LABORATORY EVALUATION OF THE PREDATION EFFICACY OF NATIVE AUSTRALIAN FISH ON *CULEX ANNULIROSTRIS* (DIPTERA: CULICIDAE)

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ABSTRACT. The introduction and establishment of fish populations can provide long-term, cost-effective mosquito control in habitats such as constructed wetlands and ornamental lakes. The predation efficacy of 7 native Brisbane freshwater fish on 1st and 4th instars of the freshwater arbovirus vector *Culex annulirostris* was evaluated in a series of 24-h laboratory trials. The trials were conducted in 30-liter plastic carboys at 25 ± 1°C under a light:dark cycle of 14:10 h. The predation efficacy of native crimson-spotted rainbowfish *Melanotaenia duboulayi* (Melanotaeniidae), Australian smelt *Retropinna semoni* (Retropinnadae), Pacific blue-eye *Pseudomugil signifer* (Atherinidae), fly-specked hardyhead *Craterocephalus stercusmuscarum* (Atherinidae), firetail gudgeon *Hypseleotris galii* (Eleotridae), empire gudgeon *Hypseleotris compressa* (Eleotridae), and estuary perchlet *Ambassis marianus* (Ambassidae) was compared with the exotic eastern mosquitofish *Gambusia holbrooki* (Poeciliidae). This environmentally damaging exotic has been disseminated worldwide and has been declared noxious in Queensland. *Melanotaenia duboulayi* was found to consume the greatest numbers of both 1st and 4th instars of *Cx. annulirostris*. The predation efficacy of the remaining Australian native species was comparable with that of the exotic *G. holbrooki*. With the exception of *A. marianus*, the maximum predation rates of these native species were not statistically different whether tested individually or in a school of 6. Based on these data, *M. duboulayi*, *H. compressa*, and *A. marianus* warrant further investigation as biological control agents in pilot field trials.

KEYWORDS   Biological control, predation efficacy, native fish, *Culex annulirostris*, schooling

INTRODUCTION

In 1925, *Gambusia holbrooki* Girard was introduced into Queensland as a mosquito-control agent (Lloyd 1986). This species has subsequently caused considerable environmental damage by displacing native Australian fish species through competition and direct predation (Arthington et al. 1986, Arthington and Marshall 1999, Ivantsoff and Aarn 1999). Therefore, alternative biological control agents to *G. holbrooki* are required to effectively control mosquito populations with minimum impact on local ecosystems.

In Brisbane, stream fish assemblages are predominantly comprised of small insectivorous and omnivorous species such as crimson-spotted rainbowfish, *Melanotaenia duboulayi* (Castelnau); Australian smelt, *Retropinna semoni* (Weber); flyspecked hardyhead, *Craterocephalus stercusmuscarum* (Günther); firetail gudgeon, *Hypseleotris galii* (Ogilby); empire gudgeon, *Hypseleotris compressa* (Krefft); Pacific blue-eyes, *Pseudomugil signifer* Kner; and estuary perchlet, *Ambassis marianus* Günther (Arthington 1992). Small, native fish species such as these are ideal candidates for mosquito control, and while these species have often been cited as effective mosquito-control agents (Hoese et al. 1980, Ivantsoff 1980, McDowall 1980, Munro 1980, Lloyd 1986), little rigorous testing has been done. Most reports of mosquito control by Australian native species do not provide any real evidence, mostly citing anecdotal examples (Cooling 1923, Hamlyn-Harris 1929). There is evidence that native *R. semoni* and *M. duboulayi* include a higher percentage of mosquito larvae in their diets than *G. holbrooki* (Lloyd 1986). Also, *P. signifer* are reported to be equally as effective as *G. holbrooki* in preying on mosquito larvae in saltmarsh pools (Morton et al. 1988). Additionally, north Queensland populations of eastern rainbowfish, *Melanotaenia splendida splendida* (Peters) and *C. stercusmuscarum*, were efficient predators of mosquito larvae in the laboratory (Russell et al. 2001).

