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Behavioural interactions between the introduced plague minnow *Gambusia holbrooki* and the vulnerable native Australian ornate rainbowfish *Rhadinocentrus ornatus*, under experimental conditions

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The impact of the plague minnow *Gambusia holbrooki* on the ornate rainbowfish *Rhadinocentrus ornatus* was investigated by comparing the behavioural response and microhabitat preferences of populations of *R. ornatus* collected from locations that are sympatric and allopatric with *G. holbrooki*. *Rhadinocentrus ornatus* populations from sympatric areas exhibited a significantly higher frequency of intraspecific chases, spent significantly more time at an intermediate depth and were nipped significantly less often by *G. holbrooki* compared to the allopatric *R. ornatus* populations. The frequency of intraspecific chases by all *R. ornatus* populations were greatest immediately following *G. holbrooki* exposure and increased further with repeated exposure. Activity levels were also significantly higher in the presence of *G. holbrooki*. *Gambusia holbrooki* and the allopatric *R. ornatus* populations showed very similar microhabitat preferences, whereas the preferences for the sympatric *R. ornatus* populations have shifted to facilitate cohabitation with *G. holbrooki*. The results suggest that sympatric populations of *R. ornatus* have evolved or developed behavioural responses to *G. holbrooki* through niche and character shifts. The implications are discussed in relation to the conservation management of *R. ornatus* and other threatened species.

Key words: behavioural interactions; *Gambusia*; introduced; invasive; native; niche shift.

INTRODUCTION

Many Australian native freshwater fishes are currently under threat from habitat destruction and the introduction of invasive pest species. Introduced fishes can directly affect native fishes *via* resource competition, predation, interference with reproduction and the introduction of parasites and diseases (Meffe, 1984; Kennard *et al.*, 2005). Exotic fishes may also contribute to reducing native fish populations indirectly by altering habitat conditions and ecosystem processes, which are often exacerbated by human activities (Arthington *et al.*, 1983).

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The destruction of aquatic habitats as a result of land clearance for housing development is considered to be a major factor contributing to the increase in exotic fish species in many waters around Australia (Arthington *et al.*, 1983; King & Warburton, 2007). This is because many introduced fish species have the ability to thrive in degraded aquatic habitats and their potential effect on aquatic ecosystem structure is magnified due to their wide environmental tolerances and high densities (Kennard *et al.*, 2005).

Through its widespread introduction, *Gambusia holbrooki* Girard has devastated several native species worldwide (Belk & Lydeard, 1994; Barrier & Hicks, 1994; Rincón *et al.*, 2002). A highly documented example is of the reduction of the native Sonoran topminnow *Poeciliopsis occidentalis* (Baird & Girard) in the U.S. (Meffe *et al.*, 1983; Meffe, 1985). In the early 1920s, *G. holbrooki* was introduced into Australia as part of a mosquito control programme. Its distribution now encompasses much of the country from Adelaide to Cairns (Arthington *et al.*, 1983; Arthington & Lloyd, 1989; Lloyd, 1990; Arthington & Marshall, 1999) where it favours aquatic vegetation in warm, slow flowing and still freshwater habitats (Casterlin & Reynolds, 1977; Pen & Potter, 1991). *Gambusia holbrooki* is an extremely successful invader and continues to expand into new areas owing to its fast maturation rate, high behavioural plasticity and ability to tolerate a wide range of environmental variables (Arthington *et al.*, 1990; Pen *et al.*, 1993). *Gambusia holbrooki* was incorrectly identified in previous literature as *Gambusia affinis* (Baird & Girard) in Australia (Lloyd & Tomasov, 1985).

In Australia, *G. holbrooki* has been implicated in the decline of many native fishes and amphibian species. *Gambusia holbrooki* is reported to consume eggs and hatchlings, as well as causing tail-fin damage by nipping the tails of tadpoles in several frog species (Morgan & Buttemer, 1996; Komak & Crossland, 2000). Similarly, *G. holbrooki* is implicated in the decline of the endangered green and golden bell frog *Litoria aurea* Lesson (Morgan & Buttemer, 1996; White & Pyke, 1996), as well as fish species from the genera *Mogurnda*, *Ambassis*, *Melanotaenia*, *Pseudomugil*, *Craterocephalus* and *Retropinna* (Lloyd, 1990; Morris *et al.*, 2001). Wager & Unmack (2004) suggested that the absence of juvenile *Scaturiginichthys vermeilipinnis* Ivantsoff, Unmack, Saeed & Crowley in freshwater springs at Edgbaston, central Western Queensland, was probably due to predation of their eggs or fry by *G. holbrooki*. Consequently, predation by *G. holbrooki* was listed as a key threatening process in the New South Wales Threatened Species Conservation Act 1995.

