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The effect of density on aggression between a highly invasive and native fish

Laura K. Lopez[1,2] iD | Andrew R. Davis[2] | Marian Y. L. Wong[2] iD

[1]LSA Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA

[2]School of Biological Sciences, University of Wollongong, Wollongong, NSW, Australia

Correspondence
Laura K. Lopez, University of Michigan, Biological Sciences Building, 1105 North University, Ann Arbor, MI 48109, USA.
Email: lklopez@umich.edu

Abstract

Population densities of invasive species fluctuate spatially and temporally, suggesting that the intensity of their aggressive interactions with native species is similarly variable. Although inter-specific aggression is often thought to increase with population density, it is often theorized that it should be exceeded by intra-specific aggression since conspecifics share a greater degree of resource overlap. Yet, the magnitude of intra-specific aggression is seldom considered when examining aggressive interactions, particularly those between invasive and native species. Here, we manipulated the density of the invasive eastern mosquitofish, *Gambusia holbrooki*, and observed its aggressive interactions with juveniles of the native Australian bass, *Macquaria novemaculeata* in a laboratory setting. For both species, the magnitudes of intra- and inter-specific aggression were recorded. Regardless of density, the native *M. novemaculeata* was more aggressive towards heterospecifics than *G. holbrooki* was. In addition to this, *M. novemaculeata* was more aggressive to *G. holbrooki* than towards conspecifics, at both low- and high-density conditions. In contrast, *G. holbrooki* was similarly aggressive towards *M. novemaculeata* and *G. holbrooki* at a high density, yet at low density, displayed significantly more aggression towards conspecifics than *M. novemaculeata*. These findings demonstrate the importance of considering intra-specific aggression when exploring behavioural interactions between native and invasive species.

KEYWORDS
aggression, density, *Gambusia holbrooki*, invasive, *Macquaria novemaculeata*

1 | INTRODUCTION

Biological invasions can have a significant deleterious effect on global biodiversity and ecosystem functioning (Gallardo, Clavero, Sanchez, & Vila, 2016; Holway & Suarez, 1999; Mack et al., 2000). Often invaders engage in interference competition with native species which can include contests, territoriality and nest vandalism (Amarasekare, 2002; Case & Gilpin, 1974; Human & Gordon, 1996; Zhang, Andersen, Dieckmann, & Brannstrom, 2015). The underlying commonality that unites these interactions is some form of aggression (Grether et al., 2013). Invasive species are frequently observed

to display more inter-specific aggression than native species, which is often considered to be a key factor in their success in spreading to and becoming established in novel systems (Holway & Suarez, 1999; Hudina, Hock, & Zganec, 2014; Pintor, Sih, & Bauer, 2008). The ultimate outcome of aggressive interactions may be the displacement of a native species as their access to food, shelter and reproductive partners becomes increasingly limited (Amarasekare, 2002).

The intensity and outcome of aggression between invasive and native species is frequently context dependent (Hudina, Zganec, & Hock, 2015; Jackson, Ruiz-Navarro, & Britton, 2015; Kaiser et al., 2013). The abundance of many invaders can be temporally and

spatially heterogeneous, which in turn suggests that the intensity of their aggressive interactions with natives may be similarly variable (Latzka, Hansen, Kornis, & Vander Zanden, 2016). Aggressive interactions have typically been thought to increase with the population density of an invader due to a higher rate of contact with native competitors as well as a reduction in resources relative to population size (Kaiser et al., 2013). However, the frequency of intra-specific interactions within an invasive population is also likely to increase with its density, which may lead to a reduction in inter-specific interactions due to altered contact rates, and the co-existence of invasive and native competitors (Britton, Cucherousset, Grey, & Gozlan, 2011; Kornis, Carlson, Lehrer-Brey, & Vander Zanden, 2014). Therefore, in order to predict the effects of an invader's density on native species, it is necessary to examine the relative magnitude of inter-specific and intra-specific aggression that each species may experience (Inouye, 2001).

