Research Article

Predatory behaviour of female guppies (Poecilia reticulata) in a mosquito control context: the importance of social and habitat factors

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Abstract

It is vital to consider behaviour when selecting an appropriate biological control agent, as behaviour can affect both the likelihood of the agent successfully controlling the target organism, and the chance of inadvertent negative effects on native ecosystems. Guppies, Poecilia reticulata, have been introduced widely outside of their native range for mosquito control, yet very little is known about their feeding decisions when more than one prey species is present, and how these decisions are mediated by social and physical aspects of the foraging environment. We investigated female guppy foraging behaviour in a two-prey system (Anopheles and Culex mosquito larvae). When feeding alone, female guppies displayed a preference for Culex larvae. However, the extent of preference was significantly affected by both the presence of conspecifics and cover, in a non-additive manner. This suggests that the presence of alternative prey will influence the effectiveness of guppies in biological control, as well as their potential ecological impact. The exact nature of this influence depends on the interaction between social and habitat factors.

Key words: malaria, Anopheles stephensi, prey preference, Poeciliidae, biological control, Culex quinquefasciatus, foraging

Introduction

Biological control is an often-overlooked vector for invasive species (Lockwood et al. 2013). It consists of the intentional introduction of a non-native species to control a pest species. Biological control can have unintended results, including the establishment of a pest more destructive than the original target species and/or a failure to control the target (Simberloff and Stiling 1996). Although this route accounts for a relatively small proportion of invasive species, those that do become invasive can have devastating effects, as demonstrated by the cane toad, Rhinella marina (Shine 2010), and the mosquito fish, Gambusia spp. (Pyke 2008). Of the IUCN’s “100 of the World’s Worst Invasive Alien Species”, five were deliberately introduced as biological control agents, including the two...
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mentioned above (Global Invasive Species Database 2019). Further, as biological control introductions are intentional by definition, it is an invasion route that can, in theory, be managed more effectively than most, through risk assessments, species selection criteria and policy (Messing and Wright 2006; Simberloff and Stiling 1996; Van Lenteren et al. 2003).

Animal behaviour is especially relevant to the selection of biological control agents (Lewis et al. 2006; Luck 1990), and an understanding of how behaviour affects the potential benefits and costs of introducing non-native species for biological control is vital in decision making (Heimpel and Cock 2018; Simberloff and Stiling 1996). First, it can help determine how successful a species might be at controlling a target pest: is it behaviourally equipped to persist in the new habitat? Will it consume target prey in sufficient numbers? Second, behaviour is important when evaluating the likelihood that a species might impact non-target species in natural ecosystems (Van Lenteren et al. 2003): will it spread beyond the intended area of introduction? Will it consume non-target prey species? Furthermore, behaviour does not occur in isolation—it can be affected by social and environmental conditions—which means that these questions are rarely simple to answer. For example, the presence of conspecific competitors or potential mates may influence predatory behaviour, as might the structural complexity of the habitat (Diehl 1992).

Mosquito-borne disease continues to be one of the most pervasive threats to human health worldwide; there were 219 million cases of malaria reported in 2017 across 90 countries (World Health Organisation 2017), and in 2010, the global incidence of dengue was estimated to be 390 million (Bhatt et al. 2013). Chemical control is costly, damaging to the environment and can lead to pesticide resistance in mosquitoes (Chandra et al. 2008). Biological control using larvivorous fish has been advocated as a cheap, safe and effective alternative to chemical control (Ghosh and Dash 2007; Kumar and Hwang 2006; Kusumawathie et al. 2008), yet there is very little evidence for their effectiveness in this role (Walshe et al. 2017). At the same time, there are serious ecological concerns about the use of non-native larvivorous fish for mosquito control (Azevedo-Santos et al. 2016; El-Sabaawi et al. 2016).

Here, we use the Trinidadian guppy (*Poecilia reticulata* Peters, 1859), and two species of mosquito (*Culex quinquefasciatus* Say, 1823, and *Anopheles stephensi* Liston, 1901) in India to: 1) investigate foraging behaviour of an introduced predator in a multi-prey system, and 2) assess how this behaviour might be affected by social and environmental factors. The guppy is an exceptionally well-studied invasive species which is now established in more than 70 countries worldwide, largely thanks to its introduction for the biological control of mosquitoes (and thereby mosquito-borne diseases) throughout the 20th and 21st centuries (Deacon et al. 2011). Guppies were brought to India in 1908 for the biological control
of mosquitoes. Over the past two decades, they have been extensively used in malaria control programmes (Ghosh and Dash 2007). *Anopheles stephensi* is a known vector of malaria in urban India while *C. quinquefasciatus* is a vector of human filariasis.

