



# The Influence of Parental Status on Courtship Effort in a Paternal Caring Fish

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## Abstract

It is widely assumed that caring for young limits the motivation of parents to seek additional mating opportunities. However, in situations where parental care does not involve direct provisioning of the offspring, but rather activities directed at the brood as a whole (e.g. guarding), it may be more efficient for parents to care for large numbers of young at once. This may be especially true for species with exclusive paternal care, with fathers that have recently acquired a brood of young potentially benefiting from vigorously courting prospective mates, so as to maximise their chances of attaining a large number of young to rear together. We experimentally tested this hypothesis in the three-spined stickleback (*Gasterosteus aculeatus*), a fish with male only care. Contrary to our predictions, we found no evidence of any differences in courtship between recently spawned egg-tending fathers and males that had not spawned. However, males that were permitted to spawn, but then had their eggs taken from them, courted less vigorously. Together, the results of our study suggest that the potential benefits of vigorous courtship in terms of acquiring additional young may be offset by additional costs faced by parental males.

## Introduction

In species with paternal care, both courting mates and caring for offspring can be time-consuming and energetically demanding. Such temporal and energetic limitations, in turn, can result in conflict between mating and parental effort (Magrath & Komdeur 2003). For example, in a bird, the Temminck's stint (*Calidris temminckii*), males that opt to incubate their eggs miss out on further mating opportunities (Thomson et al. 2014) whilst, in many other species, parents that are given the chance to pursue additional mates provide less care to their young (Magrath & Elgar 1997; Bjelvenmark & Forsgren 2003; Bonnevier et al. 2003; Symons et al. 2011) or even abandon them altogether (Keenleyside 1983; Townshend & Wootton 1985). However, recently, it has been highlighted that courtship and parental activities are not always incompatible with one another (Tallamy 2000, 2001; Stiver & Alonzo 2009). For instance, in species where parents perform courtship and rear offspring in the

same location, parents may be able to engage in courtship and care simultaneously (Stiver & Alonzo 2009). Moreover, even where parental and mating effort draw on the same limited resources, individuals may still opt to invest in both of these aspects of life history at the same time and sacrifice investment in other components of life history, such as somatic investment, in order to do so (Magrath & Komdeur 2003).

To date, the majority of research investigating the association between parental care and courtship effort has focused principally on how courtship of potential mates influences the quality of care individuals provide (Magrath & Elgar 1997; Bjelvenmark & Forsgren 2003; Bonnevier et al. 2003; Pampoulie et al. 2004; Symons et al. 2011). Less well understood, however, is how parental care might influence prevailing courtship levels (but see: Kraak & Groothuis 1994; Green et al. 1995; Pitcher & Stutchbury 2000). Here, the nature of parental care within a species is likely to be important. Where offspring within a brood compete for resources provided by their parent, there may be little benefit to

an individual parent in attracting new mates prior to the independence of existing offspring because intra-brood competition may cause parental care costs to scale with the number of offspring being cared for (cf. Ahnesjö 1996). By contrast, where parental care is non-depreciable (i.e. where the cost of rearing a brood is independent from the number of young in the brood; *sensu* Altmann et al. 1977), or where only modest increases in the cost of care arise from adding young to the brood, it may actually benefit individuals to rear multiple batches of young contemporaneously as this reduces the per capita cost of offspring care (see Smith 1992; Smith & Wootton 1995a). For males, in particular, which generally cannot directly control the number of young produced by a single mating, courting and mating with additional females after the acquisition of an initial clutch of young may represent the most effective strategy to increase the number of young in their brood, and thus capitalise on such economies of scale in parental care. Indeed, intense courtship could be employed by parental males in these circumstances, so that they can acquire additional clutches as soon as possible after the initial spawning, so as to maximise the overlap in the brooding period of existing and potential new clutches. Further to this, given evidence that females may even prefer males that are engaged in parental care (e.g. Thomas & Manica 2005; Lindström et al. 2006; Manica 2010; Nazareth & Machado 2010; also reviewed in: Jamieson 1995; Reynolds & Jones 1999), there may be additional incentive for parental males to court in order to capitalise on their enhanced attractiveness. Alternatively, however, it is also possible that parental males may reduce their courtship effort as the elevated attractiveness afforded by their parental status means that intense courtship is no longer necessary to outcompete rivals in mating competition.

