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Original Article

Male reproductive adjustments to an introduced nest predator

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Nest predation has a large impact on reproductive success in many taxa. Defending offspring from would-be predators can also be energetically and physiologically costly for parents. Thus, to maximize their reproductive payoffs, individuals should adjust their reproductive behaviors in relation to the presence of nest predators. However, effects of nest predator presence on parental behaviors across multiple reproductive contexts remain poorly understood, particularly in non-avian taxa. We ran a series of experiments to test how the presence of an egg predator, the invasive rockpool shrimp, *Palaemon elegans*, influences male reproductive decisions and egg survival in a species of fish with exclusive paternal care, the three-spined stickleback, *Gasterosteus aculeatus*. We found that, in the presence of shrimp, male sticklebacks were less likely to build a nest, invested less in territory defense against an intruder, and tended to fan eggs in their nest less and in shorter bouts, but did not alter their investment in courtship behavior. The predator's presence also did not affect egg survival rates, suggesting that males effectively defended their brood from the shrimp. These results show that reproducing individuals can be highly responsive to the presence of nest predators and adjust their behavioral decisions accordingly across a suite of reproductive contexts.

Key words: **courtship, egg survival, parental care, predation, reproductive plasticity, territorial aggression.**

INTRODUCTION

Many aspects of reproduction can be incredibly costly (Williams 1966; Trivers 1972; Stearns 1992). Not only does reproduction involve the expenditure of resources on gamete production and the physical act of copulation, but it is also often associated with energetically demanding behaviors, such as courtship or parental care (Stearns 1992). Moreover, resources that are available for mating, somatic expenditure, and parental effort may need to be traded against each other (Brown et al. 2004). Indeed, given the costs of reproduction and other life-history trade-offs, individuals are expected to adjust their investment in reproduction based on the expected reproductive payoff. For species with parental care, one particularly important factor that can alter the expected payoff from a reproductive event is the presence of nest predators.

Nest predation is a key factor that influences reproductive success in many species, including birds (Ricklefs 1969; Martin 1995), reptiles (Spencer 2002; Schwanz et al. 2010), and fish (Bailey 1989). Even an unsuccessful nest predation event may result in costs to the nest owner, such as energetically and physiologically demanding nest defense (Lazarus and Inglis 1978; Hinch and Collins 1991; Komdeur and Kats 1999; Steinhart et al. 2005). The abundance of potential nest predators often varies across relatively small spatial and temporal scales (King et al. 1998; Schauber et al. 2009), which can influence the expected reproductive returns for nesting individuals. Therefore, individuals are expected to accurately assess the current level of predation risk and make appropriate adjustments to their investment in reproductive behaviors (Dall et al. 2005). Many birds, for example, have been found to assess the presence of nest predators and adjust a range of behaviors, such as nesting site preferences (Forstmeier and Weiss 2004; Eggers et al. 2006; Fontaine and Martin 2006a; Peluc et al. 2008; Mönkkönen et al. 2009; Forsman et al. 2013) and parental care (Ghalambor and Martin 2002; Fontaine and Martin 2006b; Eggers et al. 2008; Emmering and Schmidt 2011; Ghalambor et al. 2013). However, adjustments of investment in other key reproductive behaviors, such as territory contests or courtship, remain less well understood. Furthermore, for most reproduction-related behaviors, including nest-site selection, the literature remains heavily focused on birds, with fewer studies on other nest-building taxa (Barber 2013).

We studied the ability of a nest-building fish, the three-spined stickleback, *Gasterosteus aculeatus*, to adjust investment across a variety of different reproductive contexts in response to the presence of nest predators. Males of this species exhibit a range of

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reproductive behaviors, including competition for mating territories through aggressive interactions with rivals, construction of a nest in their territory out of vegetation, elaborate courtship displays, fanning with pectoral fins to provide oxygen-rich water to the developing eggs, and defense of the nest from potential egg predators (Van Iersel 1953; Wootton 1976; Reebs et al. 1984; Östlund-Nilsson et al. 2006). Although these behaviors directly increase the male's reproductive success, they also involve considerable energy expenditure and may expose the male to predation or other physical harm (Stanley and Wootton 1986; Chellappa et al. 1989; Chellappa and Huntingford 1989; Wootton 1994; Smith and Wootton 1999).

