambiae*	Sabatinelli *et al.*, 1991
Maldives, Indian Ocean	Wells	Reduction in filariasis, but not clear if linked		Anophelines and Culicines	Velimirovic & Clarke, 1975
Brazil	Laboratory conditions	Females capable of eradicating number of larvae that could appear within 24hrs under natural conditions	Males incapable of eradicating all larvae. Other species (including *Poecilia sphenops*) proved more effective.	*Aedes aegytpi*	Cavalcanti *et al.*, 2007
Sri Lanka	Riverbed pools	Cheaper and more effective than chemical 'temephos' treatment.		13 species of *Anopheles*	Kusumawathie *et al.*, 2008
Cambodia	Domestic water tanks	Successfully reduced *Aedes* infestation by 79% compared with control		*Aedes aegypti*	Seng *et al.*, 2008

There are several variables that might affect the interactions between larvivorous fish and their prey, each of which I will discuss in turn below. These include the food preferences of the fish in the presence of two or more alternative prey types, the complexity of the habitat and also the social context in which they are foraging. Furthermore, given the considerable sexual dimorphism in *Poecilia reticulata*, and documented differences in feeding niches of males and females (Magurran, 1998), there may be sex differences in other aspects of their foraging behaviour.

Prey species preferences

Guppies are omnivorous generalists, and their diet may include algae, insects, small crustaceans, tubificid worms, fish eggs and larvae, and almost anything else that happens to fall into their habitat (Dussault & Kramer, 1981; Arthington, 1989). In the laboratory, most live prey items are consumed extremely readily, but it is unclear what might happen in a multi-prey system when there is a choice of more than one attractive prey source. This has particular relevance to their use as biological control agents in India, where there

are several species of mosquito present, belonging to three distinct genera – *Anopheles*, *Aedes* and *Culex*. In the state of Karnataka (see Figure 6.2), only a subspecies within the *Anopheles* genus appears to be responsible for the vast majority of the malaria incidence (Ghosh *et al*., 2005). In this study, *Culex* and *Anopheles* larvae will be used as they are abundant in the vicinity of the research facility.

Habitat complexity effects

Most natural habitats will have some form of vegetation or equivalent cover which provides prey with refuge; this can reduce visual encounter rates of prey by a predator when compared with a non-vegetated area (Crowder & Cooper, 1982; Priyadarshana *et al*. 2001). Furthermore, different prey species may utilise cover in different ways, and many will have evolved to actively seek it out (Laegdsgaard & Johnson, 2001), therefore in a multi-prey system the extent of habitat structure might affect prey selection (Anderson, 1984).

The larvae of different genera of mosquito display differences in feeding behaviour, habitat preferences (Merrit *et al*., 1992) and escape abilities (Sih, 1986), therefore it is possible that some species might be better at utilising cover for refuge from guppy predation. Such differences could also affect the impact that guppy predators have on each species in such a scenario. The larvae of the two genera of mosquito that will be examined here each have distinct morphology, posture in the water and colouration. *Anopheles* tends to be paler, and rests horizontally immediately below the water surface. *Culex* is generally darker and rests diagonally with a pronounced siphon touching the surface (Figure 6.1).

Figure 6.1: Photograph of 3rd instar *Culex* (left) and *Anopheles* (right) mosquito larvae to illustrate the difference in their typical resting positions.

Sex differences

Sex differences in guppy foraging behaviour have often been ignored, despite considerable sexual dimorphism (Dussault & Kramer, 1981). For example, Murdoch *et al.* (1975) neglect to specify which sex they tested in their widely cited and influential 'prey switching' paper. Male guppies exhibit determinate growth; after sexual maturity, growth slows considerably or stops completely. Females, conversely, continue to grow throughout their lives, and as a result mean female size is always greater than mean male size in guppy populations (Reznick & Miles, 1989; Magurran, 1998). Wild females tend to have longer feeding bouts than males (Dussault & Kramer, 1981) and spend a greater proportion of time foraging (Magurran & Seghers, 1994 *a, b*). There is also evidence of sex differences in feeding niches; female guppies tend to prefer benthic foraging, whilst males are more often found feeding in the water column (Magurran, 1998).

Social effects

Guppies are highly social fish. Shoaling tendency is present from birth (Magurran & Seghers, 1990*b*; Chapters 3 and 4 of this thesis) and is central to predator avoidance strategies in most wild populations (Seghers, 1974). Shoaling has been shown to increase foraging efficacy (Pitcher *et al*., 1982) and facilitate social transmission of foraging information (Laland & Williams, 1997); social learning also affects both mate choice

(Dugatkin & Godin, 1992) and antipredator behaviour in the guppy (Kelley *et al.*, 2003; Kelley & Magurran, 2003). Furthermore, the presence of conspecifics is likely to encourage intraspecific competition (Bertram, 1978), and persistent sneaky mating and courtship behaviours displayed by male guppies have been shown to be costly to harassed females by reducing their foraging time (Magurran & Seghers, 1994 *a*, *b*) and fecundity (Ojanguren & Magurran, 2007). Such costs can lead to harassment avoidance strategies such as increased female boldness (Piyapong *et al.*, 2009) and habitat segregation of the sexes (Darden & Croft, 2008). It follows that foraging behaviour may be affected by the presence of conspecifics and the sex of those conspecifics.

Aims

The aim of this study was to investigate whether guppies have a preference for the larvae of *Anopheles* (a potential malarial vector) or *Culex* (non-vector) mosquitoes and, if so, whether this preference is affected by the sex of the fish, the social conditions they are foraging under and/or the presence of cover. I also aimed to find out whether sex, cover and/or the presence of conspecifics affect the efficacy of guppy foraging.

I tested the following hypotheses:

1) Guppies have a feeding preference for one of the two species of mosquito larvae prey.
2) Prey consumption and prey preference differ between males and females.
3) Prey consumption and prey preference in females is affected by the presence of conspecifics
4) Prey consumption and prey preference in females is affected by the presence of cover.

6.2. Methods

6.2.1. Experimental methods

A total of 168 guppies, *Poecilia reticulata*, were collected from tanks and drains in the state of Karnataka, India (Figure 6.2) and kept in holding aquaria until required. These fish were the descendents of guppies that were originally introduced in this region for the purpose of mosquito control (S.K. Ghosh, pers. comm.).

Figure 6.2: Map of India, showing the state of Karnataka (in yellow) where experimental guppies were collected. The red dot indicates where this study was conducted, in laboratories at the National Institute for Malaria Research field station on the outskirts of the city of Bangalore.

Trials took place in aquaria (45 x 23 x 23 cm) filled with unchlorinated water and guppies were introduced to a tank at least 3 hours, and in most cases more than 12 hours (overnight), before a trial in order to settle. Each guppy was only used once as a focal fish, but some were re-used as companion fish in other trials.

Supplies of both *Anopheles* spp. and *Culex* spp. larvae were collected from local drains and ponds and ten of each placed into glass tube prior to each trial. The larvae were selected to be approximately the same size and all were late 3rd instar or early 4th instar. Subsamples of 68 larvae of each species were photographed in petri dishes before being used in the trials, and measured using Image J software. There was no difference in mean length of selected individuals between mosquito species (*Anopheles* (mean $\pm$ SE) = 0.444 cm $\pm$ 0.006; *Culex* = 0.442 cm $\pm$ 0.008; two-sample t-test: t=0.25; df=132; p=0.803).

The study consisted of two related but separate experiments. In both, the following protocol was used. Ten larvae of each species were introduced to the tank by pouring the tube into the front right corner. The time taken for the focal fish to consume the first larva was recorded. If this took longer than 15 minutes then the trial was aborted. From the first consumption, the interval between each larva consumed, and the species consumed, were also recorded. The trial continued for 10 minutes, or until all 20 larvae had been consumed. If shorter than 10 minutes then the time taken to consume all larvae was recorded (*length of feeding bout*). In the trials where companion fish were present, the number of larvae of each species remaining after 10 minutes was also recorded.

1) Social experiment

In this experiment, focal fish were assigned to one of four conditions: single female, single male, female with two female companions and female with two male companions.

2) Habitat complexity experiment

Here, only adult females were used as focal fish, as these are likely to play a more important role in biological control (Cavalcanti *et al.*, 2007) and because limited time in India meant it was not possible to repeat the design focusing on males. Fish were tested in one of three social conditions: 1) alone; 2) with two female companions; 3) with two male companions (Figure 6.3).
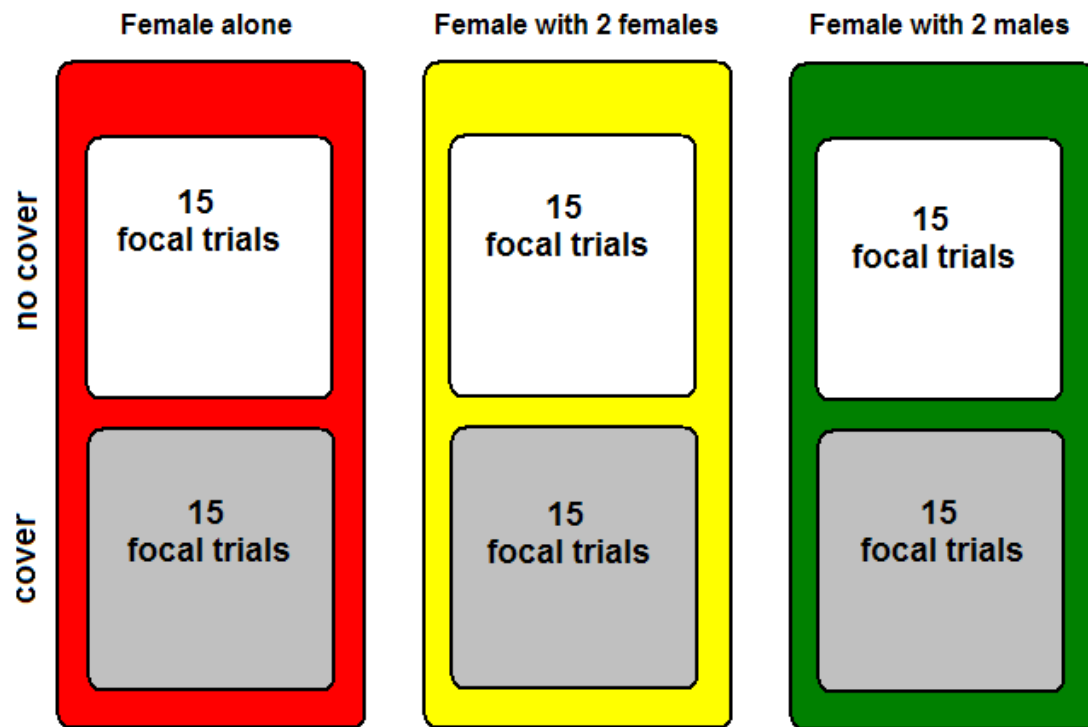
Figure 6.3: Diagram of experimental design for habitat complexity experiment.

Half of the fish in each social condition were tested in the presence of 'cover' and half without. Cover consisted of a square of green plastic (8cm x 8cm) with 'fronds' cut from the edge to the centre. This was bunched together by tying it in the centre with a piece of string (Figure 6.4).

Figure 6.4: 'cover'.

The bundle then floated on the water surface, with a few fronds dangling down into the water column. It covered a 2-dimensional area of approximately 40cm^2 and was

positioned on the right side of the tank, immediately behind where the larvae were introduced at the start of each trial. This provided an opportunity for larvae to actively seek refuge in the cover but they were not actually placed there initially.

In all trials, the following calculation was carried out for each fish and termed 'Preference for *Culex*' (P_C):

$$P_C = \# \, Culex \, larvae \, consumed - \# \, Anopheles \, larvae \, consumed$$

A P_C value of zero means that equal numbers of each species were consumed. P_C itself does not imply a significant preference for one prey, but is simply a measure of the difference in the number of the two prey types that are consumed. Means and 95% confidence limits were calculated for each condition. Where these CIs do not overlap 'zero', a significant difference (p<0.05) is highly likely.

6.2.2. Statistical methods

Where data displayed an approximately normal distribution and equal variance, t-tests or ANOVA were used as appropriate. The latter was followed by a Tukey's pairwise test where required. Non-normal data were transformed before analysis as necessary. Where data did not meet the assumption of the normal distribution even after transformation, equivalent non-parametric tests were used (i.e. a Mann Whitney U test).

6.3. Results

6.3.1. Prey consumption: social experiment

Single females consumed significantly more larvae than males during a 10 minute trial (U=186.5; N1=31; N2=29; p<0.001) (Figure 6.5), yet there was no difference in bout length (time from first larvae consumed to last larvae consumed) between the sexes (U=543; N1=31; N2=29; p=0.383) (Table 6.1), confirming a higher rate of consumption by female guppies (Table 6.1).
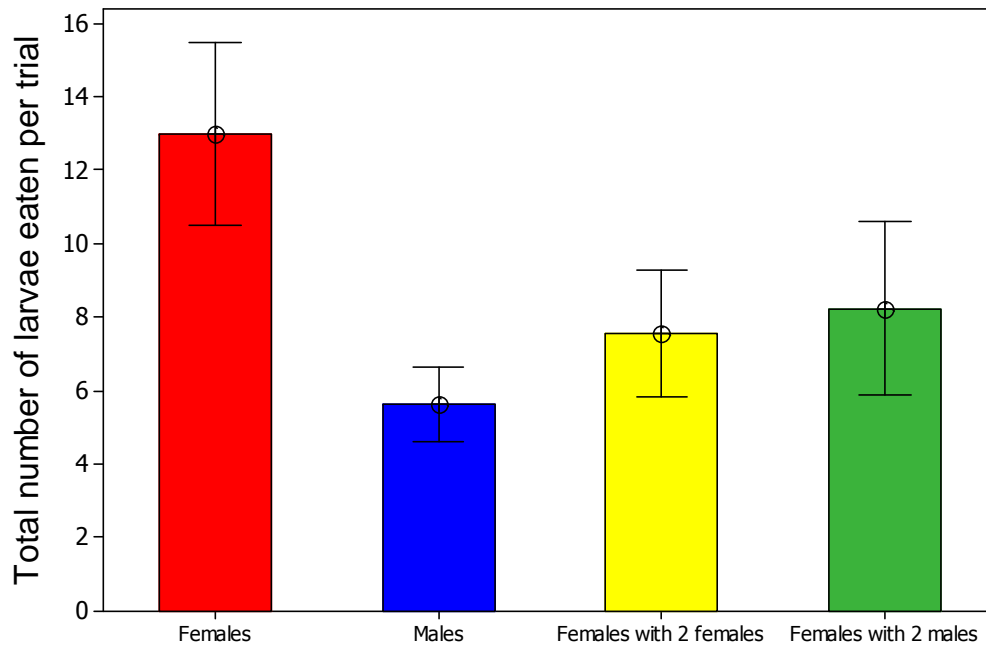
Figure 6.5: a) Total number of larvae eaten by focal fish in each condition. Bars represent 95% CIs. Conditions: Females (N=31), Males (N=29), Females with two females (N=34), Females with two males (N=25).

There was no difference in number of larvae eaten between females foraging alongside male or female companions (U=988.5; N1=34; N2=25; p=0.634), and, similarly, length of feeding bout also did not differ between the two 'social' conditions (U=1024; p=0.963), equating to a similar rate of consumption in both (Table 6.1).

Table 6.1: Consumption statistics for social experiment.

	N	Mean feeding bout length (secs±SE)	Median bout length (secs)	Consumption rate (larvae/min ±SE)	Mean % larvae consumed (±SE)	% of trials in which >95% of larvae were consumed
1 female	31	344 (±31)	332	2.9 (±0.4)	65 (±6)	39%
1 male	29	313 (±32)	328	1.4 (±0.2)	28.3 (±2.5)	0%
3 females	34	257 (±35)	202	5.3 (±1.8)	93 (±2.6)	74%
1 female + 2 males	25	242 (±34)	171	3.5 (±0.8)	84 (±5.3)	67%

The percentage of larvae eaten by all fish (focals and non-focals) during a 10 minute trial was greatest when three females were present (Figure 6.6). In this condition, 74% of trials ended with >95% of larvae being consumed. When a female and two males were present,

67% of trials resulted in >95% consumption. None of the single males consumed >95% of larvae.
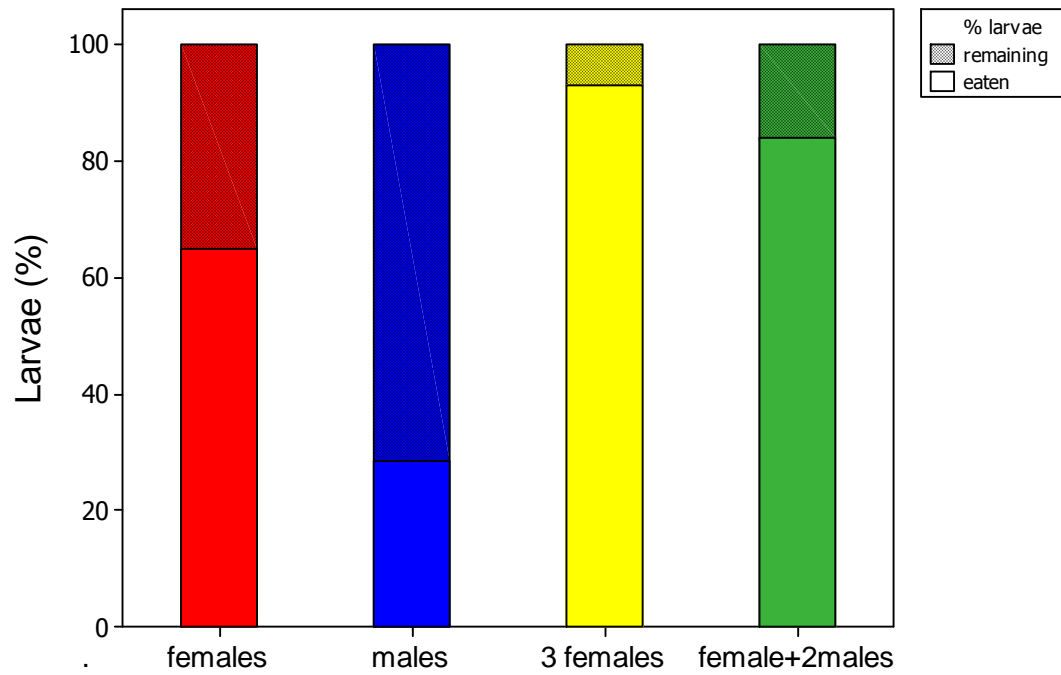
Figure 6.6: Mean % of larvae eaten and remaining between trials of each condition. In the social conditions, this includes larvae eaten by non-focal fish.
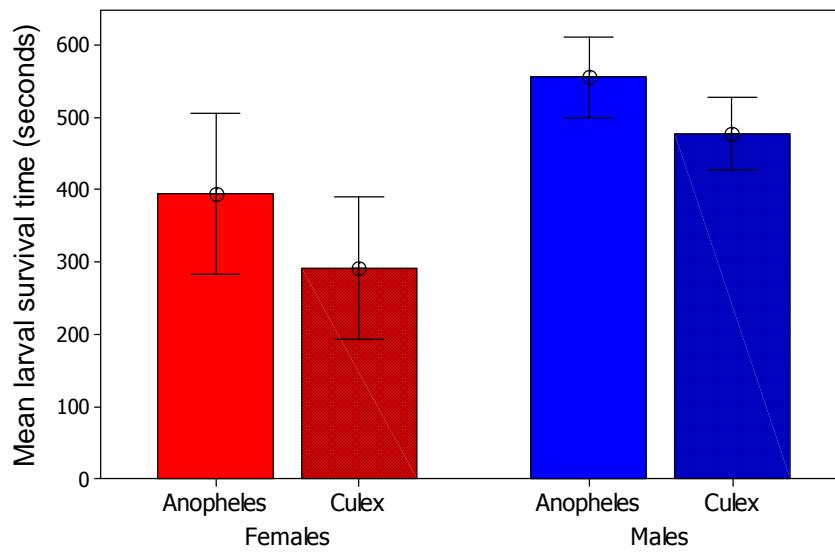
Figure 6.7: Mean larval survival time for Anopheles (A) and Culex (C) larvae in the presence of female and male guppies. Interval bars represent 95% CIs.

The larval survival time in the single fish conditions suggests that both sex of guppy ($F_{1,116}$=27.82; p<0.001) and larval species ($F_{1,116}$=4.90; p=0.029) affect larval survival time, with *Anopheles* surviving for longer in both cases, and both species surviving for longer in the presence of a male (Figure 6.7).

