

A comparison of mosquito predation by the fish *Pseudomugil signifer* Kner and *Gambusia holbrooki* (Girard) in laboratory trials

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ABSTRACT: Using larval *Cx. annulirostris* mosquitoes, this study compared the predation rate of *Pseudomugil signifer* and *Gambusia holbrooki* for four larval instars, three prey densities, and three vegetation densities. There was no significant difference in the quantity of first, second, and third instar larvae consumed by the two fish species after 24 h. There was a significant negative relationship between the predation rate and both larval instar and prey density for both fish species. In simulated vegetation trials, *P. signifer* performed marginally better than *G. holbrooki* in medium to high density vegetation (0.3 stems/cm² and 0.6 stems/cm², respectively). *Journal of Vector Ecology* 30 (1): 87-90. 2005.

Keyword Index: *Pseudomugil signifer*, *Gambusia holbrooki*, *Culex annulirostris*, native fish, mosquito control.

INTRODUCTION

Constructed wetlands are becoming increasingly common in southeastern Australia as a means of providing water treatment, water storage, and wildlife refuges. However, they are a concern for the production of pest and vector mosquito populations. In particular, *Culex annulirostris* Skuse, is a known vector of flaviviruses such as Murray Valley encephalitis virus and Kunjin virus, and alphaviruses such as Ross River virus and Barmah Forest virus, and environmentally sensitive methods of mosquito control are required to manage these pests and the threat of arboviral disease for Australian communities (Russell 1999, Russell and Dwyer 2000).

The potential of larvivorous fish in mosquito control programs has been recognised for some time and one of the most commonly used fish for this purpose has been the mosquitofish, *Gambusia holbrooki* (Girard). Introduced to Australia in the 1920s, *G. holbrooki* is now widespread and abundant in southeastern Australia (Allen et al. 2002) and has the disadvantage of adversely impacting local fauna, including native fish (Gill et al. 1999) and frogs (Morgan and Buttemer 1996, Webb and Joss 1997). A common native species in eastern Australia, the Pacific Blue-eye, *Pseudomugil signifer* Kner, inhabits both freshwater and brackish habitats and has been suggested as a potentially effective predator on mosquitoes (Durre 1991). Mosquito larvae have been reported to comprise 89% of the daytime diet of this fish in a subtropical saltmarsh (Morton et al. 1988).

The suitability of a native fish for mosquito control will depend to a great extent on its effectiveness as a predator, and this should be evaluated under laboratory conditions before field trials are undertaken (Lloyd 1986). This aim of this investigation was to determine the potential usefulness of *P. signifer* for mosquito control in freshwater wetlands. In two laboratory trials, the predation rate of the exotic *G. holbrooki*

and the native *P. signifer* was assessed in controlled laboratory conditions against various prey sizes, prey densities, and vegetation densities.

MATERIALS AND METHODS

Mosquito species

All experiments used *Cx. annulirostris* mosquito larvae from the laboratory colony at the Department of Medical Entomology, Westmead Hospital. The development of *Cx. annulirostris* larvae includes four larval instars, each increasing in size, until pupation (McDonald et al. 1977).

Fish species

Pseudomugil signifer occurs along the northeast and southeast coast drainage divisions of Australia from Cooktown, Queensland, to Ulladulla, New South Wales (Merrick and Schmida 1984). It inhabits shallows along edges of lagoons, streams, and coastal lakes in fresh to brackish water (Semple 1986, Howe 1987). *Gambusia holbrooki* occurs throughout estuarine and freshwater systems in New South Wales (NSW), South Australia, Victoria, Queensland, and parts of Northern Territory and Western Australia (McDowall 1996). It prefers shallow waters along the margins and edges of vegetation and can tolerate a wide range of salinities (McDowall 1996). Both species were collected from Homebush Bay, Sydney, NSW.

All fish were acclimatised to laboratory conditions over a two-week period before experimentation in separate holding tanks with natural lighting and temperatures of 19°-21°C and salinity of <0.5 parts per thousand. They were provided a diet of commercially available fish food twice a day and mosquito larvae or blood worms once a week.