Gambusia holbrooki is an opportunistic surface-feeding carnivore with a diet consisting of terrestrial and aquatic insects and occasionally benthic organisms (Arthington, 1989; Pen *et al.*, 1993; Stoffels & Humphries, 2003; King & Warburton, 2007). Studies have suggested that where native fishes and *G. holbrooki* coexist, competition often occurs. This is due to an extensive niche overlap in requirements for food and space, particularly when resources become scarce, which ultimately leads to competitive exclusion (Pen *et al.*, 1993; Arthington & Marshall, 1999; Bøhn & Amundsen, 2001). In addition, *G. holbrooki* is a highly aggressive species, which may cause deleterious effects in native fish species by fin nipping (Myers, 1965; Meffe, 1985). While fin nipping itself may not be lethal, damage to the skin and mucous membrane renders this fish susceptible to secondary infection (Meffe *et al.*, 1983). In a recent study by Warburton & Madden (2003), *G. holbrooki* attacked two native Australian fish species most frequently whilst competing for food. Furthermore, Howe *et al.* (1997) found that the aggressive behaviour of *G. holbrooki* was correlated with the reduced growth, feeding and reproduction in Pacific blue eyes *Pseudomugil signifer* Kner. In response to the increasing abundance and expanding distribution of *G. holbrooki*, native fish species may show competition avoidance mechanisms such as distribution and niche shifts (Lloyd, 1990; Arthington, 1991). As new areas are colonized by *G. holbrooki*, however, new interactions are initiated and intense competition may lead to local extinction.

Gambusia holbrooki has the potential to decimate native fish populations with restricted ranges, such as the native ornate rainbowfish *Rhadinocentrus ornatus* Regan (Morris *et al.*, 2001). *Rhadinocentrus ornatus* has a patchy localized distribution, occurring in coastal wallum (Banksia heathland) streams, swamps and lake habitats on the east coast of Australia from Coffs Harbour in northern New South Wales (NSW) to Fraser Island in south-east Queensland (Arthington & Marshall, 1999; Morris *et al.*, 2001; Page *et al.*, 2004). Much of the habitat that *R. ornatus* occupies also supports two vulnerable species, the honey blue-eye *Pseudomugil mellis* Allen & Ivanstoff and the oxleyan pygmy perch *Nannoperca oxleyana* Whitley (Arthington & Marshall, 1996; Pusey *et al.*, 2004). These species are currently under threat from a number of factors, including loss of habitat due to housing development, forestry and agriculture as well as resource competition with *G. holbrooki* (Arthington, 1984; Arthington *et al.*, 1994; Arthington & Marshall, 1999; Morris *et al.*, 2001). *Gambusia holbrooki* is also a known predator of the eggs and larvae of *R. ornatus* (Ivantsoff & Aarn, 1999). As a result of these threatening processes, *R. ornatus* has been nominated for protection in Queensland and NSW (Morris *et al.*, 2001; Page *et al.*, 2004). There are increasing concerns that if these threatening processes are not addressed, the current status of *R. ornatus* may shift to endangered (Arthington, 1991; Arthington & Marshall, 1999; Morris *et al.*, 2001).

The mechanistic processes underlying observed negative associations between *G. holbrooki* and Australian native fish species have received little attention and warrant both field and laboratory experimentation. The aims of this study, therefore, were to: (1) examine the activity patterns and antagonistic behaviour of *G. holbrooki* and *R. ornatus* under laboratory conditions, (2) compare and contrast the behavioural patterns of populations of *R. ornatus* living sympatrically and allopatrically with *G. holbrooki* and (3) determine habitat use by each species and the potential overlap or differentiation of habitat occupation.

MATERIALS AND METHODS

FISH COLLECTION AND STUDY SITES

Adult *R. ornatus* and *G. holbrooki* were collected over 4 days from 24 March 2007, using baited traps and seines from seven sites around Coffs Harbour and Grafton, NSW. *Rhadinocentrus ornatus* was collected from four sites containing *G. holbrooki* (sympatric) and three sites containing no *G. holbrooki* (allopatric) (Table I). The presence or absence of *G. holbrooki* at each site was determined through replicate fish

TABLE I. *Rhadinocentrus ornatus* collection site details, number of individuals and mean ± S.D. standard length (L_S)

Rhadinocentrus ornatus population	Site name	Drainage	GPS co-ordinates	Sample size	L_S (mm)
Sympatric	Urumbilum River	Orara River	30°15′56″ S; 152°58′59″ E	35	25·3 ± 4·4
Sympatric	Pine Creek, Pacific Highway	Pine Creek	30°23′53″ S; 153°01′53″ E	8	33·4 ± 5·9
Sympatric	Orara River, Watkin road crossing	Orara River	30°15′29″ S; 153°00′42″ E	15	35·7 ± 5·1
Sympatric	Sportsmans Creek	Orara River	29°28′57″ S; 152°59′22″ E	4	35·5 ± 3·4
Allopatric	Wayper Creek	Orara River	30°15′43″ S; 152°57′43″ E	35	27·9 ± 4·4
Allopatric	Lazyman Creek	Corindi River	30°02′32″ S; 153°06′39″ E	4	31·0 ± 7·6
Allopatric	Upper Pine Creek	Pine creek	30°23′18″ S; 152°57′26″ E	8	38·0 ± 6·0

surveys. *Gambusia holbrooki* was collected from the same two sites as the sympatric *R. ornatus*, as well as an additional site (Table II). The locations from which the sympatric populations were collected had been subjected to a high level of human activity resulting in open habitats dominated by invasive plants. The allopatric populations, in contrast, were collected from sites that were reasonably well preserved with limited clearing and extensive native riparian vegetation.