6.3.2. Prey consumption: habitat complexity experiment

The presence of cover did not affect the number of larvae eaten ($F_{1,79}=0.17$; p=0.679) (Figure 6.8). As before, there was no difference in focal consumption between trials with male or female companions ($F_{2,79}=2.36$; p=0.101). There was no significant interaction between the effects of social and habitat factors ($F_{2,77}=0.022$; p=0.978).
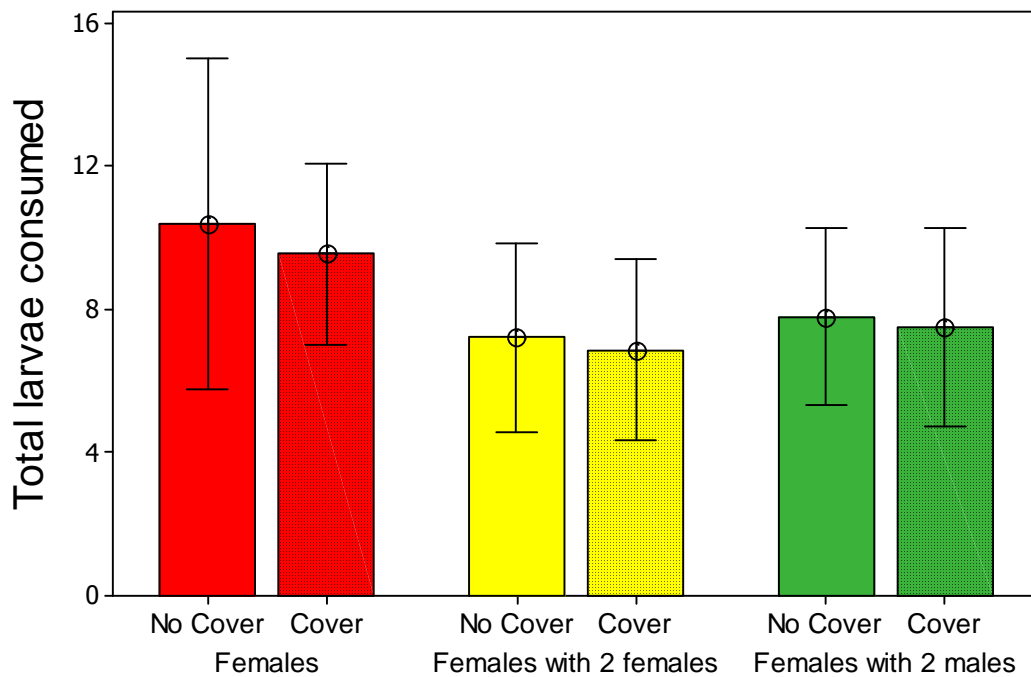
Figure 6.8: Total number of larvae consumed by female fish alone or with female or male conspecifics, and with or without cover. 95% CIs are shown. Females: no cover (N=13), cover (N=11); Females with 2 females: no cover (N=14), cover (N=15); Females with 2 males: no cover (N=14), cover (N=15).

Bout length was significantly shorter when three females were present ($F_{2,67}=12.91$; $p<0.001$; Tukey's $p<0.020$) (Figure 6.9). The presence of cover had no effect on bout length ($F_{1,67}=0.40$; $p=0.527$). There was no significant interaction between the effects of cover and social context ($F_{2,66}=2.414$; $p=0.097$).
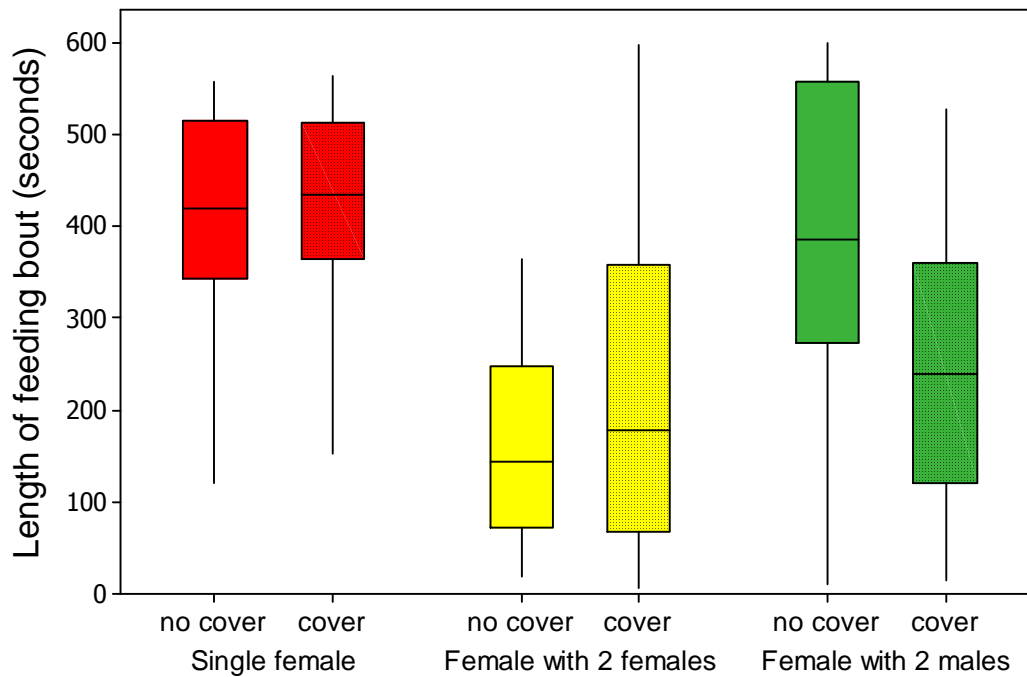
Figure 6.9: Duration of feeding bout (seconds) for female fish alone, with female or with male conspecifics, and with or without cover. Sample sizes as in Figure 6.8.

Table 6.2: Consumption statistics for habitat complexity experiment.

		N	Mean feeding bout length (secs±SE)	Median feeding bout length (secs)	Consumption rate (larvae/ min ±SE)	Mean % larvae consumed (±SE)	% of trials in which >95% of larvae were consumed
1 females	No cover	11	410(±39)	419	1.8 (±0.3)	52.3 (±10.7)	23%
	Cover	11	425 (±36)	435	1.4 (±0.2)	47.7 (±5.7)	0%
3 females	No cover	13	166 (±31)	144	4.1 (±0.9)	96.4 (±1.7)	86%
	Cover	13	185 (±50)	178	6.1 (±2.2)	88.7(±4.7)	67%
1 female +	No cover	13	380 (±51)	386	3.1 (±1.8)	78.2(±5.2)	29%
2 males	Cover	12	249 (±47)	240	3.2 (±0.6)	76.3 (±5.0)	21%

Again, the greatest depletion of larvae was seen in the three females condition, particularly in the absence of cover. In this condition 86% of trials resulted in >95% of larvae being consumed. In the presence of cover, none of the eleven single females tested managed to deplete >95% of larvae.

Despite this, cover did not have a significant effect on the number of larvae consumed by all fish in a trial ($F_{1,77}=2.62$; $p=0.110$), whereas social condition did ($F_{2,77}=25.02$; $p<0.001$). Here, all three social conditions differ significantly from each other (Tukey's: all $p<0.010$).
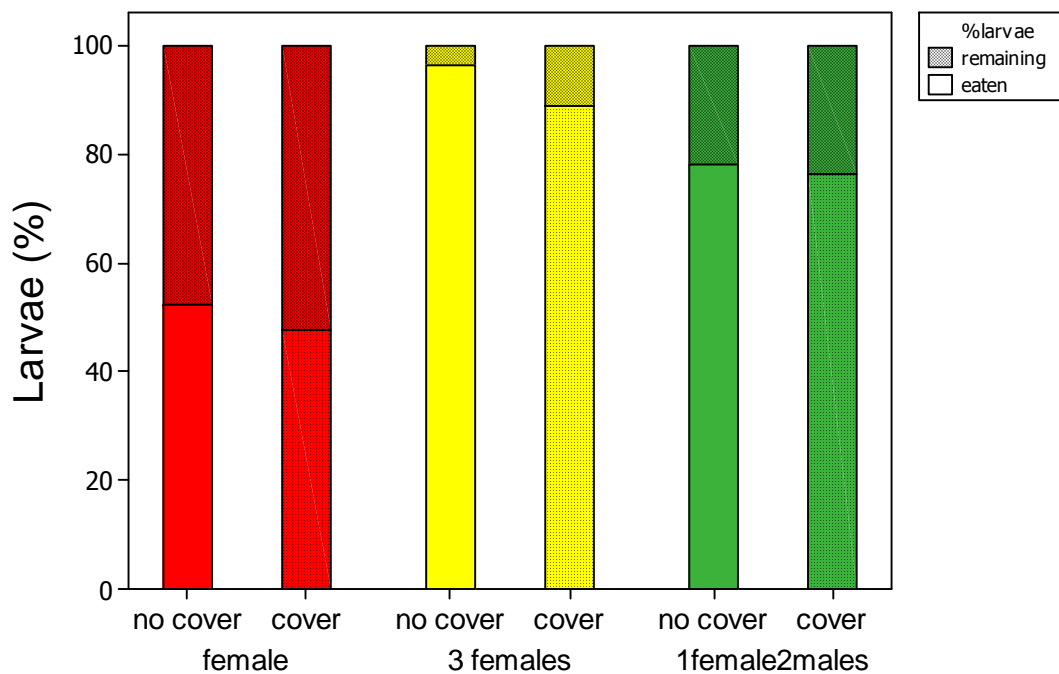
Figure 6.10: Mean % of larvae eaten and remaining between trials of each condition. In the social conditions this includes larvae eaten by non-focal fish.

6.3.3 Prey preference

Mosquito larvae of both species used the cover provided. 13% of remaining *Culex* and 15% of remaining *Anopheles* were found hiding in cover at the end of all trials.

The greatest values of P_C were in the single, no cover condition, where consistently more *Culex* than *Anopheles* were consumed. The lowest values were among single females feeding in cover (Figure 6.11).
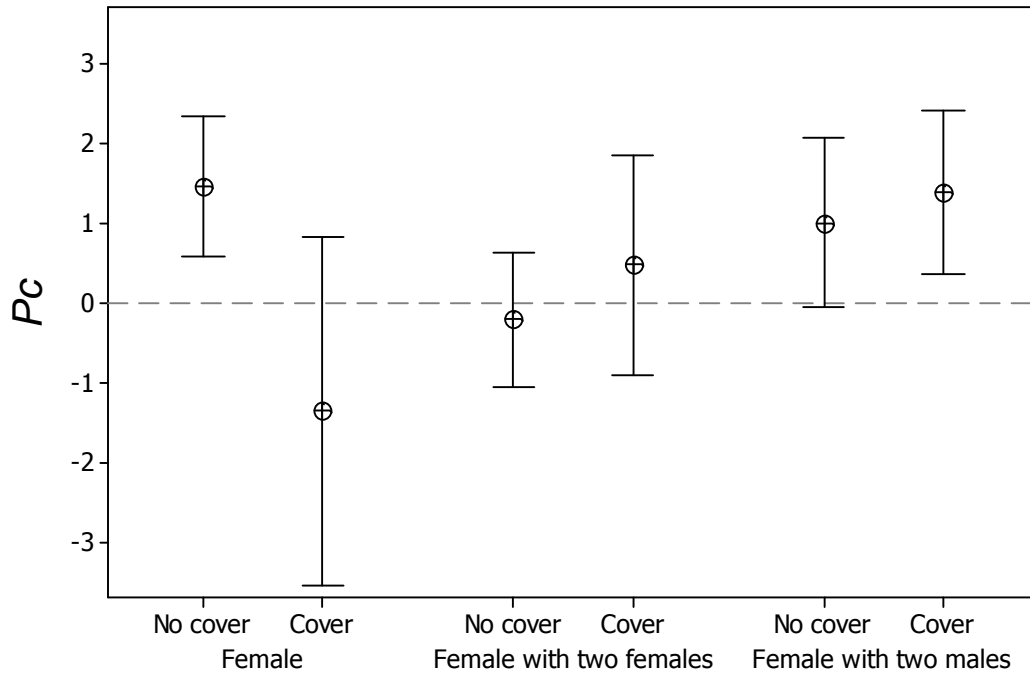
Figure 6.11: P_C values for all social and habitat conditions. The dotted line represents the expected mean if equal numbers of both species have been consumed. Bars represent 95% confidence intervals. Sample sizes as listed in Methods.

Females in the absence of cover had a preference for *Culex* (t=3.90; df=12; p=0.009), while females foraging in the presence of cover no longer displayed differential predation (t=1.39; df=13; p=0.196) (Figure 6.11).

A two-way ANOVA confirmed that variation in P_C between conditions was explained by a significant interaction between social and habitat factors ($F_{2,173}$=4.23; p=0.016) (Figure 6.12).
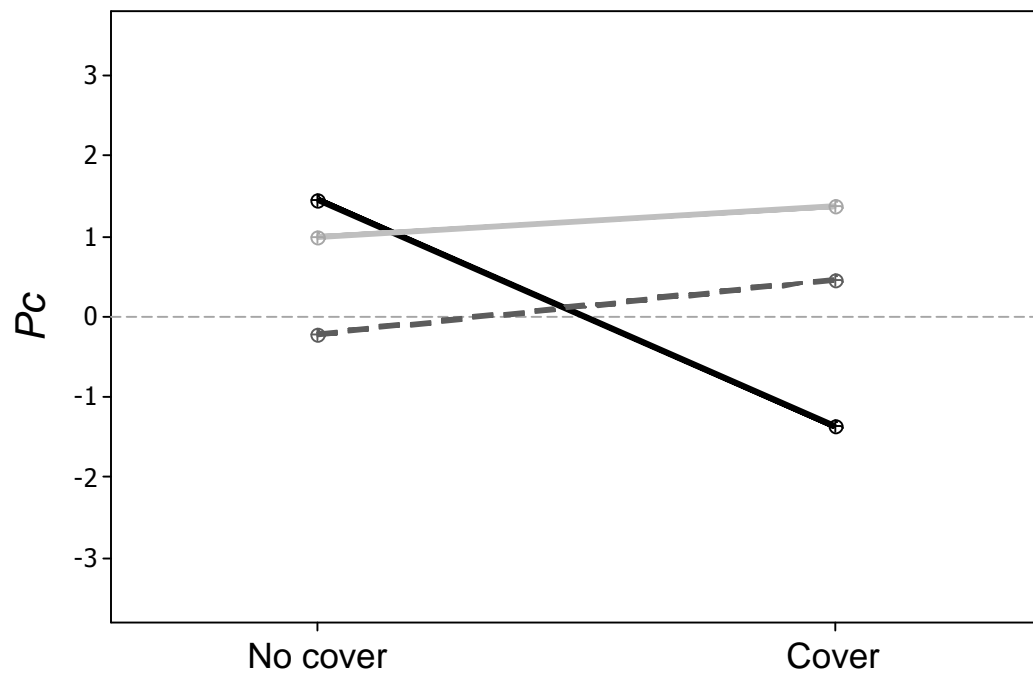
Figure 6.12: Interaction plot for P_C without and in the presence of cover. The horizontal dotted grey line represents the expected mean if equal numbers of both species have been consumed. Legend: Black = single female; Dark grey dashed= female with two female conspecifics; Light grey= female with two male conspecifics.
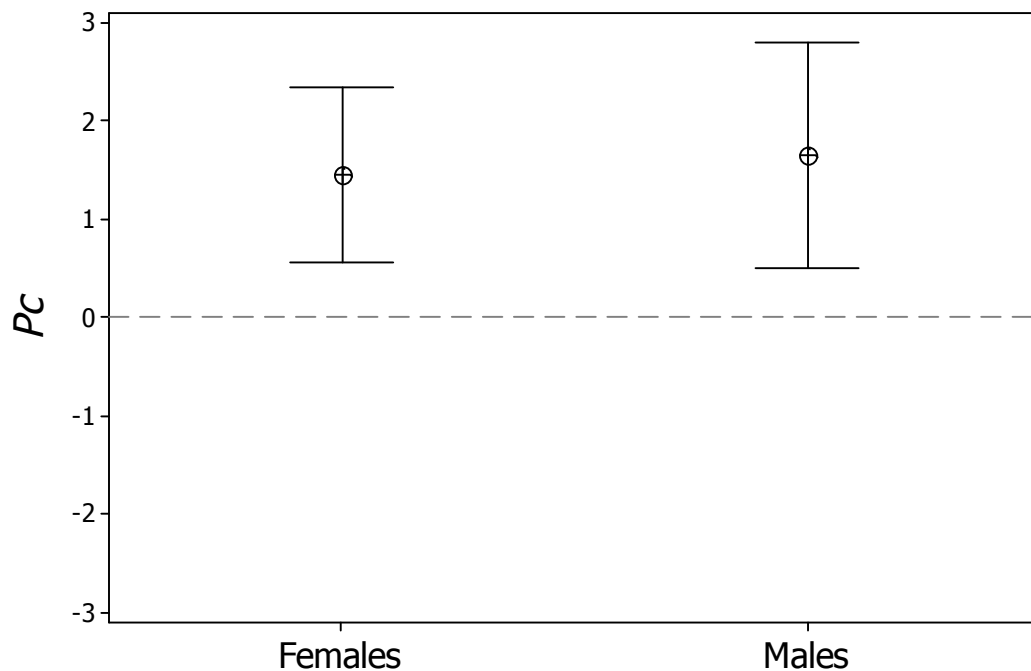
Figure 6.13: P_C values for female and male guppies foraging alone. The dotted grey line represents the expected mean if equal numbers of both species have been consumed. Symbols above and below the corresponding coloured means (joined up) represent upper and lower 95% confidence intervals. (Females: N=44; Males: N=29).

There was no difference in P_C between males or females foraging alone (two-sample t-test: t=-0.285; df=71; p=0.777). Both sexes consumed more *Culex* than *Anopheles* (Figure 6.13).

6.3.4 Results summary

Prey consumption:
- Females consume larvae at a faster rate than males
- The sex of conspecifics did not significantly affect consumption rate of focal foraging females
- More larvae in total were eaten and feeding bouts shorter when three females were foraging together

- The presence of cover did not significantly affect the number of larvae eaten

Prey preference:

- A preference for *Culex* found in both females and males when foraging alone, without cover
- A significant interaction between social and habitat factors explains variation in prey preference

6.4. Discussion

Female guppies eat more larvae and at a faster rate than males. The number of larvae eaten by a focal female was the same regardless of whether companions were male or female and whether in the presence or absence of cover. Feeding bout length (time between 1st and last consumption within a trial) for focal fish was also the same regardless of whether male or female companions were present. The total number of larvae consumed was greater in the three female condition. This was the case in both experiments.

Sex differences

Guppies of both sexes were capable of consuming 3rd and 4th instar *Culex* and *Anopheles* larvae, and do so eagerly. As might be expected due to their size, females were more efficient foragers than males, consuming significantly more larvae despite there being no difference in length of feeding bout. Elias *et al*., (1995) also found that female guppies consumed nearly double the quantity of *Culex* larvae than males did. I observed that males sometimes struggled with the *Culex* larvae, apparently due to the protruding siphon present in this species. This added handling time, but did not usually stop the eventual consumption of the prey. When females were in the presence of companions the difference in number eaten compared with the single male forager was no longer evident, probably due to rapid depletion of larvae by companions limiting the maximum number that could be consumed by the focal female fish.

Prey preference

There was a marked preference for *Culex*, the non-vector species, when fish of either sex were feeding alone. However, this preference seems to disappear under more naturalistic conditions. Both the social environment and the complexity of the habitat are capable of affecting prey preference in a non-additive manner, with the effect of cover depending on the social context.

There are a number of possible explanations for a social effect on prey preference. It is possible that in the presence of two females, increased competition may lead to reduced discrimination between prey species by the focal female. Alternatively, a greater number of fish in the arena may mean that the larvae were more active either as a behavioural response to predators or simply because there was an increased predator-prey encounter rate. The latter seems more likely as larvae of both species tended to remain still until the predator made contact, at which point they would move – potentially attracting the attention of all three predators (Sih, 1986). Whereas the diagonal resting position of *Culex* larvae means that this species is likely to be relatively more conspicuous when still, *Anopheles* tends to remain cryptic until it is prompted to move.

Habitat complexity differences

The preference for *Culex* in single fish was also negated by the addition of a small amount of cover in the tank. There was no evidence that one species of prey was using the cover more than the other, but it is possible that any difference may have been undetectable given the experimental design. It may, however, be that the refuge provided by the cover was more beneficial for previously quite conspicuous *Culex* larvae, than for the already more cryptic *Anopheles*. Baber and Babbitt (2004) investigated the effect of habitat structure on *Gambusia holbrooki* prey preference. They suggested that less active prey types might gain a better advantage from habitat structure than more active species, highlighting the importance of behavioural differences between alternative prey species to the effect of habitat complexity. Several studies have found that increased habitat heterogeneity, and the presence of refugia, can reduce predatory impact in aquatic communities (Bechara *et al*., 1993; Diehl, 1992). Savino and Stein (1989) emphasised

that the relationship between habitat complexity and predation efficacy is not always straightforward, as it depends critically on how the habitat is used by both predators and prey.