Guppies are highly social fish. Shoaling increases foraging efficacy (Pitcher et al. 1982) and facilitates social transmission of foraging information (Galef and Giraldeau 2001; Laland and Williams 1997). Persistent sneaky mating and courtship behaviours displayed by male guppies have been shown to be costly to females by reducing their foraging time (Magurran and Seghers 1994) and fecundity (Ojanguren and Magurran 2007). Such costs can lead to harassment-avoidance strategies such as increased female boldness (Piyapong et al. 2010) and habitat segregation of the sexes (Darden and Croft 2008). It follows that their foraging behaviour may be affected by the presence of conspecifics and the sex of those conspecifics. At the same time, vegetation or other refugia can reduce visual encounter rates of prey by predators in most natural habitats (Alexander et al. 2015; Briand and Cohen 1987; Crowder and Cooper 1982; Priyadarshana et al. 2001) and many prey species actively seek refuge in cover (Laegdsgaard and Johnson 2001). Since different species of mosquito larvae vary in feeding behaviour (Yee et al. 2004), habitat preferences (Merritt et al. 1992) and escape abilities (Sih 1986), it is possible that some species might be better at utilising cover for refuge from guppy predation. Accordingly, in a multi-prey system, the extent of habitat structure has the potential to influence prey preference (Anderson 1984) and thus modulate the predatory impact that guppies have on the species present.

Here we ask whether female guppies, sourced from a population introduced for mosquito control, exhibit preferences for mosquito prey species, and whether these preferences are mediated by the prevailing social and/or physical conditions. Our null hypothesis is that female guppies are equally likely to consume the larvae of *Anopheles* and *Culex* mosquitoes, and that this lack of selectivity will be maintained despite variation in the social and/or physical environment.

**Materials and methods**

*Experimental methods*

This study took place at the ICMR - National Institute for Malaria Research Field Station in Bangalore, India.

*Fish*

Guppies were collected from water tanks and drains in the state of Karnataka using dip nets, 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 (SKG, pers. obs.).
Tables
Table 1. Number of replicate trials in each treatment combination.

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<th>Female alone</th>
<th>Female with two female companions</th>
<th>Female with two male companions</th>
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<td>No Cover</td>
<td>13</td>
<td>14</td>
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<tr>
<td>With Cover</td>
<td>11</td>
<td>15</td>
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Mosquito larvae

Supplies of both *A. stephensi* and *C. quinquefasciatus* larvae were collected from local cement tanks, drains and ponds. All were late 3rd or early 4th instar. Subsamples were photographed and measured using Image J software (Schneider et al. 2012). A two-sample t-test confirmed no significant difference in mean length of selected individuals between mosquito species (*A. stephensi* (mean ± SE) = 4.44 mm ± 0.06; *C. quinquefasciatus* = 4.42 mm ± 0.08; t_{132} = 0.25, p = 0.803).

Experimental set up

Trials were conducted in aquaria (45 × 23 × 23 cm), each filled to a depth of 18 cm with unchlorinated water. Fish (focal and companion where applicable) were introduced to each experimental aquarium the night before the trial in order to settle, although in some cases just a few hours was sufficient. Guppies that were observed to be skittish or freezing on the bottom of the aquaria were not used in trials. Each guppy was only used once. We chose to test only female guppies in this study as we were interested in habitat and social factors rather than sex differences, and males typically consume significantly fewer prey items than females due to their smaller size (Elias 1995). Focal female guppies were observed in each of three social treatments: 1) female alone; 2) female with two female companions; 3) female with two male companions. Companions were chosen at random from a stock tank.

Two habitat treatments were also tested in each of the social conditions: 1) without cover; 2) with cover. This resulted in a full factorial design (Table 1). Cover consisted of a square of green plastic (8 cm × 8 cm) with “fronds” cut from the edge to the centre. This was bound with string and covered a 2-dimensional area of approximately 40 cm² (see Figure 1). In “with cover” trials it was positioned on the right-hand side of the tank, floating with all fronds partly or fully submerged and immediately behind where the larvae were introduced at the start of each trial; this allowed larvae to actively seek refuge in the cover.

Each day, each aquarium was allocated to one of the six treatments sequentially; the sequence was shifted each day of the study to avoid potential systematic bias associated with any aquarium or location within the laboratory. Each trial began with ten *Culex* and ten *Anopheles* larvae being introduced from a glass tube into the front right corner of the aquarium simultaneously. If fish did not feed within 15 minutes of larvae
being introduced then the trial was aborted. The trial continued for 10 minutes after the first larva was consumed, or until all twenty larvae had been consumed if this took less than 10 minutes. The number of each species of larvae consumed by the focal fish was recorded. Companion fish were also able to consume prey, and prey were not replenished during the trial. At the end of the “cover” trials, the number of larvae remaining inside and outside of the cover was recorded.

