

Angry when outnumbered: Behavioural aggression in *Gambusia holbrooki* is conditional upon temperature and relative abundance.

Jessica Pink¹, Anthony Moore¹, Tyrie Starrs¹, Mark Lintermans², Christopher J. Fulton¹.

¹Evolution, Ecology & Genetics, Research School of Biology, Australian National University, Canberra ACT 2601

²Institute for Applied Ecology, University of Canberra, Canberra ACT 2601



Study species (photo credits): a) *Gambusia holbrooki* female (Chris Appleby), b) juvenile *Macquaria ambigua ambigua* (Gunther Schmida), c) *Hypseleotris klunzingeri* (Australian New Guinea Fishes Association NSW).

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Glossary of Terms

Abiotic: Non-living chemical and physical factors in the environment.

Agonistic: Associated with conflict.

ANOVA: A statistical test comparing sample means, full name Analysis of Variance.

Behavioural aggression: Aggressive behaviour by one individual displayed towards another individual that can consist of various physical attacks or threats.

Benthic: Associated with the bottom of aquatic environments.

Biotic: Of or relating to living organisms.

Brood: A group of young that are produced or hatched at the same time.

Caudal fin: The tail fin of a fish.

Congener: Organisms belonging to the same genus as each other.

Conspecific: Organisms belonging to the same species as each other.

Ecosystem: An interdependent biological system involving interactions and linkages between living organisms and their physical, chemical and biological surroundings.

Eradicate: The removal of all the individuals of a particular species (animal or plant) from a given environment.

Fecundity: The number of eggs produced.

Family: A taxonomic group containing one or more genera.

Genus (pl. genera): A taxonomic category ranking consisting of one or more species.

Gestational period: Period of development from conception to birth.

Heterospecific: Organisms that belong to different species.

Interference competition: Competition where certain behaviours such as aggression directly affect other individuals or exclude them from accessing a common resource.

Interspecific: Pertaining to actions between two or more separate species.

Intraspecific: Pertaining to actions between two or more individuals of a single species.

Invasive species: Species that become established in habitats outside their native range. Also known as alien, introduced or non-native species.

Juvenile: Not fully grown or developed individual (immature).

Littoral: Part of a waterway that is located close to the shoreline.

Microhabitat: A small localized habitat within a broader ecosystem.

Mono-specific: A group containing only one species.

Native: Species that is indigenous to a particular habitat, or region; occurs naturally.

Niche: A species' individual habitat and the ecological opportunities it exploits.

Ontogenetic stage: State of physical development of an individual.

Philopatric: Behaviour of remaining at, or returning to, one's original birthplace.

Photoperiod: The amount of time an organism is exposed to light in one day.

Recruitment: The number of juvenile fish joining the adult population.

Refuge: Areas where animals can seek shelter from predation, weather conditions, desiccation or extreme temperature/salinity.

Sexually dimorphic: Males and females of the same species that have different appearances.

Species: A group of organisms that can breed and produce fertile offspring.

Turbidity: A measure of the transparency or clarity of water. High turbidity is associated with muddy water.

Zooplankton: Animal constituent of plankton.

Executive Summary

Invasive species are a cause of major ecological and economic concern worldwide. In order to limit or prevent harm caused by invasive species, we must first understand the susceptibility of an ecosystem to invaders, as well as the biological traits that make a successful invader. One trait often linked to success in invasive species is behavioural aggression. This is where individuals aggressively defend or obtain a resource from others, which is a form of interference competition. Aggressive interactions between a native and invasive species can cause the displacement of the native species into a poorer niche and can lead to physical injuries, depressions in growth, survival and reproductive success, and even local extinctions.

Gambusia holbrooki is a fish native to North America, but now considered one of the worst invasive freshwater species in the world. Introduced to Australia in 1925 as a potential mosquito control agent, where *G. holbrooki* is now present in almost every major drainage, including the Murray-Darling Basin. Previous research has documented threats that *G. holbrooki* pose towards many native species, including amphibians, invertebrates and other fish. *Gambusia holbrooki* possesses several traits that enhance its invasion success including a high tolerance for a range of physical, chemical and biological conditions, a generalist diet that enables easy shifts between a range of available food resources, and high fecundity. *Gambusia holbrooki* has also been shown to be an extremely aggressive species, with such aggression and its effects thought to be modulated by temperature, salinity, species and size.

Considering the threat *G. holbrooki* poses to Australian native species, there are considerable efforts to eradicate or control its spread as much as possible. If such efforts are to be effective, we must understand the fine-scale aspects of *G. holbrooki*'s invasion dynamics. Here we explored how the behavioural aggressiveness of *G. holbrooki* changes according to their relative abundance to native species, in order to understand how they interact with native fish when reaching new territories, and/or respond to any attempts to reduce their abundance. To do this, we quantified the nature and extent of interactions between *G. holbrooki* and two species native to the Murray-Darling Basin: *Macquaria ambigua ambigua* (golden perch) and *Hypseleotris klunzingeri* (western carp gudgeon). We explored how interactions changed with modifications of temperature, species and relative abundance using wild-caught *G.*

holbrooki from the ACT, wild-caught *H. klunzingeri* from Victoria and hatchery-reared juvenile *M. a. ambigua* from NSW.

We found that *G. holbrooki* displayed high levels of aggression towards native fish conditional upon all the factors examined. Specifically, we found that:

- *Gambusia holbrooki* were highly aggressive towards both *M. a. ambigua* and *H. klunzingeri*;
- Predation of small *H. klunzingeri* by *G. holbrooki*;
- Aggressive interactions per individual *G. holbrooki* towards native fish increased when outnumbered by native species.
- Aggressive interactions changed in a stepwise fashion, with higher levels of attack by *G. holbrooki* occurring at and above 25 °C; and
- *Gambusia holbrooki* react differently towards the two native species, chasing and biting *M. a. ambigua* but mostly only chasing *H. klunzingeri*.

Overall, our results suggest intense behavioural aggression may aid the speed of *G. holbrooki* invasions into new habitats, by allowing small numbers of *G. holbrooki* to successfully and quickly apply interference competition for resources and to gain favourable habitats for population expansion. We find that the extent of *G. holbrooki* aggression is species-dependent, and when combined with the temperature trends, this information sheds light on the potential ramifications of any eradication attempts on this species (discussed below).

Recommendations

Given *Gambusia holbrooki* has already invaded most of Australia's major drainage systems, our recommendations are phrased largely towards understanding the current impacts on native fish and the implications of any *G. holbrooki* control and eradication efforts. It should be noted that these recommendations are made based on our small-scale, species-specific interactions between invasive *G. holbrooki* and the native freshwater species, *Macquaria ambigua ambigua* and *Hypseleotris klunzingeri* in the Murray-Darling Basin. Further research may reveal additional or modifying recommendations for other native species and spatial scales.