Each population was transported in isolation to Macquarie University in well oxygenated water. Some mortality, however, occurred resulting in a low sample size of *R. ornatus* for some of the populations. This may be due to high levels of stress in these populations and requires further examination. Each population was housed separately in 150 l semi-natural aquaria at 24° C for a period of 2 weeks. Light was provided by fluorescent tubes on a 12L:12D cycle. Within the first week, fish were fed a combination of live bloodworms (chironomid larvae), white worms (*Anguillula silusiae*) and slowly weaned onto commercial flake foods.

BEHAVIOURAL OBSERVATION EXPERIMENT

Rhadinocentrus ornatus individuals were frequently observed in small shoals in the field, therefore, four fish of both *R. ornatus* and *G. holbrooki* were used for each trial in these experiments. The shoals used in the observation experiments contained mixed-sex individuals of both species to simulate the natural conditions, and observations were carried out in a large 1240 × 640 × 300 mm aquarium. To measure depth preference of *R. ornatus*, a series of white perspex steps were placed in the aquarium

TABLE II. *Gambusia holbrooki* collection site details, number of individuals, sex composition, mean ± S.D. standard length (L_S) and number of males and females

Site name	Drainage	GPS co-ordinates	Sample size	L_S (mm)	Number of males	Number of females
Urumbilum River	Orara River	30°15′56″ S; 152°58′59″ E	35	21·1 ± 3·1	12	23
Pine creek, Pacific Highway	Pine Creek	30°23′53″ S; 153°01′53″ E	35	25·2 ± 5·2	14	21
Corindi Creek	Corindi River	30°01′18″ S; 153°11′30″ E	35	24·0 ± 4·1	17	18

providing three different depths: shallow (0·1 m), intermediate (0·3 m) and deep (0·5 m) (Fig. 1). Observations of *R. ornatus* behaviour were made before, during and after the introduction of *G. holbrooki* to the test arena.

All fishes were deprived of food for 48 h prior to the experiment. Four *R. ornatus* selected from either the sympatric or allopatric *G. holbrooki* populations were introduced to the aquarium and left to settle for 15 min. The behaviour and depth preference of a focal individual was then recorded for 5 min. The behavioural variables recorded included incidences of aggression between individuals such as chasing and fin nipping, as well as the proportion of time spent in the shallow, intermediate or deep areas in the aquarium. A chase was defined as when one fish made a rapid movement towards another fish, whereby the fish being approached swam away. Nipping was defined as when a fish responded to the behaviour by another fish with a short jolt. All observations were recorded using the EthoLog 225 programme (Ottoni, 2000). The level of activity by *R. ornatus* was also recorded as the number of times each individual moved between the three depth levels. These observations were repeated until all four fish in the shoal had been observed (*i.e.* 20 min).

After initial observations were made on *R. ornatus* shoals, four *G. holbrooki* were randomly selected from a population originating from the same drainage as the four *R. ornatus* and introduced to the tank. The *G. holbrooki* were allowed to settle for 15 min, and then a single fish flake was placed on the water's surface in a randomly chosen location to encourage competitive interactions for limited resources (Warburton & Madden, 2003). During this time, the behavioural observations outlined above were repeated every 5 min for each *R. ornatus*, including incidences of aggression between *R. ornatus* and *G. holbrooki*. Following the completion of these behavioural observations, all *G. holbrooki* were removed and the four *R. ornatus* were observed again for a further 5 min each. To control for any behavioural effects that may have resulted from an increase in fish density, rather than those effects resulting specifically from *G. holbrooki* exposure, a further treatment was included in the experiment. In this treatment, the behavioural observations were repeated by adding four *R. ornatus* individuals rather than four *G. holbrooki*. Four shoals of *R. ornatus* ($n = 16$) from both the sympatric and allopatric populations were used for these latter observations.

Equal numbers of both *G. holbrooki* and *R. ornatus* were used for all the behavioural observations, and none of the fish were used more than once. In total, 14 shoals were used for the sympatric *R. ornatus* ($n = 56$) population treatment and 11 shoals were used for the allopatric *R. ornatus* population treatment ($n = 44$). In order to determine if naïve populations of *R. ornatus* alter their behaviour in response to repeated exposure to *G. holbrooki* (*i.e.* learning), each shoal of *R. ornatus* was exposed to *G. holbrooki* once a week for 4 weeks.

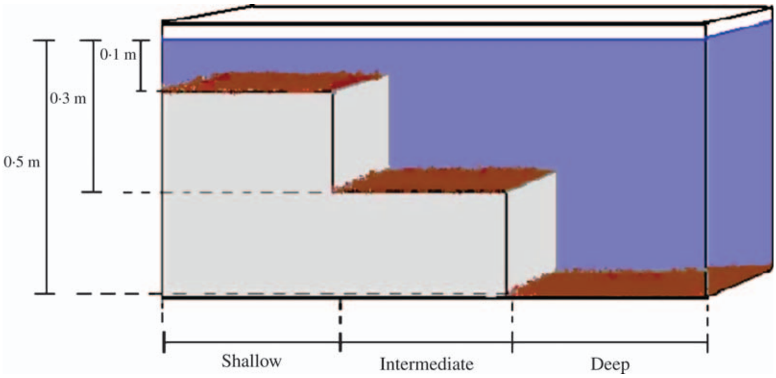

Fig. 1. Experimental tank set up for experiment 1 showing shallow (0·1 m), intermediate (0·3 m) and deep (0·5 m) depths.