Although there are a number of native Australian fish species that are potentially more effective mosquito-control agents than *G. holbrooki*, careful evaluation of local Brisbane species is required. Some investigators have compiled lists of desirable characteristics or presented steps for evaluating efficacy of local fish species as larvivores (Ungureanu et al. 1981, Cech and Moyle 1983, Ahmed et al. 1988). In addition to a number of other factors, they recommend quantitative bioassays of field-collected fish to directly assess their relative efficacy in consuming mosquito larvae. Traditionally, the predation rate of individual fish is tested in the laboratory and the resulting consumption assumed to be the maximum consumption rate of this species. However, there is also evidence to suggest that the predation efficacy of individual fish of some species may increase when feeding in a school (Baird et al. 1991, Ranta and Kaitala 1991,

Foster et al. 2001). A number of the species tested, such as *R. semoni, M. duboulayi, A. marianus,* and *P. signifer,* are known schooling species (Ivanstoff 1980, McDowall 1980, Munro 1980) and in an effort to determine maximum predation rates of each species, schools of fish in addition to individuals were evaluated. Our study, therefore, provides the essential laboratory assessment to determine which species are worthy of field assessment. *Culex annulirostris* Skuse is a major vector of arboviruses in Australia and breeds in permanent and temporary freshwater (Russell 2002).

MATERIALS AND METHODS

Collection and maintenance of local freshwater fish

Adult *M. duboulayi* (mean total length and SD of 74.30 ± 3.57 mm), *R. semoni* (46.88 ± 3.49 mm), *C. stercusmuscarum* (40.46 ± 5.74 mm), *H. galii* (36.61 ± 7.13 mm), and *H. compressa* (58.76 ± 4.35 mm) were collected from Kholo Creek, Brisbane (27°32′S, 152°51′E). Subadult *M. duboulayi* (40.31 ± 2.06 mm) were collected from Little Nerang Dam, Gold Coast (28°09′S, 153°17′E). *Pseudomugil signifer* (34.37 ± 2.23 mm) and *A. marianus* (49.63 ± 3.65 mm) were collected from the brackish reaches of the North Pine River, Brisbane (27°17′S, 153°01′E). A Department of Primary Industries permit (General Fisheries Permit PRM02079 F) was required for collection of *G. holbrooki* (31.18 ± 5.13 mm) from Kedron Brook, Brisbane (27°24′S, 153°03′E). Individuals were collected using a 40- × 40-cm, 1.2-mm-mesh scoop net (Jones's Tackle Pty. Ltd., Brisbane, Australia) and 25- × 25- × 46-cm, 2-mm-mesh bait traps (Mossop's Tackle Pty. Ltd., Brisbane, Australia). Specimens were transported to the laboratory in aerated 15-liter buckets. For those species collected from freshwater, 4 g/liter of table salt was added to the water to prevent bacterial and parasite infection and reduce the effects of stress, osmotic imbalance, and surface damage during transport (Legget 1995). In the laboratory, fish were maintained in 42- × 34- × 2-cm glass aquaria equipped with biological filtration at 25 ± 1°C. Fish were fed Wardley's tropical food flakes® (Wardley Corporation, Secaucus, NJ) and mosquito larvae daily and were acclimated for at least 14 days prior to experimentation.

Mosquitoes

The 1st and 4th instars of *Cx. annulirostris* used in this study were reared from egg rafts obtained from a colony maintained at the Queensland Institute of Medical Research (QIMR). The colony was originally established in 2001 from *Cx. annulirostris* adults collected from Boondal, Brisbane.

General

The laboratory methods applied in this study are a modification of those detailed by Panicker et al. (1985), Homski et al. (1994), and Russell et al. (2001). Whereas Homski et al. (1994) ran predation trials in 2.5-liter glass jars, we utilized 30 liters of tap water held in white plastic containers filled to a depth of 0.5 m. The increased volume/water depth was chosen to allow species to forage at the appropriate water levels according to previously stated preferences: bottom (*H. compressa*), midwater (*H. galii*), surface (*R. semoni, G. holbrooki*), or all levels (*M. duboulayi, A. marianus, P. signifer, and C. stercusmuscarum*). The tap water was aerated, treated with Prime® water conditioner (Seachem Laboratories Inc, Stone Mountain, GA) to remove chlorine and chloramine compounds lethal to fish, and left to stand for 24 h before use. Individual test specimens were removed from the holding aquaria and distributed randomly into test containers. The trials were conducted at 25 ± 1°C under a light:dark cycle of 14:10 h. For each trial, the fish were then held in the containers for a 24-h acclimation period to standardize hunger levels and ensure all gut contents had been evacuated and known numbers of *Cx. annulirostris* added. After 24 h, the fish were removed from the containers, the total length and wet weight for each individual recorded and remaining larvae (dead or alive) were counted and consumption calculated.