The current study investigated whether male three-spined sticklebacks will adjust their investment in reproductive behaviors in the presence of the rockpool shrimp, *Palaemon elegans*, an omnivore and opportunistic predator of small prey (Forster 1951; Persson et al. 2008), including fish eggs (Forster 1951; personal observations). Due to its foraging habits, this shrimp is a threat to fish eggs and small fry but not adult sticklebacks. In the Baltic Sea (where this study was conducted), it is non-native (Katajisto et al. 2013), although the local sticklebacks naturally co-occur with two other littoral shrimp species, the brown shrimp (also known as the sand shrimp), *Crangon crangon* (Candolin et al. 2016; Kuprijanov et al. 2017), an opportunistic egg predator (Oh et al. 2001; Lehtonen et al. 2013, 2018), and the Baltic prawn, *Palaemon adspersus* (Candolin et al. 2016; Kuprijanov et al. 2017).

In a series of controlled experiments, we examined whether male sticklebacks, in the presence of rockpool shrimp, altered their investment in the after key behaviors: nest building, territorial contests, courtship of females, and the fanning of developing eggs. Based on the expected payoffs, we predicted that males should respond to the presence of nest predators in their immediate surroundings by reducing their investment in the above reproductive behaviors.

MATERIALS AND METHODS

Collection and maintenance

Three-spined sticklebacks and rockpool shrimp were collected between 10 May and 14 July 2014 from multiple sites within 5 km of Tvärminne Zoological Station, which is situated on the Finnish coast of the Baltic Sea (59°50.7′N; 23°14.9′E). Animals were collected from shallow water (depth: 0.2–0.8 m) using minnow traps, plexiglass traps, and seine nets, and transported back to the station on a boat in 40 l coolers, at a maximum density of 50 individuals per cooler. Sticklebacks were sorted by sex using nuptial coloration (blue eyes, red throats for males, and dark stripes for females; Van Iersel 1953; Rowland et al. 1991) and gravidity (females have a distended abdomen when ripe with eggs). Male sticklebacks, female sticklebacks, and shrimp were held in separate 60 cm × 60 cm × 60 cm holding tanks with artificial plants for enrichment and shelter, at a maximum density of 100 individuals per tank. Fish in holding tanks were fed live opossum shrimp, *Neomysis integer*, and thawed chironomid larvae ad libitum. During experiments (see below), male sticklebacks were fed 5 chironomid larvae per day, except where otherwise noted. Rockpool shrimp subsisted mostly on algae and detritus, with stickleback eggs added to supplement their diet.

Experiment 1: nest building

To investigate the effect of the presence of rockpool shrimp on stickleback nest-building behavior, we placed focal male sticklebacks showing nuptial coloration into individual 22 cm × 26 cm × 30 cm tanks. Each male was given a shallow nesting dish of 11 cm diameter, filled with sand to act as a nest substrate, and an excess amount (7 g wet mass, accuracy: ±0.1 g) of filamentous green algae, *Cladophora glomerata*, as material for nest construction (Candolin 1997). This nesting dish was placed at the front of the tank, with an artificial plant at the back of the tank for enrichment and shelter. We then randomly allocated each male (and hence tank) to either "shrimp" or "no shrimp" treatment (n = 29 for each treatment), with five rockpool shrimp introduced to the tanks in the shrimp treatment.