It is often theorized that behavioural interactions between conspecifics should be stronger than those between heterospecifics, particularly at high population densities (Connell, 1983; Forrester, Evans, Steele, & Vance, 2006; Munday, Jones, & Caley, 2001). This is because conspecifics share a higher degree of resource overlap and may be viewed as greater rivals than heterospecifics (Connell, 1983; Forrester et al., 2006; Munday, 2004). For example, both the coral dwelling bridled goby (*Coryphopterus glaucofraneum*) and goldspot goby (*Gnatholepis thompsoni*) were reported to be at least twice as aggressive towards conspecifics than towards heterospecifics (Forrester et al., 2006). However, support for this prediction is overwhelmingly provided by studies of species in their natural ranges (Connell, 1983; Goldberg & Barton, 1992; Mangla, Sheley, James, & Radosevich, 2011), neglecting the possibility that the behaviour of invasive species which are by definition outside of their native ranges may be inherently different to that of native species (Hansen et al., 2013; Latzka et al., 2016). For example, Argentine ant (*Linepithema humile*) populations are known to display reduced intra-specific aggression between nests outside compared to within their native ranges (Tsutsui, Suarez, Holway, & Case, 2000). The result of lowered intra- compared with inter-specific aggression may be reduced levels of population self-regulation (Pintor et al., 2008). Alternatively, invaders could be similarly aggressive to hetero- and conspecifics which may also reduce population self-regulation to a degree (Sale, 1977). However, since the relationship between intra- and inter-specific aggression and density remains poorly studied, there is no current consensus as to which model invaders, or the native species they interact with, may conform to.

Here, we quantified the magnitude of inter- and intra-specific aggression between the native Australian Bass, *Macquaria novemaculeata*, Steindachner, 1966, and the invasive eastern mosquitofish, *Gambusia holbrooki*, Girard, 1859, and how both forms of aggression are mediated by *G. holbrooki* density. Since its introduction into Australia in the 1920s, *G. holbrooki* has spread to all states and territories (Pyke, 2008). Its aggressive behaviour, including fin-nipping, is frequently cited as a key mechanism behind its success as an invader (Carmona-Catot, Magellan, & Garcia-Berthou, 2013; Pyke,

2008; Sutton, Zeiber, & Fisher, 2013). Not only does the density of *G. holbrooki* vary spatially, but also populations of the invader are also known to experience seasonal fluctuations, with densities being highest following breeding in early autumn and lowest in spring (Barney & Anson, 1921).

Juveniles of the native *M. novemaculeata* are stocked extensively throughout the East Australian drainage system to support populations no longer able to reach estuaries and breed due to habitat modification (Cameron, Baumgartner, Bucher, & Robinson, 2012). Adult *M. novemaculeata* are piscivorous and have been observed to attack *G. holbrooki* in a captive setting (Grigaltchik, Ward, & Seebacher, 2012), suggesting that juvenile *M. novemaculeata* may also attempt to consume *G. holbrooki*. However, interactions between juvenile *M. novemaculeata* and *G. holbrooki* have not been recorded thus far, despite there being a number of factors which suggest that recently stocked *M. novemaculeata* may be vulnerable in the presence of the invader. Since stocked juvenile *M. novemaculeata* are hatchery reared, they are potentially naïve to *G. holbrooki* and unlike wild fingerlings which have had previous experience with *G. holbrooki* and may have adapted their behaviour to avoid interactions with the invader. Therefore, although they may not necessarily receive more aggression than wild fingerlings, they may be less adept at responding to *G. holbrooki*. In addition to this, *G. holbrooki* will have prior residence over the newly released *M. novemaculeata* which may infer a competitive advantage to the invader (Peeke, Sippel, & Figler, 1995). Considering these factors, it is possible that *G. holbrooki* could significantly impede the conservation of *M. novemaculeata* by inflicting injury and increasing the mortality rate of juvenile *M. novemaculeata* used in stocking programmes.

Overall, we expected that the strength of aggressive interactions would vary depending on the species (invasive vs. native), type of aggression (intra vs. inter) and density of animals (high vs. low). We predicted that (a) *G. holbrooki*, as an invasive species in this context, will be more aggressive towards heterospecifics than *M. novemaculeata* would be to heterospecifics. Following on from this, we also predicted that (b) the native *M. novemaculeata* will be more aggressive to conspecifics than heterospecifics at high density. Finally, since invasive species may display unique inter- and intra-specific interaction dynamics, that (c) *G. holbrooki* would will be more aggressive towards heterospecifics than conspecifics at high density.