In terms of management implications we recommend:

- Listing of *G. holbrooki* on the noxious aquatic pest list for every Australian state and territory be maintained, based on the negative effects on native species via behavioural aggression and predation;
- The likely success and impact of any eradication attempt be carefully assessed, given that partial reductions in *G. holbrooki* abundance may result in increased levels of aggression and negative impacts on existing native species living in the same area(s);
- Timing control or eradication measures to coincide with periods of lower water temperatures (less than 20 °C) as *G. holbrooki* aggression levels will be lowest at this time while removal is undertaken;
- Employing a combination of eradication measures, such as rotenone application and drying techniques, to ensure maximum efficiency in the removal of *G. holbrooki* individuals, as even a few remaining individuals can still be severely detrimental to the remaining native fishes;
- Investigating whether behavioural aggression eventually leads to drops in survivorship of *M. a. ambigua* juveniles, as this would constitute evidence that *G. holbrooki* can negatively impact recruitment success in a native species;
- Further field studies to determine whether predation of *H. klunzingeri* by *G. holbrooki* occurs in the wild, as this would illustrate a previously unknown stress to *H. klunzingeri* populations and highlight the urgency of conservation measures;

- Examine the influence of habitat structure and complexity to determine whether the presence of habitat and its complexity ameliorates or exacerbates aggressive interactions;
- Examine the influence of density per area to determine if aggressive behaviour decreases with increasing space;
- Explore behavioural aggression by *G. holbrooki* in cold-water populations (e.g. species from alpine lakes) to determine whether decreased aggression occurs at 20 °C or whether decline in aggression occurs at lower temperatures, if at all;
- Explore if selection for cold tolerance is occurring as *G. holbrooki* invades areas with colder climates (e.g. the ACT or Kosciusko National Park). *Gambusia holbrooki* could be sampled from warmer versus colder climates and exposed to cold thermal shock.
- Determine whether there is a body size threshold above which native species receive little or no aggression from *G. holbrooki*; and
- Investigate long-term sub-lethal affects of *G. holbrooki* behavioural aggression on the health and fecundity of native species.

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Introduction

Non-native species invasions are a major concern worldwide (Vitousek *et al.* 1996; Sala *et al.* 2000; Pimentel *et al.* 2005). The number of invasive species that successfully become established in new environments is quite small and only a handful of these detrimentally affect the native ecosystem (Lodge 1993; Mack *et al.* 2000; Moyle and Marchetti 2006; Davis 2009). However, those that do are a major threat to national economies, ecosystems and human health (Vitousek *et al.* 1996; Mack *et al.* 2000; Simberloff 2001; Nentwig 2007). They can cause major economic losses in agriculture, fisheries and forestry industries; and the costs associated with efforts to eradicate them or mitigate their effects are large (Mack *et al.* 2000; Pimentel *et al.* 2005). To prevent or limit the damage imposed by invasive species, it is important to understand the susceptibility of an ecosystem to invaders and the biological traits that make a species a successful invader.

Behavioural aggression is one mechanism through which invasive species impose negative effects upon natives to gain access to new habitats and resources (Holway and Suarez 1999; Amarasekare 2002). Interference competition via behavioural aggression allows an individual to aggressively defend or obtain a resource, such as the best territories, reproductive habitats and foraging habitats, and often leads to the displacement of competitively inferior animals into poorer niches (Schoener 1983; Melville 2002). If the aggressive behaviour is particularly fierce it can also result in serious physical injuries, depressions of growth rate, decreased survival, reduced reproductive success and even localised or total extinction of a native species (Howe *et al.* 1997; Laha and Mattingly 2007).

The effects of behavioural aggression are widespread, and have been documented in many animals, including reptiles (Bolger and Case 1992; Melville 2002; Polo-Cavia *et al.* 2011), birds (Ingold 1998; MacNally and Timewell 2005; Brazill-Boast *et al.* 2011) and fish (Glova 1986; Marchetti 1999; Gunckel *et al.* 2002; Taniguchi *et al.* 2002; McMahan *et al.* 2007; Brookes and Jordan 2010). In terms of limiting access to habitats, high levels of aggression towards Gouldian finches, *Erythrura gouldiae*, from Long-tailed finches, *Poephila acuticauda*, have been found to displace Gouldian finches from the best cavity nesting sites and cause a reduction in brood size by up to 57% in the displaced species (Brazill-Boast *et al.* 2011). Similarly, in laboratory studies it was found that alien Spotted tilapia, *Tilapia mariae*, residents were able to

acquire 80% of new territories, defend 100% of their acquired territories, and usurp up to 30% of the resident native *Lepomis* Sunfish territories by aggressive expulsion (Brookes and Jordan 2010). In terms of foraging, Polo-Cavia *et al.* (2011) found that invasive Red-eared slider turtles, *Trachemys scripta elegans*, initiated >81% of aggressions against native terrapins during feeding activities, leading to the invasives ingesting a greater percentage of the supplied food than the native turtles present. Likewise, a study of invasive and native Japanese stream salmonids found 1+ year old Rainbow trout, *Oncorhynchus mykiss*, aggressively dominated age-0 Masu salmon, *Oncorhynchus masou*, initiating more aggressive interactions and winning 93% of them. This leads to the relegation of age-0 Masu salmon to less favourable foraging positions within the stream resulting in reduced foraging efficiency and growth (Taniguchi *et al.* 2002). Although age-0 Masu salmon were able to aggressively dominate age-0 Rainbow trout in aquarium experiments, the positive indirect effect of age-1 Rainbow trout on age-0 Masu salmon is thought to mediate this and thus this fine scale dynamic helps facilitate the invasion of Rainbow trout into streams (Taniguchi *et al.* 2002).

Behavioural studies on animal ‘personality’ have found aggression often correlates with traits such as boldness and activity (Pintor *et al.* 2008; Biro *et al.* 2011; Pruitt *et al.* 2011), potentially increasing the likelihood of successful establishment of invasive species by altering interactions with native species (Cote *et al.* 2011). This correlation can be seen in the biased dispersal of aggressive male Western bluebirds, *Sialia mexicana*, to an invasion front where they aggressively displace a close congener, whilst philopatric males were found to be significantly less aggressive (Duckworth and Badyaev 2007).

Biotic and abiotic factors can strongly influence the nature and extent of aggressive interactions, leading to complex interactions between species. As such, elucidating the exact mechanisms of interaction can be difficult in the field. Factors such as reproductive condition, size (MacNally and Timewell 2005), temperature, ontogenetic stage (Barrier and Hicks 1994; Rincón *et al.* 2002), habitat structure, (Basquill and Grant 1998; Hasegawa and Maekawa 2008) habitat quality (MacNally and Timewell 2005) and relative habitat preferences can all play important roles in the strength and impact of behavioural aggression between invaders and natives. Larger body size has generally been associated with an increased ability to dominate and exclude

individuals via aggression. For example, MacNally and Timewell (2005)'s study on bird assemblages found that of all instances of aggression between heterospecifics, only 5.5% were initiated by a smaller individual. A similar pattern has also been seen in freshwater fish, where size is often used to establish dominance hierarchies and regulate aggressive interactions (Turner 1994; Nakano *et al.* 1998; Usio and Nakano 1998; Marchetti 1999; Moran 2007). Exceptions do occur where a smaller individual may dominant a larger one via superior behavioural aggression (Nakano 1995; Rincón *et al.* 2002). Temperature has also been shown to influence the level of aggression in fish (Rincón *et al.* 2002; Biro *et al.* 2011), reptiles (Crowley and Pietruszka 1983; Herrel *et al.* 2007), spiders (Pruitt *et al.* 2011) and insects. For example, for the dragonfly, *Pachydiplax longipennis*, aggressive interactions during foraging were positively correlated with temperature (Baird and May 2003)

***Gambusia holbrooki*: Traits Driving Invasion Success**

One of the most invasive vertebrate species in the world today is Eastern gambusia, *Gambusia holbrooki*. *Gambusia holbrooki* are a small fish, native to northern Mexico and southern USA (Wooten and Lydeard 1990). In the early twentieth century *G. holbrooki* and the closely related *G. affinis* were introduced around the world to control mosquito-borne diseases via predation upon mosquito larvae, a task at which they have been relatively unsuccessful (Karolak 2006; Kumar and Hwang 2006; Rowe *et al.* 2008). Often considered together, these two fish species are now the most widespread introduced freshwater species in the world, inhabiting every continent besides Antarctica and *G. affinis* is listed in the world's 100 worst invaders and top eight worst fish invaders (Lowe *et al.* 2000; Pyke 2005).