To stimulate nest building, we exposed each male for a 10 min period every day to a gravid female in a transparent jar (volume: ~500 mL) with a mesh lid to allow the transmission of olfactory cues. Olfaction has previously been shown to be important in stickleback mating (Reusch et al. 2001; Mclennan 2003; Häberli and Aeschlimann 2004; Heuschele and Candolin 2007). We checked all tanks every 12 h to determine whether the male had built a nest. After nest construction, males were weighed to the nearest 0.01 g on an electronic balance and measured with a ruler to the nearest 1 mm. We also measured the width of the nest entrance at its widest point (to the nearest 0.5 mm), before carefully removing the nest from the dish, drying the nest in the sun for 2 weeks, and then weighing the dried nest on an electronic balance (Candolin and Salesto 2006). Males were given 7 days to complete their nest, with previous studies (Candolin and Salesto 2006; Wong et al. 2012; Tuomainen and Candolin 2013) indicating that males that do not construct a nest during this time are unlikely to build a nest at all. If a male did not construct a nest during this time (n = 12) or nest data were lost (n = 2), the male was weighed and measured but no nest measures were available for further analyses (see below).

Experiment 2: territory defense

We also investigated whether rockpool shrimp affected the investment of male sticklebacks in territory defense against conspecifics. This experiment had a repeated measures design with two trials. In the first trial, we placed males that showed breeding coloration into individual 22 cm × 26 cm × 30 cm tanks. Each tank had a nesting dish (as per experiment 1) in the front half of the tank, and an artificial plant at the back. On construction of a nest, we removed the plant (to enhance visibility in the tank), and randomly allocated the males to either a "shrimp" or "no shrimp" treatment (n = 15 males for each treatment), with 5 rockpool shrimp added to each tank in the former. After 24 (±2) h, we placed a circular mirror (15 cm diameter, non-magnifying) into the tank for ten minutes, with the mirror positioned against the wall of the aquarium that was farthest from the nest and facing towards the nest so that the reflection of the focal male acted as a simulated territorial intrusion by a rival male. Previous research, including in sticklebacks, have used a variety of approaches to study aggression in fish, from the use of live stimuli to artificial (dummy) models (reviewed in Rowland 1999). After careful consideration of the strengths and weaknesses of each approach (Rowland 1999; Balzarini et al. 2014) and the repeated measures design of our experiment, we opted to use the mirror test, which has been successfully used to study aggression in sticklebacks for over 70 years (for example, Tinbergen 1951). In particular, the use of a mirror as a stimulus allowed us to match the resource holding potential and motivation of the intruder to the level of the focal male, which is relevant because these factors are known to affect investment in territorial conflicts (Barlow et al. 1986; Lindström 1992). We then recorded the time that elapsed

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until the male performed the first bite, as well as the number and rate of bites (after initiation of biting), towards the mirror. Bites are well established as a measure of territorial aggression in sticklebacks (Wootton 1971; Huntingford 1977; Rowland and Bolyard 2000).

After the completion of the first trial, each male was subjected to the other treatment (that is, we added 5 shrimp to the "no shrimp" tanks and removed the shrimp from the tanks that had been assigned to the "shrimp" treatment in the first trial). After another 24 h, we once again presented the mirror for ten minutes and recorded the bites towards it. This second trial followed the same protocol as the first one. On completion of the second trial, we removed males from their tanks, and weighed and measured them.

Experiment 3: courtship

To determine whether the presence of rockpool shrimp altered the investment of male stickleback in courtship, we employed another experiment with a repeated measures design. Here, we placed each focal male showing breeding coloration into an individual 34 cm × 38 cm × 30 cm tank, with a nesting dish at the front left of the tank, and an artificial plant at the back right. On nest construction, we randomly allocated each male to either a shrimp or no shrimp treatment (n = 15 males per treatment), with 10 rockpool shrimp added to each shrimp treatment tank. Due to the larger tank set up required for this experiment, we used 10 shrimp to maintain similar shrimp densities in this and the first two experiments. After 24 (± 2) h, we placed a gravid female, inside a transparent tube (volume: ~500 mL) with perforations to allow the transmission of olfactory cues, in the front right of the tank. To allow the female to acclimate, we initially covered the tube with a larger, non-perforated, opaque tube for 5 min, after which it was removed, exposing the female to the male. After a 1 min interval to allow the male in the tank to detect the presence of the female, we recoded the behavior of the male for 5 min. Specifically, we recorded the number and type of courtship acts performed by the male. We categorized the courtship acts using the following three functional categories: courtship bites, displays (consisting of zig-zags and leads), and nest tending (consisting of fanning, creeping through the nest, and nest gluing). It was possible to define each courtship act as a discrete behavior with a clear start and endpoint. For example, rapid consecutive bites towards the female, with no clear break in between each bite, were recorded as one act. Most forms of courtship were relatively stereotyped for each individual and thus one act represented approximately the same amount of investment each time it was recorded. The one exception to this pattern was fanning, which continued for variable periods of time. To account for this, we counted the number of seconds the male spent fanning. After the 5 min exposure period, we removed the female and its tube.