Sabatinelli *et al*. (1990) found that guppies consumed a greater number of *Anopheles* and *Aedes* larvae than *Culex* larvae in trials in Grande Comore and Mayotte. However, they do not mention rates of consumption, and the low absolute number associated with *Culex* may be due to the fact that the same instar in this genus can often be larger than those of other species, and so satiation may be reached after consuming fewer individual larvae. In this study the larvae were size-matched, but if instar alone rather than size was used to match the two species then on average 3rd instar *Culex* is, for example, larger than 3rd instar *Anopheles* and this may have affected the results.

Box 6.1: Further work

A next step would be to repeat experiments like these but using larger, mesocosm tanks to better represent the containers that guppies are introduced to in villages for mosquito control.

Rate of depletion of a set number of mosquito larvae could be measured at different densities of fish and with and without vegetation. It would also be interesting to examine guppy predation pressure on different instars of larvae. To best imitate a control scenario, a large number of larvae could be introduced to the tanks as 1st instars, and the number that survive to the pupal stage recorded.

It is apparent that the sex of the forager, the social environment and habitat complexity are each capable of affecting foraging behaviour and prey use. Although single fish display a preference for *Culex*, the non-vector species, it is interesting that this preference seems to disappear under more naturalistic conditions. Even when a preference was apparent, the non-preferred larvae were nevertheless readily consumed, supporting the continued investigation of guppies in malaria control.

These findings highlight the potential effects that a multi-prey system might have on the effectiveness of biological control measures using guppies. One effect might be that guppy populations are be able to persist for longer even once the target prey has been successfully eradicated or reduced (Manna *et al*., 2008), which could be beneficial from a biological control perspective as it would mean that fish would not have to be frequently replenished. However, it might simultaneously render the fish less likely to bring the target prey population under control if they are also feeding on other prey species, depending upon their relative preference for the target prey. For example, Symondson *et al*., (2006) found that the presence of alternative prey species reduced the efficacy of predatory beetles in the control of slug populations. Alternative prey species have been found to have a similar effect on the biological control of Colorado potato beetles by predatory bugs (Koss & Snyder, 2005). Another effect might be a greater negative ecological impact once established in the wild, as their impact might not be restricted to the pest species. Further analyses and experiments will help determine the exact nature of these interactions and their implications for the use of guppies as biocontrol agents of malaria (see Box 6.1).

There is a huge gap in our knowledge of the efficiency of guppies as biological control agents. This study has confirmed that taking previously neglected factors such as social context and habitat complexity into account is likely to increase the ecological validity of studies, vital when using the findings to inform decisions as important as whether to introduce guppies for the purpose of biological control.

Chapter 7

General Discussion
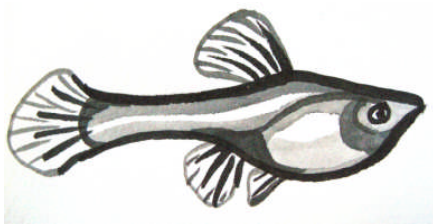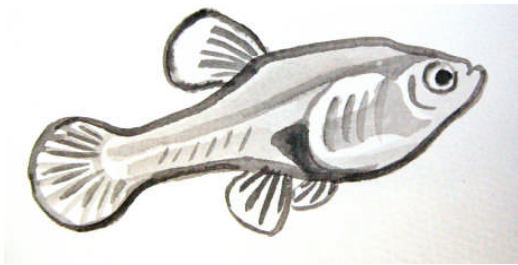
7.1 Summary and synthesis

In this section I will summarise the main findings of each chapter, with reference to the key questions outlined in Chapter 1. I will then explore the implications of these findings in the light of the aims of this thesis.

Key Question 1: What is the non-native distribution of the guppy?

The worldwide email survey proved an effective means of gathering information regarding the distribution of the guppy; revealing that it is now established in at least 73 countries outside of its native range.

Key Question 2: What are the most common modes of introduction for the guppy?

The survey also enabled the collation of information regarding rationale behind, and origins of, introductions – information that is notoriously difficult to source. Mosquito control schemes and the release of unwanted aquarium fish are the two primary routes of introduction; it appears that both contribute equally to the spread of guppies worldwide.

Key Question 3: What is known about the negative impacts of guppy introductions?

The collation of information allowed knowledge gaps to be identified; two of the most pressing being the scarcity of scientific evidence for negative effects of guppy introductions and similarly of scientific evidence for mosquito control efficacy.

Key Question 4: Do single females routinely establish viable populations?

The mesocosm studies confirmed that single female guppies are capable of establishing populations, and that these populations can persist and retain behavioural viability over several generations.

Key Question 5: Are guppies from wild populations that tend to have more 'r-selected' traits more likely to establish new populations?

The first mesocosm study suggested that founders with two very different evolutionary histories, those from habitats with 'high' or 'low' predation regimes, were equally good at establishing persistent populations.

Key Question 6: Are polyandrous females more successful colonisers?

The second mesocosm study suggested that even monandrous females were extremely successful at establishing persistent and behaviourally viable populations.

Key Question 7: Do populations founded by polyandrous females display more variation in terms of male colouration, courtship and newborn behaviour?

There was no evidence that polyandrous-founded populations had higher levels of behavioural variation, despite displaying a significantly greater diversity of male colouration.

Key Question 8: How effective are guppies as mosquito control agents?

Neither chapter 5 or 6 can claim to have answered the question that was initially set out. It is clear that a great deal more research is required before this will be possible. However, these findings make an important contribution to an under-studied area that can be built upon by future studies. The first foraging study did not find evidence of prey-switching in guppies, questioning the validity of previous studies that have been used to advocate the introduction of generalist predators to stabilise prey populations. The second foraging study revealed a preference for the non-vector mosquito larvae in a two-prey system. However, an interaction between social and habitat factors had a significant effect on this preference, tending to reduce strength of preference under more naturalistic conditions. Similarly, in the first study, the presence of males reduced the strength of preference for the preferred prey.

Synthesis of findings

The aim of this thesis was to examine the guppy in the light of its invasive success. We now have a much more complete picture of its worldwide distribution, and I have identified specific areas where significant gaps in our knowledge of invasive guppies remain. It is clear that an association with humans, both as pets and biological control agents, has been critically important to their success, although the huge range revealed by the worldwide survey suggests that this is paired with a remarkable opportunism and

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adaptability. Building upon what was already known about a species that is so well studied, both in its native habitat and in the laboratory, the ability to capitalise on introduction events and found populations has now been unequivocally demonstrated in a context relevant to an introduction scenario.

Guppies are ideally suited to establishing and persisting in small water tanks. Results from Chapters 3 and 4 suggest that this is most likely due to their reproductive abilities, which allow them to rapidly increase in numbers from very few founders at any time of year. They also suggest that such severe demographic bottlenecks have remarkably few negative effects on phenotypic traits such as antipredator behaviours. Most poeciliids are generalist predators and, as such, are able to persist on alternative prey even after the target species for biological control is at low density or eradicated. This is demonstrated by the mesocosm populations in Chapter 3, which persisted for two years (at which point the experiment was terminated) despite receiving no food supplements, presumably by opportunistically consuming a combination of algae and falling insects. Both Chapters 5 and 6 also suggested the presence of some flexibility in prey preference depending on social or habitat factors.

By favouring traits that improve the ability for guppies and other poeciliids to adapt to changes in their native environment, natural selection has generated a family of extremely successful invaders. A high degree of behavioural flexibility and life history plasticity, a broad and flexible diet (Dussault & Kramer, 1981; Arthington, 1989) and a number of reproductive specialisations, most notably ovoviviparity and sperm storage (Winge, 1937) are all strategies that are likely to have evolved in response to the constantly changing environment of ephemeral shallow water pools and streams inhabited by poeciliids. Thibault & Schultz (1978) suggested that this environment has led to the evolution of what they term a 'generalist' reproductive mechanism in the guppy, which is representative of 85% of poeciliids. Ovoviviparity ensures that developing eggs are not abandoned in unsuitable habitats as conditions change (Chesser *et al.*, 1984), and sperm storage minimises the problem of Allee effects (Taylor & Hastings, 2005).

These characters have important consequences for two of the central themes of this thesis; the establishment of guppies in natural environments and their suitability as mosquito control agents. This is partly because many of the traits associated with good invaders are also those associated with good predatory biological control agents. For example, Symondson *et al*. (2002) suggested that a good biological control agent should possess the following three attributes:

- A rapid colonisation ability to keep pace with temporal and spatial disturbances.
- Temporal persistence, maintaining numbers even when pest populations decline.
- Opportunistic feeding habits which allow rapid exploitation of attacks by resurgent pests.

These same characteristics are also likely to mean that populations can reproduce quickly and spread outside of the area of initial introduction, becoming invasive. As well as encouraging temporal persistence in periods of low target species density, their generalist habits may also render guppies less effective in the presence of the target species (Symondson *et al*., 2002; Manna *et al*., 2008). For example if a preferred alternative prey species is available, their ability to control the target species may be impaired (Chapters 5 and 6). Meanwhile, they may be having an unwanted impact on native prey and, as a result, on the rest of the ecosystem (Howarth, 1991). This explains why so many species that are introduced for biological control purposes become pests themselves (Howarth, 1991) and emphasises the need for careful consideration of both species and habitat factors when selecting suitable agents.

Aims

The aim of this final chapter is to examine the findings described above in relation to the rest of the Poeciliidae, which includes other notorious invaders such as *Gambusia affinis*. I will also place the findings in the context of invasive species risk assessments and screening measures. Finally, the potential costs and benefits of introducing poeciliids for biological control purposes will be considered and some recommendations for invasive species policy will be proposed.

7.2 Poeciliidae: an invasive family?

Most species introduced to a new habitat will either fail to thrive or be unable to establish a self-sustaining population (Williamson & Fitter, 1996*b*). The documented success of introduced poeciliids worldwide, however, suggests that this family of freshwater fish is particularly likely to do both of these things. FishBase (www.fishbase.org) lists 18 poeciliid species as 'established' or 'probably established' outside their native range (Froese & Pauly, 2010) (Table 7.1), and together they are responsible for 11% of fish species on the Global Invasive Species Database. This includes being represented by *Gambusia affinis* on their list of 'One Hundred of the World's Worst Invasive Alien Species' (http://www.issg.org/database).

It is not uncommon for certain taxonomic groups to be over-represented among invasive species (García-Berthou, 2007); for example a large number of the world's invasive plants belong to relatively few families and genera; Asteraceae, Poaceae, *Mimosa, Acacia* and *Cyperus* being the best represented (Mack *et al*., 2000). Most poeciliids tend to be small with short generation times, non-seasonal reproduction and large brood sizes, but population-level differences in these traits do not appear to affect founding success in the guppy (Chapter 3). Other 'invasive traits' found in poeciliids include phenotypic plasticity (Carvalho *et al*., 1996), polyphagy (Arthington, 1989), eurytopy (Casatti *et al.*, 2006), ovoviviparity and sperm storage (Courtenay & Meffe, 1989). The latter two reproductive characteristics are shared by most members of the Poeciliidae (Chesser *et al.*, 1984), and indeed Chapters 3 and 4 suggest that specialisations such as these, that allow a rapid increase in population size from low numbers of founders, are of greatest important to establishment success. Of course, most of the 293 species of poeciliid worldwide are not recorded outside of their native range despite sharing similar life history and reproductive traits with their invasive relatives (Froese & Pauly, 2010). This is likely to be at least partly due to an absence of opportunity, rather than implying that they lack particular traits (Rowley *et al.*, 2005).

There is a strong bias among invasive species towards those taxa that are associated with humans; in fish, this includes those used as game, in aquaculture or in the aquarium trade (García-Berthou, 2007; Alcaraz *et al.*, 2005; Marchetti *et al.*, 2004*b*). Association with humans undoubtedly contributes to invasive success within the Poeciliidae; both through the aquarium trade and by their use for mosquito control. Rixon *et al.*, (2005) found that 95% of pet stores surrounding the Laurentian Great Lakes stocked guppies, making it the second most popular aquarium fish of the survey. Furthermore, of the 20 most popular, 5 were poeciliids (Rixon *et al.*, 2005). In a 1992 survey, Chapman *et al.* (1997) found that ~4 million guppies were imported to the USA during October – making *Poecilia reticulata* the most imported aquarium fish to the USA in terms of number, accounting for 26% of all imported freshwater fish. *Poecilia* is also the best represented genera among introduced poeciliids (Froese & Pauly, 2010) (Figure 7.1).
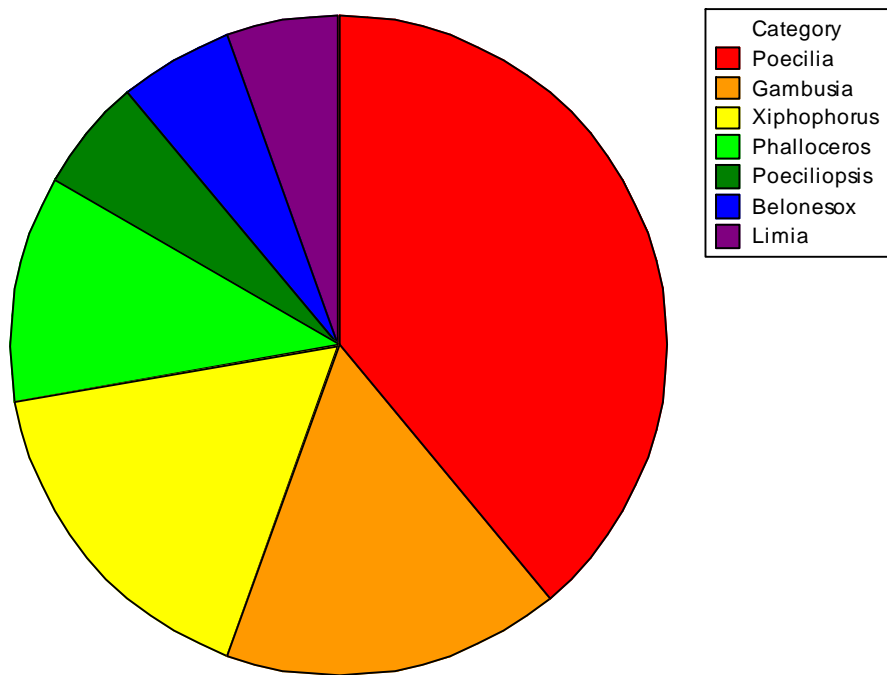
Figure 7.1: Number of poeciliid species within each genera established outside of their native range (*Poecilia*=7 species; *Gambusia*=3; *Xiphophorus*=3; *Phalloceros*=2; all others=1). Compiled using FishBase (Froese & Pauly, 2010).

Table 7.1: List of poeciliids that have been introduced outside of their native range. Compiled using FishBase (Froese & Pauly, 2010).

Species	# Intro.	# Est.	# Fail.	% Est.	Mosquito control?	Aquarium species?	Impact?	Resilience
Belonesox belizanus	1	1	0			Yes	Harmless	Medium
Cnesterodon decemmaculatus	1	0	1			Yes	Harmless	High
Gambusia affinis	77	66	2	86	Yes		Potential pest	Medium
Gambusia dominicensis	1	1	0			Yes	Harmless	High
Gambusia holbrooki	31	25	0	81	Yes	Yes	Potential pest	High
Limia vittata	2	2	0		Yes	Yes	Harmless	High
Phalloceros caudimaculatus	2	2	0		Yes	Yes	Potential pest	High
Phalloceros harpagos	1	1	0			Yes	Harmless	High
Poecilia latipinna	13	13	0	100	Yes	Yes	Potential pest	High
Poecilia latipunctata	1	1	0			Yes	Harmless	High
Poecilia mexicana	7	7	0	100		Yes	Potential pest	Medium
Poecilia petenenis	1	0	1			Yes	Harmless	Medium
Poecilia reticulata	57	47	6	83	Yes	Yes	Potential pest	High
Poecilia sphenops	11	7	2	64		Yes	Harmless	High
Poecilia velifera	6	5	0	83	Yes	Yes	Harmless	High
Poecilia vivipara	2	2	0			No	Harmless	High
Poeciliopsis gracilis	2	2	0				Harmless	High
Xiphophorus hellerii	29	26	2	90		Yes	Potential pest	High
Xiphophorus maculatus	19	16	0	84		Yes	Potential pest	High
Xiphophorus variatus	6	6	0	100		Yes	Potential pest	High

#Intro= number of countries where the species has been introduced; #Est.= number of countries where the species is listed as 'established' or 'probably established'; # Failed = number of countries where the species has been introduced but is listed as 'not established' or 'probably not established'; % Est = only presented for those that had been introduced to >5 countries. Resilience= a measure of productivity; High=minimum population doubling time of <15 months; Medium=min pop doubling time of 1.4 -4.4 years (Froese & Pauly, 2010).

7.3 Invasive species risk assessments

Half of the poeciliids that are established outside of their native range are classed as potential pests due to the existence of at least one report of negative ecosystem effects following an introduction (Froese & Pauly, 2010) (Table 7.1). Chapter 2 brought together accounts of the negative impacts of guppies on native ecosystems, including both

anecdotal reports and peer-reviewed studies. There is already a convincing body of scientific literature regarding the negative effects of predatory *Gambusia holbrooki* and *G. affinis* (Arthington, 1991; Goodsell & Kats, 1999; Mills *et al*., 2004; Morgan *et al.*, 2004), although some argue that even here more research is urgently required (Pyke, 2008). Negative impacts have also been associated with *Phalloceros caudimaculatus*, *Poecilia latipinna*, *Xiphophorus hellerii*, *X. maculatus* and *X. variatus* (Froese & Pauly, 2010; Morgan *et al*., 2004; Courtenay & Meffe, 1989).

The global ecological and economic costs of invasive species can be enormous (Pimentel *et al*., 2001). In 2004, the estimated economic costs to the USA were almost US$120 billion, with 42% of the nation's threatened or endangered species at risk primarily due to invasive species (Pimentel *et al*., 2005). The most effective means of avoiding these costs is to prevent the introduction of harmful species in the first place (Leung *et al.*, 2002; Mack *et al.*, 2000). To this end, the development of methods for identifying those species with a high risk of invasion using predictive models and screening techniques has been the subject of a great deal of research in recent years (Kolar, 2004; Kolar & Lodge, 2001, 2002; Moyle & Marchetti, 2006; Alcaraz *et al*., 2005; Ricciardi & Rasmussen, 1998; Marchetti *et al.*, 2004*a*). Such approaches have looked at a variety of biological traits that have been widely associated with invasive success as well as social, historical and taxonomic factors (Moyle & Marchetti, 2006). The ultimate aim is to identify potential invasive species before their introduction, so that appropriate legislation can prevent them from ever being imported. However, the same approach may also help to prioritise resources when dealing with species that have already been introduced, focusing on those that are most likely to persist and spread (Kolar, 2004).

One of the challenges here is that it has become increasing clear that very different factors are responsible for success at different stages of the invasion process, and as only species that succeed at every stage become invasive, all stages need to be considered when modelling invasion probability (Marchetti *et al*., 2004*b*). Despite this, the accuracy of model predictions has improved since the approach was first explored (Keller *et al.*, 2007). However, it has been argued that in order for risk assessments to be economically

viable, accuracy must be extremely high (Smith *et al.*, 1999). This is because the cost of false positives – where the introduction of economically useful species is prevented unnecessarily – will often be greater than the amount saved by successfully preventing economically damaging species from introduction. Others suggest that the precautionary principle should rule, considering that just one introduction of an invasive species can have potentially devastating effects (Simberloff, 2003). This particularly applies to aquatic introductions, where naturalisation is even more likely to be irreversible; here, it is arguable whether any intentional introductions are acceptable (Smith *et al.*, 1999).

7.4 The costs and benefits of poeciliid introductions

The case of the guppy, and that of many other introduced poeciliids, is complicated by the potential human health benefits of their introduction, which need to be weighed up against the risk of economic and ecological damage (Simberloff & Stiling, 1996). Mosquito-borne disease continues be one of the most pervasive threats to human health worldwide; malaria infects around 52 million people each year (WHO, 2003) and dengue fever a further 890,000 (WHO, 2007). Chemical control is costly, damaging to the environment and can lead to pesticide resistance in adult mosquitoes (Chandra *et al.*, 2008). Biological control using larvivorous fish has been advocated as a cheap, safe and effective alternative (Kumar & Hwang, 2006; Kusumawathie *et al.*, 2008; Dash, 2009).