HABITAT PREFERENCE EXPERIMENT

To compare habitat preference of *G. holbrooki* and *R. ornatus*, a 920 × 360 × 390 mm fish tank was divided into three sections. One section contained artificial floating cover, the middle section consisted of open water with gravel only and a third section contained artificial submerged vegetation. The artificial floating cover was made of green wool attached to floating plastic rings (250 mm diameter) and the artificial submerged vegetation consisted of strips of green plastic bags (250 × 20 mm) anchored to a plastic mesh hidden under the gravel.

Mixed-sex shoals containing four individuals of either *R. ornatus* or *G. holbrooki* were introduced to the fish tank and left to settle for 10 min. *Rhadinocentrus ornatus* individuals were selected from either the sympatric *G. holbrooki* populations ($n = 32$), or allopatric *G. holbrooki* populations ($n = 16$). *Gambusia holbrooki* individuals ($n = 16$) were selected from one of the three populations: Corindi Creek, Urumbilum River and Pine Creek at Pacific Highway. The location of each of the fishes in the experimental aquaria was then recorded every 30 s for a period of 25 min by counting the number of fishes in each of the three habitats. The position of the habitats was swapped halfway through each trial to remove any preferential bias, and the fishes were allowed to settle for 5 min before their location was recorded again.

To minimize any external disturbances, observations for both the behavioural observation and habitat preference experiments were made in a darkened room, with the only light source provided by a fixed fluorescent tube above the aquarium. The room temperature was set at 22° C, and heaters and filters were removed from the aquaria during the observations. After completing the experiments in accordance with the guidelines by the Macquarie University Ethics Committee (Ref. no. 2007/003) and NSW fisheries legislation, *R. ornatus* individuals were returned to their home tanks for further study and *G. holbrooki* were euthanased with an overdose of anaesthetic (MS-222 buffered with sodium bicarbonate).

DATA ANALYSIS

Due to the low sample sizes collected from some field populations, *R. ornatus* samples were 'lumped' together into allopatric and sympatric categories. To avoid pseudoreplication, counts taken for each fish in a shoal were summed together and behaviour was analysed at the shoal level. Highly skewed data were $\log_{10}$ transformed prior to analysis.

All behavioural observations taken before, during and after *G. holbrooki* presence, as well as over 4 weeks, were analysed with single-factor repeated measures ANOVA (StatView version 5.0.1). The frequency of nips and chases by *G. holbrooki* and *R. ornatus* as well as the difference in habitat preferences between these two species were analysed with single-factor ANOVA. Fisher's PLSD *post hoc* analysis was conducted to further scrutinize the data where appropriate. Body size was initially included as a covariate in these analyses but it was found to have no bearing on the results and was subsequently removed.

RESULTS

BEHAVIOURAL OBSERVATIONS: WEEK 1

The frequency of chases among *R. ornatus* individuals during all stages of *G. holbrooki* exposure was significantly higher in the sympatric compared to the allopatric *R. ornatus* populations [one-way ANOVA, $F_{1,23}$, $P < 0{\cdot}05$; Fig. 2(a)]. Similarly, the sympatric *R. ornatus* populations exhibited a significantly higher frequency of chases between individuals in the density control treatment (*i.e.* the addition of more *R. ornatus* conspecifics) compared to the allopatric