2 | METHODS

2.1 | Study species and acclimation

Adult eastern mosquitofish, *G. holbrooki*, (all values are $\bar{x} \pm SE$ unless stated otherwise; male, mass = 0.14 ± 0.01 g; standard length = 20.43 ± 0.20 mm; female, mass = 0.14 ± 0.01 g; standard length = 20.01 ± 0.27 mm), were collected from freshwater ponds located at the University of Wollongong campus, NSW, Australia (34°24′19″S 150°52′42″E) using a baited hand-held landing net. There was no significant difference in length of male and female *G. holbrooki* (t_{98} = 0.28, *p* = .779). While adult female *G. holbrooki*

	Macquaria novemaculeata intra-specific	Mixed	*Gambusia holbrooki* intra-specific
Low density	1 Mn 2 Mn*	1 Gh* 2 Mn*	1 Gh* 2 Gh
High density	4 Mn 2 Mn*	4 Gh* 2 Mn*	4 Gh* 2 Gh

Note: The aggression of focal fish (marked with *) was recorded. For each species, aggression from individuals in the low- and high-density mixed treatments was compared with those in the intra-specific treatments to determine whether the preference to interact with con- or heterospecific differed with density.

are typically larger than males (Pyke, 2008), fish for the present study were collected during late autumn and early winter when male and female young of the year born in spring were prevalent and fully mature females were rare (Lopez, pers. obs.). Juvenile Australian bass, *M. novemaculeata* (mass = 0.97 ± 0.03 g; standard length = 36.17 ± 0.35 mm) of mixed sex were obtained from a local hatchery (Aquablue Seafoods). Juvenile *M. novemaculeata* used in this study were similar in size to those stocked into waterways (Hutchison et al., 2006) to better assess whether *G. holbrooki* negatively impacts stocking of this native species. This species was not sexed as this can only be done post-mortem, which was not possible in this study. Furthermore, it is likely that sex-specific differences in aggression in *M. novemaculeata* have not emerged at this developmental stage (Johnsson, Sernland, & Blixt, 2001). Both *G. holbrooki* and *M. novemaculeata* are generalist species which feed on zooplankton, insect larvae and insects (Pyke, 2008; Smith, Baumgartner, Suthers, & Taylor, 2011). Piscivory has been recorded in *M. novemaculeata* as small as 67 mm, yet it is not common until individuals are longer than 100 mm (Smith et al., 2011).

To conduct the experiment, 4 recirculating aquarium systems in temperature-controlled rooms were used at the University of Wollongong. Each system consisted of 8 aquaria (60 × 30 × 30 cm) that were inter-connected and subjected to water conditions held constant at 21°C and 15 ppt salinity. Each aquarium was aerated with a bubbler, lined with 2 cm of sand and contained 1 PVC pipe (15 × 6 cm) to provide shelter. To house *M. novemaculeata*, 24 aquaria (*N* = 130 total fish) were established across 4 separate systems (*N* = 33 or *N* = 32 fish per system). For *G. holbrooki*, 8 aquaria (*N* = 140 total fish) were used across 4 separate systems (*N* = 35 fish per system). All fish remained under these conditions for the 7 days to ensure adequate acclimation. During this time, both species were maintained at a 12:12 light:dark cycle and fed an equal amount of a commercial fish pellet (New Life Spectrum Thera formula) and frozen bloodworms daily. During acclimation, intra-specific aggressive interactions were not recorded. Individuals were re-randomized when introduced from housing aquaria to observation aquaria to avoid familiarization.