We then subjected each male to the alternate treatment (from "shrimp" to "no shrimp" treatment and *vice versa*). After another 24 h (± 2) period, we placed another gravid female (size matched to the previous female within 2 mm) into the tank and again measured the male's courtship behavior, after the same protocol as in the first trial. After the completion of the second trial, we weighed and measured all males. Four males did not exhibit any courtship behavior during one or both trials and were therefore excluded from the analyses.

Experiment 4: parental care

To investigate the effect of the presence of rockpool shrimp on stickleback parental care, we placed focal male sticklebacks showing breeding coloration into 34 cm × 38 cm × 30 cm tanks, 1 male per tank. Each tank had a nesting dish in the front left corner, and an artificial plant in the back right. All tanks were checked twice a day for the construction of a nest. If the male constructed a nest outside the dish, we carefully lifted the nest by hand and relocated it onto the dish. We then checked the tank the next day to determine whether the male had accepted the nest relocation by having anchored the nest into the sand substrate of the dish.

Once the male had established a nest, we introduced a gravid female to the tank to spawn with the male. During this period, we checked the tanks every 30 min to determine whether spawning had occurred. A female that failed to spawn within 3 h was removed and replaced with a new gravid female. After spawning, we weighed and measured both the male and the female and then returned only the male into the tank. We left the eggs in the nest to harden (at least 2 h; Candolin et al. 2008) before taking the nest and eggs out of the tank. The eggs were carefully removed from the nest using forceps, rested on paper towel for 10 s to remove excess water, weighed, reinserted into the nest (*sensu* Candolin et al. 2008), and then immediately reintroduced into the tank. Consisted with previous findings (Candolin et al. 2008), all males readily re-accepted the return of their nest and eggs.

We gave all males the following day to make any repairs to their nests, and then randomly allocated each male to either the shrimp or no shrimp treatment (n = 23 males per treatment). Again, as with experiment 3, due to the larger tank set up required for this experiment, 10 rockpool shrimp were introduced to tanks in the shrimp treatment to control for any potential effects of predator density across the different experimental assays. We observed each tank for 10 min on the first, third, and fifth day after spawning, recording the number and duration of fanning bouts, as well as the number of attacks (bites and chases) towards shrimp. Males were fed 3 thawed chironomid larvae per day during this period. Two males in the shrimp treatment abandoned their nest within the first couple of days and were therefore excluded from the analysis.

On day 6 after spawning, we removed the eggs from the nest and re-weighed them (with one data point missing due to a human error), which allowed us to determine the extent of egg loss/survival during the parental care period.

The research detailed in this paper complies with all relevant State and Federal laws and was approved by the Biological Sciences Animal Ethics Committee of Monash University (BSCI/2014/05).

Statistical analyses

All statistical analyses were conducted using R 3.1.1 (R Development Core Team 2014). For each model, the presence of shrimp and any covariates (see below) were included as fixed effects, and the date that the replicate commenced was incorporated as a random effect (to account for temporal biases). For the experiments with a repeated measures design, we also incorporated the ID of the focal male as a random effect.

We used linear mixed models (LMMs) for the analyses of normally distributed data (nest entrance size and nest weight in experiment 1, the total number of bites and bite rate after the first bite of the mirror in experiment 2, and the change in egg mass and body mass in experiment 4). For the analyses of count data that were not normally distributed (number of courtship acts in experiment 3, seconds spent fanning in experiment 4), we used generalized linear mixed models (GLMMs) with a Poisson distribution and a logarithmic link function. Where necessary, to account for over-dispersion in the dataset, we included

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the observation (that is, trial ID) as a random effect. To analyze the probability of nest construction through time in experiment 1, and time until the first bite towards the mirror in experiment 2, we conducted a survival analysis using a Cox Proportional Hazards mixed-effects model.