Predation efficacy on 4th instars of *Cx. annulirostris*

Two hundred 4th instars of *Cx. annulirostris* were added to each of 7 treatment and 3 control containers. Males and females of each fish species were tested separately to determine possible differences in predation efficacy between sexes. Both adult and subadult *M. duboulayi* were tested. If all larvae were consumed by a species within the initial test, the individuals were retested, using increments of 50 larvae until the maximum predation rate had been determined. Data was also expressed as number consumed per gram of body weight.

Predation efficacy on 1st instars of *Cx. annulirostris*

One thousand 1st instars of *Cx. annulirostris* were added to each of 5 treatment and 3 control containers and tested over 24 h for predation by males of the 8 species. Again, when all larvae were consumed, numbers were increased in increments of 1,000 larvae until the maximum predation rate was determined. Data was also expressed as number consumed per gram of body weight.

Effects of intragroup competition on predation efficacy of native fish

For comparison with individual predation rates, schools of 6 male fish were tested under the same conditions as individual tests using 4th instars of *Cx. annulirostris*. To allow for possible increased predation rate due to schooling, each group of 6 fish was offered 12 times the mean number of larvae eaten in individual predation trials. Each experiment was replicated 5 times using different fish in each trial. The number of larvae consumed per fish per school was then calculated based on the assumption that individuals within each school consumed equal numbers of larvae.

Statistical methods

To correct for natural larval mortality in treatments, predation efficacy data was normalized with respect to the controls using Abbott's formula (Abbott 1925). The effect of fish species on predation efficacy was tested using a 1-way analysis of variance with all pairwise comparisons analyzed by Tukey honestly significantly different test (Tukey 1953) (SigmaStat for Windows, version 2, Jandel Corporation). The effect of fish gender and intragroup competition on predation efficacy within species was tested using a *t*-test. Raw data was squareroot transformed to satisfy the assumptions of normality and equal variance.

RESULTS

Predation efficacy on 4th instars of *Cx. annulirostris*

Of the 8 species evaluated, only *G. holbrooki* ($t = -5$, df $= 12$, $P < 0.001$) and *H. galii* ($t = 3.49$, d.f. $= 12$, $P = 0.005$) exhibited gender differences in predation efficacy (Table 1). Female *G. holbrooki* consumed greater numbers of *Cx. annulirostris* than males, whilst male *H. galii* consumed greater numbers than did females. When reanalyzed relative to body weight, no such differences were apparent. When the predation rates of males and females were combined for each species, adult *M. duboulayi* consumed significantly higher numbers than all other species tested and, not surprisingly, subadult *M. duboulayi* (F $= 51.99$, df $= 8$, $P < 0.001$) (Table 1). Subadult *M. duboulayi* consumed significantly higher numbers than all remaining species except *R. semoni*, which consumed significantly higher numbers than *P. signifer* and *H. galii* (F $= 51.99$, df $= 8$, $P < 0.001$). The predation rate of *G. holbrooki* was comparable with the remaining 6 native species evaluated (F $= 51.99$, df $= 8$, $P < 0.001$).

Relative to body weight (Table 1), subadult *M. duboulayi* consumed significantly higher (F $= 26.28$, df $= 8$, $P < 0.001$) numbers of 4th instars of *Cx. annulirostris* than *H. compressa*, *A. mari-*