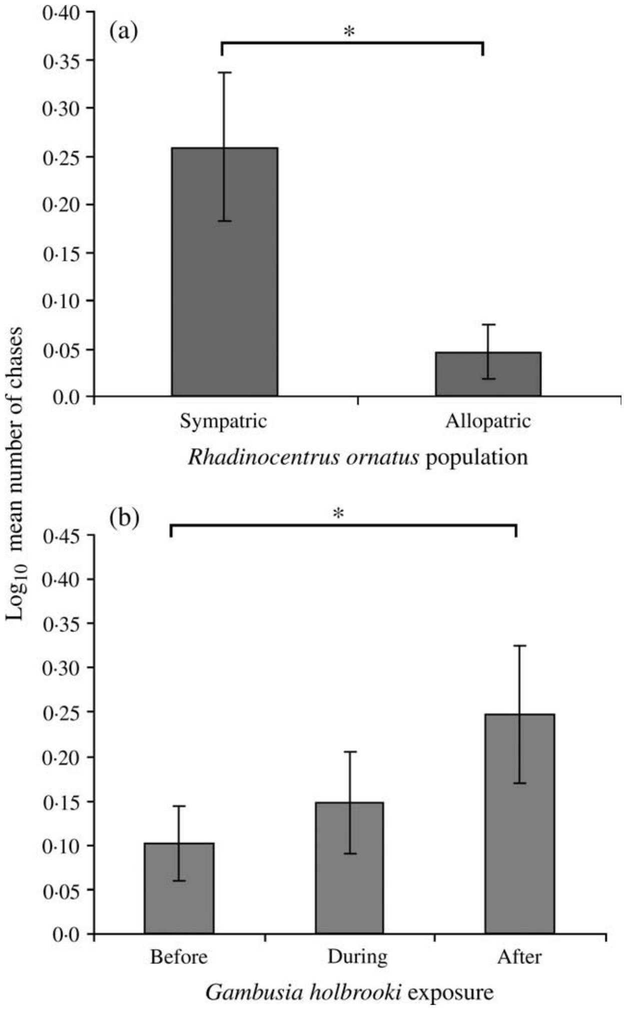

FIG. 2. Log_{10} mean ± S.E. frequency of intraspecific chases by *Rhadinocentrus ornatus* from (a) the sympatric and allopatric populations over the duration of the experiment and (b) all *R. ornatus* populations before, during and after *Gambusia holbrooki* exposure, during week 1 (*, $P < 0{\cdot}05$).

R. ornatus populations (one-way repeated measures ANOVA, $F_{1,6}$, $P < 0{\cdot}05$). The frequency of chases between individuals in all *R. ornatus* populations increased from the periods before to after *G. holbrooki* exposure [Fisher's PLSD, $P < 0{\cdot}05$; Fig. 2(b)]. Nipping by *R. ornatus* was also observed in the sympatric *R. ornatus* populations, however, it did not occur frequently enough to analyse.

During *G. holbrooki* exposure, the allopatric *R. ornatus* populations were nipped by *G. holbrooki* more frequently than the sympatric *R. ornatus* populations (one-way ANOVA, $F_{1,23}$, $P < 0{\cdot}05$; Fig. 3). There was no significant difference in the frequency of *G. holbrooki* chases directed at *R. ornatus* between the allopatric and sympatric populations. Casual observations suggested that male *G. holbrooki* were more aggressive than females.

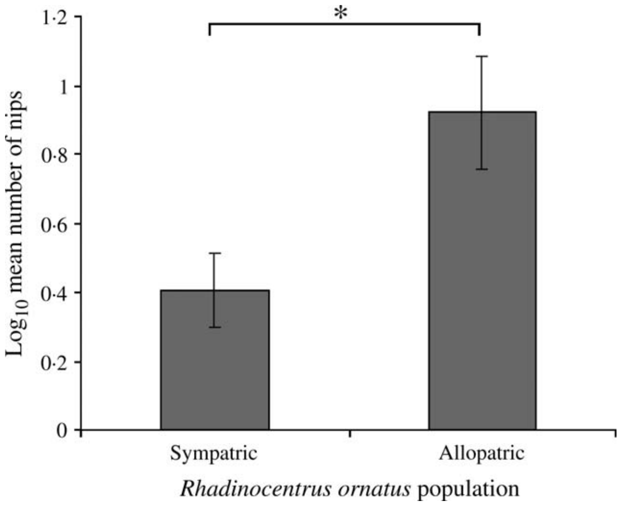

FIG. 3. $\log_{10}$ mean ± S.E. frequency of *Gambusia holbrooki* nipping *Rhadinocentrus ornatus* from the allopatric and sympatric populations during *G. holbrooki* exposure in week 1 (*, $P < 0·05$).

For simplicity, only the results for the amount of time *R. ornatus* spent in the intermediate depth are presented since this depth was most indicative of the overall depth preferences by *R. ornatus* and best illustrate their change in behaviour in response to *G. holbrooki*. There was a significant difference between the periods before, during and after *G. holbrooki* exposure in the amount of time each population spent in the intermediate depth (one-way repeated measures ANOVA, $F_{2,23}$, $P < 0·05$). Based on the *post hoc* analysis, the sympatric *R. ornatus* populations spent significantly more time in the intermediate depth during and after *G. holbrooki* exposure compared to before *G. holbrooki* exposure (Fisher's PLSD, both $P < 0·01$; Fig. 4). While there was no difference between the *R. ornatus* populations in the amount of time they spent in the intermediate depth before and during *G. holbrooki* exposure, a significant difference was revealed after *G. holbrooki* exposure (one-way ANOVA, $F_{1,23}$, $P < 0·05$).

There was no significant difference in *R. ornatus* activity levels between *R. ornatus* populations, nevertheless, activity levels varied depending on the presence of *G. holbrooki* (one-way repeated measures ANOVA, $F_{2,23}$, $P < 0·01$). *Rhadinocentrus ornatus* activity levels increased significantly during *G. holbrooki* exposure and decreased after *G. holbrooki* exposure (*post hoc* Fisher's PLSD test, $P < 0·001$ and $P < 0·05$, respectively; Fig. 5).