Gambusia holbrooki was first introduced into Australia in 1925 (early literature refers to *G. affinis* in Australia), and is now present in all Australian states and territories and most major drainage divisions (Rowe *et al.* 2008). Exacerbating their spread has been the alteration of flow patterns in rivers by installation of dams and weirs, which has helped create favourable habitats for them (Rehage *et al.* 2005; Rowe *et al.* 2008). Documented threats to native species include predation on eggs, larvae and young of various animals and zooplankton (Arthington 1991; Ivantsoff and Aarn 1999; Baber and Babbitt 2003; Blanco *et al.* 2004), and forms of interference competition that have led to habitat shifts and population decreases for amphibians, invertebrates, and fish in

invaded habitats around the world (Meffe *et al.* 1983; Arthington 1991; Howe *et al.* 1997; Pyke and White 2000; Hamer *et al.* 2002; Rincón *et al.* 2002; Karolak 2006).

Gambusia holbrooki has three life history attributes that are important to its immense success in colonizing new territories. Firstly, *G. holbrooki* has a high tolerance for a range of physical, chemical and biological conditions including temperature, salinity, high turbidity and low dissolved oxygen levels (Meffe 1991; Pyke 2005; Karolak 2006; Alcaraz *et al.* 2008; Macdonald and Tonkin 2008). Secondly, it is a generalist predator and has been shown to eat a large range of aquatic micro and macro invertebrates, algae and plant material (Pen *et al.* 1993; Blanco *et al.* 2004; Pyke 2005) and is opportunistic and able to shift easily between available food sources (Pen *et al.* 1993). Thirdly *G. holbrooki*'s reproductive strategy lends itself to rapid colonization, as females give birth to highly developed live young, can be multiply-inseminated and can store sperm in their oviducts for months after their last mating, allowing them to reproduce without the recent presence of a male (Wourms 1981; Pyke 2005). Their short gestational period, large number of broods and large brood size gives *G. holbrooki* a huge reproductive potential that can enable rapid evolution to suit new habitats (Pyke 2005; Macdonald and Tonkin 2008; Rowe *et al.* 2008).

Behavioural aggression has also been cited as a trait used by *G. holbrooki* in colonizing new areas (Pyke 2005; Macdonald and Tonkin 2008). Aggression in this species has been characterized as chasing, pushing, thrusting, lunging, biting, and fin-nipping, often resulting in the removal of portions of fins and other body parts from their victims (Howe *et al.* 1997; Lintermans and Osborne 2002; Karolak 2006; Rowe *et al.* 2007). Notably, such aggressive behaviour is often directed at fish larger than *G. holbrooki*, which rarely attain greater than 60mm total length (Rincón *et al.* 2002; Rowe *et al.* 2007, Macdonald and Tonkin 2008). Aggression by *G. holbrooki* has been found to negatively affect native species. For instance, studies on the interactions between *G. holbrooki* and two native Spanish toothcarps, *Aphanius iberus* and *Valencia Hispanica*, in Europe found that an increase in agonistic attacks by *G. holbrooki* led to decreased feeding rate and disruption of reproductive behaviour (Rincón *et al.* 2002). Similarly, Barrier and Hicks (1994) found that *G. holbrooki* showed more aggression towards New Zealand Black mudfish, *Neochanna diversus*, than vice versa, with the highest rates of aggression by *G. holbrooki* directed towards mudfish fry and juvenile mudfish. Notably, such aggression was found to reduce prey

capture rates in mudfish fry and juveniles in the presence of *G. holbrooki* aggression, causing juvenile mudfish to adopt more benthic positions in the habitat (Barrier and Hicks 1994). In laboratory settings, the negative effects of *G. holbrooki* have been found to vary with temperature (Rowe *et al.* 2007; Priddis *et al.* 2009), salinity (Alcaraz *et al.* 2008), species and size (Barrier and Hicks 1994; Rincón *et al.* 2002).

One area that has received little attention in previous research is the importance of relative abundance of individuals (invasive species to native species) for behavioural aggression in both invasive species in general and *G. holbrooki* in particular. Information regarding the behaviour of *G. holbrooki* in situations of differing abundance relative to native species would be invaluable to our understanding of how they manage to establish themselves so consistently and successfully in new territories. By determining whether *G. holbrooki* can impose early behavioural aggression in ecosystems where they are initially outnumbered (early point of invasion), a mechanism for their continued success could be inferred.

Here we aimed to quantify the nature and extent of aggressive interactions between the invasive species *G. holbrooki* and two freshwater species, juvenile *Macquaria ambigua ambigua* (golden perch) and *Hypseleotris klunzingeri* (western carp gudgeon). Both these species are native to the Murray-Darling Basin (MDB) in Australia, throughout which *G. holbrooki* is now present. Our intention was to explore how *G. holbrooki* interact with native species at roughly similar body size to their own, when introduced to a common environment at differing relative abundances. As such, we specifically examined the number and nature of attacks between *G. holbrooki* and these two native species under differing ratios of invasive to native individuals, and determined whether temperature plays a role in modifying this aggressive behaviour. Our working hypothesis was that *G. holbrooki* individuals would display higher levels of aggression when outnumbered by native fish in order to gain access to new habitat. We used our findings to explore what this may mean for controlling the spread and impact of this species in Australian waterways.

Materials & Methods

Study species

Gambusia holbrooki are a small, sexually dimorphic fish, with deeper bodied females that can reach 50-60mm in length and smaller and slimmer males averaging 35mm

(Pyke 2005; Lintermans 2007; Rowe *et al.* 2008). They show a clear preference for slow moving or still water, such as large slow moving rivers, creeks, ponds, backwaters, wetlands, lakes, reservoirs, channels and bores, accessing these habitats via both deliberate introductions and flood induced spread (Arthington *et al.* 1983; Lintermans 2004; Lintermans and Osborne 2002; Rowe *et al.* 2008). Within these habitats they tend to occupy the shallow surface waters (within 15cm of the surface) and littoral margins of the water bodies (Lintermans and Osborne 2002; Pyke 2005; Lintermans 2007; Rowe *et al.* 2008) and prefer warmer temperatures (around 31-35°C (Pyke 2005)). *Gambusia holbrooki* are opportunistic and generalist predators (Ivantsoff and Aarn 1999; Stoffels and Humphries 2003; Pyke 2005; Rowe *et al.* 2008). Spawning occurs between spring and autumn, most frequently during the warmest months, and is influenced by both photoperiod and temperature (Pyke 2005; Lintermans 2007; Macdonald and Tonkin 2008; Rowe *et al.* 2008).

Macquaria ambigua ambigua (golden perch) are a large freshwater fish of the Percichthyidae family. For the purposes of brevity, *Macquaria ambigua ambigua* will hereafter be referred to as *M. a. ambigua*. They are native to Australian freshwater river systems and occur in the tributaries of the Murray-Darling system where they are an important recreational species (Allen *et al.* 2002; Lintermans 2007). They are long-lived, moderate to large species, averaging around 45cm in length (Allen *et al.* 2002). Their preferred habitats include backwaters and billabongs that have warm, turbid and sluggish waters but they can be found throughout the MDB in a range of riverine habitats (Allen *et al.* 2002; Lintermans 2007). They migrate upstream to spawn during spring and summer in temperatures of 23-26°C (Tonkin *et al.* 2006; Lintermans 2007).

Hypseleotris klunzingeri (western carp gudgeon) are a small freshwater fish of the Eleotridae family. It is small, around 40-50 mm total length, slender and laterally compressed (Allen *et al.* 2002; Lintermans 2007). It occurs naturally in Australian rivers, especially lowland habitats throughout the MDB and their preferred habitat is in littoral vegetation surrounding slow flowing bodies of water including dams, lakes, streams, creeks and billabongs (Allen *et al.* 2002; Lintermans 2007). Spawning occurs in late spring to early summer in water temperatures exceeding 22.5°C (Allen *et al.* 2002; Lintermans 2007).