Fish are most often introduced into enclosed, manmade water bodies such as wells, troughs and water storage tanks, as these tend to be the most common breeding sites for mosquitoes around human settlements (Sabatinelli *et al.*, 1991; Seng *et al.*, 2008; Cavalcanti *et al.*, 2007). Unfortunately, floods and monsoons facilitate escapes, some of which result in self-sustaining populations that eventually spread and cause damage to natural ecosystems. An obvious compromise would be the use of native larvivorous fish, given the assumption that translocations within the same region might be less likely to cause ecological damage (Fletcher *et al.*, 1993; Arthington, 1991). However, it is often difficult to source species that are capable of establishing persistent populations, particularly in small ponds, wells and troughs (Tiwari, 1989; Chandra *et al.*, 2008) – something that guppies excel at (Chapters 3 and 4).

Currently the use of biological control is largely unregulated; most countries lack specific legislation relating to biological control introductions (Simberloff & Stiling, 1996). It can be argued that if fish are successful at reducing malarial incidence, in effect saving lives, then this outweighs the risk of ecological impact. Bioeconomic models set out to evaluate this more quantitatively, and some have been designed to take into account the costs and benefits of species introductions and compare these with the costs of management and control; incorporating direct monetary impacts as well as those with non-market values such as ecological damage and human health benefits (Choquenot *et al.*, 2004; Leung *et al.*, 2002). However, such models can only be used with confidence if information regarding the costs and benefits is available. This thesis identified effectiveness as biological control agents and extent of negative ecological effects as two important gaps in our knowledge of introduced guppies; expanding what is known in these areas will enable more useful cost benefit analyses to be applied to poeciliid introductions.

7.5 Conclusions and recommendations

Emerging as an issue of great concern is the release of pet fish (Duggan *et al.*, 2006; Padilla & Williams, 2004). Chapters 3 and 4 clearly demonstrated that it takes only a few guppies, or even a single individual, to found a self-sustaining population. Given their shared reproductive traits, other poeciliids are likely to also possess this ability (Chesser *et al.*, 1984). As aquarium release fish are usually introduced directly into natural habitats, and given that this route of introduction is notoriously difficult to regulate, it seems that reducing the number of aquarium releases would be an effective target for minimising the negative impact that introduced poeciliids have on native species and ecosystems (Copp *et al.*, 2005*a*). One approach could comprise of increasing public awareness about invasive species (Copp *et al*., 2005*b*). This could be achieved by educating fish owners about ethically and environmentally sound alternatives to releasing unwanted fish into the natural environment, such as returning them to the vendor for re-sale, donating them to public institutions with display tanks, or by performing humane euthanasia using iced water (USGS, 2010). It may also be beneficial to introduce legislation, requiring pet shop owners to provide their customers with more information

on which species of ornamental fish are invasive and their potential ecological impacts if released (Chang *et al*., 2009; Duggan *et al*., 2009; Padilla & Williams, 2004).

This thesis has demonstrated for the first time that single female guppies are capable of routinely establishing new populations. It seems that this ability is not dependent on polyandry and also does not seem to be affected by variation in life history characteristics between distinct wild populations. Guppies may still have a role to play in reducing mosquito-borne disease (Ghosh *et al*., 2005; Kusumawathie *et al.*, 2008). However, the findings of this thesis emphasise the critical importance of further research to explore the factors that might affect both their efficacy as mosquito control agents and their impact on natural ecosystems.

7.6 Future research

Chapter two identified the impact of guppy introductions and the effectiveness of the guppy in mosquito control as two important gaps in our knowledge. I will also discuss preliminary work that has already begun using mesocosms and computer simulations to extend the investigations of colonisation ability in the guppy, and how these might develop in the future.

The impact of guppy introductions

Reports of the negative impacts of guppy introductions are largely anecdotal and extremely varied, including reductions in diversity, declines in particular species and habitat degradation. However, it is extremely difficult to examine the effect of a guppy introduction in isolation from the many other temporally correlated factors that also have the potential to affect the ecosystem. The negative effects of *Gambusia*, the introduced predatory poeciliid, are well documented. The guppy is less aggressive and predatory than *Gambusia*, but its rapid reproduction means that it might compete for food and space with native species, and its broad diet means that even when introduced to control a specific target species, it is likely to have an impact on non-pest aquatic invertebrates. Both of these could lead to ecosystem-wide effects. Such impacts could be explored either using a mesocosm set up or by conducting natural experiments (see Chapter 2, Box

2.1). Findings would help inform future risk assessments and decisions regarding the introduction of poeciliids for mosquito control and would leave aquarists better informed about the implications of releasing pet fish into natural habitats.

The effectiveness of the guppy in mosquito control

The effectiveness of the guppy in the control of mosquitoes remains controversial. A useful extension of the studies described in this thesis would be mesocosm tank experiments (see Box 6.1), which would help bridge the gap between behaviour observed by individual fish in the laboratory and findings from villages where guppies have been introduced. These could be used to pinpoint the conditions and optimum number of fish that should be introduced or maintained in water containers for maximum biological control efficiency. The most important measure of success in such experiments would be the number of mosquito larvae that survive long enough to pupate, rather than focusing on predation at particular instars as in most laboratory studies.

Colonisation ability

This thesis has demonstrated that the guppy has a remarkable ability to colonise. The mesocosms resemble tanks into which they might be introduced for mosquito control, but it would be interesting to examine how this colonisation ability is affected in a more naturalistic scenario - such as those including interspecific competitors or predators. An extension of the mesocosm study described in Chapter 3 is already underway at the University of the West Indies in Trinidad. Here different founding ratios of *Poecilia sphenops* (another invasive poeciliid) and guppies have been introduced into tanks, with the aim of examining the importance of competition to colonisation success in both species.

The extensive body of work relating to the evolutionary ecology of the guppy provides an ideal opportunity to investigate the evolutionary processes involved in invasions. Here, I demonstrated that phenotypic traits were largely unaffected by a severe demographic bottleneck. It would be interesting to investigate whether such a bottleneck might reduce

the ability of the population to adapt and persist in changing conditions relative to a population that has not recently undergone a demographic bottleneck.

Population Viability Simulations

One tool that lends itself to the investigation of such a well-studied invasive species is population viability analysis. Although most often applied to modelling the extinction probabilities of endangered or reintroduced species, it equally has the potential to be applied to establishment probabilities of introduced invasive species. 'Vortex' (Lacy *et al.*, 1993) is freeware produced by the Chicago Zoological Society and allows the input of parameters relating to life history characteristics, and other statistics such as number of founders, mortality rate and inbreeding coefficients, which it uses to generate probabilities of establishment and persistence over a set time frame.

It is possible to take advantage of the huge body of existing knowledge concerning the guppy in order to make important advances in the study of invasive species in general. The application of population viability analysis illustrates just one of the ways in which this can be capitalised upon, as such simulations are critically dependent on the parameter values being both available and accurate, which is not the case for most invasive species. I hope that this thesis marks the start of what becomes a productive branch of poeciliid research, with others taking advantage of what I believe is a unique opportunity to make progress in the field of invasion biology.

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Appendices

Appendix I

Email questionnaire

Subject: Invasive Guppies: Worldwide Survey

Dear [insert name],

We are hoping you might be able to help us with our work on invasive freshwater fish.

Specifically, we are keen to document the distribution and origin of invasive guppies (*Poecilia reticulata*) worldwide. Although this freshwater species is native to Trinidad and northern South America, it is now found in every continent – with the exception of Antarctica. They have been introduced for a variety of reasons, including their deliberate introduction as mosquito control agents and their incidental introduction as unwanted aquaria fish. Similarly, mosquitofish (*Gambusia* spp.), have also been widely introduced in order to reduce mosquito populations.

Despite much being known about the fish themselves, very little is known about the origins and reasons behind the introductions and the spread of these invasive species. We hope to construct a comprehensive database combining information from around the world to enhance our understanding of invasive guppies and mosquitofish, and of invasive species in general.

We would be extremely grateful if you could spare a few minutes to answer the following questions:

1)   Are you aware of the presence of guppies or mosquitofish in streams, rivers and ponds in [insert country/region]? (If not, please proceed to question 5). If possible, please specify regions or particular locations.

2)   If so, are the fish found throughout the region, or in localised parts only?

3)   Do you know anything about the origin of the introduction(s)? For example, when, where and/or why they were introduced?

4)   Are you aware of any negative effects on habitats, ecosystems or other fauna in areas where the fish are found (e.g. predation on/competition with native species)?

5)   Can you think of any other information regarding guppies or mosquitofish in your region that might be of interest to us?

6)   Finally, if you can suggest any useful contacts that may help us with our enquiries we would be very grateful if you could let us know their name and/or contact details.

Our goal is to produce an online map showing the contemporary distribution of guppies and we will of course acknowledge your help in contributing records (including negative ones) to it.

Many thanks for your help,

With best wishes,

Amy Deacon and Anne Magurran

http://www.st-andrews.ac.uk/~guppy/

Appendix II

Photographs of guppies from introduced populations

A collection of photographs of guppies from introduced populations around the world.

a)

b)

c)

d)

e)

f)
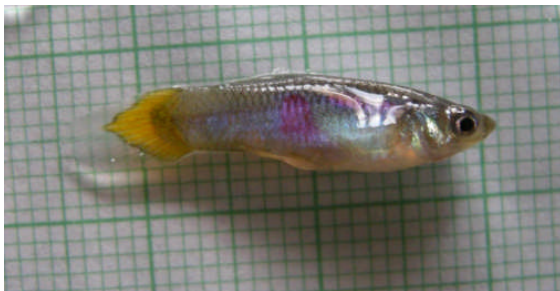
Figure III: a) male *P. reticulata* from Lake Baringo, Rift Valley, Kenya. b) Female with offspring from Lake Naivasha, Rift Valley, Kenya; c) Male and d) female specimens from Malaysia; e) Male from Karnataka, India and f) a tank of guppies in a garden in Kerala, India (the owner told me they were gathered from a local stream); g) Male and h) female specimens from the island of Rodrigues, Western Indian Ocean; i) Male and j) female guppies from a recently founded feral population in Tefe, Brazil; k) Sample of guppies and i) a male guppy from Brazzaville, Congo. Photographs courtesy of Dr Rob Britton (a, b), Amir Ahmad (c, d), Dr Olaf Weyl (g, h), Professor Anne Magurran (i, j) and Dr Victor Mamonekene (k, l).

Appendix III

Continent-by-continent summary of introduced guppy populations

i) Africa

Countries present

Algeria (#108b), Comoros Islands (#60), Democratic Republic of the Congo (#108a), Ghana (#14), Kenya (#10), Madagascar (#54), Mauritius (#43), Mayotte(#60), Morocco (#108c), Namibia (#18), Nigeria (#28), Republic of the Congo (#108a), Reunion (#60; #112c), Rodrigues (SABIF), South Africa (#22; #37; #51), Uganda (#64; #94f), Zambia (Froese & Pauly, 2009).

Countries absent

Despite a self-sustaining population in a hotel pond in Lilongwe, **Malawi**, which has been there since at least the late 1980s, guppies have not been found in streams and ponds in the north of the country. However, southern Malawi has been less thoroughly sampled (#4).

Guppies also appear to be absent from **Egypt** (#59), **Eritrea** (#65) and **Gabon**. This is despite extensive sampling in the latter (#64).

Origins

In 1988, guppies were found for sale at a local fish market in **Madagascar** (Lever, 1996). Reports suggest that they were naturalised here by the early 1960s, probably originating from aquarium releases. They are now found in lakes and ponds on the Parc Zoologique et Botanique de Tsimabaza in Antananarivo and Parc Zoologique d'Ivoloina near Toamasina, and also in the low altitude crater lakes of the Massif d'Ambre in the extreme North, and the middle and lower reaches of the streams draining its northern and eastern slopes (#54).

Guppies were introduced to **Namibia's** interior water bodies from an unknown source for ornamental purposes (#18) and are now thought to persist at Kuraman Oog and Lake Otjikoto sinkholes, although it is not known if these populations have since spread (De Moor & Bruton, 1988).

In 1912 guppies were reportedly imported to Jonkershoek fish hatchery in **South Africa** from Barbados for mosquito control purposes, but failed to survive the winter (#37). Since then, fish have been frequently escaped or been released by aquarists (#22), possibly with some also being released for the purpose of mosquito control (#37). Populations appear to be reasonably localised (#22), and their presence in natural water bodies appears to remain restricted to Northern Natal (Kranzkloof and Kenneth Stainbank nature reserves) and Empangeni, Zululand. De Moor and Bruton (1988) predicted that populations would spread to penetrate the floodplains of Umfolozi, Mkuze, Phongolo, Maputo, Incomati and the Limpopo river with potentially harmful consequences (#25; de Moor & Bruton, 1988).

Ugandan guppies were introduced from the USA in 1948 to control mosquitoes (Lever, 1996). In lakes and large ponds this proved unsuccessful, as they were heavily predated upon by larger native fish, however, they soon became established in small streams and ditches (#68). Populations are reported from a number of streams several kilometres apart, indicating that they may be widespread. Locations include Bugungu stream that flows to Jinja, near the source of the Nile, as well as a number of Budongo forest streams flowing into Lake Albert, a giant papyrus swamp in Kibale National Park and also in several crater lakes (#94). In the 1970s they were abundant in Lake Victoria near Jinja, especially near cremation ghats, although this situation may have changed since Idi Amin expelled the Asian community (#54). In 1956, guppies were introduced from Uganda to **Kenya** for the purpose of mosquito control (Courtenay, pers. comm.; Welcomme 1981). Here, they established populations in the Tana River and Lake Naivasha and also in Lake Baringo (Lever, 1996; #10).

Guppies were introduced to **Nigeria** for mosquito control during the colonial period (#28) and then again in 1972 for teaching ecology (Courtenay, pers. comm.; Welcomme, 1981). They are now are found around Lagos and Port Harcourt in the South, with indications that they may be moving inland, as they have already been found at least 30km from Lagos (#28). In Ghana, guppies occur in drains and small streams all over the country, having being introduced as mosquito control agents (#14). Populations are found throughout the Niayes zone along the coast from Dakar to St Louis in Senegal, after being introduced for mosquito control. However, they remain largely restricted to ponds and tanks with little opportunity to reach rivers (#15).

Guppies were introduced to Mauritius in around 1920, and soon became established in streams, ponds, lakes and marshes (Lever, 1996). Populations from South America were also introduced to the island of **Reunion** in the 1950s (#60), and then subsequently a population was transferred to the island of **Mayotte** in 1980 for the purpose of a mosquito control study. Similarly, in 1988, guppies were also introduced to **Grande Comore** for the same purpose, primarily added to mosque cisterns and washing basins. They proved hugely successful in this role and were later also introduced to Moheli and Anjouan, where they have spread through the water course (#60). Guppies have also been collected from the island of **Rodrigues** (SABIF, 2009).

Guppies were found in streams around Kitwe, **Zambia** in 1980, and it is possible that a permanent population may have established in Kafue swamp. They also exist in aquaculture ponds near Lake Kariba, but persistence outside of the ponds is considered unlikely (Thys van den Audenaerde**,** 1994).

In Central Africa, guppies were introduced to Brazzaville, **Republic of Congo** in 1985 during a malarial control workshop and are now found in the marshy areas and channels that surround the city. However, they do not seem to have spread beyond these localised areas and have also had little impact on mosquito abundance, probably due to high levels of cover in these channels. They have also been recorded from the Nsele river near Kinshasha in the **Democratic Republic of Congo** (#108a).

They are also reported as present in **Algeria** and **Morocco**, although little is known about these introductions (#108).

ii) Asia

Countries present

Hong Kong (#57), India (Lever, 1996; Raghavan *et al*., 2008), Indonesia (Lever, 1996), Israel (#77), Japan (#106), Malaysia (#6), Pakistan (#42), Russia(#11), Saudi Arabia (Froese & Pauly, 2009), Singpore (Lever, 1996; Khoo *et al*., 2002), Sri Lanka (Lever, 1996), Taiwan (Liang *et al.*, 2008), Thailand? (Nico *et al*. 2007), United Arab Emirates (Bartley, 2006).

Countries absent

They were introduced to **Bangladesh** from Thailand in 1957 for research purposes, but it is unknown if they managed to subsequently establish in the wild. Although present in **Laos** along parts of the Lao-Thai border, guppies have to be restocked annually in order to persist overwinter and continue to be a mosquito-control agent (fishbase, 2009). Guppies were also reportedly introduced to **Myanmar** unsuccessfully (Lever, 1996).

There are no reports of guppies in the wild in **China**, despite being a very common aquarium species here (#120).

Guppies are bred on fish farms at Abu Al Kasib, south of Basrah in **Iraq** and although no feral populations are known, they may be or become established (#99). They are also popular as aquarium fish in Iran, but no invasive populations are known (#16). Ongoing sampling of fish biodiversity in Lebanon has not revealed any guppies as of yet (#17) and there is also no recorded presence in Kyrgystan (#61) or Turkey (#67).

Origins

Feral populations are established in **Russia**, as a result of aquarium releases. They are restricted to areas with artificially warmed water, and are found near a number of towns

and cities including Moscow, and also in drainages of the Volga and Upper Dom Rivers (#11).

In **Israel**, guppies probably arrived as discarded pets, as the ornamental fish trade is prolific in Southern Israel (#77; #80). Populations can now be found in a small isolated spring near Kibbutz Kfar Szold in Upper Galilee (#81) and also in the Beit Shean valley in northern Israel, where they are most likely present in both natural and artificial habitats (#77; #80). Guppies are also established in the United Arab Emirates and Saudi Arabia but very little is known about the origins of these feral populations (Bartley, 2006).

Guppies were first imported to Madras, **India**, in 1908-1909 by Major Selley, but reportedly all died out (Chandra *et al*. 2008). However, a thriving population was discovered in 1946 in a temple water tank, from which they were subsequently transferred to other localities in Madras. Now they can be found in ponds in Ranchi, Bihar, Rameshwaram, Thanjuvar, Madras city, Malabar, Guddapah, Kurnool and elsewhere in Southern India – as well as having been recorded in the Kulbhor River near Loni in Maharashtra (Lever, 1996). They have also been recorded in the Chalakudy river in the Western Ghats of Kerala (Raghavan *et al*., 2008).

They are also present in **Pakistan**, with a distribution which is restricted to coastal areas (#42).

Guppies were introduced to **Sri Lanka** in the 1920s and 1930s (#86; Lever, 1996) in order to control mosquitoes (#91). They are found in many river and lagoon locations around the south of Sri Lanka – all in the wet zone (#86), and are abundant in highly polluted canals in cities such as Galle, Colombo and Kandy (Welcomme, 1988).

Many parts of **Japan**, such as Northern Kyoto are too cold for guppies to persist. However, localised populations have been reported from the Ryukyu Islands and several other areas where there are hot springs (for example Honshu and Kyushu). As they are an

extremely popular aquarium species here it is likely that introduced populations are the descendents of released pets (#106).

The guppy is said to be 'firmly established' in the waterways of **Singapore**, having been first introduced in 1937 from South America for the purpose of mosquito control (Chou & Lam, 1989; Khoo *et al*., 2002). Since then, both mosquito control and released ornamental guppies have probably contributed to feral populations (Ng *et al*., 1993). Rapid urbanisation beginning in the 1980s has led to the fragmentation of feral populations in Singapore (Khoo *et al*., 2002).

Guppies are widespread in ditches, canals and drain systems throughout **Malaysia** (#6) originating from both aquarium releases (#55) and introductions from Venezuela, Brazil and Singapore for mosquito control (Ang *et al*., 1989). They have been seen or collected in Kuala Lumpa (#6), Penang Island, the northern part of Langkawi island, eastern Terengganu, De Bana, Bahau, Negeri Sembilan and central Pahang Peninsula (#55). In Indonesia, guppies were introduced for mosquito control in 1920, and soon escaped into streams and ponds around Bandung, West Java (Lever, 1996).

In **Hong Kong**, guppies are thought to have been introduced in the 1940s by aquarists, although it could have been earlier than this. They are now ubiquitous, although some believe that they may be on the decline as a result of the increasing dominance of *Gambusia* (#57). Feral populations of guppies have also been recorded in **Taiwan**, probably originating from ornamental releases as they are extremely popular pets here (Liang *et al.*, 2008).

In 1920 guppies were introduced to **Indonesia** as a mosquito control measure. However, they escaped into ponds and open waters in Bandung, West Java and have instead competed with plankton feeding native species, whilst failing to have an impact on the mosquito problem (Eidman, 1989).

iii) Australasia

Countries present

Australia (Northern Territory (#3), Queensland) (Lever, 1996), Cook Islands (Lever, 1996), Fiji (Lever, 1996), French Polynesia (Lever, 1996), Guam (Lever, 1996), New Caledonia (Lever, 1996), New Guinea (#2), New Zealand (Lever, 1996), Palau (Lever, 1996), Papua New Guinea (Lever, 1996), Philippines (#26), Samoa (Lever, 1996), Vanuatu (Lever, 1996).