BEHAVIOURAL OBSERVATIONS WITH REPEATED *G. HOLBROOKI* EXPOSURE

The differences between populations tended to be consistent from weeks 1 to 4, therefore, only the results of interest are presented here. The frequency of chases between *R. ornatus* conspecifics after *G. holbrooki* exposure increased after each exposure to *G. holbrooki* (one-way repeated measures ANOVA, $F_{3,23}$, $P < 0·05$). *Post hoc* analysis suggests that the frequency of chases after

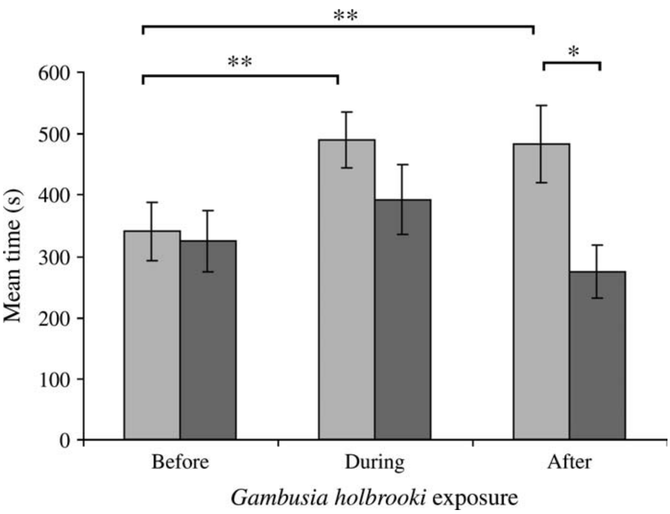

FIG. 4. Mean ± S.E. time *Rhadinocentrus ornatus* spent in the intermediate depth from the sympatric (▨) and allopatric populations (■) before, during and after *Gambusia holbrooki* exposure, during week 1 (*, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$).

G. holbrooki exposure were significantly higher in weeks 3 and 4 compared to week 1 (Fisher's PLSD, $P < 0{\cdot}01$ and $P < 0{\cdot}05$, respectively; Fig. 6).

HABITAT PREFERENCE

The habitat preference of the three populations of *G. holbrooki* did not differ significantly from one another (ANOVA, $F_{2,9}$, $P > 0{\cdot}05$) and were combined

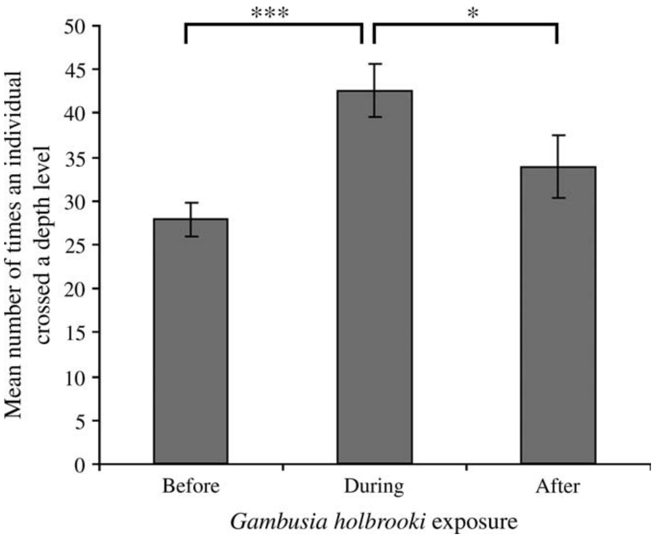

FIG. 5. Mean ± S.E. *Rhadinocentrus ornatus* activity levels from all *R. ornatus* populations before, during and after *Gambusia holbrooki* exposure during week 1 (*, $P < 0{\cdot}05$; ***, $P < 0{\cdot}001$).

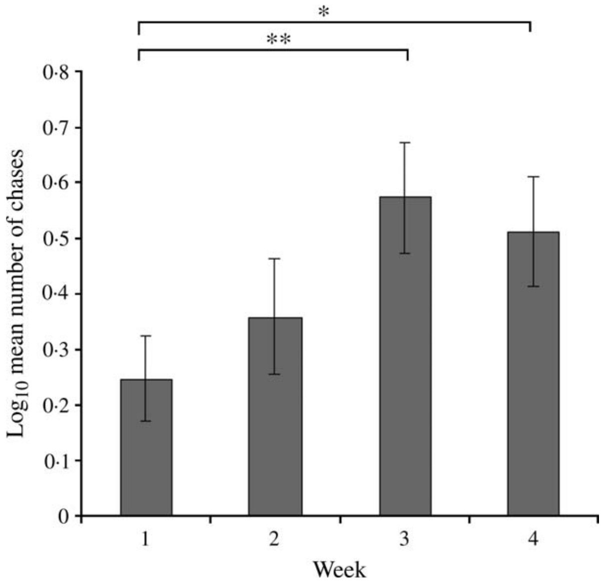

FIG. 6. Log_{10} mean ± S.E. frequency of intraspecific chases by *Rhadinocentrus ornatus* from all *R. ornatus* populations after *Gambusia holbrooki* exposure over 4 weeks (*, $P < 0.05$; **, $P < 0.01$).

for clarity. The difference in the mean number of *G. holbrooki* and *R. ornatus* from the sympatric and allopatric populations occurring in the floating cover differed significantly (one-way ANOVA, $F_{2,21}$, $P < 0.001$; Fig. 7). The sympatric *R. ornatus* populations spent significantly more time in the floating cover