Gambusia holbrooki, *M. a. ambigua* and *H. klunzingeri* coexist with one another within different habitats of the MDB, including in ephemeral habitats such as floodplains, which are an important part of both native species' life cycle (Allen *et al.* 2002)

Collection and housing

Gambusia holbrooki were collected from Lake Ginninderra and Bruce Ponds, ACT, via dip and seine nets, between February and April 2010 by research staff at the Australian National University. *Macquaria ambigua ambigua* juveniles were sourced from the Uarah Fish Hatchery, Grong Grong NSW, between February and May 2010. A mixture of *H. klunzingeri* covering a full range of sizes were collected by seine net from billabongs located on the flood plains of the Ovens River near Peechelba, Victoria in March 2010. Average weights of *G. holbrooki*, *H. klunzingeri* and *M. a. ambigua* were recorded (Table 1).

Fish were transported to the aquarium facility at the Australian National University, Canberra, where they were housed in mono-specific, mixed sex groups of 20-30 individuals in 60L glass aquaria. Aquaria contained 4 mm diameter cleaned river gravel substrate to a depth of 2.5 cm and no refuges. Conditioned Canberra tap water (matured for 5 days to allow chlorine to dissipate) was used with 50g of Aqua Master "Sure Start" salts (Aristopet Pty. Ltd. Eagle Farm QLD) added. Tanks had airstone-powered under gravel filtration systems, a 12:12 hr light:dark regime and were maintained at treatment temperatures (20, 25 or 30 °C) via a combination of controlled room heating and submersible 200W heaters. Fish were fed thawed blood worms twice daily to satiation. All fish had a minimum of 5 days of acclimation in holding tanks prior to their use in behavioural experiments.

Experimental Design

Five separate treatments were run, involving a combination of *G. holbrooki* to native fish ratios and temperatures. Firstly, to explore how *G. holbrooki* varied their aggression according to changes in the numbers of natives present, eight individuals of *G. holbrooki* were placed with varying numbers of juvenile *M. a. ambigua* (16, 8, 4 and 0) into a tank and their behavioural interactions over a set period (see below) at a water temperature of 25 °C were recorded. This was repeated five times for each ratio. Following this, we explored the interactive influence of temperature and ratio on the

aggressive interactions between *G. holbrooki* and juvenile *M. a. ambigua* across seven ratios where total density was kept at twenty fish per tank (*G. holbrooki* : native; 4:16, 8:12, 10:10, 12:8, 16:4, 20:0, 0:20). Each ratio was replicated six times across each of the three temperatures of 20, 25 and 30 °C. Finally, to explore how such aggression may vary across species, we repeated the seven-ratio constant density trials with *H. klunzingeri* at 30 °C.

Table 1: Summary of experimental parameters - species, temperature, ratios, replications and mean (\pm S.E) mass for *G. holbrooki*, *M. a. ambigua* and *H. klunzingeri*.

Species	Temperature (°C)	Ratios (<i>G. holbrooki</i> :native)	Replication per ratio	Mean mass (g) \pm standard error	
				<i>G. holbrooki</i>	Native
<i>G. holbrooki</i> & <i>M. a. ambigua</i>	25	8:16, 8:8, 8:4, 8:0	5	0.30 \pm 0.01	1.35* \pm 0.02
	30	4:16, 8:12, 10:10, 12:8, 16:04, 20:0, 0:20	6	0.31 \pm 0.01	0.67 \pm 0.02
	25	4:16, 8:12, 10:10, 12:8, 16:4, 20:0, 0:20	6	0.33 \pm 0.02	0.51 \pm 0.08
	20	4:16, 8:12, 10:10, 12:8, 16:4, 20:0, 0:20	6	0.27 \pm 0.01	0.70 \pm 0.02
<i>G. holbrooki</i> & <i>H. klunzingeri</i>	30	4:16, 8:12, 10:10, 12:8, 16:4, 20:0, 0:20	6	0.37 \pm 0.02	0.32 \pm 0.01

**Macquaria ambigua ambigua* suffered a large mortality event prior to experiment and more juvenile individuals had to be sourced from the Uarah Fish Hatchery. These juveniles were unavoidably larger than those used in other experiments

To conduct the interaction experiments, we used three test aquariums of 19.7cm x 50.0cm x 22.4cm dimensions, which had the back and sides covered in opaque black plastic to avoid interactions among adjacent tanks. The experimental tanks had the same water source as the holding tanks with no substrate or refuges and were maintained at the experimental temperature using a 50 W glass water heater prior to filming. Three digital high definition hard disk drive cameras (Sony HDR-SR5) were mounted on tripods and placed 1 m away from the front of each tank, with opaque screens placed between the cameras and the tanks to allow observers to enter and exit the room freely. Incandescent lighting was mounted 1m above the tank to ensure adequate light.

Each tank was divided in half by a solid, white Perspex divider. Each group of test species were placed into the experimental tank on either side of the dividing barrier

and allowed 15 minutes of equilibrium time, before the video camera started recording and the plastic barrier was removed. A total of 40 minutes of interactions were then recorded, whereupon the fish were removed, euthanized in ice water, weighed (to nearest 0.01g) and placed in frozen storage. Fish that had eaten another fish were euthanized and dissected and both predator and prey fish were weighed.

Each replicate 40-minute series of footage was separated into three, 5-minute subsections (0-5, 15-20 and 30-35 minutes) for analysis by a single observer (TS), who recorded all interactions between fish and placed them into four categories: Bite – one fish biting another; lunge – attempted mating by male *G. holbrooki*; chase – fish tried to catch another for purposes of a bite or lunge (note that if multiple fish were involved, they were recorded as separate chasing events); and predation – event where one fish consumed another.

Statistical analyses

Number of attacks (bite, lunge, chase and predation) were summed across the three five minute sections of footage and these values were divided by the number of *G. holbrooki* present to derive the number of attacks per *G. holbrooki* individual. The mean, standard deviation, standard error and 95% confidence limit were then calculated across all experimental replicates for all combinations of species, ratios and temperatures. Relative abundance analysis was carried out for the constant *G. holbrooki* vs. varying ratio of *M. a. ambigua* at 25 °C s using a 1-way ANOVA (ratio as fixed factor) for each behaviour (bite, lunge and chase). Effects of temperature were then explored for *G. holbrooki* vs. *M. a. ambigua* interactions across three temperatures via 2-way ANOVA (temperature and ratio as fixed factors) for each behaviour (bite, lunge and chase). Cross-species comparisons were carried out between experiments at 30 °C (*G. holbrooki* vs. *M. a. ambigua* and *G. holbrooki* vs. *H. klunzingeri*) using a 2-way ANOVA (species and ratio as fixed factors) for each behaviour (bite, lunge and chase).

Results

Overall 18,161 separate attacks were recorded across the 47 hours of digital video analysed. *Gambusia holbrooki* initiated the majority of attacks (96.1%) and of those 74.4% were against heterospecifics and 25.6% towards conspecifics. Of the attacks initiated by native individuals (3.9% of total) 72.7% were directed at heterospecifics

and 27.3% at conspecifics. The most frequent form of aggressive interaction was chasing, accounting for 65.7% of attacks, followed by 31.4% and 2.82% of attacks being bites and lunges, respectively. Predation events accounted for <0.1% of all attacks (only 10 records), and occurred only between *G. holbrooki* and *H. klunzingeri* (attacker and victim respectively; Table 2). *Gambusia holbrooki* that ate the native fish were larger individuals (>0.48g; pop mean = 0.37g; Table 2), and *H. klunzingeri* individuals that were consumed were the smallest individuals (<0.09g; pop mean = 0.32g; Table 2). Note that predation events were not analysed further as so few were recorded.