We examined a range of potential covariates during model selection, including standard length, condition (the residual of the regression of weight by length for all sticklebacks tested in the experiment), time until the first bite (in the analysis of bite rate), and order (in the experiments with repeated measures). Model selection was accomplished by optimizing the Akaike Information Criteria (AIC), a penalized likelihood score estimating the amount of information lost by the model (Akaike 1974). When models differed by <2 units in their AIC scores, the more parsimonious model was chosen. All models were fit using residual maximum likelihood.

RESULTS

Experiment 1: nest construction

Males took more time to construct a nest in the presence of shrimp (Figure 1, Table 1), with 9 and 3 males not building a nest at all in the presence and absence of shrimp, respectively. However, shrimp had no significant effect on nest entrance width (shrimp treatment: 5.1 ± 0.2 mm [mean ± SE], n = 19; no shrimp treatment: 5.2 ± 0.2 mm, n = 24; LMM: t_{35} = −0.3852, P = 0.70) or the weight of the nest (shrimp: 1.30 ± 0.12 g, n = 20; no shrimp = 1.45 ± 0.11 g, n = 24; LMM: t_{36} = −1.077, P = 0.29).

Experiment 2: territory defense

The time it took for the male to start biting the mirror did not differ between the shrimp and no shrimp treatments (Cox Proportional Hazards mixed model, z = −0.229, P = 0.82, n = 30), with 19 out of 30 males biting within the 600 s observation period both in the presence and absence of shrimp, taking 167 ± 34 s and 149 ± 34 s, respectively, until the first bite.

Considering only males that directed bites towards the mirror during both trials (n = 17), the total number of bites towards the mirror had a marginally non-significant tendency to be lower in the presence of shrimp (LMM: t_{15} = −1.902, P = 0.077), with males starting to bite earlier exhibiting more bites (LMM: t_{15} = 2.279, P = 0.038). Bite rate after the first bite was lower in the presence of shrimp (LMM: t_{15} = -3.785, P = 0.002; Figure 2), with the biting rate over the remaining time being lower for males that commenced biting earlier (LMM: t_{15} = −4.094, P = 0.001).

Table 1

Cox Proportional Hazards mixed-effects model of the daily probability of nest construction, for stickleback in the presence or absence of shrimp (n = 29 males per treatment)

	Estimate	SE	z	P
Shrimp	−0.72	0.49	−2.25	0.025
Standard length	1.02	0.77	1.33	0.18
Condition	−7.85	4.11	−1.91	0.057

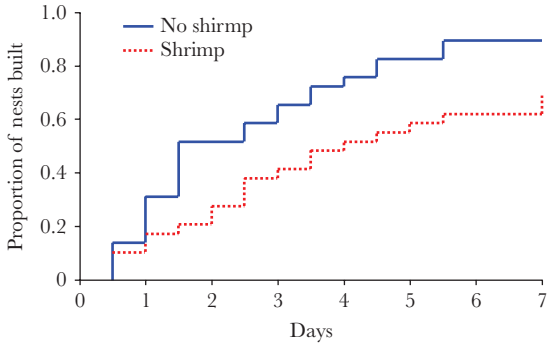

Figure 1
The progress of nest building in the presence (n = 29) and absence (n = 29) of shrimp.

Experiment 3: courtship

For males that courted in both trials (n = 26), the presence of shrimp did not affect the number of courtship acts directed towards females (GLMM: z = 0.63, P = 0.53; Figure 3). The number of courtship acts of each category was also unaffected by the presence of shrimp, though nest tending was positively related to the body condition of the male, and the number of zig-zags and lead displays increased in the second trial of the repeated measures design (Table 2).