Table 1. Mean number expressed as Abbott's adjusted (±SD) and per gram of body weight of fourth instar of *Culex annulirostris* consumed by 7 native and 1 introduced fish species individually or in a school of 6.[1]

| Fish species[2] | Male | | Female | | Combined | | Schooled |
	Mean number (±SD)	Mean number (±SD) per g body weight	Mean number (±SD)	Mean number (±SD) per g body weight	Mean number (±SD)	Mean number (±SD) per g body weight	mean number (±SD)
A. m.	46.7^c,d (20.7)	34.8^c,d (14.7)	52.2^c (11.8)	34.9^c,d (9.5)	49.5^c,d (16.5)	34.9^d (11.9)	75.3^a (12.0)
C. s.	38.3^d,e (11.2)	102.2^b (35.3)	44.3^c,d (24.3)	76.4^b,c (23.1)	41.3^c,d (18.5)	89.3^b,c (31.6)	62.5^a (14.5)
H. c.	44.9^c,d,e (28.7)	21.8^d (12.4)	47.7^c,d (23.8)	20.7^d (8.4)	46.4^c,d (25.1)	21.2^d (10.0)	79.2^a (19.0)
H. g.	54.9^c,d (32.7)	72.0^b,c (43.4)	16.7^d (7.3)	59.9^b,c (23.6)	35.8^d (30.2)	66.0^c (34.2)	68.2^a (10.5)
M. d. (adult)	319.9^a (14.2)	83.0^b (9.8)	237.4^a (102.0)	82.8^a,b (34.6)	278.7^a (82.0)	82.9^b,c (24.4)	NT
M. d. (subadult)	101.9^b (16.6)	150.7^a (16.6)	119.3^b (29.5)	133.1^a (31.8)	112.0^b (24.0)	142.6^a (25.3)	92.7^a (19.9)
P. s.	29.0^d,e (13.2)	60.4^b,c (27.5)	23.3^c,d (7.4)	57.2^b,c (18.3)	26.0^d (10.4)	58.7^c,d (22.1)	19.9^b (8.4)
R. s	75.1^b,c (13.8)	96.2^a,b (22.3)	65.4^b,c (33.5)	111.3^a,b (55.5)	70.3^b,c (25.1)	103.8^a,b (41.4)	99.2^a (31.4)
G. h.	18.8^e (7.1)	105.0^a,b (52.3)	64.9^b,c (27.8)	137.3^a (60.2)	41.8^c,d (30.9)	121.1^a,b (56.7)	NT

[1] Column means followed by the same letter were not significantly different (*P* > 0.05). NT, not tested.
[2] *A. m.*, *Ambassis marianus*; *C. s.*, *Craterocephalus stercusmuscarum*; *H. c.*, *Hypseleotris compressa*; *H. g.*, *Hypseleotris galii*; *M. d.*, *Melanotaenia duboulayi*; *P. s.*, *Pseudomugil signifer*; *R. s.*, *Retropinna semoni*; and *G. h.*, *Gambusia holbrooki*.

Table 2. Comparison of the mean number (Abbott's adjusted [±SD]) of first instar *Culex annulirostris* consumed by 7 native and 1 introduced fish species versus the mean number consumed per gram of body weight.[1]

Fish species	Male			
	Mean number (±SD)		Mean number (±SD) per g body weight	
Ambassis marianus	744.8[c,d]	(348.0)	565.2[c,d]	(311.6)
Craterocephalus stercusmuscarum	855.9[b,c]	(268.5)	1,475.5[c]	(526.1)
Hypseleotris compressa	402.0[d]	(217.3)	236.0[d]	(133.5)
Hypseleotris galii	829.6[c,d]	(128.6)	1,052.1[c,d]	(363.1)
Melanotaenia duboulayi (subadult)	7,164.5[a]	(628.4)	9,708.5[b]	(1,239.0)
Pseudomugil signifer	581.5[c,d]	(325.1)	984.2[c,d]	(593.6)
Retropinna semoni	863.6[b,c]	(236.9)	1,328.8[c]	(392.7)
Gambusia holbrooki	1,648.1[b]	(414.8)	15,026.5[a]	(5,328.9)

[1] Column means followed by the same letter were not significantly different ($P > 0.05$).

anus, H. galii, C. stercusmuscarum, adult *M. duboulayi* and *P. signifer. Retropinna semoni* and *G. holbrooki* were also efficient larvivores, consuming significantly higher ($F = 26.28$, df $= 8$, $P < 0.001$) numbers of 4th instars of *Cx. annulirostris* than *H. compressa, A. marianus, H. galii,* and *P. signifer. Craterocephalus stercusmuscarum, H. galii,* and adult *M. duboulayi* consumed significantly ($F = 26.28$, df $= 8$, $P < 0.001$) higher numbers of 4th instars of *Cx. annulirostris* than either *H. compressa* or *A. marianus* ($P < 0.001$).