Focal fish were measured to the nearest mm at the end of each trial; there was no significant difference in female size between the treatments (Female SL (mean ± SE) = 24 mm ± 0.38; F_{(2,81)} = 0.371, p = 0.691). A t-test confirmed that males were significantly smaller than the female guppies in our trials (Males SL = 18 mm ± 0.37; t_{(58)} = 9.11, p < 0.001). This difference is assumed to represent the natural sexual dimorphism in the source population, as focal fish were selected at random.

**Statistical analyses**

The following calculation was conducted for each focal fish and termed “Preference for *Culex*” ($P_C$):

$$P_C = \frac{\text{# Culex larvae consumed} - \text{# Anopheles larvae consumed}}{}$$

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. It was chosen over alternative measures of prey preference because the experimental design in this case did not include

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*Figure 1.* Cover comprised of a square of green polythene cut into “fronds” and tied in the centre. This floated just below the surface during “with cover” trials. Photo by A. Deacon.
replenishment of prey (i.e. it represented the natural situation where food is a limited resource) and therefore it would be misleading to present an index based on density. Means and 95% confidence limits were calculated for each treatment.

All data were approximately normally distributed according to the Kolmogorov-Smirnov test and displayed homogeneity of variance according to Levene’s test. A two-way ANOVA was applied to the data to explore the effects of both social and habitat factors, including an interaction term.

Results

The greatest values of $P_C$ were in the single, no cover treatment, where consistently more *Culex* than *Anopheles* were consumed. The lowest values were among single females feeding with cover (Figure 2).

A two-way ANOVA confirmed that variation in $P_C$ between treatments was explained by a significant interaction between social and habitat factors ($F_{2,77} = 5.03; p = 0.009$; Supplementary material Table S1; Figure 2). Specifically, while the presence of cover decreases the proportional consumption of *Culex* by single females, this effect disappears in the presence of conspecifics. Females foraging alongside two males and in the presence of cover, consumed significantly higher proportions of *Culex* than *Anopheles*.

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.
Discussion

Our results show that the prey preferences of guppies, introduced for the purpose of biological control, are mediated by the interaction between the social and physical environment. Female guppies displayed a marked preference for *Culex* over *Anopheles* larvae when feeding alone without cover. However, this preference disappears when females are foraging in the presence of conspecifics and, furthermore, the introduction of cover alters this social effect.

The measure of preference in this study is a conservative one, as we did not replenish prey during trials. This meant that in cases where the majority of prey were consumed, even if one prey species was consistently consumed first, this information will be played down in the index. Alternative indices of preference (e.g. those described in Cock 1978) were less appropriate as they are not equipped to deal with depletion. However, even using this relatively conservative measure of preference we still detected significant preferences within treatments. It is also important to note that trials were very short (10 minutes), therefore even without replenishment this could be considered a snapshot of an ecologically-valid scenario for guppies in a biological control context.

Competition

We found that the female preference for *Culex* disappeared in the presence of conspecifics. It is possible that in the presence of companions, increased competition may have led to reduced discrimination between prey species by the focal female (Bertram 1978). However, the behavioural response of the prey is likely to also be important here, especially when predator density is higher. Sih (1986) found that mosquito larvae responded to predators by moving less, and that this response varied between species. It is thus possible that in this case the additional predators are affecting the relative detectability of the two prey species. The larvae of the two genera of mosquito examined here each have distinct morphology, posture in the water and colouration. *Anopheles* tend to be paler, and rest horizontally immediately below the water surface. *Culex* are generally darker and rest diagonally with a pronounced siphon touching the surface (Merritt et al. 1992). Associated behavioural differences are likely, and have been demonstrated between other mosquito species (e.g. Skiff and Yee 2014), but were not measured here.

An alternative explanation for the change in preference strength in the presence of conspecifics is that when three fish are present, the preferred prey depletes faster. As time progressed this would lead to fewer opportunities for the focal fish to select the preferred prey and reduce the apparent strength of preference. Females are likely to be stronger competitors as their consumption rate of mosquito larvae is nearly double
that of males (Elias 1995). The fact that the treatment with three female fish showed the greatest change in mean preference from that of the single fish supports both explanations. Further studies on the behaviour of the mosquito larvae at different predator densities, and recording the species consumed by companions as well as the focal fish, would help discriminate between these explanations.

**Sexual harassment**

Interestingly, the addition of cover in the male companion treatment led to focal females once again displaying a preference for *Culex*. We cannot rule out the possibility that this could be because males consumed more *Anopheles* in these trials, although trials testing males alone (in the same set up as presented here) suggest that males also display a preference for *Culex* (Deacon 2010) so this seems unlikely. Another explanation is that the cover enabled females to spatially separate themselves from males and therefore be relatively unaffected by their presence. Such spatial segregation has been documented in wild populations in which sexual harassment drove females into suboptimal habitats (Darden and Croft 2008). A useful follow up study would be to record the distances between companions and their locations within the aquaria during foraging trials, as this would help interpret these differences.