2.2 | Experimental design

To examine inter- and intra-specific aggression in *G. holbrooki* and *M. novemaculeata* under low and high densities of *G. holbrooki*, we established 3 experimental treatments: (a) mixed, in which aquaria contained both *G. holbrooki* and *M. novemaculeata* (*N* = 20 groups), (b) *M. novemaculeata* intra-specific, in which aquaria contained only *M. novemaculeata* (*N* = 20 groups) and (c) *G. holbrooki* intra-specific where only *G. holbrooki* were present in aquaria (*N* = 20 groups). Within all treatments, 2 density levels were established—low density (containing 3 fish in total) and high density (containing 6 fish in total; Table 1). For the mixed treatment, the number of *G. holbrooki* was 1 for the low-density level (*N* = 10 groups) and 4 for the high-density level (*N* = 10 groups), while the density of *M. novemaculeata* was held constant at 2 individuals (Table 1). The total number of *M. novemaculeata* and *G. holbrooki* in the intra-specific treatments was the same as the total number of both species (combined) in the corresponding low- and high-density mixed treatments (Table 1).

We used a form of response surface design developed to isolate the relative levels of intra- and inter-specific aggression (Forrester et al., 2006; Inouye, 2001). The behaviour of one focal individual in the *G. holbrooki* single-species treatment and multiple focal individuals in the other treatments was recorded. Behavioural interactions among focal individuals were not recorded in this study. Focal individuals were randomly selected. The aggression displayed by individuals to heterospecifics was compared with that directed to conspecifics in the mixed- and single-species treatments, respectively. For example, we recorded aggression displayed by the sole *G. holbrooki* in the low-density mixed treatment compared with that of the sole focal *G. holbrooki* towards non-focal individuals in the low-density *G. holbrooki* single-species treatment. These observations show how inter- and intra-specific aggression varied independently of overall fish density. This is contrasted against levels of inter- and intra-specific aggression displayed by focal *G. holbrooki* in the high-density mixed and *G. holbrooki* single-species treatments to ascertain how animal density affects the relative aggression displayed towards con- and heterospecifics. Since the goal of the experiment was to examine how the density of an invasive species affects intra- and inter-specific aggression, we solely manipulated densities of *G. holbrooki* and not *M. novemaculeata* in the mixed treatments.

Due to the time of year in which *G. holbrooki* were collected, fish densities in local populations were relatively low (Lopez, pers. obs.). We were unable to consistently collect enough males or females to use a single sex throughout the experiment, and the

relative abundances of the sexes changed over the course of the experiment. For this reason, we used both sexes in mixed groups in the high-density intra-specific and mixed treatments. In the high-density intra-specific and mixed treatments, the densities of focal male and female *G. holbrooki* varied so that replicates contained either 4 males (*n* = 2), 4 females (*n* = 2), 2 males and 2 females (*n* = 2), 3 males and 1 female (*n* = 2) or 1 male and 3 females (*n* = 2). In both the low-density intra-specific and mixed treatments, the number of replicates containing male and female focal *G. holbrooki* was equal (*n* = 5 male, *n* = 5 female). Wild densities *G. holbrooki* were estimated following collections made over time in the source ponds, giving an approximation of seasonal changes in density. The overall densities of individuals used in the study were based on estimates of seasonal low and high *G. holbrooki* densities in Wollongong water bodies relative to the aquaria size used in this study (Lopez, pers. obs.).

2.3 | Behavioural observations

Following an acclimation period of 2 weeks, *M. novemaculeata* and *G. holbrooki* were captured using hand nets, measured using hand-held callipers, (mm standard (SL) ± 0.1 mm) and weighed with an electronic balance (grams (g) ± 0.1 g). To enable the identification of individuals and to control for any effect of sex, size and reproductive status, each fish was tagged with fluorescent polymer elastomer tags (yellow, green, red, pink and orange; Northwest Industries Ltd) which was injected subcutaneously into the dorsal musculature (Malone, Forrester, & Steele, 1999).

In the mixed and *G. holbrooki* intra-specific treatments, 1 *G. holbrooki* (low density) or 4 *G. holbrooki* (high density) were introduced into a test aquarium (60 × 30 × 30 cm) 24 hr prior to the addition of 2 focal *G. holbrooki* or *M. novemaculeata* (Table 1). In the *M. novemaculeata* intra-specific treatment, 1 *M. novemaculeata* (low-density level) or 4 *M. novemaculeata* (high-density level) were introduced into a test aquarium 24 hr prior to the addition of 2 focal *M. novemaculeata* (Table 1). This order of residency was intended to simulate the temporal occurrence of species in the event of fish stocking whereby *G. holbrooki* is present prior to the introduction of *M. novemaculeata*.