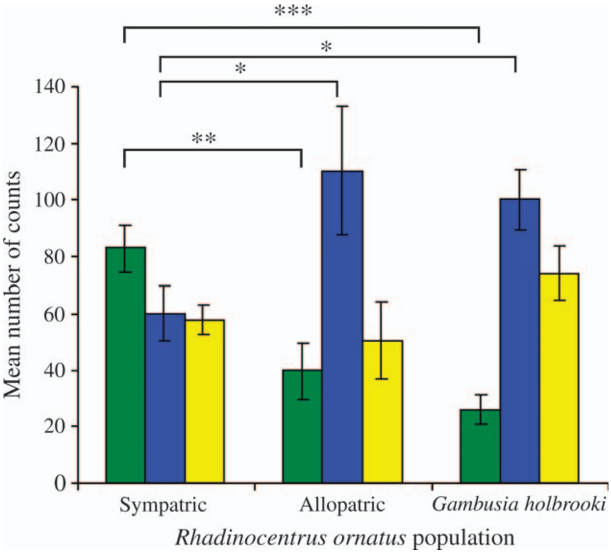

FIG. 7. Mean ± S.E. number of observations when *Rhadinocentrus ornatus* from the sympatric ($n = 32$) and allopatric populations ($n = 16$) and *Gambusia holbrooki* ($n = 16$) occurred in the floating cover (🟩), open water (🟦) and submerged vegetation (🟨) (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

compared to both *G. holbrooki* and the allopatric *R. ornatus* populations (*post hoc* Fisher's PLSD test, $P < 0.001$ and $P < 0.01$, respectively). The difference between *G. holbrooki* and the number of *R. ornatus* from the sympatric and allopatric populations occurring in the open water habitat type was also significant (one-way ANOVA, $F_{2,21}$, $P < 0.05$). *Post hoc* analysis revealed that both *G. holbrooki* and the allopatric *R. ornatus* populations utilized the open water habitat more frequently than the sympatric *R.ornatus* populations (Fisher's PLSD, both $P < 0.05$). There was no significant difference between populations with respect to their occupation of the submerged vegetation.

DISCUSSION

The results of this study suggest that *G. holbrooki* can directly affect *R. ornatus* populations in a number of ways. First, nipping and chasing by *G. holbrooki* can lead to the displacement of *R. ornatus* in the water column resulting in missed feeding opportunities. This is because both *G. holbrooki* and *R.ornatus* feed on aquatic and terrestrial invertebrates at the water surface (Bayly *et al.*, 1975; Arthington & Marshall, 1999; King & Warburton, 2007). The increased activity levels and aggression observed in all *R. ornatus* populations were attributable to *G. holbrooki* exposure and not just the addition of food or a change in fish density. Other studies have shown that the presence of *G. holbrooki* increased activity levels and aggression in native species (Meffe, 1985; Howe *et al.*, 1997; Warburton & Madden, 2003). This constant harassment may lead to increased stress levels, susceptibility to secondary infections, excess energy expenditure and have additional indirect fitness costs including increased susceptibility to predators.

The manner in which *R. ornatus* responded to *G. holbrooki* varied depending on the degree of exposure each population was exposed to in the wild. The higher frequency of attacks by *G. holbrooki* on allopatric *R. ornatus* may be due to a lack of avoidance response in these populations. Allopatric populations spent more time close to the surface, which may have invoked a greater number of interactions with *G. holbrooki*, especially when food was present. The sympatric *R. ornatus* populations, in contrast, spent more time in the intermediate depth both during and after *G. holbrooki* exposure and were nipped less frequently. Thus, *R. ornatus* living in sympatry with *G. holbrooki* displayed a niche shift thereby reducing the number of agonistic interactions with *G. holbrooki*. Studies that have shown that when *G. holbrooki* and native fish species occur together, *G. holbrooki* frequently occurs at the surface and displaces other species to the deeper regions of the tank (Warburton & Madden, 2003).

The results from the habitat preference experiment also support the notion that *R. ornatus* from sympatric populations may utilize alternative microhabitats to avoid interactions with *G. holbrooki* and to reduce the amount of niche overlap between the species. Allopatric populations showed very similar habitat preferences to *G. holbrooki*, preferring open habitats that provide maximum access to the water surface where both species forage on terrestrial insects (Casterlin & Reynolds, 1977; Arthington *et al.*, 1983; King & Warburton, 2007). The similarity in habitat preferences between the allopatric *R. ornatus* populations and *G. holbrooki* suggests that high competitive interactions would occur following introduction. Competition between species often leads to resource partitioning or competitive exclusion (Pen *et al.*, 1993; Arthington & Marshall, 1999; Bøhn & Amundsen, 2001). In contrast to the allopatric populations, the habitat preferences of *R. ornatus* from the sympatric populations differed from *G. holbrooki* showing a strong preference for floating cover. These observations are consistent with those of other studies, indicting a habitat shift induced by exposure to *G. holbrooki* (Belk & Lydeard, 1994; Moloney, 2002; King & Warburton, 2007). When taken together, it appears that niche shifts may be a common response to *G. holbrooki* invasions but may pave the way for coexistence in syntony (Rincón *et al.*, 2002).