Countries absent

Feral guppies have never been reported from Tasmania (#5, #40, #41), New South Wales (#8), or Australian Capital Territory (#9) probably due to unsuitable temperatures (#40).

In **Tonga**, *P. mexicana* is used as a successful mosquito control agent and guppies do not appear to be present (#36). In the **Maldives** there are no freshwater bodies that are inhabitable by fish, but guppies have been introduced to household wells for mosquito control. Formerly this was a widespread practice, but a move towards closed wells and alternative water sources in urban areas now limits their use (#13).

Origins

There have been multiple introductions of guppies to **Australia**, but they were most probably first introduced around 1910, during a Colonial Office effort to control mosquitoes in various colonies in the tropics (Lindholm *et al.*, 2005). By 1929 the guppy was included on a list of introduced mosquito-eating fish in the country, and it is thought that they were further spread for mosquito control during the Second World War (Lindholm *et al.*, 2005). These introductions were compounded by unintentional introductions in the 1960s by aquarists (#38), and at least one population in the North Johnstone river almost certainly originated from an old aquaculture facility (#56). Most populations have reverted to wild colouration, but occasionally 'delta tail' males are found, suggesting re-releases of aquarium stock (Lever, 1996). Today, guppies are found in Queensland and the Northern Territory. Within Queensland they are widely distributed and found in locations including Cairns, Innisfail, Ingham, Mackay, Rockhampton and in Ban Ban Springs near Gayndar (Lever, 1996). They are also found locally near Barambah

springs near Burnett River and further south. (see Lindholm *et al.* 2005). Guppies were formerly widespread around Brisbane, but in 1981 were reduced to a relic population in Seven Hills Creek (Lever, 1996).They are typically not abundant and tend to be restricted to slow flowing margins of streams, usually in disturbed habitats. In the Northern Territory, guppies are restricted to the Darwin area, where they are found as localised populations in the Ludmilla creek and sporadically elsewhere in the suburbs (#3). It has been observed that guppies appear to be spreading southwards, whilst Mosquitofish are encroaching northwards, and will be of great interest to observe what happens in areas where they overlap (#21).

It is unclear exactly when guppies were introduced to **New Zealand**, but they were certainly one of the earliest warm-water introductions here and there has been a population around Reporoa, central North Island, possibly since as early as the 1920s (Lever, 1996). They also used be found in the Waipahihi on the North Island, but are now confined to a few small geothermal streams of the volcanic plateau near Reporoa from where it periodically spreads downstream to occupy waters along the margins of the cooler Waikato river (#53; McDowall, 1999). The range in these streams seems not to be expanding (Froese & Pauly, 2009).

Guppies were reported from Port Moresby, **Papua New Guinea** in 1967, and have since spread to the Waigani swamp, which is part of the Laloki River system. They are believed to be descendents of released aquarium fish (Lever, 1996).

The guppy is the most widespread Poeciliid in **Fiji**, having most likely originated from aquaria releases (#1). It is now found on the main islands of Viti Levu and Vanua Levu in at least 50% of catchments, but has not yet reached Taveuni (#1). In Eastern Viti Levu it is heavily preyed upon by *Hypseleotris guentheri* (Froese & Pauly, 2009).

Guppies are present in streams and ponds in the **Cook Islands** (#2), and are particularly common on Rarotonga but are also present on islands of Atiu, Mauke and Mitiaro and

probably Mangaia. Anecdotally it seems that they were introduced pre-1980 for mosquito control (#63).

Vanuatu (#1), **New Caledonia** (#1), **Palau** (#2), **French Polynesia** (#2), **Guam** (#2), **Samoa** (#2) and **New Guinea** (#2) all support populations of introduced guppies in streams and ponds. In most cases their origin is unknown, although in Vanuatu and New Caledonia it is likely to have been the result of aquarium releases and in Palau it was introduced by the Japanese for mosquito control between 1914 and 1944 (Lever, 1996). In French Polynesia, guppies are found on the islands of Tahiti, Moorea and Tubauï (FishBase, 2009).

Guppies were introduced to the **Philippines** from Hawaii in 1905 for mosquito control purposes (#26, #70) and are now found in streams, rivers, canals and stagnant water bodies all over the country, especially on the islands of Luzon, Visayas and Mindanao. Specifically, they are known from Lake Taal, Lagu Lagu and Hilosig Creeks, eastern Tunga and the Ambacan River at Makinhas and Butihan, Leyte (#26).

vi) Central America and Caribbean

Countries present

Barbados (#44), Costa Rica (#97; #101), Cuba (Lever, 1996), Dominican Republic (Lever, 1996), Guadaloupe (Lever, 1996), Haiti (Lever, 1996), Jamaica (Lever, 1996), Martinique (Lim *et al.*, 2009), Nicaragua? (unidentified *Poecilia* spp., #93), Panama (#83), Puerto Rico (Lever, 1996), US Virgin Islands (Nico, 2009).

Countries absent

Countries where guppies have not been found despite the occurrence of considerable sampling effort include the **Bahamas** (#94), **Belize** (#98), **Honduras** (#96) and **El Salvador** (#84). However, unidentified species of Poeciliid have been reported from El Salvador, at Ichanmichen, Zacatecoluca, La Paz, and it is possible that these include *P. reticulata* (#82).

Origins

Guppies have been reported from the streams, rivers and polluted ditches of San Jose and the Cartago region of **Costa Rica** since 1962 (#101), having most probably been introduced from Venezuela (#101), but have not been reported from more rural or lowland locations (#97). It is assumed that populations originated from aquarium releases (#101).

First reports of guppies on **Barbados** date back to 1861 and although they are thought to have been introduced, there is a small possibility that guppies might be native here (#44). They are abundant in streams and ponds throughout the island.

Guppies were released onto the Isla de la Juventud, **Cuba**, for the purpose of mosquito control at an unknown date (Lever, 1996).

Puerto Rican guppies are thought to have been accidentally been introduced from aquaria in 1935, and in 1946 they were found in Adjuntas, Aibonito, Comerio and Cayey where they were reported to prey upon *Schistosoma mansoni* (Courtenay pers. comm.; Lever, 1996). Today they are one of the three most ubiquitous fish on the island, occurring in 50 of 81 sites sampled and at a mean density of 2,911 fish per hectare (#94; #95). It is not known when guppies were introduced to **Haiti** and the **Dominican Republic** but they are now common in lowland and mountain streams (#94).

Guppies in **Jamaica** are likely to have originated from the extensive local aquarium trade (#66) and are now found in localised pockets throughout the island in lowland streams. Other scientists have failed to come across guppies during sampling (#94).

In **Panama**, guppies were released for mosquito control during the construction of the canal (1900-1912) under the orders of the Canal authorities, and are now found in many rivers and lakes (#83).

iv) Europe

Countries present

Albania (Crivelli, 1995), Canary Islands (Lever, 1996), Czech republic? (Holcik, 1991) Germany? (#119), Hungary? (Holcik, 1991; #72) The Netherlands (#92; #72), Slovakia (#79), Spain (Elvira & Almodóvar, 2001).

Countries absent

Guppies are thought to be absent from the **UK**, **Iceland** (#112), **Montenegro** (#90), **Cyprus** (#88), **Greece** (#85), **Italy** (#24,#29,#30), **Portugal** (#69, #74,#73) and the **Azores** (#71).

In **Italy** they are commonly for aquarium trade in the Lazio region, but have not ever been found during river sampling. Other than artificially or geothermally heated stretches of water, winter conditions would probably not be suitable for guppies (#24, #29, #30). The same is true of **Portugal** (#69, #74, #73).

In 1963 and 1965, two populations were established in **England**. Both were in locations where water temperature was elevated as a result of factory discharge and have since died out (Lever, 1996; Courtenay pers. comm).

In **Germany**, guppies have been introduced by aquarists to a few specific locations, but such populations are not considered to be self-sustaining, more likely relying on re-introductions (#119).

Reports of guppies near Ljubljana in **Slovenia** are thought to be a case of misidentification, and in any case are not present at this location any more (#72).

Origins

In Europe, the majority of introductions have been as inadvertent aquaria releases. The climate is generally not warm enough for guppies to overwinter, and most self sustaining populations are associated with artificially or geologically elevated water temperatures, for example in thermal ponds or in streams near to power stations. For this reason they

tend to be very localised, with little potential for spread. It also appears that reports from some countries are of a particularly anecdotal nature, and this may be due to the ephemeral nature of populations in such a climate.

In 2000, aquarists released guppies in the Mijares river, **Spain** and they are now considered to be an acclimatised introduced species here (Elvira & Almodóvar, 2001). In 2002 there was also a record of another population located between Barcelona and Valencia, but it is not known if it has persisted (#75).

Guppies have also been found at some thermal ponds around Bojnice, **Slovakia** and also self-sustaining in the Teply Potok creek (for a 2km stretch), after being released by aquarists most likely in the 1960s (#79).

Ten locations for populations have been recorded in the **Netherlands** (#87), including in the Noordzeekanaal, as a result of aquarium releases, most of which are associated with warm water outlets near power plants. However, the climate is too cool to allow any spread from these localised populations (#92).

There are some anecdotal reports of guppies present at an industrial site near Koeln, **Germany** (#72). There are also reports of established populations in **Albania** (Crivelli, 1995), the **Czech Republic** (Holcík, 1991), and in **Hungary** (Holcík, 1991), although they are very localised and rarely found. Guppies in Hungary arrived primarily from ornamental releases but also from mosquito control efforts; the first introductions here took place in 1924 (Holcík, 1991).

vi) North America

Countries present

Canada (Courtenay, pers. Comm), Mexico (Lever, 1996), USA (Arizona, California, Colorado, Conneticut, Florida?, Hawaii, Idaho, Missouri, Montana, Nevada, New Mexico, Texas, Utah, West Virginia, Wisconsin, Wyoming) (Lever, 1996; Nico, 2009).

Countries absent

Domestic guppies have been introduced several times in dairy and poultry waster lagoons in California for the purpose of mosquito control. However they seem to be unable to overwinter and thus are not considered to be permanently established (Courtenay, W. pers. comm.). In the past guppies have been collected in Florida, thought to be associated with aquarium releases. However, none have been found in recent years and they have never been considered to be permanently established here (#78, #45).

Although they are found in several different states in Mexico, guppies seem to be absent from the Yucatan peninsula despite heavy and frequent sampling. Perhaps because *Poecilia velifera* was introduced as a mosquito-control agent here instead (#103).

Origins

In the USA and Mexico guppies have been released primarily as unwanted aquaria fish (Lever, 1996), but populations in California and Hawaii probably originate from a combination of mosquito control and aquaria releases.

In the USA the main cause of guppy introductions is the release of aquarium fish, aside from some of the California releases which were for mosquito control (Nico, 2009). It is thought the Nevada population was probably established in 1957 (Deacon *et al*., 1964) and it was still persisting in Clark County in 1980 (Courtenay & Deacon, 1982). In Texas they are found in a stenothermal ditch draining into the San Antonio river (Nico, 2009).

The guppies in Hawaii were introduced to Oahu in 1922, and became established in streams flowing through Honolulu as well as on the windward side of the island. Subsequent introductions took place in Kauai, Maui and Molokai, and thriving populations now exist on all five major islands (Lever, 1996).

A localised population of guppies near some hotsprings in Alberta, **Canada** was thought to have died out in 1976, however, Courtenay found they were still present in 1995 (W. Courtenay, pers. comm.).

Many introductions have taken place in **Mexico**, largely in the form of aquarium stocks being released. Locations include Parras and Monterrey as well as several parts of the Rio Balsas (#103) and upper Rio Panuco around Lago de Chapala, Laguna Cortes and Todos Santos, Cabo San Lucas and also in Baja California (Lever, 1996). Despite being numerous, populations seem to be relatively localised (#103). In Mexico, guppies have been found both in straight domestic sewage and also in pesticide-polluted sewage (#76).

v) South America

Countries present

Brazil (#23), Colombia (Lever, 1996; Welcomme, 1988), Paraguay (#49), Peru (Lever, 1996; Welcomme, 1988).

Countries absent

Despite being a popular aquarium fish in **Uruguay**, guppies are unable to survive winter temperatures (#47). Feral guppies are also not known in **Argentina** (#48) or **Chile** (#102).

They were introduced to Santa Cruz, **Bolivia** in the 1970s by a Japanese corporation to reduce mosquito populations, and for a time were widespread throughout the city. However, during the late 1980s, rapid urban growth cut off the streams and the fish disappeared (#62).

Origins

Guppies were introduced to **Peru** in 1940 for mosquito control (Welcomme, 1988) and in 1958 were found in creeks leading to beaches of Lima and around the southern parts of the city. They also occur in the swamps of La Villa near Lima (#34), in the puddles and small streams of Iquitos, in rivers from Tumbes to Ica, in the jungle (northeast) upper river Mayo in Departamento San Martin and in the jungle (central), Satipo, Dept. Junin (#35; Ortega & Vari, 1986; Ortega et al., 2007). They are also found around the Peruvian Amazon basin and in areas along the coast until Ecuador. In the cooler, more arid South, guppy distribution and spread is limited (#34). **Colombian** guppies were also first

introduced in 1940 as mosquito control agents, and in 1981 were reported as 'widespread throughout warmer zones', especially the Magdalena and Orinoco watersheds (Courtenay pers, comm.; Welcomme, 1988).

A major introduction of guppies to **Brazil** occurred at the start of the 20th century, but they have probably been continuously introduced both for mosquito control and by aquarium hobbyists since then (#23). They are now abundant around urban and degraded areas, such as Rio de Janeiro and Sao Paulo and their surrounds as well as in urban areas of Amazonas state (#100). Localised populations of guppies are found in parts of **Paraguay**, including at Asuncion bay, San Lorenzo and San Bernardino, where they persist even in street drains and polluted streams (#49).

Appendix IV

List of countries reporting the presence or absence of guppies

Country	Guppies?	Date of first introduction	Purpose
Albania	Yes		
Algeria	Yes		
Australia	Yes	1960s	MC+AQ
Barbados	Yes	1861	
Brazil	Yes	1900s	MC+AQ
Cambodia	Yes		MC+AQ
Canada	Yes		AQ
Canary Islands	Yes		
Colombia	Yes	1940	MC
Comoros	Yes	1988	MC
Cook Islands	Yes		MC
Costa Rica	Yes	1962	AQ
Cuba	Yes		MC
Czech Republic	Probably		
DR of Congo	Yes	1985	MC
Dominica	Yes		MC+AQ
Dominican Republic	Yes		
Fiji	Yes		AQ
French Polynesia	Yes		
Germany	Yes		AQ
Ghana	Yes		MC
Guadeloupe	Yes		MC
Guam	Yes		
Haiti	Yes		
Hawaii	Yes	1922	MC
Hong Kong	Yes	1940s	AQ
Hungary	Probably		
India	Yes	1908	MC
Indonesia	Yes	1920	MC
Israel	Yes		AQ
Jamaica	Yes		AQ
Japan	Yes	1974	AQ
Kenya	Yes	1956	MC
Madagascar	Yes	1960s	AQ
Malaysia	Yes		MC+AQ
Martinique	Yes		
Mauritius	Yes	1920	
Mayotte	Yes	1980	MC
Mexico	Yes		AQ
Morocco	Yes		
Namibia	Yes		AQ
Netherlands	Yes		AQ
New Caledonia	Yes		AQ
New Zealand	Yes	1920s	
Nigeria	Yes	Colonial & 1972	MC
Pakistan	Yes		
Palau	Yes	1914	MC
Panama	Yes	1900	MC
Papua New Guinea	Yes	1967	AQ
Paraguay	Yes		

Country	Guppies?	Date of first introduction	Purpose
Peru	Yes	1940	MC
Philippines	Yes	1905	MC+AQ
Puerto Rico	Yes	1935	AQ
Republic of the Congo	Yes		
Reunion	Yes	1950s	MC
Russia	Yes		AQ
Samoa	Yes		
Saudi Arabia	Yes		
Senegal	Yes		MC
Singapore	Yes	1937	MC+AQ
Slovakia	Yes	1960s	AQ
South Africa	Yes	1912	MC+AQ
Spain	Probably		
Sri Lanka	Yes	1925	MC
Taiwan	Yes		AQ
Thailand	Probably	1948	
Uganda	Yes		MC
United Arab Emirates	Yes		
US Virgin Islands	Yes		MC
USA	Yes	1957	MC+AQ
Vanuatu	Yes		AQ
Zambia	Yes	1980	AQ
Argentina	No		
Azores	No		
Bahamas	No		
Bangladesh	No		
Belize	No		
Bolivia	No	1970s	MC
Chile	No		
Cyprus	No		
Ecuador	No		
Egypt	No		
El Salvador	No		
Eritrea	No		
Gabon	No		
Greece	No		
Honduras	No		
Iceland	No		
Iran	No		
Iraq	No		
Italy	No		
Kyrgystan	No		
Malawi	No	1980s	AQ
Maldives	No		
Montenegro	No		
Myanmar	No		
Nicaragua	Possibly		
Portugal	No		
Rodrigues	No		
Slovenia	No		
Tasmania	No		
Turkey	No		
UK	No		
Uruguay	No		

AQ=Aquarium releases; MC=Mosquito control.

Appendix V: Table of respondents and summary of responses in relation to guppies.

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
Algeria								
108c	Prof. Jos Snoeks	Senior Scientist & Curator of fishes Zoology Department, Royal Museum for Central Africa, Belgium.	jsnoeks@africamuseum.be	Yes	NFI	NFI	NFI	NFI
Argentina								
48	Pablo Ribla	Aquarist	pecesargentinos@neuf.fr	No	NA	NA	NA	NA
Australia								
3	Helen Larson	Curator of Fishes, Museum and Art Gallery of the Northern Territory, Darwin.	Helen.larson@nt.gov.au	Yes	Darwin – Ludmilla creek and sporadically in suburbs.	NFI	No studies have been done.	The museum holds various specimens of guppies from Australia and S.E. Asia.
2	David Boseto	Freshwater fish biologist, University of the South Pacific, Fiji.	dboseto@yahoo.com	Yes	NFI	NFI	NFI	NFI
5	Leon Barmuta	Senior Lecturer in Zoology, University of Tasmania	Leon.Barmuta@utas.edu.au	No	NA	NA	NA	NA
8	Graham Pyke	Senior Fellow, Australian Museum, Sydney	Contactable from: http://australianmuseum.net.au/staff/graham-pyke/	No	New South Wales	NA	NA	NA
9	Michael Jennions	Australian National University, Canberra.	michael.jennions@anu.edu.au	No	Canberra region	NA	NA	NA
33	Rob Brooks	University of New South Wales	rob.brooks@unsw.ed.au	Yes	New South Wales and Queensland. In many streams, but localised within streams	NFI	Unaware	NFI

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
38	Kevin Warburton	University of Queensland	k.warburton@uq.edu.au	Yes	Queensland coastal areas	Around 1960s, probably unintentional aquaria releases	Impacts not known	Numbers increasing in Brisbane area.
40	Tim Farrell	Inland Fisheries Service, Tasmania	Tim.Farrell@ifs.tas.gov.au	No	Tasmania	NA	NA	NA
41	Grant Scurr	NRM North, Tasmania	Grant.Scurr@dpiw.tas.gov.au	No	Tasmania	NA	NA	NA
56	Brad Pusey	Griffith University	bpusey@westnet.com.au	Yes	Northern Australia and wet tropics region. Specifically in North Johnstone River. Widespread but not abundant.	Aquarium releases.	NFI	Usually associated with riparian degradation in margins and submerged pastures.
Austria								
72	Paul Veenvliet	See 'Slovenia'	See 'Slovenia'	No	There have been guppies and mosquito fish (species not specified) in the past in a thermal stream in Villach, Austria. No longer present due to predation by introduced *Hemichromis guttatus*. Now there are populations of *H. guttatus*, *Amatitlania nigrofasciata* (=*Cryptoheros nigrofasciatus*), *Ancistrus* "sp.3", and *Xiphophorus maculatus* x *X. helleri* hybrids. This is a well known "dump-site" for unwanted fish; single individuals of nearly any other aquarium fish species can be found alongside native fish. The local vegetation consists of various aquarium plants (mainly *Vallisneria spiralis* and *Cryptocoryne* spp.).	NFI	NFI	NFI
Bahamas								
94a	Brian Langerhans	Assistant Professor, North Carolina State University	langerhans@ou.edu	No, despite extensive sampling.	NA	NA	NA	Other poeciliids present
Barbados								
44	Angela Fields	Lecturer, University of the West Indies, Cave Hill.	afields@uwichill.edu.bb	Yes	Ponds throughout the island.	Described in1861 (De Philippi) but cannot exclude possibility that are native.	NFI	NFI

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
Belize								
98	Peter Esselman	Aquatic Ecologist, University of Michigan.	esselman@umich.edu	No	NA	NA	NA	Commonly sold as pets, so future introductions possible.
Bolivia								
62	Gonzalo Flores	Programme officer, FAO	fao-bo@fao.org	No	NA	NA	NA	Introduced by Japanese company for mosquito control in 1970s and persisted until late 1980s.
Brazil								
23	Paulo Buckup	Museu Nacional Quinta da Boa Vista, Rio de Janeiro	buckup@acd.ufrj.br	Yes	Particularly abundant in urban and degraded areas, such as Rio de Janeiro, but also widespread throughout in small streams.	Major introduction at start of 20th Century for mosquito control, but suspected continuous introduction by fish hobbyists as well as mosquito control.	Fieldwork indicates strong negative correlation with native fish diversity. Predictive but not cause and effect.	Many specimens in scientific collections in Brazil, usually with GPS locality data. Managesa database at www.mnrj.ufri.br
100	Anne Magurran	Professor of Ecolgy and Evolution, University of St Andrews	aem1@st-andrews.ac.uk	Yes	Found localized around Tefe, and also at Tabatinga, Manaus and Sao Paulo.	Pet shop releases at Tefe.	Unknown.	
Cambodia								
105	So Nam	Adjunct Professor and Director, Inland Fisheries Research and Development Institute (IFReDI)	sonammekong2001@yahoo.com	Yes	Mondulkiri in Northeastern Cambodia in the Srepok river basin.	Aquarium releases and mosquito control	Likely to be competition for food & habitat with native species.	
Cayman Islands								
94d	Brian Langerhans	See 'Bahamas'	See 'Bahamas'	No	NA	NA	NA	NA
Chile								
102	Brian Dyer	Universidad del Mar, Valpariso.	bdyer@udelmar.cl	No	NA	NA	NA	Unable to survive in south due to temperatures, but also no reports from north.