Observations commenced after a 30-min acclimation period followed by a 5-min habituation to the presence of the observer. Only one person (LKL) observed the fish to avoid potential issues with observer bias. Each focal individual was observed for 10 min during which time the number of aggressive (approaches, chases and nips) behavioural interactions between other individuals was recorded (Flood & Wong, 2017; Matthews & Wong, 2015; O'Mara & Wong, 2015). To account for diurnal variation in behaviour, the same fish were observed once in the morning (0900–1200) and once in the afternoon (1300–1600). As no significant variation in the frequency of aggressive behaviours between the AM and PM observation periods were found for neither *M. novemaculeata* (GLMM, Time; $F_{1,152}$ = 0.68, *p* = .41) nor *G. holbrooki* (Time; $F_{1,169}$ = 0.01, *p* = .99), the number of discrete aggressive behaviours performed by each individual in the

AM and PM observation periods was summed, giving data for 20 min of observation time for each focal individual.

2.4 | Statistical analysis

A number of *G. holbrooki* died following interactions with *M. novemaculeata* in the low (*n* = 1)- and high (*n* = 6)-density mixed treatments (out of a total 50 *G. holbrooki* in mixed conditions), and so behavioural data collected from these individuals were excluded from all subsequent analyses.

All statistical analyses were conducted in RSTUDIO version 3.5.1 (R Core Team, 2016) using the MASS package. We used generalized linear mixed models (GLMMs) with negative binomial distributions which accounted for overdispersion in the count data (Dellinger, Zhang, Bell, & Hellmann, 2018; Zuur, Ieno, Walker, Savaliev, & Smith, 2009).

The first model compared whether *M. novemaculeata* or *G. holbrooki* were more aggressive to heterospecifics in the low- and high-density mixed conditions of the experiment. It included density (binomial: high or low), species (binomial: *G. holbrooki* or *M. novemaculeata*) as fixed effects and an interaction between density and species. We also included body length (continuous) as a covariate in the model to control for variation due to size and tank ID as a random effect to control for the non-independence of aggression and variation in *G. holbrooki* sex ratios. Fish ID was not included in any model since we did not use a repeated measures approach.

To compare whether *M. novemaculeata* and *G. holbrooki* showed different levels of aggression to con- or heterospecifics at low- and high-density conditions, we ran two separate models, one for each species. Both models included density and treatment (binomial: mixed or intra-specific) and the interaction between these fixed effects. Once again body length was included as a covariate and tank ID as a random effect. For the model, comparing the levels of aggression displayed by *G. holbrooki*, sex (binomial: male or female), was also included as a factor to control for any variation in aggression arising from sex. Non-significant interaction terms were removed from all models, and since sex was not a significant factor in any model, it was removed from the final analysis. Following application of the Bonferroni correction, the alpha value was adjusted to 0.016 for the all models. Post hoc analyses were conducted using the package lsmeans.

2.5 | Ethical note

At the conclusion of the experiment, *G. holbrooki* were euthanized using clove oil since it is illegal to release an invasive species under NSW law. *Macquaria novemaculeata* were used in another experiment covered by a separate protocol. Animal collection and husbandry, collection of observational data and euthanasia protocols were approved by the University of Wollongong Animal Ethics Committee (protocol AE14/07) and adhered to the Scientific Collection guidelines (permit No. P13/0011-1.3) of the NSW Department of Primary Industries.

3 | RESULTS

Regardless of density level, *M. novemaculeata* was more aggressive towards heterospecifics than *G. holbrooki* was (Figure 1, Table 2). At low density, *M. novemaculeata* were approximately twice as aggressive towards heterospecifics than *G. holbrooki* was, while at high density, *M. novemaculeata* were approximately 4 times more aggressive towards heterospecifics than *G. holbrooki*. Only *M. novemaculeata* was more aggressive towards heterospecifics in high- than low-density conditions as there was no significant difference in aggression from *G. holbrooki* in the high- and low-density conditions (Figure 1, Table 2).