Here, we set out to empirically test the influence of parental status on the courtship of male three-spined sticklebacks (*Gasterosteus aculeatus*). During the breeding season, male sticklebacks compete for territories and construct nests from sediment and plant material (Östlund-Nilsson 2006). Males then attract passing females to their nest using elaborate zigzag courtship displays (Wootton 1976; Rowland 1994). If a male is successful in his efforts, the female will deposit her eggs within his nest. The male then becomes the sole care provider of the eggs, defending them from potential predators as well as fanning them with his pectoral and caudal fins to provide ventilation (van Iersel 1953; Whoriskey & Fitzgerald 1994; Östlund-Nilsson 2006). Male sticklebacks are capable of caring for the young of multiple females at once (Kraak et al. 1999a,b), and it seems likely that the cost of guarding

multiple clutches of young is not substantially greater than that of defending a single clutch (Perrone & Zaret 1979; Lazarus & Inglis 1986; Smith & Wootton 1995a). Moreover, although competition for dissolved oxygen may be increased when males rear multiple clutches simultaneously (van Iersel 1953; Reeb et al. 1984; Coleman & Fischer 1991; Bakker et al. 2006; see also: Perrin 1995), it appears that males can compensate for this with modest changes in fanning behaviour and loss of energetic resources relative to the total cost of brood care (van Iersel 1953; van den Assem 1967; Sargent & Gebler 1980; Coleman & Fischer 1991). Taken together, this suggests that it is likely to be more efficient for males to rear multiple clutches simultaneously rather than sequentially in this species. Therefore, we predict that male sticklebacks that have recently acquired an initial clutch of eggs will court more vigorously than non-parental males, as intense courtship may help these recently spawned egg-tending males quickly acquire additional clutches that they can then rear alongside their existing eggs, thereby increasing the reproductive pay-offs for providing care with minimal additional parental investment (cf. Jamieson & Colgan 1989; Jamieson et al. 1992). To date, few studies have directly compared measures of mating effort between parental and non-parental males, and among the few that have the previous breeding experience of non-parental males did not always match that of parental individuals (Jamieson & Colgan 1989; Goldschmidt et al. 1993). Consequently, the effect of parental status may potentially be influenced by differences in prior breeding experience. Thus, to enable us to disentangle these two phenomena, we compared the courtship behaviour of parental male sticklebacks with both non-parental males that had been given the opportunity to court but not spawn with a female as well as non-parental males that had been permitted to spawn but subsequently had their eggs removed.

## Methods

This study was approved by the Monash University Biological Sciences Animal Ethics Committee (permit BSCI/2014/15) and complies with all the relevant laws of Finland and Australia for research involving live vertebrates.

## Collection and Pre-experimental Housing

We collected three-spined sticklebacks from the littoral zone of the Baltic Sea near the Tvärminne Zoological Station (59°50.7'N, 23°15.0'E) using minnow

traps between May and July 2014. Fish were transported back to the station in 50L coolers, segregated by sex based on hints of nuptial coloration (males have blue eyes and red throats) and housed in 200L stock aquaria (~50 fish per tank) until their use in experiments. These stock aquaria, as well as experimental aquaria, were situated in an outdoor aquarium facility exposed to ambient outdoor light (~18.5-h light per day) and temperature conditions (measured mean = 13°C, range = 10–19°C) and provided with continuous flow-through of sea water. Fish in the stock aquaria were fed daily on chironomid larvae and *Neomysis* shrimp *ad libitum*.