Predation efficacy on 1st instars of *Cx. annulirostris*

Subadult *M. duboulayi* were found to be the most effective predator of *Cx. annulirostris* 1st instars, consuming significantly higher numbers than all other species tested ($F = 83.85$, df $= 7$, $P < 0.001$) (Table 2). Conversely, *H. compressa* were the least effective predator of 1st instars of *Cx. annulirostris,* consuming significantly less ($F = 83.85$, df $= 7$, $P < 0.001$) than *M. duboulayi, R. semoni,* and *C. stercusmuscarum. Pseudomugil signifer, H. galii,* and *A. marianus* were intermediate to, but not statistically different from, these 2 groups. The introduced *G. holbrooki* consumed significantly higher numbers ($F = 83.85$, df $= 7$, $P < 0.001$) than *H. compressa, P. signifer, H. galii,* and *A. marianus.*

Subadult *M. duboulayi* consumed significantly higher ($F = 68.75$, df $= 7$, $P < 0.001$) numbers of 1st instars of *Cx. annulirostris* larvae per gram of body weight than all other native species tested. *Hypseleotris compressa* was least effective, consuming significantly less ($F = 68.75$, df $= 7$, $P < 0.001$) than *M. duboulayi, C. stercusmuscarum,* and *R. semoni.* The introduced *G. holbrooki* consumed significantly higher numbers ($F = 68.75$, df $= 7$, $P < 0.001$) than all other species tested.

Effects of intragroup competition on predation efficacy of native fishes

Intragroup competition within a school of fish (Table 1) did not significantly increase the number

of 4th instars of *Cx. annulirostris* larvae consumed by male *M. duboulayi* ($t = 0.87$, df $= 10$, $P = 0.41$), *R. semoni* ($t = -1.87$, df $= 9$, $P = 0.1$), *P. signifer* ($t = 1.15$, df $= 9$, $P = 0.28$), *C. stercusmuscarum* ($t = -2.08$, df $= 17$, $P = 0.053$), *H. galii* ($t = -1.03$, df $= 10$, $P = 0.327$), and *H. compressa* ($F = 3.65$, df $= 10$, $P = 0.09$). Individual *A. marianus* consumed significantly higher numbers of 4th instars of *Cx. annulirostris* larvae within a school than when tested alone ($t = -5.1$, d.f. $= 10$, $P < 0.001$).

DISCUSSION

This is the 1st evaluation of Australian native fish as mosquito predators utilizing *G. holbrooki* as a comparison. The predation efficacy of various species of *Gambusia* have been evaluated prior to their use in numerous mosquito-control programs worldwide (Menon and Rajagopalan 1977, Yadav et al. 1992, Homski et al. 1994). In comparison, native species performed favorably, some consumed greater numbers of mosquito larvae, i.e., *M. duboulayi* and *R. semoni,* while others demonstrated similar predation rates to those of the introduced species.

Yadav et al. (1992) and Jayasree et al. (1992) used laboratory tests for evaluating the predation efficacy of candidate biological control agents, presenting a predatory index, which was defined as the number of larvae consumed per gram of body weight. As size-related sexual dimorphism has been noted in both *G. holbrooki* and *H. galii* (Hoese et al. 1980), use of the predatory index was considered appropriate in evaluating performance.

In addition to high predation efficacy, species that do not discriminate on the basis of instar are favored for operational use. In this case, populations will feed continuously without periods of prey switching or starvation due to the absence of a preferred instar. However, instar discrimination may still be desirable if the preference is for later instars, as a predilection for early instars may fail to reduce mosquito densities below that caused by expected natural mortality of the population (Service 1995).

