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Behavioral responses to, and fitness consequences from, an invasive species are life-stage dependent in a threatened native fish

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ABSTRACT

Native wildlife are impacted by invasive species in numerous ways and will be more vulnerable if they cannot recognize the threat posed by an invader. Impacts, however, are generally assessed for a single life stage and without consideration of behavioral responses. This limits knowledge of the mechanisms underpinning the threats of invaders and the responses that could help or hinder native animals to mitigate this threat.

We conducted a series of experiments to examine if the threat of an invader and behavioral responses by a native animal are life-stage dependent. Our focal species were a widespread invasive (*Gambusia holbrooki*) and a threatened native Australian freshwater fish (*Galaxiella pusilla*). We show that the threat of, and behavioral responses to, the invader vary across life-cycle stages. *Gambusia holbrooki* had different effects on *G. pusilla*: inhibiting reproduction and consuming larvae but not eating eggs or reducing adult growth and survival. Although larval *G. pusilla* avoided visual cues from *G. holbrooki*, native predators and conspecific adults, they did not avoid olfactory cues from *G. holbrooki*, which is maladaptive considering the predation risk. In addition, adult *G. pusilla* did not avoid any *G. holbrooki* cues, providing further evidence of maladaptive behavior.

Our study is one of the first comprehensive evaluations of how the threats of an invader to a native species, as well as the responses to this threat, are life-stage dependent. We use our empirical results to develop a general framework for understanding the mechanisms by which invasives threaten native biota, and highlight how this can be used to help assess and mitigate the threat of invaders.

1. Introduction

The introduction of species outside their natural range is a key threat to biodiversity, with introduced species linked to the declines and extinction of many species globally (Mollot et al., 2017). While preventing the spread of introduced species is important, identifying and evaluating the impacts of species already present outside their natural range is essential to minimising their damage to native wildlife.

While some native species benefit from introductions of non-native species (Rodriguez, 2006), many suffer reduced fitness due to competition (Human and Gordon, 1996), habitat modification (Byers et al., 2010), predation (Fritts and Rodda, 1998) and infectious diseases (Peeler et al., 2011). These interactions often reduce survival or growth, but can also impact breeding (Gribben and Wright, 2006). Some species can adapt to these threats, for example, *Daphnia* grow bigger to escape predation from invasive invertebrates (Gillis and Walsh, 2017). However, failing to respond adaptively can lead to extinction (Strauss et al., 2006).

Native species will be more impacted if they do not perceive introduced species as a threat (Polo-Cavia and Gomez-Mestre, 2014), and the lack of a shared evolutionary history can mean native species are less likely to behave adaptively. While some species evolve appropriate responses given a sufficient period of co-existence, the failure to do so can lead to an evolutionary trap (Schlaepfer et al., 2005), when the environment is rapidly altered, and formerly reliable cues are no longer associated with adaptive outcomes (Schlaepfer et al., 2002). The threatened amphibian, *Rana draytonii*, provides a clear example (D'Amore et al., 2009). Body size is the main cue used in mate selection, as females are the largest native frogs in California and size positively correlates with fecundity. However, the introduction of the larger *Rana catesbeiana* has rapidly altered the size distribution of frogs so choosing mates based on size now often leads to interspecific amplexus and has become maladaptive. Maladaptive responses can increase extinction risk, and native species may also become trapped by cues used for

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threat detection or prey selection (Sih et al., 2010). Despite the importance of both fitness impacts and behavioral responses, surprisingly few studies have investigated both factors simultaneously (although see Gomez-Mestre and Diaz-Paniagua, 2011; Thawley and Langkilde, 2017).

The susceptibility of a native species to the impacts of invasive species may change across their life cycle (reviewed by Langkilde et al., 2017). For example, individuals may grow to a size where predation is not a threat (Ling and Willis, 2005). Conversely, as in the case of *Rana draytonii*, the presence of an introduced species might not be detrimental until the native species reaches sexual maturity. As adaptive behavior requires a level of avoidance relative to the risk (Sih et al., 2010), the appropriate behavioral response to an invasive species may also vary for different life stages of a native species. Due to the lack of evolutionary history, recognizing the threat of exotic species often relies on prior experience rather than an evolved innate response (Carthey and Blumstein, 2018), further demonstrating the potential for variation in susceptibility across the life cycle. Therefore, a comprehensive assessment of the threat of an invader requires evaluating the fitness costs and behavioral responses across all life-cycle stages of a native species. While some examples exist showing ontogenetic changes in behavior related to predation risk (e.g. Langkilde et al., 2017), research on the impacts of invasive species generally focuses on a single life stage and behavior is rarely considered (Fig. 1). This is particularly true for aquatic ecosystems where the potential for invasive species to cause evolutionary traps has been the focus of little work (Hale and Swearer, 2016); a key knowledge gap considering traps can compromise population persistence (Hale et al., 2015b).