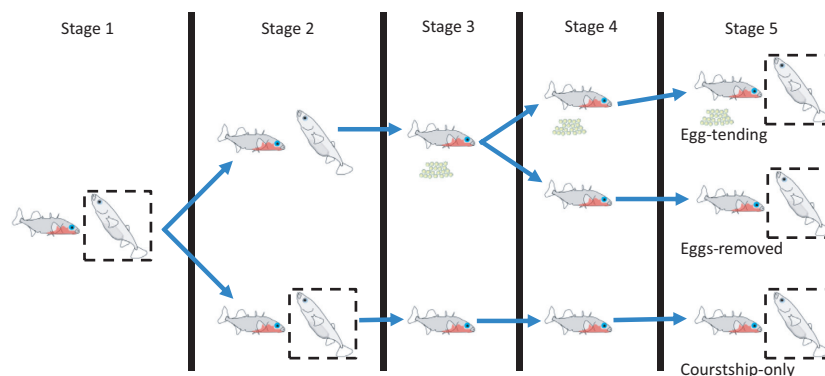
### Nest Building

We began each replicate by removing a single male from the stock tank, measuring his standard length and weight, and then placing him alone in an experimental aquarium (40 × 40 × 40 cm). Each aquarium was supplied with a (14 cm diameter) round dish filled with sand and 7 ± 0.1 g of filamentous green algae (*Cladophora*) to enable the male to construct a nest (Candolin 2000b). We checked experimental aquaria daily for a completed nest, which we identified by the appearance of a distinct tunnel with an exit (van Iersel 1953). Any males that had not constructed a nest after 3 days were excluded from the experiment and returned to the sea. During the nest-building phase, and for the rest of experimentation, individual males were fed three chironomid larvae

per day, which is sufficient for males to complete multiple consecutive parental cycles (Candolin 2000a).

### Baseline Courtship Assay

To allow us to obtain a baseline measure of the courtship intensity of individual male sticklebacks, we assayed the courtship effort of each male the day after it had completed nest construction. To assay male courtship, we exposed each male to a ready-to-spawn (as indicated by her distended abdomen: Wootton 1984) female (standard length: 58 ± 4 mm [ $\bar{x}$  ± SD]; mass: 2.8 ± 0.6 g) selected from a stock tank (Fig. 1: Stage 1). The female was held inside a (10 × 7.5 × 40 cm) clear acrylic container positioned inside the corner of the experimental aquarium during this assay. This container was perforated with tiny holes and filled with sea water to a depth slightly greater than the water level of the experimental aquarium. As a result of this water flow was primarily from the container holding the female to the experimental aquarium with the male, encouraging the male to receive female olfactory cues whilst reducing the amount of olfactory cues reaching the female. After the female was introduced into her holding container, we allowed the female five min to acclimate. During this time, visual contact between the sexes was prevented by placing an opaque acrylic barrier around the female container. Immediately following this acclimation period, we removed the opaque barrier, thereby permitting visual contact between the



**Fig. 1:** Schematic of the experimental procedure. In stage 1, male sticklebacks that had constructed a nest were given the opportunity to court a ready-to-spawn female that was held within a clear, perforated receptacle. A baseline measure of male courtship effort was recorded during this initial assay. In stage 2, males were either permitted to continue courting the female whilst she remained in the receptacle or the female was released from the receptacle thereby making spawning possible. Following this, males in stage 3 had either acquired eggs as a result of spawning or had no eggs as a result of not being given the opportunity to spawn. In stage 4, half of the males that were spawned had their eggs experimentally removed, creating three treatments: 'egg-tending', 'eggs-removed' and 'courtship-only' males. Finally, in stage 5, males in each of the treatments were permitted to court a new female, enabling their courtship to be assayed (not shown is the fact that this assay was conducted twice with a separate female each time). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

male and female. We then observed the behaviour of the male for 10 min quantifying his courtship behaviour by counting the number of zigzag dances performed by the male. A zigzag dance involves rapid side-to-side movements of the male whilst approaching the female (for a detailed description see: Wootton 1976, 1984 and references within). Counts of zigzag dances were chosen as the measure of courtship effort, as they were the most clearly discernible and frequently performed courtship behaviour (see also: Sevenster-Bol 1963; van Iersel 1953) and are also repeatable (Dzieweczynski & Forrette 2015). Moreover, there is evidence to suggest that males that engage in greater numbers of zigzag dances are more successful at attracting females to mate (von Hippel 2000; but see: Rowland 1995) and engaging in more rapid zigzag dances may augment the effectiveness of other attractive male traits too (Künzler & Bakker 2001).