Because *P. signifer* and *G. holbrooki* are schooling fish (Moyle and Cech 1982, E. Howe, CSIRO, personal communication 1998), they were chosen randomly and tested

in groups of five in each replicate container for both experiments. Fish were returned to their aquaria upon completion of each treatment with a minimum of two weeks before the next treatment began. The experiments were conducted in a temperature-controlled environment (20° to 22°C) with natural lighting. A measuring tube similar to that of Litvak (1983) was used to measure fish without injury. Measurements were taken from the snout tip to the base of the tail.

Statistics

For both experiments, logistic regression analyses (SPSS for Windows, Version 10.0) were used to determine the independent predictors of the proportion of larvae consumed after 24 h. In this study, logistic regression analysis (or logit analysis) was applied to estimate the risk factor R on a dichotomous outcome factor Y (larva eaten, $Y = 1$; larva not eaten, $Y = 0$) as measured by the odds ratio. By using this method, it is possible to adjust for many confounding variables X (species, larval instar, larval density, and vegetation density) simultaneously. The problem is to estimate the effect associated with a risk factor (eaten vs. not eaten). The odds ratio is usually chosen as the measure of risk factor (or treatment) effect because it is simply related to the parameters of the logistic regression model.

Prey density and larval instar

The fish were starved for 24 h and placed in 1 liter containers (12 cm x 18 cm) 30 min before experimentation. Each fish species was exposed to each of the four larval instars (first, second, third, and fourth instar) at three separate densities of mosquito larvae (50, 100, and 200 larvae per tray) with three replicates at each density. A total of 45 fish was used at each larval density tested. Counts of mosquito larvae were made after 24 h. The same group of five fish was not used in any two successive treatments. The mean standard length of *P. signifer* and *G. holbrooki* used in all four treatments was 16.11 mm (sd = 2.67 mm) and 14.85 mm (sd = 2.71 mm), respectively.

Impact of vegetation density on predation

Experimental trays with a surface area of 55 cm x 37.5 cm and a gravel substrate shaped to a slope of 1:5 to simulate a pond bank contained approximately 21 liters of conditioned water with a maximum water depth of 10 cm. Stems of *Juncus acutus* Linnaeus were used to simulate emergent vegetation. They were placed within a fixed area along the shallow side of the tray in a grid pattern covering approximately one-third of the surface area. One hundred and fifty fourth instar larvae were placed in each tray 30 min prior to introduction of the fish. Fish were starved for 24 h before testing and were exposed to three vegetation densities: 100 stems (0.15/cm²), 200 stems (0.3/cm²), and 400 stems (0.6/cm²) with nil stems as a control and four replicates of each density. The minimum distance between stems decreased from approximately 1.8 cm at the low density to 0.90 cm at the high density. The number of larvae remaining after 24 h was recorded. The mean standard length of all *G. holbrooki* and *P. signifer* we used

was 20.58 mm (sd = 1.41 mm) and 21.08 mm (sd = 1.53 mm), respectively.

RESULTS

Prey density and larval instar

After 24 h, 100% of the first, second, and third instar larvae were consumed by both fish at the low and medium prey densities (data not shown). The percentage predation for *G. holbrooki* and *P. signifer* at the highest density (200 larvae) is shown in Figure 1. Mean predation rates on first, second, and third instar larvae by *G. holbrooki* and *P. signifer* were greater than 90%. However, the mean predation rate by both fish on fourth instar larvae was lower than 45%. As larval density increased, fish consumed more larvae, but this increase was not proportional to the change in density with mean predation rates of both species lower at high prey densities (Figure 2). Both species consumed approximately 85% of the lowest larval density; however, *G. holbrooki* and *P. signifer* consumed 35% and 50% of larvae, respectively, at the highest density. The odds of a larva being eaten by *P. signifer* were 1.75 times greater than those of being eaten by *G. holbrooki* ($P < 0.001$, 95% C.I. 1.47-2.08), after adjusting for instar and density. At the medium density, the odds that a larva was consumed by either fish decreased by a factor of 0.34 ($P < 0.001$, 95% C.I. 0.23-0.49), and at the highest density they decreased by a factor of 0.10 ($P < 0.001$, 95% C.I. 0.07-0.14).