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
China								
120	Chen Xiao-Yong	Associate Professor, Chinese Academy of Sciences, Yunnan.	chenxy@mail.kiz.ac.cn	No	NA	NA	NA	Very common in aquarium tanks but not seen in wild.
Comoros								
60	Sixte Blanchy	Agence française de l'adoption, Paris.	sixte.blanchy@agence-adoption.fr	Yes	NFI	1988, by Dr Blanchy, for mosquito control experiments	NFI	Only effective as control agents in cisterns and wells, not in natural habitats.
108e	Prof. Jos Snoeks	See 'Algeria'.	See 'Algeria'.	Yes	NFI	NFI	NFI	NFI
Cook Islands								
63	Gerald McCormack	Director, Cook Islands Natural Heritage Trust	Gerald@nature.gov.ck	Yes	Common on Rarotonga; present on Atiu, Mitiaro. Not reported on Mangaia but probably present.	Anecdotal reports suggest mosquito control. Pre-1980.	NFI	
2b	David Boseto	See 'Australia'	See 'Australia'	Yes	NFI	NFI	NFI	NFI
Costa Rica								
101	William Bussing	University of Costa Rica	wbussing@biologia.ucr.ac.cr	Yes	Not as common as 40 years ago. San Jose streams and gutters in the highlands. Never in lowlands.	Assume aquarium introductions	Mainly found in contaminated streams. In a few places may compete with native poeciliids and rivulus.	
97a	William McLarney	Aquatic Biologist	anaiinc@dnet.net	Yes/No	Meseta central/urban centre of CR back in 1968. Now works in rural areas but no guppies there.			
Cyprus								
88	Constantinos Moustakas	Dept of Fisheries and Marine Research	cmoustakas@dfmr.moa.gov.cy	No	No native freshwater species apart from European Eel as streams tend to be seasonal	NA	NA	NA
Democratic Republic of Congo								
109	Victor Mamonekene	Universite Marien Ngouabi	mamonekene@hotmail.com	Yes	NFI	NFI	NFI	NFI
108a	Prof. Jos Snoeks	See 'Algeria'.	See 'Algeria'.	Yes	In the Nsele, an affluent of the River Congo close to Kinshasa.	NFI	NFI	NFI

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
Dominica								
110b	Donald Stewart	Professor SUNY College of Environmental Science and Forestry	djstewart@esf.edu	Yes	Three different locations	Probably initially mosquito control and then spread aided by aquaria releases	Effects not studied.	
Dominican Republic								
94b	Brian Langerhans	See 'Bahamas'	See 'Bahamas'	Yes	A mountain stream	NFI	NFI	NFI
Ecuador								
110a	Donald Stewart	Professor SUNY College of Environmental Science and Forestry	djstewart@esf.edu	No	25 years of sampling in Napo River Basin	NA	NA	NA
Egypt								
59	Abdel Rahman	Senior Aquaculture Scientist, Worldfish Centre, Egypt.	aelgamal@worldfish-eg.org	No	NA	NA	NA	NA
El Salvador								
82	Enrique Barraza	Ministerio de Medio ambiente y Recursos Naturales El Salvador	eulaliabarr@yahoo.com ebarraza@marn.gob.sv	No	None in Lake Coatepeque, Sapo or Ilopango.	NA	NA	NA
84	Licenciado Jose Arturo Nunez	Departamento de Ambiente Corte suprema de justicia	jarnu2003@yahoo.com	No	NA	NA	NA	NA
Eritrea								
65	Moeketsi Mokati	FAO representative, Eritrea.	Moeketsi.Mokati@fao.org	No	NA	NA	NA	NA
Ethiopia								
20	Abebe Getahun	Addis Ababa University	Abebe12002@yahoo.com	None known	NA	NA	NA	NA

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
Fiji								
1	Aaron Jenkins	Senior Program Officer, Wetlands International-Oceania	apjenkins@connect.com.fj	Yes	On Viti Levu and Vanau Levu in at least 50% of catchments. Not on Taveuni - yet.	Likely to be aquarium releases	Where poeciliids are found there has been decline in water quality and native species richness compared with non-invaded sites. Probably water quality gives livebearers a competitive advantage. Negative correlation between native forest cover and presence of invasives (i.e. poeciliids and cichlids).	No justification for introduction of livebearers on any Pacific island - native species exist that are in fact better mosquito larvae predators.
2c	David Boseto	See 'Australia'	See 'Australia'	Yes	NFI	NFI	NFI	NFI
French Polynesia								
112b	Philippe Keith	Muséum national d'Histoire Naturelle, Paris	keith@mnhn.fr	Yes	1920s by Mr Guild de Maraa. Likely to be both for MC and AQ now.	NFI	NFI	NFI
2d	David Boseto	See 'Australia'	See 'Australia'	Yes	NFI	NFI	NFI	NFI
Gabon								
64	Carl Hopkins	Professor, Cornell University	cdh8@cornell.edu	No	Despite extensive sampling.	NA	NA	NA
Germany								
119	Carsten Nowak	Conservation Research Section, Senckenberg Research Institute, Gelnhausen	Carsten.Nowak@senckenberg.de	Yes, possibly.	in very few regions, specific locations unknown	Aquaria releases	Not self-sustaining, probably maintained by repeated introductions, negative effects unlikely.	NFI

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
Ghana								
14	Hederick Dankwa	Water Research Institute, Ghana.	hrdankwa@hotmail.com	Yes	All over - especially in drains and small streams	Not sure when, but were introduced for mosquito control	Not known.	Believes they are effective biocontrol agents
Greece								
89	Leonidas Varnakas	Technologist in Aquaculture and Fisheries, Hellenic Centre for Marine Research, Institute of Inland Waters, Athens.	louisvard@gmail.com	No	No documented occurrence.	NA	NA	NA
Guam								
116b	Brent Tibbatts	Division of Aquatic and Wildlife Resources, Guam Department of Agriculture.	brent.tibbatts@gmail.com	Yes	17 rivers and 3 lakes or impoundments	Probably pet trade in 1960s	No consequences seen due to direct competition, but possibly linked to disease outbreak in native species (*columnaris, Lernea*)	Guppies are allowed to be imported. Gambusia are not allowed (by the Department of Agriculture). Mollies, platies and swordtails are also allowed, but are not as widespread as guppies and *Gambusia*.
2e	David Boseto	See 'Australia'	See 'Australia'	Yes	NFI	NFI	NFI	NFI
Hawaii								
117a	Richard MacKenzie	Research Ecologist, Institute of Pacific Islands Forestry, USDA Forest Service, Hawaii	rmackenzie@fs.fed.us	Yes	Not in remote streams. Tend to be in systems near humans.	1920s for mosquito control, but likely to have been additional aquarium releases.	Previous work suggests parasites from poecilliids now affect native gobies and eleotrids.	Currently investigating ecosystem effects using experimental flumes. Wetlands where invasive guppies, mollies and *Gambusia* dominate are strongly correlated with total nitrogen (increasing with density of invasives), as tend to eat lots. All three species excrete more N than natives. Hoping to publish this soon.

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
Honduras								
96	Wilfredo Matamoros	The University of Southern Mississippi Department of Biological Sciences Ichthyology Lab.	wilmatamoros@yahoo.com	No	Despite 5 years of sampling.	NA	NA	NA
Hong Kong								
57	David Dudgeon	**Professor, Division of Ecology & Biodiversity, University of Hong Kong.**	ddudgeon@hku.hk	Yes	Ubiquitous	Mosquito control, 1940s.	No	Suspect that *Gambusia* has eradicated *P. reticulata* when both introduced to same place.
Hungary								
72	Paul Veenvliet	See 'Slovenia'	See 'Slovenia'	Yes	Population reported at Hevis (Balaton).	NFI	NFI	NFI
Iceland								
114	Skuli Skulason	Professor of Fish Biology, Hólar University College, Iceland.	skuli@holar.is	No	No reports, asked many colleagues.	NA	NA	NA
India								
19	Vijay Anand	A Rocha India	india@arocha.org	Not aware	NA	NA	NA	NA
Iran								
16	Afshin Afzali	Department of Fisheries, Faculty of Natural Resources, University of Tehran.	afshin_azali2002@yahoo.com	No	No information available suggesting it is wild in Iran	NA	NA	NA
99b	Brian Coad	Canadian Museum of Nature, Ottawa, Canada	bcoad@mus-nature.ca	No	NA	NA	NA	NFI
Iraq								
99a	Brian Coad	See 'Iran'.	See 'Iran'.	No	NA	NA	NA	Bred on fish farms at Abu Al Kasib (south of Basra) and may become established.

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
Israel								
77	Arik Diamant	Director, National Center for Mariculture, Israel Oceanographic and Limnological Research Institute.	diamant@agri.huji.ac.il	Yes	In Asi Stream, Beit Shean Valley, northern Israel	Probably discarded pets.	Not aware, but likely.	Fancy guppies cultured in Arava valley in southern israel, for export to Europe.
80	Menachem Goren	Department of Zoology, Tel Aviv University	GorenM@tauex.tau.ac.il	Yes	Assi river (=Amal river).	Probably discarded pets.		
81	Dani Golani	Department of Evolution, Systematics and Ecology, The Hebrew University of Jerusalem	dgolani@cc.huji.ac.il	Yes	Small isolated spring near Kibbutz Kfar Szold in Upper Galilee.			*Poecilia velifera* found there also
Italy								
24	Lorenzo Tancioni	University of Rome 2	tancioni@uniroma2.it	Not known	NA	NA	NA	NA
29	Marco Seminara	University of Rome 1	marco.seminara@uniroma1.it	No	NA	NA	NA	NA
30	Marcello Bazzanti	University of Rome 1	marcello.bazzanti@uniroma1.it	No	But has heard some reports to the contrary.	NA	NA	NA
Jamaica								
66	Eric Hyslop	University of the West Indies, Jamaica	eric.hyslop@uwimona.edu.jm	Yes	Not widespread, just in a few isolated pockets	Aquaria releases	NFI	NFI
94e	Brian Langerhans	See 'Bahamas'	See 'Bahamas'	No	NA	NA	NA	NA
Japan								
106	Dr Reiji Masuda	Associate Professor, Fisheries Research Station, Kyoto University	Reiji@kais.kyoto-u.ac.jp	Yes	Ryukyu islands and other hot spring areas.	Popular aquarium species so likely to have been released	Not known	*P. sphenops* is alsp reported from Muroran, Hokkaido, Northern Japan.

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
107	Dr Mizuki Matsunuma	Kagoshima University Museum, Kagoshima	k1139853@kadai.jp	Yes	Hot springs: Aso region, Kumamoto prefecture, Ibusuki region, Kagoshima as well as Ryukyu islands south of Amami-oshima island, and Ogasawara islands. Also recorded from Nitanda and Amori rivers, and cities of Ibusuki and Kirishima, as well as rivers in Kikai island and Amami-oshima.	Mosquito control, First records in Kagoshima province were in 1974. Also may originate from aquarium fish.	Probably predate upon or compete with native *Oryzias latipes* - esp. in Okinawa island.	*Xiphophorus*, *P. mexicana* and *P. sphenops* also all recorded
Kenya								
10	Rob Britton	Scientist on Rift Valley Lakes Project; Senior Lecturer in Conservation Ecology, Bournemouth University	rbritton@bournemouth.ac.uk	Yes	Localised in freshwater lakes. Not aware of any river populations, yet less sampling of rivers than lakes, so remains a possibility.	No. (wild-type in morphology - has photos)	Stable isotope analysis suggests that guppies rely on detrital component of particulate organic matter - other species reliant on zoo/phytoplankton which means competition unlikely.	NFI
108d	Prof. Jos Snoeks	See 'Algeria'.	See 'Algeria'.	Yes	NFI	NFI	NFI	NFI
Kyrgyzstan								
61	Raymon van Anrooy	Fishery Officer, FAO, Sub-regional Office for Central Asia, Turkey.	Raymon.VanAnrooy@fao.org	No	NA	NA	NA	NA
Lebanon								
17	Michel Bariche	Assistant Professor, Marine Biology & Ichthyology, Biology Department, American University of Beirut.	michel.bariche@aub.edu.lb	Not known	NA	NA	NA	NA

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
Madagascar								
54	Paul Loiselle	Curator of Freshwater Fishes New York Aquarium	ploiselle@wcs.org	Yes	Restricted to low altitude crater lakes of the Massif d'Ambre in extreme North and to the middle and lower stretches of the streams draining its northern and eastern slopes. Also lakes and ponds in botanic garden and zoo in Antananarivo…	No. However, were reported as naturalised in 1960s by Kiener. Most likely caused by irresponsible aquarists.	Guppies and *Gambusia* share responsibility for extirpating *Pachyanchax sakaramyi* from the Massif d'Ambre.	
Malawi								
4	George Turner	Professor of Evolutionary Biology and Biodiversity, Dept of Biological Sciences, University of Hull	g.f.turner@hull.ac.uk	No	Only in hotel pond. Have looked for them in streams and ponds but none found.	NA	NA	NFI
Malaysia								
6	Mazlan A. Ghaffar	Associate Researcher, Marine Ecosystem Research Centre, Universiti Kebangsaan Malaysia	mag@pkrisc.cc.ukm.my	Yes	Throughout localities in every state in Malaysia - clear water ditches, small canals, irrigation canals and home drainage systems.	Not known	Not known	Used as feed fish by aquarists
55	Amir Ahmad	University of St Andrews, UK	aa452@st-andrews.ac.uk	Yes	Collected from Penang Is., Langkawi Is., Terengganu (eastern part) and Pahang (central) Peninsular Malaysia. On Peninsular Malaysia, found in many parts. Additional site 17/10/08 - De Bana (stream), near Bahau (town), in Negeri Sembilan (state).	Probably ornamentals, not recorded as for mosquito control.	No record. Tend to occupy places uninhabited by native species or seem to co-exist in other places.	Found in many different habitats, such as monsoon drains in towns, small ditches, streams, creeks, shallow streams, high altitude streams (e.g. Cameron Highland, Pahang >700m). Females grow bigger at high altitudes. Has more data, and attaches photos.

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
Maldives								
13	Hasan Shakeel	Senior Biologist, Marine Research Centre, Maldives.	hshakeel@mrc.gov.mv	Not in natural water bodies	Found only in enclosed household wells, no freshwater bodies that are inhabitable by freshwater fish.	Introduced as aquarium fish and for mosquito control.	No	No established pops in natural habitats. Use of biocontrol diminished as urban centres use closed wells instead of traditional open wells.
Marianas								
116c	Brent Tibbatts	See 'Guam'.	See 'Guam'.	No	NA	NA	NA	NA
122	Steve McKagan	Fisheries Biologist, Saipan.	smckagan@hotmail.com	No	NA	NA	NA	Suspected that there might be guppies, but genetic tests indicate otherwise: *Gambusia* and *P. latipinna* present.
Mauritius								
7	Dr Emily Hardman	Former Scientific Coordinator, Shoals Rodrigues.	Not available	No	No studies on freshwater environments of Rodrigues at all - only a few permanent rivers so doubtful.	NA	NA	NA
43	Chandani Abbadoo	Senior Lecturer, Faculty of Science, University of Mauritius.	chandani@uom.ac.mu	Yes	NFI	NFI	NFI	also *Xiphophorus* present
Mexico								
76	Salvador Contreras-Balderas	Professor Emeritus, Department of Biology, Universidad Autonoma de Nuevo Leon,(Monterrey	saconbal@gmail.com	Yes	several basins and at several localites (see papers) - maybe more as many have yet to be sampled.	No	Yes, appear related to species extirpation/diminishing pops or assemblages. Usually presence related to water overextraction (causing warming & salinisation) and pollution, or other invasives.	*P. reticulata* has been collected twice in straight domestic sewage, one of which included agricultural (pesticide-containing) discharges. Also, see attached/sent papers

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
103	Juan Schmitter-Soto	El Colegio de la Frontera Sur, Chetumal.	juan.schmitter-soto@fulbrightmail.org	Yes	Found in Subbasin Tepalcatepec-Infiernillo of river Balsas basin, Michoacan. None found in Yucatan despite much sampling. Tend to be concentrated close to headwaters at Taretan and San Vicente.	NFI	T-I basin has more exotics than natives. Tilapia probably worse impact, and *Pterygoplichthys*. Hard to demonstrate impact. Cenotes tend to be species-poor.	In Yucatan native species *P. velifera* was introduced for mosquito control in 1930s and 1940s. He is impressed by *P. mexicana* colonisation ability of places such as puddles on streets - where it matures at small sizes. If *P. reticulata* does same, success as invader is no wonder.
104a	Michael Tobler	Department of Wildlife and Fisheries Sciences, Texas A&M University.	michi.tobler@gmail.com	Yes	Rio Pihuamo, near Pihuamo, Jalisco, Mexico (19.263,-103.370). Localised.	Unknown, but there is a tropical fish store in the village of Pihuamo	Other invasive species in region more important.	Guppies not present in great abundance
Micronesia								
116d	Brent Tibbatts	See 'Guam'.	See 'Guam'.	No	NA	NA	NA	NA
117b	Richard MacKenzie	See 'Hawaii'.	See 'Hawaii'.	Yes	Not in remote streams- tend to be in streams near human settlements.	Mosquito control. Not sure of when.	NFI	NFI
Montenegro								
90	Danilo Mrdak	University of Montenegro.	danilomrdak@gmail.com	No				
Morocco								
32	Abdelhamid Azeroual	North African Network for the Wetlands	azeroual67@yahoo.fr	No	NA	NA	NA	NA
108b	Prof. Jos Snoeks	See 'Algeria'.	See 'Algeria'.	Yes	NFI	NFI	NFI	NFI
Mozambique								
115	Olaf Weyl	Senior Aquatic Biologist, SAIAB	o.weyl@saiab.ac.za	no	NFI	NFI	NFI	NFI
Namibia								
18	Daniel Okeyo	Co-ordinator of Aquaculture, University of Fort Hare	dokeyo@ufh.ac.za	Yes	Has been introduced to the Kuraman Eye and Lake Otjikoto.	Introduced from unknown source for ornamental purposes	Does not pose a threat currently to indigenous species.	Suggested many references.
108f	Prof. Jos Snoeks	See 'Algeria'.	See 'Algeria'.	Yes	NFI	NFI	NFI	NFI

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
Netherlands								
87	Elsa Brokkelkamp	Projectmedewerker vissen, Stichting RAVON	e.brokkelkamp@ravon.nl	Maybe	Old records, need verification. Map provided.			
92	Henrik de Nie		hwdenie@planet.nl	Yes	Very localised to powerplants	Probably aquaria releases.	NFI	NFI
72	Paul Veenvliet	See 'Slovenia'	See 'Slovenia'	Yes	There have been various rumors of guppy populations in NL and I have several times asked around on forums and at field- biologist's meetings. Only one population could be confirmed: at Ijmuiden (Hoogovens).	The origin is certainly aquarium releases.		In the 1980's I visited the site several times and once managed to find and catch a single guppy. This was an adult female with a red tail fin. I believe that this was a released aquarium fish, not a representative of a feral population. Recently I heard that guppies are still/again present at this site.
New Caledonia								
112a	Philippe Keith	See 'French Polynesia'.	See 'French Polynesia'.	Yes	Including the Loyaute islands.	Thought to be during 2nd world war by Americans for mosquito control: 1942-1945	NFI	Some authors doubt origin
1c	Aaron Jenkins	See 'Fiji'.	See 'Fiji'.	Yes	NFI	NFI	NFI	No justification for introduction of livebearers on any Pacific island - native species exist that are in fact better mosquito larvae predators.
2f	David Boseto	See 'Australia'	See 'Australia'	Yes	NFI	NFI	NFI	NFI
New Guinea								
2h	David Boseto	See 'Australia'	See 'Australia'	Yes	NFI	NFI	NFI	NFI
New Zealand								
53	Bob McDowall	National Institute of Water and Atmospheric Research	b.mcdowall@niwa.cri.nz	Yes	Only in geothermally heated water bodies.	Aquaria releases	NFI	NFI

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
Nicaragua								
93	Ronald Escoto	INPESCA, Nicaragua	jescoto@inpesca.gob.ni	No	NA	NA	NA	NA
Nigeria								
28	Babatunde Olaosebikan	Babatunde Olaosebikan, Federal College of Freshwater Fisheries Technology.	badolax@yahoo.com	Yes	Lagos and Port Harcourt areas in the Southern region of Nigeria.	Likely to be during colonial period for mosquito control.	No studies, so impact unknown. However, hardy nature means tend to occupy areas where indigenous fish cannot.	In Lagos, found in polluted sewer waters, without any obvious distress - although some seem to have swollen stomachs
Pakistan								
42	Najam Ul Huda Khan	Coordinator Survey Programme, Pakistan Wetlands Programme	nhkhan@wwf.org.pk	Yes	Only in the coastal regions	NFI	NFI	NFI
Palau								
116a	Brent Tibbatts	See 'Guam'.	See 'Guam'.	Yes	NFI	NFI	NFI	NFI
2g	David Boseto	See 'Australia'	See 'Australia'	Yes	NFI	NFI	NFI	NFI
Panama								
83	Licenciado Lorenzo Becerra	Jefe del Programa Acuícola de Panamá	lbvpa@yahoo.com	Yes	Found in most water bodies in the country.	Released to control mosquitoes	None known.	Released during construction of canal between 1900-1912, under the authority if the sanitary authorities of the canal. Included Summary of exotic species found in Panama and origins of these.
Paraguay								
49	Pablo Ribla	See 'Argentina'	See 'Argentina'	Yes	Localised parts, including Asucion Bay and San Lorenzo - even in streets where it rains a lot.	NFI	NFI	NFI
Peru								
34	Roland Bucher	Fishbase collaborator, Peru.	rolbuch@terra.com.pe	Yes	Peruvian Amazon basin and coastal areas of Peru. Swamps of La Villa near Lima and in 1958 in freshwater creeks on beaches of Lima. Also in puddles and small streams in Iquitos - not in larger streams due to abundance of other fish.	NFI	NFI	Probably occurs along the coastal area up to Ecuador. Conditions less favorable to the south.