Table 2. Statistics for predation events during experiment: temperature, ratio, predator, predator mass, prey and prey mass.

Temperature (°C)	Ratio	Predator	Predator mass (g)	Prey	Prey mass (g)
30	4:16	<i>G. holbrooki</i>	0.7494	<i>H. klunzingeri</i>	0.0242
30	4:16	<i>G. holbrooki</i>	0.5194	<i>H. klunzingeri</i>	0.0301
30	8:12	<i>G. holbrooki</i>	0.5453	<i>H. klunzingeri</i>	0.0576
30	8:12	<i>G. holbrooki</i>	0.9673	<i>H. klunzingeri</i>	0.0462
30	10:10	<i>G. holbrooki</i>	1.0904	<i>H. klunzingeri</i>	0.0351
30	10:10	<i>G. holbrooki</i>	1.0904	<i>H. klunzingeri</i>	0.0711
30	12:08	<i>G. holbrooki</i>	1.2812	<i>H. klunzingeri</i>	0.0890
30	12:08	<i>G. holbrooki</i>	0.5962	<i>H. klunzingeri</i>	0.0353
30	12:08	<i>G. holbrooki</i>	0.4839	<i>H. klunzingeri</i>	0.0448
30	16:04	<i>G. holbrooki</i>	0.7914	<i>H. klunzingeri</i>	0.0485

Bite attacks per *G. holbrooki* individual occurred significantly more for ratios where *G. holbrooki* were outnumbered (8G : 16N) or were at an equal abundance (8G : 8N) to *M. a. ambigua* compared with when it outnumbered *M. a. ambigua* (8G : 4N) ($F_{2,12} = 5.947$, $p = 0.016$; Fig. 1). This pattern was visible for chase attacks but was not significant ($F_{2,12} = 2.576$, $p = 0.117$). This was also evident in analyses carried out on both the temperature (Fig. 2) and species (Fig. 3) effect, with number of chases and bites significantly decreasing with the changes in abundance of *G. holbrooki* from being outnumbered to numerically dominant (Table 3, Table 4).

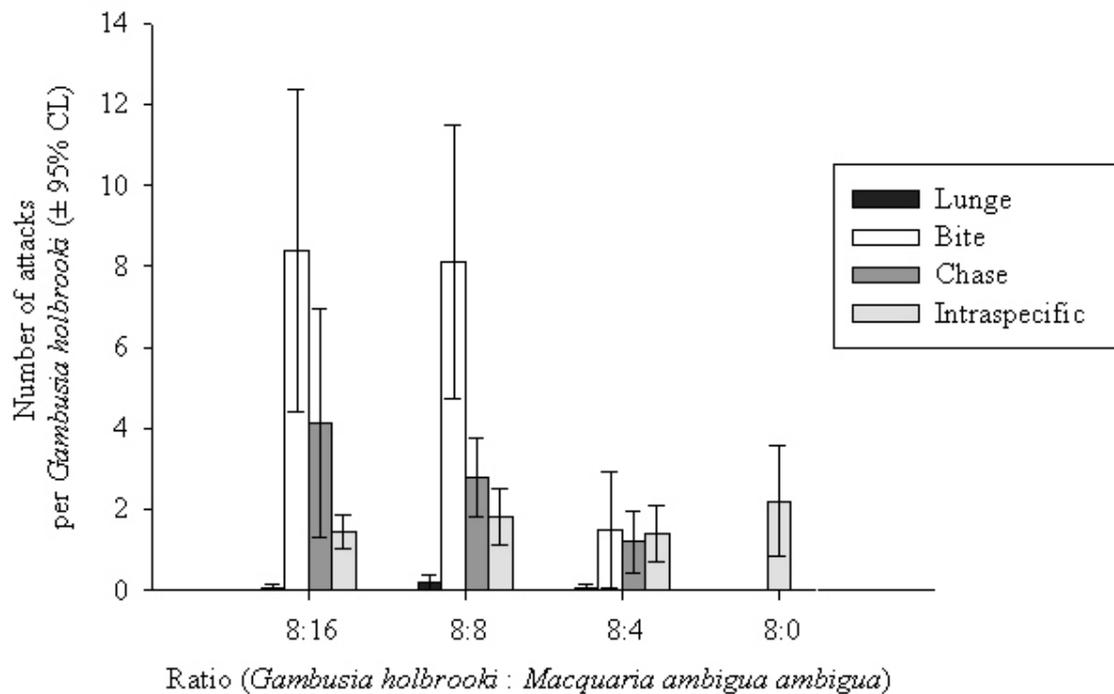


Figure 1. Mean number of attacks (lunge, bite, and chase) by *Gambusia holbrooki* against *Macquaria ambigua ambigua* when interacting at 25°C and at varying ratios of invasive to native fish. Intraspecific attacks were between *G. holbrooki* only and show total attacks (summation of lunges, bites and chases).

Temperature significantly influenced the number of aggressive interactions between *G. holbrooki* and *M. a. ambigua* (Fig. 2, Table 3). Numbers of bites and chases between 25 and 30 °C for all ratios are similar, but there were significantly fewer attacks of either kind at 20 °C, where attack numbers were extremely low (<2 attacks on average per *G. holbrooki* individual over the observation period). The significant interaction term between ratio and temperature for both chase and bite (Table 3) is most likely due to the unchanging nature of attacks at 20 °C, irrespective of the ratio of *G. holbrooki* : native, compared to the correlation between number of attacks and ratio seen at 25 and 30 °C (Fig. 2).