Experiment 4: parental care

Male sticklebacks in the presence of shrimp averaged 11.8 ± 1.3 (n = 21) attacks (bites and chases) on shrimp over the 10 min

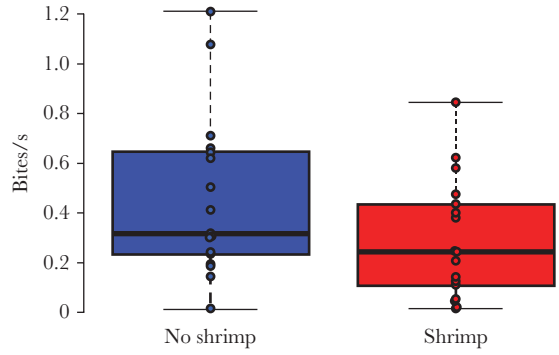

Figure 2
The rate of bites towards an intruder (a mirror) after initiation of the aggressive behavior (n = 17 males used in both treatments). Box plots show all quartiles and the circles indicate raw data points.

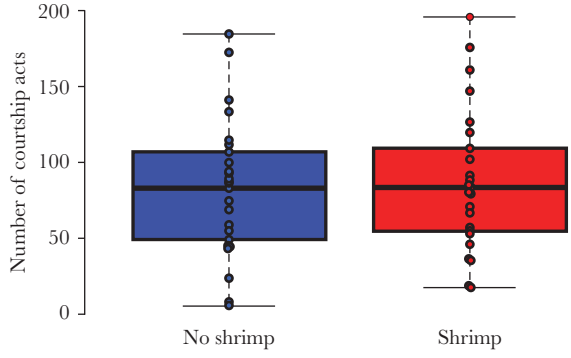

Figure 3
The number of acts of courtship in the presence and absence of shrimp (n = 26 males). Box plots show all quartiles and the circles indicate raw data points.

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Table 2

Generalized linear mixed models for the number of courtship acts in each category (courtship bites, displays, or nest tending), in the presence or absence of shrimp (n = 26 males)

Courtship type	Effect	Estimate	SE	z	P
Courtship bites	Shrimp	−0.02	0.19	−0.10	0.92
Displays	Shrimp	0.43	0.30	1.42	0.15
	Order	0.64	0.30	2.10	0.036
Nest tending	Shrimp	−0.49	0.53	−0.94	0.35
	Condition	3.22	1.43	2.21	0.028

observation period. There was a tendency for males to spend less time fanning their eggs in the shrimp (38 ± 7 s, n = 21) than no shrimp (62 ± 9 s, n = 23) treatment (GLMM: z= −1.911, P = 0.056). Similarly, the average fanning bout length had a marginally non-significant tendency to be lower in the presence (11.8 ± 1.3 s, n = 19) than the absence (16.7 ± 1.5 s, n = 23) of shrimp (LMM: t_{39} = −1.906, P = 0.064). There was no difference in the weight of egg mass lost during the experiment between the treatments (shrimp: 0.15 ±0.02 g, n = 20; no shrimp: 0.20 ± 0.05 g, n = 23 LMM: t_{41} = 0.902, P = 0.37).

DISCUSSION

This study found that the presence of rockpool shrimp, potential predators of stickleback eggs, altered the reproductive investment of parental male three-spined sticklebacks. Specifically, males were less likely to construct a nest, bit less vigorously at a simulated conspecific intruder, and tended to fan eggs in their nest less and with shorter bouts in the presence of shrimp. However, males were not found to adjust their level of courtship to the presence of shrimp.

This study is the first to show that the presence of potential nest predators can affect the probability of nest construction in fish. Specifically, we found that male sticklebacks were more reticent to build a nest when shrimp were present, which was likely compounded by the considerable energetic costs of nest construction (Stanley and Wootton 1986). Indeed, under such circumstances, males may be better off relocating or postponing their nest-building until conditions are more favorable (see Candolin and Salesto 2006). Many birds (Forstmeier and Weiss 2004; Fontaine and Martin 2006b; Mönkkönen et al. 2009; Emmering and Schmidt 2011; Parejo and Avilés 2011; Forsman et al. 2013), and turtles (Spencer 2002; Spencer and Thompson 2003), for example, preferably use nesting sites where the numbers of nest predators are lower, indicating that this is a taxonomically widespread response. Interestingly, however, previous studies on another species of fish, the sand goby, showed that males did not alter their probability of nest construction in the presence of either native (Lehtonen et al. 2013) or novel (Lehtonen et al. 2018) nest predators. Hence, there may be fundamental differences among different fish species in the benefits gained from reallocating resources needed in a breeding attempt to the growth and survival, or in their ability to adjust nest-building behavior, in response to predators. The underlying taxonomic, life-history, physiological, and environmental reasons for these differences warrant further investigation.