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
35	Hernan Ortega	Museo de Historia Natural Universidad Nacional Mayor de San Marcos	hortega@terra.com.pe	Yes	Most of country, in rivers from Tumbes to Ica (coast), in jungle (northeast), upper river Mayo in Dep. San Martin, jungle central, Satipo, Dep. Junin and other sites.	1940s for malarial control	Negative in central jungle as they displace the native ichthyofauna and at least one family suffered intoxication when eating them.	See Ortega & Vari, 1986; Ortega *et al.*, 2007.
Philippines								
26	Rafael D Guerrero	Philippine Council for Aquatic and Marine Research and Development	aquabios@laguna.net	Yes	Found throughout the country especially in the major islands of Luzon, the Visayas and Mindanao.	Introduced from Honolulu, Hawaii in l905.	More beneficial than destructive, controlling aquatic vermin and feeding carnivorous cultured fishes and invertebrates. Considered a nuisance by some.	He once did a study on the effect of Derris root powder on *P. reticulata.*
70	Christine Casal	Research Analyst, FishBase Project, The WorldFish Center.	c.casal@cgiar.org	Yes	Luzon and Visayas, and perhaps also Mindanao	Introduced from Hawaii in 1905.	Pest in milkfish ponds	
111	Joie Matillano	College of Environmental Science and Forestry, State University of New York.	joiemati@yahoo.com	Yes	NFI	1980s or even earlier. To control mosquitoes initially and now also from aquaria	NFI	Established wild populations are used as food for carnivorous aquarium species
Portugal								
69	Filipe Ribeiro	Lisbon University	fmribeiro@fc.ul.pt	No	NA	NA	NA	NA
71	Ricardo Serrao Santos	University of the Azores	ricardo@notes.horta.uac.pt	No	NA	NA	NA	NA
73	Vitor Almada	Professor, Instituto Superior de Pscologia Aplicada	valmada@netcabo.pt	No	NA	NA	NA	NA
74	Karim Erzini	University of Algarve	kerzini@ualg.pt	No	NA	NA	NA	NA

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
Puerto Rico								
95	Tom Kwak	North Carolina State University	tkwak@ncsu.edu	Yes	Ubiquitous - most common introduced species; found at 51 sites.	No	Not studied, but possibly affects invertebrate and native fish communities.	where found, was found at high densities
94c	Brian Langerhans	See 'Bahamas'.	See 'Bahamas'.	Yes	NFI	NFI	NFI	NFI
Republic of the Congo								
108g	Prof. Jos Snoeks	See 'Algeria'.	See 'Algeria'.	Yes	Marshy area around Brazzaville	NFI	NFI	NFI
109	Victor Mamonekene	Universite Marien Ngouabi	mamonekene@hotmail.com	Yes	Channels and marshes of Brazzaville.	Released during 1985 during a workshop on malaria control	No noticeable effects on native fish or on malaria. Channels full of grass and rubbish giving plenty of cover.	None in Point Noire.
Reunion								
112c	Philippe Keith	See 'French Polynesia'.	See 'French Polynesia'.	Yes	NFI	After 1908 from Mauritius for mosquito control.	NFI	NFI
Russia								
11	Nina Bogutskaya	Zoological Institute, Russian Academy of Sciences	office@zin.ru	Yes	In areas of artificially warmed water in a number of towns and cities.	Unintentional introduction by aquarium release	NFI	Some suggested references.
Samoa								
2i	David Boseto	See 'Australia'.	See 'Australia'.					
Senegal								
15	Anis Diallo	Centre de Recherches Oceanographiques de Dakar Thiaroye.	anis_diallo@hotmail.com	Yes	In 'Niayes zone' - an ecological region along the coast from Dakar to St Louis. Particularly in ponds used to feed vegetable culture.	Introduced to control malaria and bilharzia.	Fish only known in enclosed areas where no way to reach river or sea.	Would be useful to check whether still present. Current status unknown.
108c	Prof. Jos Snoeks	See 'Algeria'.	See 'Algeria'.	Yes	NFI	NFI	NFI	NFI

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
Slovakia								
79	Vlado Kovac	Department of Ecology, Faculty of Natural Sciences, Comenius University	kovac@fns.uniba.sk	Yes	Isolated to thermal ponds in the vicinity of Bojnice, and in the Teply Potok creek (here are self sustained)	It is said that aquarists released specimens in the 1960s, which then spread - to locations including the TP creek	None known.	Also present in various local ponds, but have a temporary existence in most cases.
Slovenia								
72	Paul VeenVliet	Institute Symbiosis	paul.veenvliet@zavod-symbiosis.si	No, probably not.	Various rumours of guppies in Netherlands- Ijmuiden (Hoogovens) from aquarium releases. Rumours of guppies in a thermal spring near Ljubljana are likely to be the result of species misidentification. Recently only native *Squalius cephalus* seen.	NFI	NFI	It is possible that there were guppies here at some day in the past, but it is not a suitable habitat (cold, no hiding places, predatory native fish present) and any occurrence must have been for a very short time only.
South Africa								
22	Sean Marr	Freshwater Research Unit Department of Zoology University of Cape Town	Sean.marr@uct.ac.za	Yes	provides excel spreadsheet - more localised than *Gambusia*.	Aquarium releases	Very limited study of freshwater fish - not been looked at in western cape.	Also swordtails present due to aquarium releases.
25	Steven Lowe	Department of Ichthyology and Fisheries Science (DIFS), Rhodes University	S.Lowe@ru.ac.za	no	Works on Breede, Oliphants-Doring and Kouga river catchments and has never seen guppies or *Gambusia*	NFI	NFI	NFI
37	Kit Magellan	Rhodes Postdoctoral Fellow, Department of Ichthyology and Fisheries Science (DIFS), Rhodes University	k.magellan@ru.ac.za	Yes	Documented in localised parts but this may not reflect their actual distribution.	Probably aquaria trade, but also possibly mosquito control	There is no research into this as of yet. Have submitted research proposal on the subject, waiting to hear about funding.	Attached map.
51	Paul Skelton	Managing Director, SAIAB	p.skelton@ru.ac.za	Yes	Fairly localised; recorded in KwaZulu Natal and also inland. See map in De Moor & Bruton (1998 - attached)	Escapees from ornamental fish farms	Not aware of studies locally.	Recommends SAIAB database.

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
Spain								
75	Emili Garcia-Berthou	Professor of Ecology, Institut d'Ecologia Aquàtica Universitat de Girona	emili.garcia@udg.es	Yes, possibly.	Only one record (Doadrio, 2002), in Miljares/Millars, between Barcelona and Valencia. Unsure if still there.	NFI	NFI	Also established in Canaries, apparently (provides a reference).
Sri Lanka								
86	Ramani Shirantha	PhD student, Department of Zoology University of Kelaniya, Sri Lanka	shirantha@kln.ac.lk	Yes	Negombo lagoon, Kalani river basin, Colombo and surburbs, Kalu river, Mahaaweli river basin, Mahaweli uppermost catchment, Walawe river.	Between 1925-28 introduced as mosquito control by State anti-malarial campaign, and then again in 1970s to control *Culex* in Colombo. Have now spread to upper areas as well as initial lowlands.	Yes - possibly threaten riverine biodiversity - seems they selectively feed on larvae other than mosquitoes and have invaded natural habitat of the indigenous fish *Davario malabaricus* (Danio).	Included map.
91	Priyantha Epa	Senior Lecturer Department of Zoology University of Kelaniya, Sri Lanka	epa@kln.ac.lk	Yes	Mainly in highly polluted canals in major cities (Galle, Colombo, Kandy). Not in natural freshwater streams.	Introduced to control mosquitoes but find ornamentals in wild in some areas.	Environmentalists say that guppies impact native invertebrates - no scientific evidence	Cultured as an ornamental fish - climate very suitable to growth and breeding.
Thailand								
118	Nonn Panitvong	Thailand Fish & Nature Explorer	npanitvong@hotmail.com	Yes	Bangkok, national parks such as Koh Yai and Thong PaPhum NP in Western Thailand.	Mosquito control by Government, probably.	Compete with *Aplocheilus panchax* and *Oryzias danceana* on outskirts of Bangkok. Impede shrimp harvesting when found in these ponds. Appear to be fewer native species over last 5 years.	Seem to fare better in running water, rather than stagnant ponds. Do better in degraded habitats.

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
Tonga								
36	Jon Harding	Canterbury University, New Zealand	jon.harding@canterbury.ac.nz	No	NA	NA	NA	NA
Turkey								
67	Guler Ekmekci	Hacettepe University	gulere@hacettepe.edu.tr	No	NA	NA	NA	NA
Uganda								
68	Wandera S B	Reserch Scientist NaFIRRI	sb@firi.go.ug	Yes	widespread in streams. Have been found in Bugungu stream that flows into Napolean gulf at Jinha near source of Nile. Also in Budongo forest. As these two locations are 300km apart, seems likely also found elsewhere.	1940s and 1950s for mosquito control -failed at this as mosquitos still rampant.	Probably minimal.	guppies thrive in places where mosquitos aren't found anyway = could use native fish as controllers in stagnant waters instead.
94f	Brian Langerhans	See 'Bahamas'.	See 'Bahamas'.	Yes	Rare, but in some crater lakes are abundant. Found in papyrus swamps in kibale national park.	NFI	NFI	NFI
54b	Paul Loiselle	See 'Madagascar'.	See 'Madagascar'.	Yes	There was a thriving pop of guppies in Lake Victoria (Jinja, Uganda) in the 1970s - near cremation ghats, but when Amin expelled asian community this may have changed…			
Uruguay								
47	Daniel Carnevia	Instituto Nacional de Pesca, Montevideo, Uruguay.	carnevia@pes.fvet.edu.uy	no	In aquariums, but can't survive the winter (15C)	NA	NA	Other, native, species used as biocontrol agents instead.
US Virgin Islands								
113a	Bill Loftus	Professor of Aquatic Ecology and President of Florida International Aquatic Research & Communication, LLC	bill_loftus@usgs.gov	Yes	Only found at one location on St John.	Local people introduced for mosquito control.	Low numbers so negligable effect likely.	NFI

Reference code #	Name	Institution	Contact details	Guppies?	Locations	Origin	Negative effects	Additional Information
USA								
113b	Bill Loftus	See 'US Virgin Islands'.	See 'US Virgin Islands'.	No				
45	Paul Shafland	Non-native Fisheries Laboratory	paul.shafland@myfwc.com	Yes (Florida) but not considered to be 'established'.	A few collected in isolated catches -no reports for 15 years though. Has poor survival rate in Florida. Places reported= Hillsborough & Palm Beach Counties. Hillsborough thought to be non-self-sustaining and Palm Beach pop throught to have died in drought in late 1970s.	Likely to be illegal aquarium releases/unwanted pets or fish-farm ditches.	NFI	See Courtenay et al. 1974, Courtenay & Hensley, 1978 and Courtenay et al. 1984. Summarised with quotes.
104b	Michael Tobler	See 'Mexico'.	See 'Mexico'.	Yes	San Marcos river, near Martindale, Caldwell County, Texas.	likely from aquarium stocks	NFI	NFI
46	Walter Courtenay	USGS Fishery Biologist	wcourtenay@usgs.gov	Yes	NFI	NFI	NFI	Provides information from Welcomme 1981 and other publications.
Vanuatu								
1	Aaron Jenkins	See 'Fiji'.	See 'Fiji'.	Yes	NFI	Not sure - likely to be aquarium releases.	NFI	No justification for introduction of livebearers on any Pacific island - native species exist that are in fact better mosquito larvae predators.
Zambia								
50	Cyprian Katongo	East Africa Water Association	katongo1960@yahoo.co.uk	Not aware.	NFI	NFI	NFI	NFI

Appendix VI: *Gambusia*: Table of respondents and summary of responses

Reference code #	Name	Institution	Contact details	*Gambusia*?	Locations	Origin	Negative effects	Additional Information
Australia								
3	Helen Larson	Curator of Fishes, Museum and Art Gallery of the Northern Territory, Darwin.	Helen.larson@nt.gov.au	Yes	Used to be in Railway Dam, and likely to be populations awaiting breakout. Eradicated from John Hayes Rockhole in Central Australia and Ilparpa swamp in Alice Springs.	NFI	None known	The museum holds various specimens of *Gambusia* from Australia and S.E. Asia.
5	Leon Barmuta	Senior Lecturer in Zoology, University of Tasmania	Leon.Barmuta@utas.edu.au	Yes (*G. affinis*)	Tasmania. Wetlands in Tamar estuary near Launceston.	Probably early 1990s - illegally. Probably to 'control mosquitoes'.	No studies.	IFS wants eradication and to prevent further spread (media campaign).
8	Graham Pyke	Senior Fellow, Australian Museum, Sydney	Contactable from: http://australianmuseum.net.au/staff/graham-pyke/	Yes (*G. holbrooki*)	New South Wales, widely distributed and almost ubiquitous along coastal plain.	1928 from East coast of USA via Italy.	Eat frogspawn and tadpoles. Small native fish absent from areas where *Gambusia* are present in high densities.	Difficult to eradicate, even from small, isolated ponds.
9	Michael Jennions	Australian National University, Canberra.	michael.jennions@anu.edu.au	No	Mainly in the immediate area of Canberra and then in farm dams.	NFI	NFI	Just starting to work on a *Gambusia* project
33	Rob Brooks	University of New South Wales	rob.brooks@unsw.ed.au	Yes	Several drainage canals, quite localised.	NFI	Lots of effects reported in literature.	NFI
38	Kevin Warburton	University of Queensland	k.warburton@uq.edu.au	Yes	Queensland coastal areas and localised areas in Western Australia and Northern Territory	1920s for mosquito control.	Associated with declines of many native fish. Aggressive, especially towards small fish; chase & nip fins causing secondary infections. Can occur in huge numbers. Aggression increases activity and conspecific aggression by rainbow fish.	Lab studies indicate that coexisting introduced swordtails may exacerbate impacts of *Gambusia*. .

Reference code #	Name	Institution	Contact details	*Gambusia*?	Locations	Origin	Negative effects	Additional Information
40	Tim Farrell	Inland Fisheries Service, Tasmania	Tim.Farrell@ifs.tas.gov.au	Yes (*G. holbrooki*)	Tasmania. Found in Legana area (east Tamar) of NE Tasmania in 1991, attempted eradication but re-established population discovered in 2001 in Wetlands reserve. Eradication reattempted and currently being evaluated. Recent surveys show sporadic distribution throughout lower reaches of Tamar estuary.	Late 1980s by human vector.	Concerned about effect on threatened golden bell frog, *Littoria raniformis.*	NFI
41	Grant Scurr	NRM North, Tasmania	Grant.Scurr@dpiw.tas.gov.au	Yes (*G. holbrooki*)	Tasmania. Specific sites for permanent populations: Tamar Islands Wetland Reserve, the 'Landfall' property on the east Tamar opposite Tamar Island and a site at Windermere between the West Tamar Highway and Tamar estuary.	In 1980s illegally introduced from Queensland; partly for personal dam mosquito control and partly for mischief making.	Clear absence of tadpoles in *Gambusia*-infected areas (i.e. Levee at wetland reserve - tadpoles only on *Gambusia*-free side).	NFI
Bahamas								
94a	Brian Langerhans	Assistant Professor, North Carolina State University	langerhans@ou.edu	No. Despite extensive sampling.	NA	NA	NA	Other poeciliids present
Belize								
98	Peter Esselman	Aquatic Ecologist, University of Michigan.	esselman@umich.edu	No	NA	NA	NA	Commonly sold as pets, so future introductions possible.
Chile								
102	Brian Dyer	Universidad del Mar, Valpariso.	bdyer@udelmar.cl	Yes	Central region=everywhere. Found at Loa river and Mataquito river (coordinates in email)	Mosquito control	Negative effects, tend to do best in disturbed habitats.	.
China								
120	Chen Xiao-Yong	Associate Professor, Chinese Academy of Sciences, Yunnan.	chenxy@mail.kiz.ac.cn	Yes	Especially south of Yangtze River	NFI	competes with native fish for food and space	NFI
Costa Rica								
101	William Bussing	University of Costa Rica	wbussing@biologia.ucr.ac.cr	No	NA	NA	NA	Only native species (*G. nicaraguensis*)
97a	William McLarney	Aquatic Biologist	anaiinc@dnet.net	No	NA	NA	NA	NA

Reference code #	Name	Institution	Contact details	*Gambusia*?	Locations	Origin	Negative effects	Additional Information
Cyprus								
88	Constantinos Moustakas	Dept of Fisheries and Marine Research	cmoustakas@dfmr.moa.gov.cy	Yes (*G. affinis*)	Throughout region.	First in 1939 from Syria to reduce mosquitoes, but then introduced all over.	None observed as no native species - actually help maintain carnivores for aquaculture	NFI
Egypt								
59	Abdel Rahman	Senior Aquaculture Scientist, Worldfish Centre, Egypt.	aelgamal@worldfish-eg.org	Yes	NFI	NFI	NFI	NFI
El Salvador								
82	Enrique Barraza	Ministerio de Medio ambiente y Recursos Naturales El Salvador	eulaliabarr@yahoo.com ebarraza@marn.gob.sv	Maybe – not certain of identification	NFI	NFI	NFI	NFI
84	Licenciado Jose Arturo Nunez	Departamento de Ambiente Corte suprema de justicia	jarnu2003@yahoo.com	No	NA	NA	NA	NA
Eritrea								
65	Moeketsi Mokati	FAO representative, Eritrea.	Moeketsi.Mokati@fao.org	No	NA	NA	NA	NA
Fiji								
1	Aaron Jenkins	Senior Program Officer, Wetlands International-Oceania	apjenkins@connect.com.fj	Yes (*G. affinis*)	Most common on Viti Levu.	Introduced for mosquito control from an unknown source in the 1930s.	Where poeciliids are found there is decline in water quality and native species richness. Probably water quality gives livebearers a competitive advantage.	Negative correlation between native forest cover and presence of invasives (e.g. poeciliids and cichlids). No justification for introduction of livebearers on any Pacific island - native species exist that are in fact better mosquito larvae predators.
2c	David Boseto	See 'Australia'	See 'Australia'	Yes (*G. affinis* and *G. holbrooki*)	NFI	NFI	NFI	NFI

Reference code #	Name	Institution	Contact details	*Gambusia*?	Locations	Origin	Negative effects	Additional Information
Greece								
89	Leonidas Varnakas	Technologist in Aquaculture and Fisheries, Hellenic Centre for Marine Research, Institute of Inland Waters, Athens.	louisvard@gmail.com	Yes	Inland waters.	First documented in 1927 for antimalarial control. Came from Italy and France. Introduced first into Macedonia then spread.	Competes with *Valencia letourneuxi*, Shows aggression and fin-erosion. May also compete with *Aphanius fasciatus* (no evidence).	NA
85	Dimitra Bobori			Yes	All surface waters - both *G. holbrooki* and *G. affinis*. Anywhere with stagnant/low flow waters - even in marshes of several islands	Introduced repeatedly between 1927-1937 from France and Italy by Greek ministry of public health for biocontrol of mosquito larvae.	Competes with native species *Valencia letourneuxi* - an endemic to N. Greece.	
Guam								
116b	Brent Tibbatts	Division of Aquatic and Wildlife Resources, Guam Department of Agriculture.	brent.tibbatts@gmail.com	Yes	15 rivers and 5 lakes or impoundments	Mosquito control		Not currently allowed to be imported.
Honduras								
96	Wilfredo Matamoros	The University of Southern Mississippi, Department of Biological Sciences	wilmatamoros@yahoo.com	No	Despite 5 years of sampling.	NA	NA	NA
Hong Kong								
57	David Dudgeon	Professor, Division of Ecology & Biodiversity, University of Hong Kong.	ddudgeon@hku.hk	Yes	Throughout.	Mosquito control, 1970s.	Predates upon tadpoles & small fish. Tends not to invade pristine habitats. Seems to be responsible for elimination of *Oryzias curvinotus*.	Suspect that *Gambusia* has eradicated guppies when both introduced to same place. *Gambusia* 'toxic' to anything it shares an environment with.