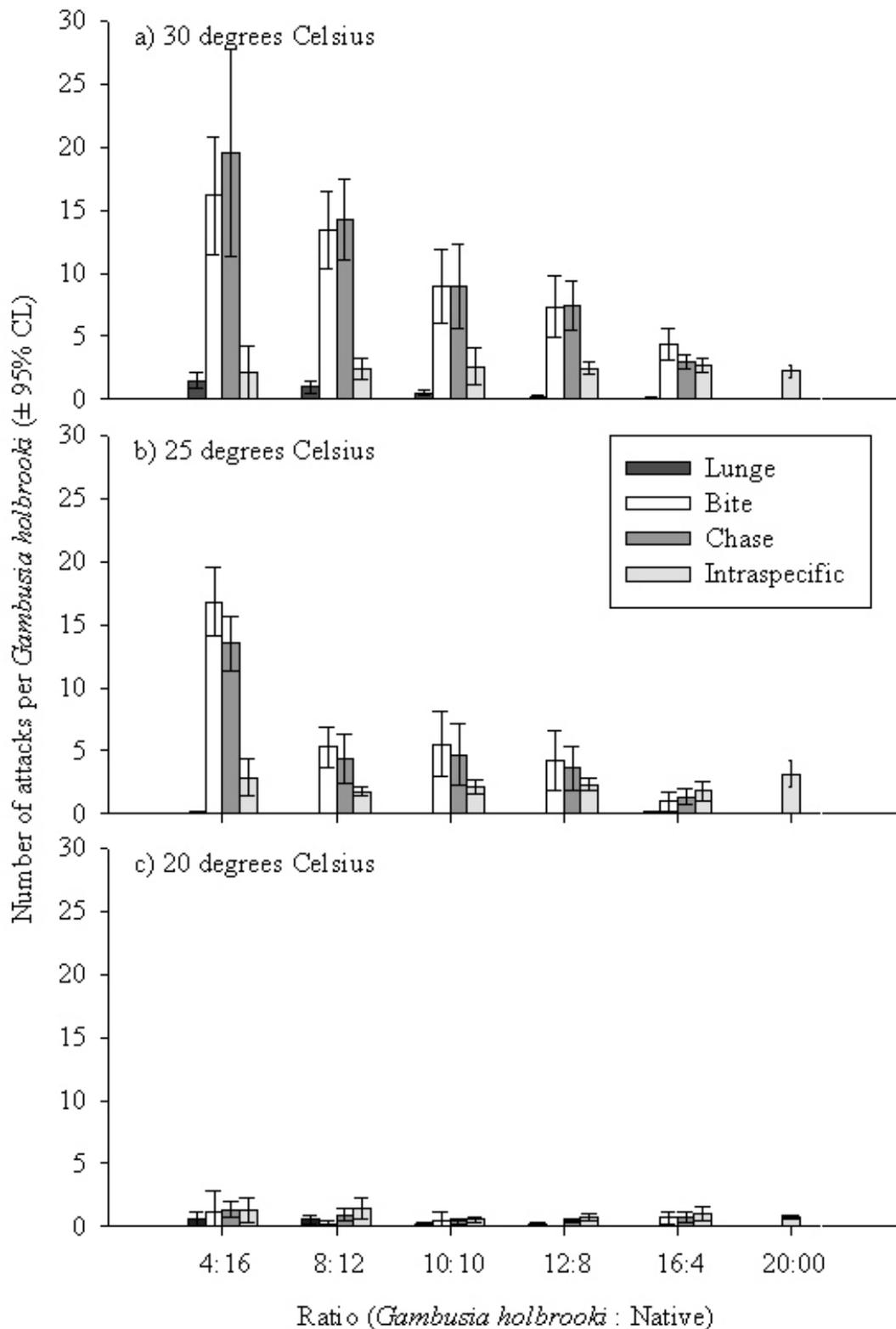


Figure 2. Mean number of attacks (lunge, bite, and chase) by *Gambusia holbrooki* against *Macquaria ambigua* when interacting at six ratios of *G. holbrooki* to native fish and at (a) 30 °C (b) 25 °C (c) 20 °C. Intraspecific attacks were between *G. holbrooki* only and show total attacks (summation of lunges, bites and chases).

Table 3. Summary of 2-way ANOVAs with temperature and ratio as determining factors and (a) bite, (b) chase, and (c) lunge as independent variables.

Effect	SS	DF	MS	F	p
(a) Bite					
Temperature	1382	2	690.9	90.97	<0.01
Ratio	902	4	225.5	29.69	<0.01
T * R	499	8	62.4	8.21	<0.01
(b) Chase					
Temperature	1456	2	728	62.63	<0.01
Ratio	983	4	245.7	21.14	<0.01
T * R	523	8	65.4	5.63	<0.01
(c) Lunge					
Temperature	5.352	2	2.676	20.9	<0.01
Ratio	5.146	4	1.287	10.05	<0.01
T * R	4.342	8	0.543	4.24	<0.01

Aggressive behaviour by *G. holbrooki* also differed against the two native species of *M. a. ambigua* and *H. klunzingeri* at 30 °C (Fig. 3), with *G. holbrooki* directing significantly more bite attacks towards *M. a. ambigua* than *H. klunzingeri*. The number of chases was significantly different between the two species but this appears to be driven by results from the 10G : 10N ratio as attacks are similar between species for all other ratios. The results at this ratio also appear to be driving the interaction term between species and ratio for chases, although this term is only marginally significant (Table 4). The interaction term for bites is highly significant (Table 4). When *G. holbrooki* is at high abundance compared with native species, individuals direct attacks at both species at a similarly low rate. However, as its relative abundance decreases with respect to the native species there is an increase in the overall number of attacks of which significantly more are directed towards *M. a. ambigua* compared with *H. klunzingeri* and this is likely driving the significant interaction term.

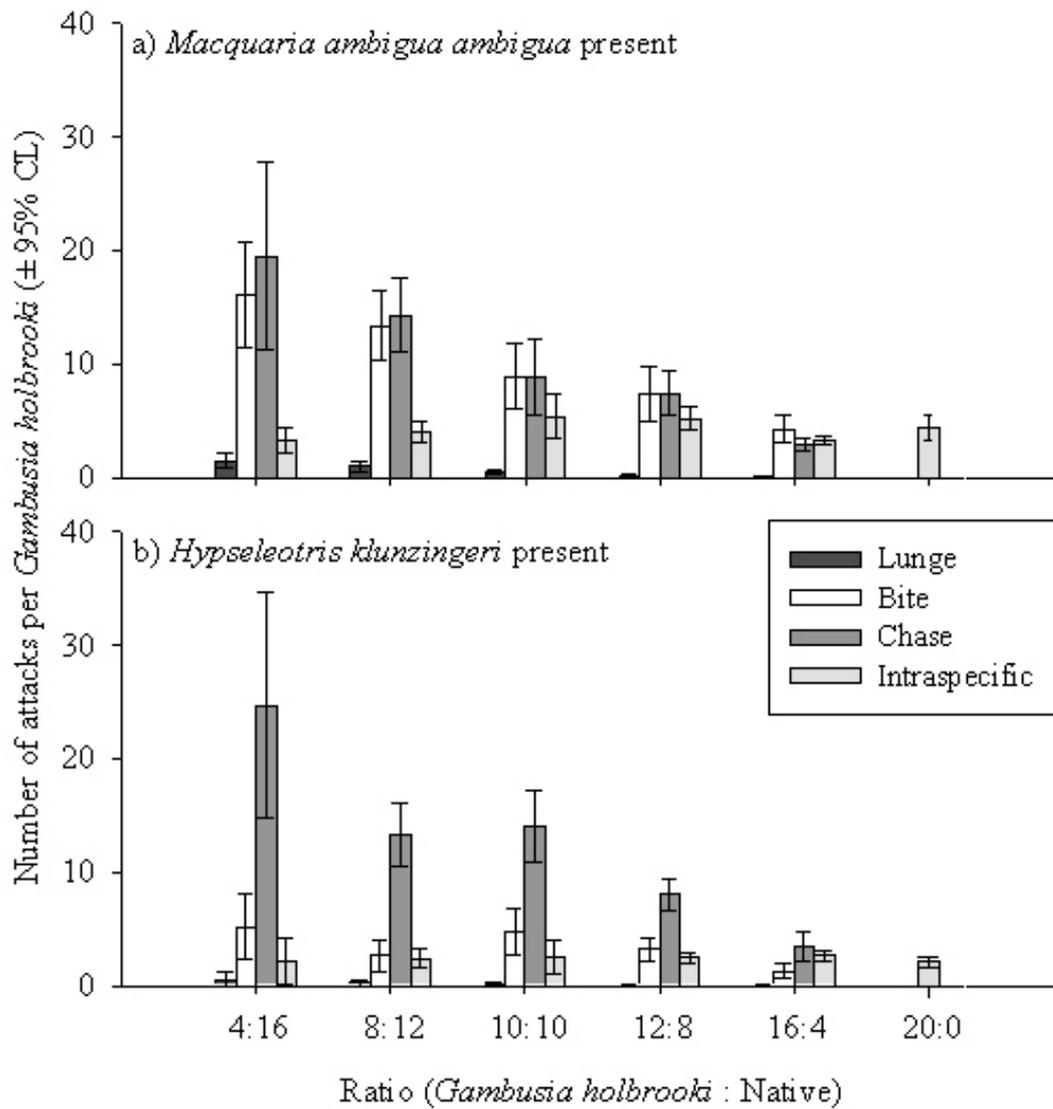


Figure 3. Mean number of attacks (lunge, bite, and chase) by *Gambusia holbrooki* at 30 °C and six ratios of *G. holbrooki* against a) *Macquaria ambigua ambigua* and b) *Hypseleotris klunzingeri*. Intraspecific attacks were between *G. holbrooki* only and show total attacks (summation of lunges, bites and chases).