Because neither species consumed all the fourth instar larvae at 24 h, data were analysed separately to determine significant differences between the species. Fish species and larval density were independent predictors of the proportion of larvae consumed. The odds of a larva being eaten by *P. signifer* were 1.51 times higher than the odds of a larva being consumed by *G. holbrooki* ($P < 0.001$, 95% C.I. 1.26-1.82) after adjusting for larval density. For every doubling in larval density, the odds that a larva was eaten by *G. holbrooki* decreased by a factor of 0.51 ($P < 0.001$, 95% C.I. 0.46-0.58). For every doubling in larval density, the odds that a larva was eaten by *P. signifer* decreased by a factor of 0.57 ($P < 0.001$, 95% C.I. 0.51-0.64).

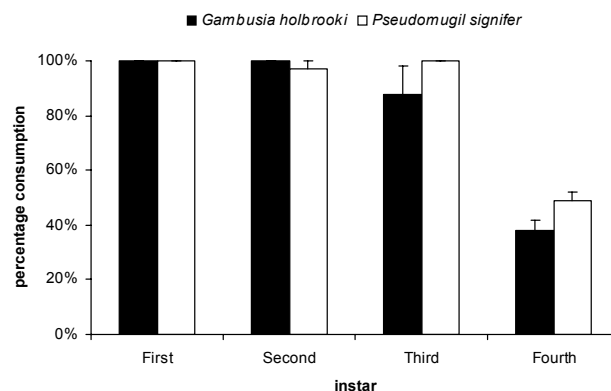


Figure 1. Percentage predation by *Gambusia holbrooki* and *Pseudomugil signifer* at 24 h for first, second, third, and fourth instar *Culex annulirostris* larvae at the highest density (200 larvae). Error bars represent standard deviation.

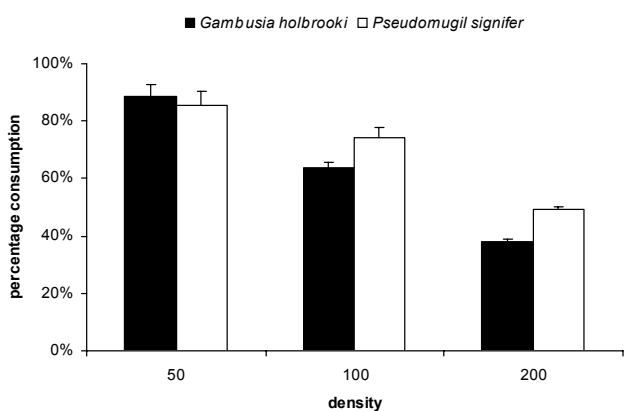


Figure 2. The percentage of fourth instar *Culex annulirostris* larvae consumed at 24 h by each fish species for the three larval densities. Error bars show standard deviation.

Impact of vegetation density on predation

In the control trays, larvae were distributed evenly around the perimeter, sometimes aggregating in the corners. In trays with low density stems, approximately half of the larvae were observed to distribute within the vegetated area. At medium and high density stems, the majority of larvae were distributed well within the vegetation and moved only occasionally into open water.

The number of larvae consumed was examined separately for each fish species because of the significant interaction between the species variable and stem density (Figure 3). *Gambusia holbrooki* responded similarly at the medium