At both density levels, *M. novemaculeata* were more aggressive towards *G. holbrooki* than conspecifics. This difference was magnified at high compared with low *G. holbrooki* densities, yet there was no significant effect of density alone and no interaction between density and treatment (Figure 2a, Table 3a). In contrast to *M. novemaculeata*, aggression from *G. holbrooki* to both *M. novemaculeata* and conspecifics at high density was minimal. At low density, however, *G. holbrooki* were significantly more aggressive to conspecifics than *M. novemaculeata* (Figure 2b, Table 3b).

4 | DISCUSSION

The relationship between the density of an invader and the intensity of aggressive interactions between invasive and native species differs greatly between study species and ecosystems (Yokomizo, Possingham, Thomas, & Buckley, 2009). Contrary to our predictions, the native species, *M. novemaculeata*, was more aggressive towards heterospecifics than was the invasive species, *G. holbrooki*. In addition to this, *M. novemaculeata* displayed more aggression to *G. holbrooki* than other *M. novemaculeata*. Also, unexpectedly, *G. holbrooki* was equally aggressive to *M. novemaculeata* and other *G. holbrooki*

at high density, although it was markedly more aggressive towards conspecifics at low density. These results demonstrate that the relative levels of intra- and inter-specific aggression, specifically from a native species towards an invader, and from a invader towards conspecifics, can shift unexpectedly with an invader's density.

Numerous invasive species have been reported to be more aggressive than the natives with which they interact (Carmona-Catot et al., 2013; Howe, Howe, Lim, & Burchett, 1997; Rincon, Correas, Morcillo, Risueno, & Lobon-Cervia, 2002). Despite this, and in contrast to our first prediction, the native *M. novemaculeata* displayed more aggression to heterospecifics than did *G. holbrooki* at high density. This finding may be related to size differences between native and invasive species in the current study. Where asymmetric
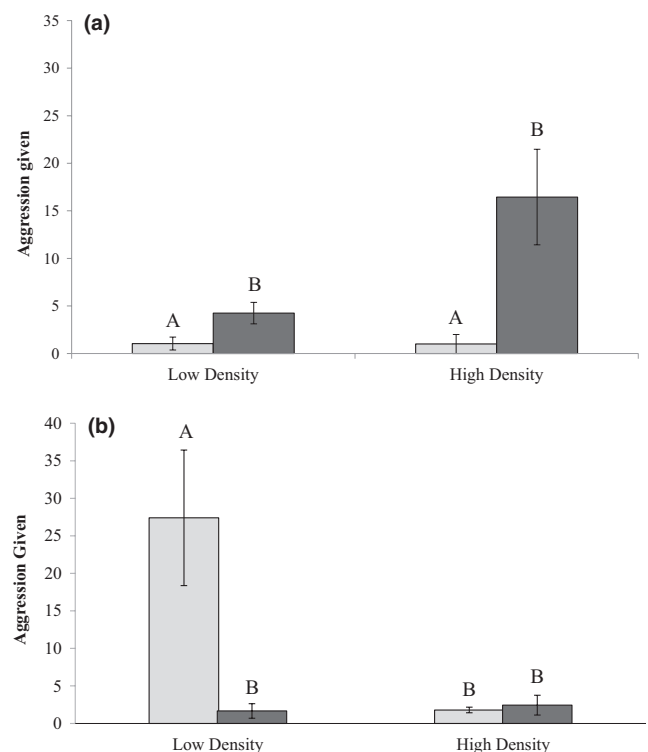
TABLE 2 Results of a generalized linear mixed model (negative binomial distribution) comparing the effects of *Gambusia holbrooki* density on inter-specific aggression from *Macquaria novemaculeata* and *G. holbrooki*

	Z_{82}	p
Density	−2.726	.006
Species	−2.722	.006
Density × species	1.806	.071
Length	0.026	.979