Table 4. Summary of 2-way ANOVAs with species and ratio as determining factors and (a) bite, (b) chase, and (c) lunge as independent variables.

Effect	SS	DF	MS	F	p
(a) Bite					
Species	652.3	1	652.3	65.97	<0.01
Ratio	415.3	4	103.8	10.5	<0.01
S * R	184	4	46	4.65	<0.01
(b) Chase					
Species	3.077	1	3.077	18.48	<0.01
Ratio	7.306	4	1.827	10.97	<0.01
S * R	1.896	4	0.474	2.85	0.033
(c) Lunge					
Species	65	1	64.6	1.94	0.17
Ratio	2404	4	600.9	18.05	<0.01
S * R	97	4	24.4	0.73	0.575

Reciprocal attacks by natives against *G. holbrooki* were comparatively low, accounting for only 2.9% of total attacks. Chasing was the most common of these aggressions against *G. holbrooki* by natives with only one bite recorded. *Macquaria ambigua ambigua* were more aggressive towards *G. holbrooki* than *H. klunzingeri* and they were more aggressive at 30°C than at 20 or 25°C (Table 5). Indeed, the highest numbers of attacks against *G. holbrooki* were chases by conspecifics. Levels of intraspecific attacks across ratios (Fig. 1), temperatures (Fig. 2), and species (Fig. 3) for control experiments (20G : 0N) all indicate that intraspecific aggression occurs at similar levels to interspecific aggression at ratios where *G. holbrooki* outnumber the native species.

Table 5. Ranked table of average attacks (bite, lunge, and chase) against *Gambusia holbrooki* by *Macquaria ambigua ambigua* when interacting at three temperatures and varying ratios. Rows are ranked from highest to lowest mean number of chasing attacks and attacks that occurred less than twice on average are excluded.

Attacker	Temperature (degrees Celsius)	Ratio (G:M)	Type of Attack		
			Bite	Lunge	Chase
<i>M. a. ambigua</i>	30	10:10	0.2	-	17.5
<i>M. a. ambigua</i>	30	12:08	-	-	16.8
<i>M. a. ambigua</i>	30	4:16	-	-	15.7
<i>M. a. ambigua</i>	30	8:12	-	-	13.8
<i>M. a. ambigua</i>	20	12:08	-	-	3.3
<i>M. a. ambigua</i>	20	8:12	-	-	3.2
<i>M. a. ambigua</i>	20	4:16	-	-	3.0

Discussion

Behavioural aggression can be a pivotal trait driving the establishment and invasion success of a new species into a native community. Here we document the exceptionally strong behavioural aggression of the invasive species *G. holbrooki* against two native Australian freshwater fish species. Moreover, we provide the quantitative evidence that *G. holbrooki* escalate their individual rates of attack against natives when outnumbered by native fishes compared to when they are numerically dominant. Since *G. holbrooki* attack fish that are both larger and smaller than themselves, this aggressive strategy may be a pivotal trait for the invasion success and establishment of *G. holbrooki* in waterways around the world. We also report the first case, to our knowledge, of predation by *G. holbrooki* upon native *H. klunzingeri*.

Increased aggression when outnumbered is likely to be a key factor in the rapid invasion and establishment of *G. holbrooki* in new habitats. Indeed, *G. holbrooki* has established new reproductive populations with high success after almost all documented introductions (96.8% success in introduced areas (Garcia-Berthou *et al.* 2005)). Possessing a suite of traits spanning wide environmental tolerances to high fecundity plays a central role in the ability of *G. holbrooki* to colonise a wide range of habitats. Intense behavioural aggression may be the driving force behind the speed of these invasions, since *G. holbrooki* appear to adapt their aggressive behaviour according to their relative abundance. By exploring a typical sequence of invasion, we envision that when *G. holbrooki* are outnumbered in the early phases of invasion the vanguard individuals impose considerable stress and employ interference competition for resources to quickly gain favourable habitats for population expansion. Once *G. holbrooki* gain numerical dominance in their environment, they avoid expending energy on aggressive behaviour. Interestingly, this variable response is quite rapid, based on the fact that our experiments revealed these differences when *G. holbrooki* were placed into aquaria with different relative abundances of natives and responded accordingly within minutes. Rapid and flexible behavioural aggression is one trait that likely explains the speed and invasion success of *G. holbrooki*, similar to that seen in the invasion of Western bluebirds back into their former territory in North America (Duckworth and Badyaev 2007)

These findings are important in the consideration of eradication techniques. Techniques such as the use of chemicals and manual measures such as dip-netting,

bait traps and drying treatments used in small contained waterways have proved successful for *G. holbrooki* eradication on occasions. These include the use of drying treatments in Alice Springs, rotenone treatment in Canberra (Lintermans and Rutzou 1990) or the combination of drying treatments and rotenone application in New Zealand (Rowe *et al.* 2008; Macdonald and Tonkin 2008). However, in larger, more open waterways or small waterways where eradication techniques are unsuccessful, the presence of *G. holbrooki* in small numbers following an unsuccessful eradication is unlikely to eliminate stress on native populations since we have shown that numbers as low as four *G. holbrooki* can contribute a significantly large number of attacks when outnumbered by native species. For example, the use of bait traps targeting *G. holbrooki* in their preferred microhabitat has been suggested as a method for reducing the abundance of *G. holbrooki* close to spawning times for native fish and amphibians in spring and summer (Brookhouse and Coughran 2010). However this technique does not completely eradicate *G. holbrooki* and although it will reduce their abundance, the potential for increased aggression by *G. holbrooki* means that the total levels of aggression towards the native species will not change by much. Successful control attempts would require both biological and hydrological expertise in the area and a coordinated approach using multiple techniques to achieve complete eradication.

Ambient temperature was a strong modulator of behavioural aggression in *G. holbrooki*, since they were significantly more aggressive towards native species at warmer temperatures (i.e. at and above 25 °C). Such temperature-dependent behavioural aggression has been reported for other species such as spiders, where female *Anelosimus studiosus* were shown to increase activity, responsiveness and aggression towards prey at higher temperatures (Pruitt *et al.* 2011). Here we found that aggressive interactions by *G. holbrooki* against native *M. a. ambigua* followed a step-wise function, with similarly high numbers of attack at 25 and 30 °C, dropping away to very low numbers of attack at 20 °C. We know that being ectothermic, fish metabolism and sensory capabilities are linked to the ambient water temperature (Wootton 1998). Given that aggressive acts such as biting, chasing or lunging require metabolic energy, it is therefore logical that at higher temperatures more energy will be available and levels of aggression can increase.

Temperature-dependent behavioural aggression has been seen previously in *G. holbrooki*. They were seen to be less aggressive towards two species of Spanish toothcarps when the temperature was lower, with minimal aggression seen at 14 °C compared with 22 and 26 °C, where a significantly higher number of aggressive interactions were documented against one species (Rincón *et al.* 2002). Similarly, interactions between *G. affinis* and Inanga, *Galaxias maculatus*, in New Zealand were shown to be influenced by temperature, with both mortality and the extent of caudal fin loss (a result of fin nipping) being related to temperature and significantly more occurring as temperature increased (Rowe *et al.* 2007). The interaction between *G. affinis* and least Chub, *Iotichthys phlegethontis*, had a positive relationship with temperature with virtually no attacks occurring at 10 and 15 °C and significantly more aggression being seen at 20 and 25 °C (Priddis *et al.* 2009). We know the preferred temperature of *G. holbrooki* is around 31-35 °C (Pyke 2005) and that they have a very large temperature tolerance (Macdonald and Tonkin 2008). Here we document a likely threshold of 20-25 °C around which dramatically higher and lower levels of aggression are displayed by local Canberran populations of *G. holbrooki* against native fish species. Populations of *G. holbrooki* have been recorded at higher altitudes in the MDB (e.g. up to 1300m, Lintermans unpublished data) and future research using samples from these thermally acclimated populations would likely find that colder adapted populations have increased aggression at temperatures under 25 °C when compared to the population from this study and populations from warmer habitats. It was noted that a large proportion of *G. holbrooki* used were surviving for extended periods in ice slurry at the end of the experiment, suggesting a potential for these local populations to have adapted to colder water temperatures.