Reference code #	Name	Institution	Contact details	*Gambusia*?	Locations	Origin	Negative effects	Additional Information
Iran								
16	Afshin Afzali	Department of Fisheries, Faculty of Natural Resources, University of Tehran.	afshin_azali2002@yahoo.com	Yes	Widely introduced - found in all inland waters especially still ones.	First introduced in 1922-1930 to Ghazian marsh of Caspian littoral. Then samples from this habitat transferred to over 3000 permanent water bodies around the country.	Predates on eggs and young of natives and competes for food.	Iranian *Gambusia* were *G. holbrooki* but possible that some also *G. affinis*. Photo provided. In some habitats, e.g. the south of Iran (Hormuz basin), Gambusia live with native cyprinodontiformes (*Aphanius dispar*) and both predate on mosquito larvae.
99b	Brian Coad	Canadian Museum of Nature, Ottawa, Canada	bcoad@mus-nature.ca	Yes	Throughout region	Deliberately introduced.	No direct evidence, but assumed to compete.	NFI
Iraq								
99a	Brian Coad	See 'Iran'.	See 'Iran'.	No	Throughout region.	Deliberately introduced.	No direct evidence, but assumed to compete.	NFI
Israel								
77	Arik Diamant	Director, National Center for Mariculture, Israel Oceanographic & Limnological Research Institute.	diamant@agri.huji.ac.il	Yes	In Asi Stream, Beit Shean Valley, northern Israel.	Introduced, probably by the British, 100 years ago to combat *Anopheles* mosquitoes - malaria was endemic at that time.	Not aware, but likely.	NFI
80	Menachem Goren	Department of Zoology, Tel Aviv University	GorenM@tauex.tau.ac.il	Yes	In all water systems throughout Israel apart from Ein Feshkha, an isolated oasis at northwest of Dead Sea.	100 years ago, probably by British - to combat malaria	Not aware, but likely.	NFI
81	Dani Golani	Department of Evolution, Systematics and Ecology, The Hebrew University of Jerusalem	dgolani@cc.huji.ac.il	Yes	Every inland body of water	NFI	NFI	NFI

Reference code #	Name	Institution	Contact details	*Gambusia*?	Locations	Origin	Negative effects	Additional Information
Italy								
12	Massimiliano Scalici			**Yes**	Widespread in Mediterranean regions	Malarial control, introduced between 1965 and 1970		
24	Lorenzo Tancioni	University of Rome 2	tancioni@uniroma2.it	Yes	At local scale, distribution corresponds with potamal stretches including estuarine zones. In coastal lagoons, confined to fresh and brackish water areas. Also found in aquatic lentic lakes and ponds. This all means that *Gambusia* has a patchy distribution.	NA	NA	NA
29	Marco Seminara	University of Rome 1	marco.seminara@uniroma1.it	Yes (*G. holbrooki*)	Littoral vegetated zones of lakes and ponds. Rarely streams and rivers. As most lakes are in north (80%) and central (16%) then distribution is skewed accordingly	*Anopheles* sp. control in 1925-1930.	NFI	Land reclamation after WWII expanded distribution to drainage channels and basins with no *Anopheles* - such as Veneto, Emilia-Romagna, Tuscany, Latium, Campania and Sardinia.
30	Marcello Bazzanti	University of Rome 1	marcello.bazzanti@uniroma1.it	Yes	Some ponds and lakes in Latium region, near Rome. Believe is expanded to several lowland regions with discontinous presence.	1922 as biological control for *Anopheles*.	Low species richness of zooplankton and benthic invertebrates (see paper).	NFI
Jamaica								
66	Eric Hyslop	University of the West Indies, Jamaica	eric.hyslop@uwimona.edu.jm	Yes (*G. holbrooki*)	Localised.	Aquaria releases likely.	Likely hybridisation with native species	NFI
Japan								
107	Dr Mizuki Matsunuma	Kagoshima University Museum, Kagoshima	k1139853@kadai.jp	Yes (*G. affinis*)	Entirety of Southern Japan	Mosquito control, 1970s in Kagoshima.	Probably predate upon or compete with native *Oryzias latipes* - esp. in Okinawa island.	*Xiphophorus*, *P. mexicana* and *P. sphenops* also all recorded

Reference code #	Name	Institution	Contact details	*Gambusia*?	Locations	Origin	Negative effects	Additional Information
Kenya								
10	Rob Britton	Scientist on Rift Valley Lakes Project; Senior Lecturer in Conservation Ecology, Bournemouth University	rbritton@bournemouth.ac.uk	No	NA	NA	NA	NA
108d	Prof. Jos Snoeks	See 'Algeria'.	See 'Algeria'.			NFI	NFI	NFI
Kyrgyzstan								
61	Raymon van Anrooy	Fishery Officer, FAO, Sub-regional Office for Central Asia, Turkey.	Raymon.VanAnrooy@fao.org	Yes	Chu and Kara-Darya river basins	NA	NA	NA
Lebanon								
17	Michel Bariche	Assistant Professor, Marine Biology & Ichthyology, Biology Department, American University of Beirut.	michel.bariche@aub.edu.lb	Yes	Litani river, Ammik Marsh (Litani), Quaraoun dam (Litani).	In the 1960s, for mosquito control.	None reported.	NFI
Madagascar								
54	Paul Loiselle	Curator of Freshwater Fishes New York Aquarium	ploiselle@wcs.org	Yes	Ubiquitous, other than a *Gambusia*-free zone between the Mananjeba basin southwards to the Anjingo in the north-west.	Yes. First introduced in 1929 by Dr J Legendre for malarial control. Subsequent intros include Lake Alaotra in 1940 and Lake Itasy in 1950.	Played a major role in driving endemic *P. sakaramyi* and *Pantanodon sp.* close to extinction, and likely caused the extinction of *Pantanadon madagascariensis*.	No obvious impact on malaria or mosquitos. Can also be partly blamed for dramatic decline in *Bedotia tricolor* and undescribed congeners in upper Manampatrana and Sambava rivers, as well as the decline of Paratilapia and Ratsirakia in Lake Alaotra and central highlands, and may have also contributed to the extinction of *Ptychochromoides itasy.*

Reference code #	Name	Institution	Contact details	*Gambusia*?	Locations	Origin	Negative effects	Additional Information
Malaysia								
6	Mazlan A. Ghaffar	Associate Researcher, Marine Ecosystem Research Centre, Universiti Kebangsaan Malaysia	mag@pkrisc.cc.ukm.my	Yes	Throughout.	Not known	Not known	Used as feed fish by aquarists
Maldives								
13	Hasan Shakeel	Senior Biologist, Marine Research Centre, Male', Maldives.	hshakeel@mrc.gov.mv	Not in natural water bodies	Found only in enclosed household wells. No freshwater bodies that are inhabitable by freshwater fish. However, found throughout in aquariums and wells	Introduced as aquarium fish and for mosquito control.	No	No established pops in natural habitats. Use of fish to control mosquitoes diminished as urban centres use closed wells instead of traditional open wells.
Mauritius								
43	Chandani Abbadoo	Senior Lecturer, Faculty of Science, University of Mauritius.	chandani@uom.ac.mu	Yes (affinis)	NFI	NFI	NFI	Also *Xiphophorus* present
Mexico								
76	Salvador Contreras-Balderas	Professor Emeritus, Department of Biology, Universidad Autonoma de Nuevo Leon	saconbal@gmail.com	Yes	Several basins and at several localites (see papers) - maybe more as many have yet to be sampled.	No	NFI	*G. affinis* has been confused until recently with *G. speciosa* at several locations (Miller, 2006). - see papers attached to email
Micronesia								
116d	Brent Tibbatts	See 'Guam'.	See 'Guam'.	Yes	Mortloks	NFI	NFI	NFI
Morocco								
32	Abdelhamid Azeroual	North African Network for the Wetlands	azeroual67@yahoo.fr	Yes	All moroccan rivers, caught it in Sebou, Ziz and Loukkos.	In 1920s originally, by French colonists. Then again in 1950s by American soldiers.	Competition with Moroccan loach, *Cobitis Maroccana* in Sebou & Loukkos, and with *Aphanius saourensi*s in Saoura (Algeria)	NFI

Reference code #	Name	Institution	Contact details	*Gambusia*?	Locations	Origin	Negative effects	Additional Information
Namibia								
18	Daniel Okeyo	Co-ordinator of Aquaculture, University of Fort Hare	dokeyo@ufh.ac.za	No	NA	NA	NA	NA
New Zealand								
52	Graham Wallis			Yes	North island, especially warmer northern regions.	NFI	NFI	NFI
53	Bob McDowall	National Institute of Water and Atmospheric Research	b.mcdowall@niwa.cri.nz	Yes (*G. affinis*)	Widespread in northern NZ.	1930s for mosquito control	International concern, but no specific studies in NZ.	See McDowall (1999) and McDowall (2004).
Nigeria								
28	Babatunde Olaosebikan	Babatunde Olaosebikan, Federal College of Freshwater Fisheries Technology.	badolax@yahoo.com	Yes	Lagos and Port Harcourt areas in the Southern region of Nigeria.	Likely to be during colonial period for mosquito control.	No studies, so impact unknown. However, hardy nature means tend to occupy areas where indigenous fish cannot.	In Lagos, found in polluted sewer waters, without any obvious distress - although some seem to have swollen stomachs
Pakistan								
42	Najam Ul Huda Khan	Coordinator Survey Programme, Pakistan Wetlands Programme	nhkhan@wwf.org.pk	Yes (*G. affinis*)	Foul waters in lower Sindh, Punjab and Southwestern parts of Balochistan.	Malaria control in late 1920s from USA	Competition with local fauna but usually found in marginal waters that hold few other fish.	NFI
Peru								
35	Hernan Ortega	Museo de Historia Natural Universidad Nacional Mayor de San Marcos	hortega@terra.com.pe	Yes	Discovered in ponds raising crustaceans in Tumbes.	1980s, associated with importation of shrimp.	NFI	NFI
Philippines								
26	Rafael D Guerrero	Philippine Council for Aquatic and Marine Research and Development	aquabios@laguna.net	Yes	Found throughout the country especially in the major islands of Luzon, the Visayas and Mindanao.	Introduced from Honolulu, Hawaii in l905.	More beneficial than destructive - controlling aquatic vermin & feeding carnivorous cultured fishes & invertebrates.	Considered a nuisance by some.

Reference code #	Name	Institution	Contact details	*Gambusia*?	Locations	Origin	Negative effects	Additional Information
111	Joie Matillano	Environmental and Forest Biology, College of Environmental Science and Forestry, State University of New York.	joiemati@yahoo.com	Yes	NFI	1980s or even earlier. To control mosquitoes.	NFI	NFI
Portugal								
69	Filipe Ribeiro	Lisbon University	fmribeiro@fc.ul.pt	Yes	Widespread.	From USA to southern Italy by ship in 1920s for mosquito control. 12 released into local pond in Valencia, Eastern Iberia. First record in Portugal was in Vouga drainage in 1939, and by 1950s was present in lower Tejo, near Lisbon.	Yes	NFI
Puerto Rico								
95	Tom Kwak	North Carolina State University	tkwak@ncsu.edu	No	NA	NA	NA	NA
South Africa								
22	Sean Marr	Freshwater Research Unit Department of Zoology University of Cape Town	Sean.marr@uct.ac.za	Yes	provides excel spreadsheet - widespread	Mosquito control	Very limited study of freshwater fish - not been looked at in western cape.	public perception is that *Gambusia* control mosquito populations.
25	Steven Lowe	Department of Ichthyology and Fisheries Science (DIFS), Rhodes University	S.Lowe@ru.ac.za	Not seen, but heard reports	Works on Breede, Oliphants-Doring and Kouga river catchments and has never seen guppies or *Gambusia*	NFI	NFI	NFI

Reference code #	Name	Institution	Contact details	*Gambusia*?	Locations	Origin	Negative effects	Additional Information
37	Kit Magellan	Rhodes Postdoctoral Fellow, Department of Ichthyology and Fisheries Science (DIFS), Rhodes University	k.magellan@ru.ac.za	Yes	Documented in localised parts but this may not reflect their actual distribution.	Either introduced in the 19th Century by an American immigrant, or to the Eastern cape in 1970s by Eastern Cape Conservancy. Both for mosquito control.	There is no research into this as of yet. Have submitted research proposal on the subject, waiting to hear about funding.	Provided map.
51	Paul Skelton	Managing Director, SAIAB	p.skelton@ru.ac.za	Yes	Fairly localised; recorded in KwaZulu Natal and also inland. See map in De Moor & Bruton (1998 – provided)	Yes, see reference (escapees from ornamental fish farms)	Not aware, but studies have not been carried out locally, as far as aware.	Recommends SAIAB database.
Thailand								
118	Nonn Panitvong	Thailand Fish & Nature Explorer	npanitvong@hotmail.com	Yes	Bangkok, national parks such as Koh Yai and Thong PaPhum NP in Western Thailand.	Mosquito control by Government, probably.	Compete with *Aplocheilus panchax* and *Oryzias danceana* on outskirts of Bangkok. Make shrimp harvesting harder when found in these ponds. Appear to be fewer native species over last 5 years.	Do better in degraded habitats. Seem to fare better in running water, rather than stagnant ponds.
Tonga								
36	Jon Harding	Canterbury University, New Zealand	jon.harding@canterbury.ac.nz	No	NA	NA	NA	NA
Turkey								
67	Guler Ekmekci	Hacettepe University	gulere@hacettepe.edu.tr	Yes	Throughout region.	NA	NA	NA
USA								
78	Mac Kobza			Yes (*G. holbrooki*)	Native and introduced	Introduced in some places for mosquito control. Now chemicals are used instead.		Lab is looking at other exotics including Mayan cichlid and African jewelfish.

Reference code #	Name	Institution	Contact details	*Gambusia*?	Locations	Origin	Negative effects	Additional Information
97b	William McLarney			Yes	Scattered around watershed. Mainly in Lake Emory at Franklin and the lower watershed of Cowee Creek downstream of Franklin.	Ornamental plant business introduced to control mosquitoes. Probably also arrived with plants and people from Florida. Probably multiple introductions.	No, numbers are very low.	Not sure which species, is slight possibility of being native but unlikely. Likely to mainly be *G. holbrooki*.

NA= not applicable; NFI= no further information.

Appendix VII

Worked example of complementarity analysis

Hypothetical 'Mesocosm A' contains a population consisting of 3 males (Figure A). These are each photographed and their black and orange colour spots scored on a spreadsheet (Table A) according to the system illustrated in Figure 4.2.

A matrix was then created, and each pair of fish compared with each other (Table B). For example, Male 1 and Male 2 together possess 7 different markings (S_{jk}), 4 of which are unique to either one of them ($\underline{U}_{jk}$). Their complementarity value is therefore 0.57 (4/7). Once this has been calculated for every possible pairwise comparison within the sample, an average complementarity value is calculated. In this case:

$$\sum C_{jk} / N = C$$

$$C = (0.57 + 0.17 + 0.71) / 3 = 0.483$$

N = number of comparisons

The end result is a mean C value for each mesocosm, and values from tanks founded by singly mated and multiply mated females can be compared.
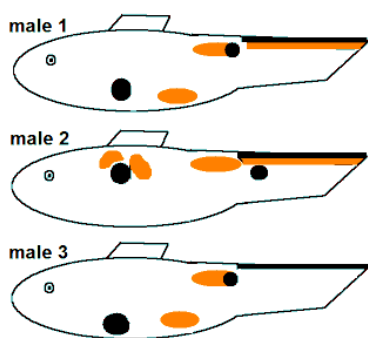
Figure A: Sample of males from hypothetical Mesocosm A.

Table A: Spreadsheet layout for recording presence of black and orange markings.

	Male 1	Male 2	Male 3
B1			
B2			
B3			
B4	X		X
B5		X	
B6			
B7			
B8	X		X
B9			
B10			
B11			
B12	X	X	X
O1			
O2			
O3			
O4	X	X	X
O5			
O6			
O7			
O8			
O9	X		X
O10			
O11			
O12	X	X	

Table B: Matrix

	Male 1			Male 2		
	U_{jk}	S_{jk}	C_{jk}	U_{jk}	S_{jk}	C_{jk}
Male 1						
Male 2	4	7	**0.57**			
Male 3	1	6	**0.17**	5	7	**0.71**

Appendix VIII

Table VIII: MANOVA analysis of variation with condition (single or multiply mated female founded populations) for a) newborn behaviours and b) courtship behaviours. CV=coefficient of variation.

a)

Multivariate tests	Wilks' λ	Df		F	p
Condition (single or multiple)	0.814	4, 7		0.399	0.804
Between-subjects tests	**SS**	**Df**	**MS**	**F**	**p**
Condition (single or multiple)					
evasion ability CV	18.372	1	18.372	0.165	0.693
activity CV	274.524	1	274.524	0.149	0.708
time in cover CV	202.118	1	202.118	0.193	0.669
reaction distance CV	14.403	1	14.403	0.090	0.771
Error terms					
evasion ability CV	1114.758	10	111.476		
activity CV	18469.248	10	1846.925		
time in cover CV	10446.246	10	1044.625		
reaction distance CV	1608.562	10	160.856		
Total					
evasion ability CV	27044.653	12			
activity CV	88188.379	12			
time in cover CV	35843.958	12			
reaction distance CV	33427.761	12			

b)

Multivariate tests	Wilks' λ	Df		F	p
Condition (single or multiple)	0.989	3, 17		0.064	0.978
Between-subjects tests	**SS**	**Df**	**MS**	**F**	**p**
Condition (single or multiple)					
sigmoids CV	0.991	1	0.991	0.000	0.985
following CV	134.932	1	134.932	0.159	0.695
thrusts CV	1.727	1	1.727	0.001	0.970
Error terms					
sigmoids CV	54539.368	19	2870.493		
following CV	16138.909	19	849.416		
thrusts CV	23132.737	19	1217.512		
Total					
sigmoids CV	555039.393	21			
following CV	133945.227	21			
thrusts CV	214643.861	21			

Appendix IX

Individual plots of % Daphnia consumed over each 8 day trial for conditions one (C1, a single female), two (C2, a female with two males) and three (C3, a female with two other females).

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C3, Fish 1   C3, Fish 2   C3, Fish 3

C3, Fish 4   C3, Fish 5   C3, Fish 6

C3, Fish 7   C3, Fish 8   C3, Fish 9

C3, Fish 10   C3, Fish 11   C3, Fish 12

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