Recent studies have shown that the level of aggression displayed in territorial contests can be affected by a variety of external factors in a range of taxa (Elwood et al. 1998; Johansson et al. 2000; Gray et al. 2002; Killian and Allen 2008; Bergman et al. 2010), including sticklebacks (Bolyard and Rowland 1996; Candolin et al. 2008, 2014) and other fish with parental care (Lehtonen 2014; Lehtonen et al. 2015). However, as far as we are aware, the effect of nest predators on an individual's investment in territorial contests has not been previously investigated (but see Huntingford 1982 for the effect of would-be predators of adults). The results of this study provide moderate evidence that individuals are capable of adjusting their investment in territorial contests in relation to the presence of nest predators. In particular, the presence of shrimp did not affect how likely or quickly males responded to a territorial intruder, but those males that were responsive bit the intruder at a lower rate in the presence of shrimp. Given the aggressive nature of territorial interactions in this species (Van Iersel 1953; Rowland 1989), such adjustments in the intensity of aggression are likely to be influenced by energetic expenditure (Chellappa and Huntingford 1989), risk of physical injury (Dingle and Caldwell 1969; Palombit 1993; Drews 1996), or potential trade-offs between mating opportunities and parental effort (Stiver and Alonzo 2009). An individual should be willing to invest in territorial disputes only to the extent that their costs do not exceed the expected benefits of owning the territory (Parker and Rubenstein 1981; Hammerstein and Parker 1982). As the purpose of establishing a territory in male three-spined sticklebacks is to facilitate reproduction, a territory of lower reproductive value (for example, due to nest predators) should therefore impact the effort the male is willing to invest in competing for that territory. The lower biting rate by male sticklebacks in the presence of rockpool shrimp can therefore be an adaptive response to an increased risk of nest predation. Response rate towards conspecific intruders may also be lower if nest predators distract nest holders.

Given that stickleback males adjusted both their willingness to build a nest and intensity of attacks towards an intruder in relation to the presence of shrimp, their failure to adjust either the amount or type of courtship was unexpected. In particular, if the presence of nest predators will decrease the expected reproductive benefits of a territory, males could be expected to be less willing to invest in reproductive behaviors, such as courtship, when nest predators are around. The result is unlikely to be due to physiological restrictions on the plasticity of courtship because sticklebacks have demonstrated the ability to alter their level of courtship in response to a variety of other external factors (that is, rival males: Candolin 1997; Kim and Velando 2014, predation risk: Candolin 1997; Candolin and Voigt 1998, the recent loss of eggs from the nest: Deal et al. 2016, and environmental conditions: Candolin et al. 2007). Why, then, did the presence of egg predators not affect courtship in the current study?

One possibility is that the expected benefits of courtship considerably outweigh the costs, even when shrimp are present, especially after males have already spent energy in building and maintaining their nest (Stanley and Wootton 1986). The energetic costs of courtship in stickleback have not been explicitly documented, and although courtship behaviors appear vigorous, they are also quite short in duration. It is possible, therefore, that even when the expected reproductive benefits are reduced (as in the presence of shrimp), they may still be greater than the energetic costs of courtship. Moreover, males did not lose significant numbers of eggs, suggesting that the presence of a manageable risk of egg predation may not have warranted forgoing potential opportunities to reproduce. Finally, given that male sticklebacks have the option of cannibalizing some or all of their eggs to recoup their energetic investment (Rohwer, 1978), the benefits of investing heavily in courtship may be high regardless of

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the presence of egg predators. However, the conspicuous nature of stickleback courtship is thought to also increase the susceptibility of males to predation (Candolin and Voigt 1998; Johnson and Candolin 2017), meaning that the costs of courtship are influenced by predation risk. Hence, in high risk environments, sticklebacks may need to be more selective with their level of courtship. Therefore, the level of courtship adjustments to a decrease in the expected reproductive payoff, under a risk of predation to the courting male, provides an interesting avenue for further research.