Note: Non-significant interactions were backward stepwise removed ($p < .016$).
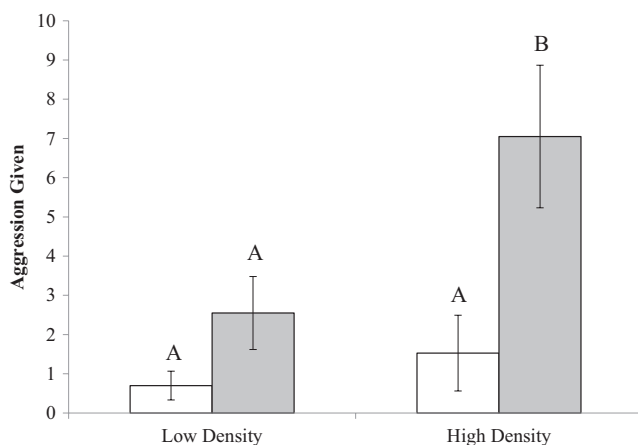
FIGURE 1 Mean (±*SE*) inter-specific aggression from *Gambusia holbrooki* (open bars) and *Macquaria novemaculeata* (closed bars) at low and high *G. holbrooki* densities towards focal heterospecifics, *n* = 10

FIGURE 2 Mean (±*SE*) aggression from (a) *Macquaria novemaculeata* and (b) *Gambusia holbrooki* to non-focal conspecifics (light grey bars) and heterospecifics (dark grey bars) at low and high *G. holbrooki* densities, *n* = 10

TABLE 3 Result of generalized linear mixed models (negative binomial distribution) testing the effects of *Gambusia holbrooki* density and treatment on (a) *Macquaria novemaculeata* and (b) *G. holbrooki* aggression

	Z_{79}	p
(a) *Macquaria novemaculeata*		
Density	−1.806	.071
Treatment	5.558	<.0001
Density × treatment	−1.890	.059
Length	2.045	.041
	Z_{92}	p
(b) *Gambusia holbrooki*		
Density	4.264	<.0001
Treatment	−0.776	.438
Density × treatment	−2.437	.015
Length	0.529	.597
Sex	1.258	.208

Note: Non-significant interactions were backward stepwise removed ($p < .016$).

aggression is observed, the larger species is often more aggressive (Little, Draud, & Itzkowitz, 2013; Moretz, 2003), and in the present study, *M. novemaculeata* were on average 1.67 times longer than *G. holbrooki*. Similarly, Garcia and Arroyo (2002) reported that the hen harrier raptor (*Circus cyaneus*) was larger and more aggressive towards heterospecifics than the Montagu harrier (*Circus pygargus*). It is possible that since *M. novemaculeata* were both larger in size and also juveniles, they had a greater energetic requirement (Bystrom, Andersson, Kiessling, & Eriksson, 2006; Bystrom & García-Berthou, 1999; Ohlberger, Mehner, Staaks, & Holker, 2012) and therefore may have placed a higher value on food when *G. holbrooki* density was high and hence exhibited higher rates of aggression towards the invader. In addition to this, larger, more mature *G. holbrooki* than those used in this study may have been more prone to aggression towards *M. novemaculeata*.

To understand the effect that density has on inter-specific aggression, it is necessary to consider the strength of intra-specific interactions (Connell, 1983; Forrester et al., 2006; Inouye, 2001). In opposition to our second prediction, *M. novemaculeata* were more aggressive to *G. holbrooki* than *M. novemaculeata* at either low or high density. This result contradicts expectations that intra-specific aggression should exceed that directed to heterospecifics. It is important to note that the majority of studies which support this concept have solely observed interactions between species in their native ranges and thus do not account for invasive-native species dynamics. Indeed, inter-specific aggression has been observed to be greater or equal to intra-specific aggression for some native species competing with invaders (Tsutsui et al., 2000; Warnock & Rasmussen, 2013). For example, Warnock and Rasmussen (2013) reported that the autochthonous bull trout (*Salvelinus confluentus*) diverted more aggression towards a high density of the non-native brook trout (*Salvelinus fontinalis*) than conspecifics. Our results demonstrate that there is a need to further explore the relative strengths of inter- and

intra-specific aggressive interactions between invasive and native species.