We use a case study to explore whether the threat of an invader and behavioral responses by a native animal vary ontogenetically. Specifically, we studied the effects of the invasive mosquitofish (*Gambusia holbrooki*) on a small fish native to south-eastern Australia, the dwarf galaxias (*Galaxiella pusilla*). *Gambusia holbrooki* is highly aggressive, one of the most widespread invaders in freshwater systems, and has been linked to the decline of numerous native fish and amphibian species (Pyke, 2008). Causes of these declines may be egg and larval predation (Ivantsoff and Aarn, 1999; Komak and Crossland, 2000), competition (Becker et al., 2005), reproductive disruption (Rincón et al., 2002) or aggressive interactions such as fin-nipping. Some species have evolved appropriate behavioral responses (Keller and Brown, 2008) but *G. holbrooki* could create evolutionary traps for others (Hale et al., 2015a). *G. pusilla* are listed as ‘vulnerable’ on both the IUCN Red List and the *Australian Environment Protection and Biodiversity Conservation Act 1999*, with population declines mostly attributed to habitat loss and degradation, and *G. holbrooki* (Saddlier et al., 2010). The two species commonly co-occur (Coleman et al., 2017), but studies of their interactions are limited to two unpublished theses (T. Westbury, W. Koster unpublished data). Halting and reversing population declines requires a thorough understanding of how *G. holbrooki* impact *G. pusilla* and how this is influenced by their environment, behavior and life history.

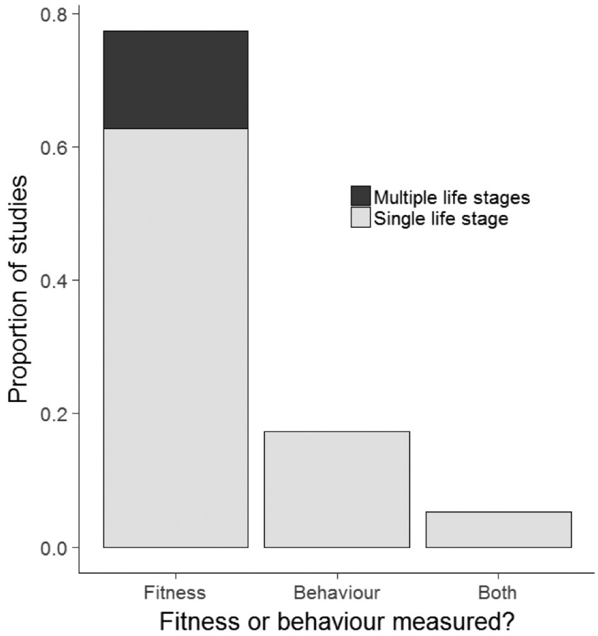

Fig. 1. The results of a review of all studies investigating the interactions between native species and introduced *Gambusia affinis* or *Gambusia holbrooki*. Combined, these species are the most widespread freshwater fish in the world and are among the planet's worst invaders (Pyke, 2008). We categorized studies according to whether they measured the impacts to the native species fitness only, behavioral responses only, or both fitness impacts and behavioral responses. Fitness impacts included reductions in growth, breeding and survival. Only 11 studies have assessed the fitness impacts across multiple life-cycle stages of a native species and none have looked at behavioral responses for more than one life cycle stage. See Supplementary material for further information.

We conducted laboratory and field-based experiments to ask: (1) what are the fitness consequences from *G. holbrooki* for *G. pusilla* eggs, larvae and adults; and (2) do *G. pusilla* larvae and adults behave adaptively when encountering *G. holbrooki*? If larval or adult *G. pusilla* suffer fitness impacts, but do not avoid *G. holbrooki*, this would be evidence for an evolutionary trap (Schlaepfer et al., 2002).

Based on impacts to other native species (Ivantsoff and Aarn, 1999; Komak and Crossland, 2000) and frequent observations of adults at sites where *G. holbrooki* are present (Coleman et al., 2017), we predicted heavy predation on eggs and larvae, but not on adult *G. pusilla*. However, we predicted *G. pusilla* may suffer sub-lethal effects (e.g. reduced growth or reproductive output) from stress, aggression or competition, as seen with similarly sized fish (Howe et al., 1997). Due to the lack of shared evolutionary history, we predicted both larvae and adult *G. pusilla* would fail to avoid *G. holbrooki*. However, as these species have coexisted for at least 70 years (McKay, 1984), *G. pusilla* (a predominately annual species: Coleman et al., 2017) may have evolved appropriate responses (e.g., the lizard *Podarcis pityusensis* acquired the ability to react to novel predators (snakes) within 10 generations; Ortega et al., 2016). *G. pusilla* could also have innate behaviors that reduce predation risk. As we found that larvae suffered high fitness costs (see Results), we also explored if they avoid native predators or adult conspecifics which are not cannibalistic. We also tested a third question: (3) are the fitness consequences for *G. pusilla* moderated by vegetation cover? Dense aquatic vegetation reduces the impact of *G. holbrooki* on some species (Baber and Babbitt, 2004), and could be a way for managers to mitigate the fitness costs on *G. pusilla* if it provides refuge from predators.

2. Material and methods

2.1. Animal collections and housing conditions

We collected *G. pusilla* eggs from O'Grady's wetland (OGR: 38°01′12″ S, 145°16′1″ E) in the south-eastern suburbs of Melbourne, Australia. Eggs were collected still attached to the macrophyte *Persicaria* on which they were exclusively found. For larval experiments we reared *G. pusilla* larvae to the age of 1–3 days, which were preflexion and moved by darting. Adult *G. pusilla*, *G. holbrooki*, as well as dragonfly larvae (Odonata) and diving beetles (Dytiscidae) that are predators of small fish (Gooderham and Tsyrlin, 2003), were collected from Centre Road Drain (CRD: 38°02′9″ S, 145°18′17″ E) approximately 3 km from OGR. All animals were housed in a temperature-controlled room (21 °C) with a 12 L:12 D light cycle, where rearing and experiments took place.