**Prey behaviour and habitat structure**

The proportion of remaining larvae found in the cover at the end of the trials was similar for both prey species; however, we did not record prey behaviour during the trial. This would be an interesting follow up study, as it may be that the refuge provided by the cover was more beneficial for conspicuous *Culex* larvae than for the more cryptic *Anopheles*. Baber and Babbitt (2004) investigated the effect of habitat structure on *Gambusia holbrooki* preference for two species of tadpole prey. They suggested that less active prey types might gain a greater advantage from habitat structure than more active species, highlighting the importance of behavioural differences between alternative prey species for the effects of habitat complexity.

Several studies have found that increased habitat heterogeneity, and the corresponding increase in refugia, can reduce predatory impact in aquatic communities (Bechara et al. 1993; Diehl 1992). 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 (Savino and Stein 1989). Again, further studies on the behaviour of the prey in this system would be insightful.

**Implications for biological control**

Our findings highlight the potential effects of a multi-prey system on the effectiveness of biological control measures using guppies. In our study,
both the simplest (females alone) and the most ecologically complex (females with males and cover) treatments revealed a preference for the species that is not a vector for malaria (Culex). This selectivity suggests that under such conditions female guppies would be unlikely to control the target species’ population. Conversely, females were relatively indiscriminate in their feeding habits in the other four treatments. The interacting effects of the social and physical environment on predatory behaviour could allow guppy populations to persist for even longer once the target prey has been successfully controlled (Manna et al. 2008), by readily consuming alternative prey. This could be beneficial from a biological control perspective as it would mean that fish would have to be replenished less frequently, if at all. However, depending upon their relative preference for the target prey, 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 – and especially if they show no evidence of preferring the target under any conditions.

Dua and colleagues (2007) found that guppies failed to deplete C. quinquefasciatus populations in a polluted drain in northern India, and used stomach content analysis to suggest that this could be explained by the presence of alternative planktonic prey. This phenomena is well-documented in other taxa, for example the presence of alternative prey species reduced the efficacy of predatory beetles in the control of slug populations (Symondson et al. 2006). Alternative prey species have a similar effect on the biological control of Colorado potato beetles by predatory bugs (Koss and Snyder 2005).

Future research

The next steps in evaluating the efficacy of guppies in mosquito control should involve more naturalistic mesocosm set ups and field-based studies. Several existing field studies seem to point towards the success of guppies as biological control agents when introduced into artificial containers. 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 1991). Similarly, malaria was virtually eradicated from several villages in Karnataka, India after the introduction of guppies into wells (Ghosh et al. 2005). However, a recent systematic review of published research into the efficacy of larvivorous fish in malarial control concluded that, despite such studies, there is currently insufficient evidence to determine whether introduced larvivorous fish have a significant effect on malarial incidence (Walshe et al. 2017). Clearly there is a need for more field-based research in this area.
As with the efficacy of guppies as mosquito control agents, their negative ecological effects are also poorly understood. However, there is growing evidence for their negative impact on native aquatic communities, via predation, competition, habitat alteration and disease (e.g. Gagne et al. 2015; Holitzki et al. 2013). Our findings emphasise that the impact of introduced guppies is unlikely to be restricted to the target pest species in the presence of alternative prey, and under inevitably variable social and habitat conditions, in the wild.

Introduced guppies come from a wide variety of sources – including wild, feral and domesticated populations (Deacon et al. 2011). The guppies studied here are thought to originate from wild-type guppies introduced to India in 1908 for malaria control (SKG, pers. obs.), which means they have coexisted with these prey species for more than a century. It would be interesting to compare the foraging behaviour and prey preferences of guppies of different origins and extents of domestication. Differences in foraging behaviour have been documented even between wild phenotypes in their native range (Bassar et al. 2010) and such variation may be relevant to both the efficacy and the ecological impact of introduced guppies.

Conclusions

There is a huge gap in our knowledge of the efficiency of guppies as biological control agents (Walshe et al. 2017), and similarly in our understanding of the ecological impacts of introduced guppies (El-Sabaawi et al. 2016). The findings presented here demonstrate that taking previously neglected factors such as social context and habitat complexity into account is likely to increase the ecological validity of studies. This is vital when using the findings to inform decisions as important as whether to introduce an exotic species to control diseases that are devastating to human populations. Further experiments will help determine the exact nature of these interactions and their implications for the use of guppies as biocontrol agents for malaria and other mosquito-borne diseases.

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