Male sticklebacks had a tendency to fan less in the presence of shrimp. This result is similar to the findings of Stein and Bell (2019), who investigated the parental care of male stickleback in the presence of *Aeshna* dragonfly naiads, which, like shrimp, pose a threat to stickleback young. In that study, males also adjusted fanning behavior in the presence of a predator but the strength of this adjustment varied among stickleback populations depending on the history of coexistence with the predator. The results of our study suggest that there may also be a trade-off between fanning and nest defense in three-spined sticklebacks, as males tended to fan in the presence of shrimp in shorter bouts and the overall fanning time was ~40% lower in the presence of shrimp, albeit these differences were marginally non-significant. Shorter fanning bouts should enable males to check their territory more frequently for nest predators. Such trade-offs between parental care and nest defense have been observed in, for instance, orange-tufted sunbirds, *Cinnyris bouvieri*, with individuals that provision the nest more being less capable of defending it (Markman et al. 1995).

In contrast to the studies that physically restricted the nest predators (for example, using perspex partitions: Smith and Wootton 1995 or tethers: Lissåker and Kvarnemo 2006), this study used unrestrained nest predators. This approach allowed us to examine whether any re-allocation of time or resources between fanning to nest defense were sufficient to prevent nest predation. Given that there was no difference in egg loss between the treatments, we can conclude that males in this study were successful in preventing significant levels of nest predation. This result is concordant with those reported in sand gobies, in which males that did not lose possession of their nest were able to prevent significant egg losses in the presence of both native and novel egg predators (Lehtonen et al. 2018). Reduced fanning time and bout length, however, may still have consequences for egg development. For instance, eggs may take longer to hatch, and therefore delay the ability of the male to remate. Given the limited length of the stickleback reproductive season, an increase in the time until re-nesting can result in lost opportunities for further breeding, ultimately resulting in decreased reproductive output.

The rockpool shrimp was first reported in our study area 11 years before this work was conducted (Katajisto et al. 2013) and has since become locally abundant (Katajisto et al. 2013; Candolin et al. 2016, 2018). Previous findings in another fish with parental care, the arrow cichlid, *Amphilophus zaliosus*, suggest that parents may engage in less adaptive responses towards novel, compared with native, nest predators (Lehtonen et al. 2012). However, in contrast to that study, we found that sticklebacks were still able to respond, presumably adaptively, by adjusting their behavior to the presence of rockpool shrimp. One possible reason for this is that sticklebacks in our population naturally co-occur with two other littoral shrimp species, the brown shrimp and the Baltic prawn, and may be able to generalize across different shrimp predators (Stein and Bell 2019). Sticklebacks and rockpool shrimp may also have interacted during their evolutionary past, with the Baltic Sea habitat having existed only for less than 10 000 years (Björck 1995).

Overall, the findings of this study support the prediction that a decrease in reproductive payoffs can lead to a decrease in a male's investment in some reproductive behaviors (Carlisle 1982; Stearns 1992). In particular, we found that males were less willing to invest in both nest building and the intensity of territorial conflict–but not in the level of courtship–in the presence of shrimp. Furthermore, the tendency for a shift in egg fanning behavior in the presence of shrimp is likely to prevent egg predation, but may come at a cost, such as an increased time until hatching. This study, therefore, contributes to an important body of evidence that individuals adjust investment in reproductive behaviors in the presence of nest predators. The findings include the first evidence of nest predator-induced alteration of investment in territory defense, as well as the first evidence in fish for a reduced probability of nest construction.

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Data availability: Analyses reported in this article can be reproduced using the data provided by Gravolin et al. (2021).

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