The sympatric *R. ornatus* populations were more aggressive than the allopatric *R. ornatus* populations possibly due to their co-occurrence with *G. holbrooki*. *Rhadinocentrus ornatus* from these populations have evolved or developed behavioural adaptations to cope with the increased competition and aggression induced by the presence of *G. holbrooki*. Likewise, intraspecific competition by *Galaxias maculatus* (Jenyns), which occur sympatrically with *G. holbrooki* in the wild, had a greater effect than interspecific competition with the exotic species (Becker *et al.*, 2005). Furthermore, the aggressive behaviour by sympatric *R. ornatus* populations provides evidence of a character shift suggesting that naïve species can cope with invasions of *G. holbrooki*. Studies have shown that changes in behavioural and morphological traits can enhance the abilities of native species to enable them to persist in invaded areas and gain access to resources (Forseth *et al.*, 2003; Strauss *et al.*, 2006). Hence, aggressive behaviour in these sympatric populations could be a type of character shift that is inherited and, or based on early experience with *G. holbrooki*, to allow them to cope with (or may be symptomatic of) *G. holbrooki* invasions. Population differences in behaviour are often heritable but further modified through learning (Brown *et al.*, 2006). Further experiments are necessary to determine whether the behaviour is heritable or developed through experience with *G. holbrooki* during ontogeny.

Intraspecific chases by *R. ornatus* from all *G. holbrooki* density populations increased over time after repeated *G. holbrooki* exposure. In the density control experiment, however, intraspecific chases by *R. ornatus* occurred only in the sympatric populations and did not change over time. This suggests that the increased aggression exhibited by all populations could only be due to *G. holbrooki* exposure. Thus, whilst the sympatric populations were consistently more aggressive, all fish respond to the presence of *G. holbrooki* in a similar way. This is most likely to be indicative of elevated stress levels. Exotic species, however, are known to modify the behavioural strategies as well as the dominance status of other species (Hardwood *et al.*, 2002; Blanchet *et al.*, 2007).

Under high selective processes such as predation or competition, the behavioural responses of fishes can evolve fairly rapidly (Meffe, 1985; Reznick *et al.*, 1997). Meffe (1985), however, suggests that native species have a limited time frame to evolve behavioural responses, particularly in the light of the explosive population increases typical of invasive species. Hence, rapid invasions of *G. holbrooki* in isolated areas containing naïve populations of *R. ornatus* may provide little opportunity for the species to evolve appropriate behavioural responses, thus increasing the probability of local extinction. The results presented herein suggest that the lack of prior experience with *G. holbrooki* renders the allopatric *R. ornatus* populations particularly vulnerable to the potential impacts of this exotic species. Likewise, tadpoles of *L. aurea*, showed no developmental and behavioural responses to *G. holbrooki* (Hamer *et al.*, 2002). However, the larvae of the common Australian frog *Limnodynastes tasmaniensis* (Günther) which coexists with *G. holbrooki* in several waterbodies has been shown to avoid *G. holbrooki* under experimental conditions (Lane & Mahony, 2002). This suggests that some species can cope with invasion under certain circumstances.

Other factors, such as habitat modification, can further threaten the survival of *R. ornatus* by producing unfavourable environmental conditions. The negative impacts of *G. holbrooki* can be exacerbated in disturbed or degraded habitats (Ling, 2004). The best management strategy for maintaining native fishes would be the eradication of *G. holbrooki* (Lydeard & Belk, 1993). Complete eradication, however, is unlikely to be achieved owing to the pervasive nature of *G. holbrooki* in Australian waters (Mills *et al.*, 2004; Laha & Mattingly, 2006). Thus, alternative strategies need to focus on promoting the coexistence of *G. holbrooki* and native fishes (Mills *et al.*, 2004). For instance, the rehabilitation of riparian habitats in disturbed habitats where *G. holbrooki* thrives, may shift the balance in favour of native species (Pusey & Arthington, 2003). *Rhadinocentrus ornatus* is commonly found in coastal wallum habitats along with a number of other vulnerable fish species, thus protection or rehabilitation of this environment is particularly urgent (Pusey *et al.*, 2004).

In summary, *G. holbrooki* can have multifaceted effects on *R. ornatus*. First, *G. holbrooki* frequently nipped and chased *R. ornatus* from all populations, with individuals from allopatric populations being particularly vulnerable. Exposure to *G. holbrooki* was also responsible for increased activity levels in *R. ornatus* and aggression in all *R. ornatus* populations increased following repeated *G. holbrooki* exposure. This was particularly the case for the sympatric *R. ornatus* populations, which exhibited a higher level of aggression during all stages of *G. holbrooki* exposure. *Gambusia holbrooki* and allopatric *R. ornatus* populations shared similar microhabitat preferences, whereas the sympatric *R. ornatus* populations have apparently shifted their preferences through evolutionary or developmental processes, enabling them to coexist with *G. holbrooki*. Thus, naïve populations may be able to cope with invasions of *G. holbrooki* as long as they are given time to evolve behavioural adaptations and find refuge from competition. Whatever strategy is employed, it is abundantly clear that managing *G. holbrooki* as a threatening process is necessary for the conservation of *R. ornatus* and other native fish species.

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