For instance, the survival rate of *Cx. annulirostris* in man-made ponds without introduced predators was estimated to be 11% (McDonald and Buchanan 1981). Also, if a biological control agent consumes early instars in preference to the later ones, the resulting reduced densities of older larvae may lessen interspecific competition, thus allowing more larvae to pupate and give rise to adults (Service 1995). Although all species consumed both 1st and 4th instars of *Cx. annulirostris,* 2 species, *H. compressa* and *P. signifer,* showed a possible preference for one instar over another. *Hypseleotris compressa* is reported to prefer larger, coarser food (Hamlyn-Harris 1929) and this was evident in the results, as this species was the least effective predator of 1st instars of *Cx. annulirostris.* In contrast, *P. signifer* was the least effective predator of 4th instars of *Cx. annulirostris* but consumed similar numbers of 1st instars as other species. The small mouthgape of *P. signifer* results in up to 100-fold increases in handling time for prey items longer than 3 mm (Booth et al. 1985). The mean length of 4th instars of *Cx. annulirostris* in the current study was 6.3 ± 0.8 mm. *Melanotaenia duboulayi* consumed high numbers of both instars, as did *A. marianus, R. semoni, C. stercusmuscarum,* and *H. galii.*

At least one species, i.e., *A. marianus,* showed a statistically significant increase in predation efficacy when used as a school. Efficacy for other schooling species, such as *M. duboulayi, R. semoni, C. stercusmuscarum, A. marianus,* and *P. signifer* did not change.

Although all species consumed mosquito larvae, the current data and available literature indicate that some species have less potential for use in mosquito-control programs than others. For instance, while *R. semoni* and *C. stercusmuscarum* were efficient larvivores, both showed poor survival during collection and transport (McDowall 1980, Russell et al. 2001). In captivity, high levels of mortality were also noted in the current study and stocks for testing were difficult to collect due to these species' patchy distributions. Conversely, *M. duboulayi, H. galii,* and *H. compressa* are hardy species, particularly *H. compressa,* which were able to tolerate very poor water quality (Cooling 1923, Hamlyn-Harris 1929). Also, *M. duboulayi* and *H. galii* are highly adaptable to laboratory and field conditions and reproduce readily in aquaria and experimental ponds, making them easy to culture and distribute (Hamlyn-Harris 1929, Hoese et al. 1980). However, *H. galii* displayed differences in predation efficacy between sexes, indicating that they may have less potential as mosquito-control agents than some of the other species. The other gudgeon tested, *H. compressa,* displayed some preference for later instars, which may prove beneficial in mosquito-control programs. In contrast, the predilection of *P. signifer* for early instars is considered a disadvantage for a mosquito-control agent. The other brackish-water species evaluated, *A. marianus,* was an effi-

cient larvivore when schooled and may have potential if released into appropriate habitats. However, this species is reported to feed predominantly on aquatic plants (Arthington 1992), and given a choice may show preference for aquatic plants over mosquito larvae in the field.

Populations of nontarget macroinvertebrates, serving as alternative food sources, may reduce the intensity of mosquito predation by fish (Hoy et al. 1972, Hurlbert et al. 1972, Kramer et al. 1988, Linden and Cech 1990, Walton and Mulla 1991, Blaustein 1992). Although *A. marianus* fed readily on mosquito larvae in our laboratory trials, gut content analysis (Arthington 1992) indicated that this species is predominantly vegetarian in nature. Cooling (1923) noted *P. signifer* is less likely to feed on mosquito larvae in nature than when observed in a laboratory (Cooling 1923). In tropical north Queensland, *M. splendida* is known to prey on tadpoles of the green tree frog, *Litoria caerulea* (White) (Hylidae) (Russell et al. 2001). In New South Wales, *H. compressa, H. galii,* and *P. signifer* reportedly prey on green and golden bell frogs, *Litoria aurea* (Lesson), eggs, fry, and tadpoles if sufficiently hungry (Pyke and White 2000). In Brisbane, *H. compressa* and *H. galii* had ingested large numbers of chironomid rather than mosquito larvae, as determined by their gut contents (Arthington 1992). These findings indicate that the next essential step in our study should be to find the response to alternative prey.

This present study demonstrates that predation rates of some Australian native fish are equal to and in some cases, i.e., *M. duboulayi,* greater than *Gambusia* species evaluated in this and similar studies worldwide (Menon and Rajagopalan 1977, Yadav et al. 1992, Homski et al. 1994). Currently, *M. duboulayi, H. compressa,* and *A. marianus* are the strongest candidates for biological control programs against *Cx. annulirostris,* especially for introduction into constructed wetlands and ornamental lakes.

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