Aggression by *G. holbrooki* also varied according to the native fish being attacked. While chasing of *M. a. ambigua* and *H. klunzingeri* by *G. holbrooki* tended to occur at the same frequency, *G. holbrooki* appeared to bite *H. klunzingeri* less frequently than *M. a. ambigua*. Notably, the *M. a. ambigua* juveniles used were approximately double the body mass of *G. holbrooki*, suggesting that *G. holbrooki* maintains high levels of aggression spanning multiple types of attack when placed with a native species larger than itself. Previous studies on aggressive behaviour have shown a general trend of larger individuals being more aggressive, both in interspecific interactions (MacNally and Timewell 2005) and intraspecific interactions (Moran 2007). Such size-related

aggression is frequently seen in the establishment of dominance hierarchies in species such as in birds (MacNally and Timewell 2005) and fish, particularly salmonids (Usio and Nakano 1998; Nakano *et al.* 1998).

Contrary to this trend, however, both here and in previous studies *G. holbrooki* have been found to show aggression against larger adversaries and have been found in several instances to chase and nip individuals that are larger than themselves (Lintermans 2007; Macdonald and Tonkin 2008). For instance, *G. holbrooki* attacked larger Spanish toothcarps frequently (Rincón *et al.* 2002), and *G. affinis* attacked both juvenile Inanga and adult Inanga approximately double their size or larger (Rowe *et al.* 2007). There are anecdotal reports of *G. holbrooki* attacking adult cod and bass species that are multiple orders of magnitude larger (Lintermans unpublished, Moore unpublished). While chasing attacks alone are likely to negatively affect native species by interfering with their normal patterns of space use, the added stress of biting attacks against juvenile *M. a. ambigua* could also cause additional stress through physical damage of fins which can lead to the potential for fungal infections to occur in wounds (Meffe 1983; Faragher and Lintermans 1997; Laha and Mattingly 2007). Whether this increased aggression was a result of size differences or species specific differences cannot be determined from our data. However, these results back up other experimental data showing *G. holbrooki*'s ability to aggressively interact with fish both larger and smaller than themselves. Future research considering the size of the native fish attacked could determine the size range over which *G. holbrooki* is prepared to aggressively attack individuals and at what size, if any, they decide the target is too large.

Perhaps most concerning about the attacks by *G. holbrooki* on juvenile *M. a. ambigua* is the potential for recruitment failure of some of the larger native fish species. Under the circumstances recorded here, juveniles of this native fish are subject to considerable stress that may have substantial ramifications for growth and survival. *Gambusia holbrooki* has been seen to reduce reproductive success of native species, such as in their interactions with Spanish toothcarps (Rincón *et al.* 2002) and the Pacific blue-eye, *Pseudomugil signifier* (Howe *et al.* 1997). Interactions between *G. holbrooki* and the Pacific blue-eye caused both reduced growth and survival in adult blue-eye and a significantly reduced fecundity and ovarian weight in females. This was hypothesised to be the result of aggressive behaviour affecting survival of

reproductive females and increasing their physiological stress levels (Howe *et al.* 1997). *Gambusia* species have been discussed as a possible cause of recruitment declines and failures in fish such as Chub (Ayala *et al.* 2007) and Barrens topminnow, *Fundulus julisia* (Goldsworthy 2005 cited in Laha and Mattingly 2007) via predation and injury at early life stages. *Hypseleotris klunzingeri* are also at risk of recruitment failure as we observed *G. holbrooki* eating *H. klunzingeri* individuals on ten occasions where only the larger *G. holbrooki* individuals and the smaller, probably juvenile, *H. klunzingeri* individuals were involved. Our results show that predation between *G. holbrooki* and small *H. klunzingeri* is possible, and if this is occurring in wild populations, which is likely due to their overlap in habitat preference, it could potentially affect the recruitment success of *H. klunzingeri* via predation at early life stages.

Results of small aquaria trials may not reflect behaviour in the wild and it is vital that experiments on a larger scale and field work under natural conditions be carried out to validate these results. The presence of only a single choice of food is also likely to influence results as prey choice and availability has been shown to affect predatory and aggressive interactions in fishes (Rincón *et al.* 2002; Paradis *et al.* 2005). A range of other factors may play a role in the nature and frequency of behavioural aggression by *G. holbrooki*, chief among those likely to be habitat structure and availability. Research looking at how spatial scale and habitat complexity plays a role in aggressive response would be beneficial. Habitat complexity potentially mitigates aggressive interactions between fish such as zebra fish, *Danio rerio*, (Basquill and Grant 1998) and between invasive and native species such as white spotted char, *Salvelinus leucomaenis*, and brown trout, *Salmo trutta* (Hasegawa and Maekawa 2008). The impacts of habitat complexity on behavioural interactions could be investigated by increasing aquaria size and by providing native species with habitat types of differing structure and complexity. Done in the presence or absence of *G. holbrooki* we could detect alterations in levels of aggression as a result of the changes in habitat. Changes in aggression depending on habitat size could also be assessed by maintaining a set number of total fish and varying the volume of the tanks. Long-term experiments aimed at quantifying the deleterious effects of long-term exposure to *G. holbrooki* aggression would be valuable. The measurement of a large range of health parameters in native fish before and after trials with and without *G. holbrooki* could

provide insight on the long-term effects of *G. holbrooki* on native populations of *M. a. ambigua* and *H. klunzingeri* in the MDB. These research areas would have potential to elucidate whether different habitats ameliorate or exacerbate aggressive interactions and whether long-term interaction with *G. holbrooki* is likely to impact on the health of native freshwater fish. This would help determine whether these species are likely to be under serious conservation threat from the continued presence of *G. holbrooki* in the MDB.

Given the high reproductive output, generalist diet and high tolerance of physical, chemical and biological conditions, *G. holbrooki* is well-placed to invade a wide range of habitats. Studies have also shown that the traits of boldness, activity and dispersal are correlated in *G. holbrooki*, potentially adding to their invasive repertoire (Cote *et al.* 2010; 2011). Here we document how these traits combined with a tendency for extreme individual aggression when outnumbered by other fish species equips *G. holbrooki* with the potential to quickly invade and establish themselves in new habitats, seemingly regardless of the fish community already in residence. Such a combination of traits goes a long way to explain how these species can invade new habitats so quickly and effectively. However, the question now is how do we use this new understanding to control the spread of this species and can we truly eradicate them once established? Our evidence suggests that if control is attempted, every individual needs to be removed, as small numbers of *G. holbrooki*, (as low as four individuals), are still able to exert significant levels of aggression on native populations. Moreover, the link between rates of aggression and temperature suggest that timing control attempts to occur at colder water temperatures will be of benefit. This would allow additional time for the full removal of the species when aggression levels are naturally low. This result supports preliminary field work in *G. holbrooki* eradication such as work by Tonkin *et al.* (2010) finding that eradication was successful when targeting individuals in early spring after being reduced to very low numbers during the natural winter season declines in population size (Pyke 2005).

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