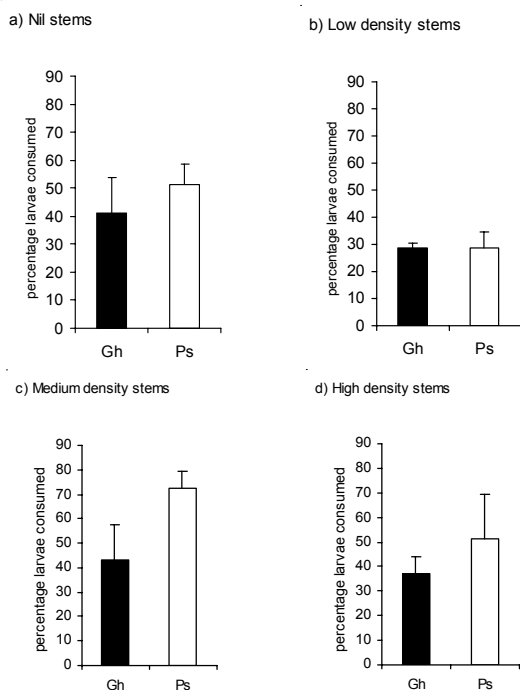


Figure 3. Percentage (%) of *Culex annulirostris* larvae consumed by *Gambusia holbrooki* (Gh) and *Pseudomugil signifer* (Ps) at a vegetation density of a) Nil stems, b) Low density stems, c) Medium density stems, and d) High density stems. Error bars show 95% confidence intervals.

($P=0.67$, 95% C.I. 0.81-1.40) and high density stems ($P=0.11$, 95% C.I. 0.60-1.06), consuming approximately 45% of the larvae compared to trays with nil stems. However, the odds of a larva being eaten by *G. holbrooki* at the low stem density decreased significantly by a factor of 0.51 ($P<0.001$, 95% C.I. 0.38-0.69) compared to the trays with no vegetation. *Pseudomugil signifer* was most effective in the medium density stems, consuming 70% of the larvae. The odds of a larva being consumed in the medium density stems were 1.86 greater ($P<0.001$, 95% C.I. 1.42-2.42) than the odds of a larva being consumed in no vegetation. However, at the low density stems the odds of a larva being eaten decreased by a factor of 0.47 ($P<0.001$, 95% C.I. 0.35-0.63) compared to trays with no vegetation.

DISCUSSION

Our results indicate that *P. signifer* holds potential as a mosquito control agent with its predation rate on all larval instars, regardless of prey density, comparable to that of *G. holbrooki*. However, both species reached a level of satiation when exposed to high prey densities or late instar mosquito larvae. The results support the findings of Booth (1980) who reported that similarities in the digestive tract histology and the relationship of body length to weight indicated comparable metabolic requirements of the two species.

Within the limitations of this investigation, *P. signifer* performed marginally better than *G. holbrooki* in experimental trays with nil stems and with medium to high density stems. *Pseudomugil signifer* consumed more larvae than *G. holbrooki* at the medium and high density stems, with a maximum mean of 70% of larvae consumed as opposed to 45% for *G. holbrooki*. Studies in North America have reported on the poor foraging ability of *G. affinis* in emergent weed habitats (Legner and Medved 1974, Walters and Legner 1980), and such is supported by the results of this experiment.

The consumption of larvae by both fish was not directly proportional to increases in vegetation density and this result may be attributed to behavioural changes by the mosquito larvae that influenced their distribution throughout the experimental trays. Larvae were readily consumed in the absence of vegetation as they clustered in the corners and distributed around the edges of the trays. However, as the density of the vegetation increased, larvae distributed themselves more evenly throughout the experimental tray. The distribution of larvae within the vegetation may have elicited an aggregative response in the fish so that the fish spent more time searching the area of highest larval density (Hassell and May 1974).

Although the results of these laboratory investigations indicate that *P. signifer* may be a useful mosquito control agent in the field, to be effective, *P. signifer* must be resilient to changes in water quality and interspecific competition. Many constructed wetlands are purposely built for storing and/or treating stormwater and wastewater runoff and, as a result, often contain polluted water that may be unsuitable for *P. signifer* populations.

The proliferation of *G. holbrooki* in urban catchments may limit the successful introduction of native fish such as *P. signifer* (Lloyd et al. 1986, Howe et al. 1997). The removal of *G. holbrooki* may need to be considered, but wetlands downstream of drainage systems will be susceptible to reintroduction of the fish. While the introduction of *G. holbrooki* is not encouraged, wetlands that support high densities of fish may benefit from the impact on mosquito populations, and management of vegetation density to increase their effectiveness as larval mosquito predators will be important.

In isolated or newly constructed wetlands, *P. signifer* may be able to establish and flourish and effect substantial larval control. Fortunately, *P. signifer* is not adversely affected by the commonly used mosquito control agent *Bacillus thuringiensis israelensis* (Brown et al. 1998), therefore this bacterial larvicide can be used readily in habitats once the fish has been introduced if supplemental control is required.

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