Considering that a disparity in body size may have mediated inter-specific aggression between *M. novemaculeata* and *G. holbrooki*, it is also necessary to consider the role of size in determining the relative magnitude of intra- and inter-specific competition for *M. novemaculeata*. If an increase in resource value at high competitor densities led to increased inter-specific aggression in this study, a comparable or greater increase in intra-specific aggression may also be expected. However, aggression is a costly behaviour and the risk of injury as a result of direct interactions typically increases for an individual with the relative body size of their competitor (Garcia & Arroyo, 2002; Herrel et al., 2009; Huntingford, Metcalfe, Thorpe, Graham, & Adams, 1990). Future studies could also benefit from exploring interactions between *M. novemaculeata* of different age classes to determine whether there are other factors aside from body size, such as social dynamics and disputes over resources, which influence intra-specific aggression in this species.

It has been hypothesized that one of the behavioural differences between species in native and non-native ranges is lower levels of intra- compared with inter-specific aggression at high densities (Pintor, Sih, & Kerby, 2009; Tsutsui et al., 2000). In contrast to our fourth prediction, however, inter- and intra-specific aggression from *G. holbrooki* was equal in high-density conditions. This may suggest that under a high density of *G. holbrooki*, con- and heterospecifics represented the same level of threat to individuals, as per the lottery model (Sale, 1977). This result contradicts previous studies which report stronger inter- than intra-specific aggression in invaders (Tsutsui et al., 2000; Warnock & Rasmussen, 2013). However, there is also evidence that invaders can be equally aggressive to con- and heterospecifics (Kalinoski, 1975) or aggressive more so towards con- than heterospecifics (Sutton et al., 2013). Considering that *M. novemaculeata* may have

been more aggressive towards heterospecifics than was *G. holbrooki* because they were larger, it follows that *G. holbrooki* may have limited its aggressive behaviour towards *M. novemaculeata* in order to reduce the risk of injury or predation.

It is important to note that aside from differences in body size potentially mediating aggression, an additional explanation of the results presented here may be that *M. novemaculeata* was driven to consume *G. holbrooki* (making the motivation predation rather than simply aggression over territory). A small number of *G. holbrooki* were killed following aggressive interactions with *M. novemaculeata*, and in a number of these cases, *M. novemaculeata* attempted to consume the invader, yet were unsuccessful due to limited gape size. It is interesting to note, however, that there was no apparent anti-predator response from *G. holbrooki* following exposure to *M. novemaculeata*. Neither species utilized shelters provided in the study, and the levels of aggression displayed by *G. holbrooki* to *M. novemaculeata* and conspecifics were all similar except for the high levels of intra-specific aggression in the low-density treatment. However, we cannot dismiss the possibility that there are multiple body size-mediated negative interactions (predation and aggression) occurring between these species. In the future, it would therefore be beneficial to track the outcome of aggressive interactions between these species over a range of size classes. This would help to determine at what stage following stocking *M. novemaculeata* are likely to receive most aggression from *G. holbrooki* and whether the release of older *M. novemaculeata* fingerlings would assist in controlling this invader in waterways. Furthermore, it could reveal whether *G. holbrooki* are a food source for adult *M. novemaculeata* in the wild, and so may provide some benefit for the native.

In conclusion, the present study demonstrates that value of measuring the relative magnitudes of inter- and intra-specific interactions when examining aggression between invasive and native species. Taken together, our findings suggest that *M. novemaculeata* may be more aggressive than *G. holbrooki* at high densities of the invader and displays more aggression when exposed to the invader than conspecifics. Therefore, where *M. novemaculeata* juveniles are released into waterbodies containing a high density of *G. holbrooki*, the invader may be more likely to experience the negative effects of these aggressive interactions, including slower growth, higher injury and mortality and increased vulnerability to predation may be observed (Zhang et al., 2015).

CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

The data sets pertaining to the present study can be found in the Appendix S1.

ORCID

Laura K. Lopez https://orcid.org/0000-0003-4818-1544
Marian Y. L. Wong https://orcid.org/0000-0001-6393-6453

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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