### Manipulating Male Parental Status

At the end of the baseline courtship assay, we randomly assigned each male to a treatment group (Fig. 1: Stages 2–4). For two of these treatments ('egg-tending' and 'eggs-removed'; see description below), males were permitted to spawn with the female that was used as the stimulus during their baseline courtship assay. After releasing the female to spawn, each experimental aquarium was checked over the course of the next 24 h, with the female removed after spawning had occurred. Following removal of the female, we then waited for the clutch of eggs to harden within the nest (Swarup 1958; Kraak & Bakker 1998). For males in the 'eggs-removed' treatment, we carefully removed the entire clutch from the nest with a pair of tweezers on the same night that spawning had occurred. Males in the 'egg-tending' group were allowed to retain their eggs, but to ensure that the disturbance of the nest was similar across treatments, we also disturbed their nest with tweezers without ultimately removing eggs.

For the third treatment of this experiment, referred to as 'courtship-only', we did not allow the males to spawn. Instead, the stimulus female from the baseline courtship assay remained confined to the container so that the male could continue courting her. We removed the female after a period of time that was comparable to the time that males in the other treatments were in contact with a female. The mean time females were left with males across treatments was  $9 \pm 6$  h [ $\bar{x} \pm \text{SD}$ ], with no significant difference between treatment groups (single-factor ANOVA:

$F_{2,71} = 1.09$ ,  $p = 0.341$ ). As with males in the other two treatments, 'courtship-only' males also had their nests disturbed with tweezers.

For the experiment, a larger proportion of males ( $n = 65$  in total) were assigned to the treatments that required spawning (i.e. 'egg-tending' and 'eggs-removed') to account for some males failing to spawn within the 24-hour period given. Males that did not spawn ( $n = 17$ ) were excluded from our main data analysis, with no further testing carried out on them. To rule out the possibility that exclusion of these males caused among treatment differences in courtship motivation, we tested (using a negative binomial generalised linear model) whether the baseline courtship level of males was related to treatment group, including those males that failed to spawn as a fourth treatment. Here, we found no evidence that the treatment group was related to the number of zigzag dances performed by males in the baseline assay (likelihood ratio test:  $\chi^2_{3,86} = 2.864$ ,  $p = 0.413$ ), suggesting that sampling bias was not an issue.

The final sample sizes for the 'egg-tending', 'eggs-removed' and 'courtship-only' treatment groups were 24, 24 and 26, respectively. The standard length of the males was  $53 \pm 5$  mm [ $\bar{x} \pm \text{SD}$ ], and their mass was  $2.0 \pm 0.6$  g, with ANOVA revealing no significant difference in size among treatments (standard length:  $F_{2,71} = 1.511$ ,  $p = 0.2278$ ; mass:  $F_{2,71} = 0.984$ ,  $p = 0.379$ ).

### Male Courtship Post-manipulation

Following manipulation of male parental status, we quantified the courtship effort of males by exposing them to additional ready-to-spawn females presented inside a clear container (Fig. 1: stage 5). These assays took place on the day following the baseline courtship assay  $18 \pm 5$  h [ $\bar{x} \pm \text{SD}$ ] (range: 6–31 h) after manipulation of male parental status, with no difference in commencement time between treatments (single-factor ANOVA:  $F_{2,71} = 0.521$ ,  $p = 0.596$ ). This timeframe was chosen as it fits within a critical window whereby it is long enough after the spawning of males (where this occurred) that males have recovered their courtship drive (van Iersel 1953; Wootton 1976) whilst still being close enough to the time of the initial spawning that the putative benefits of concurrent clutch rearing should be large for 'egg-tending' males.

The procedure for acclimating and exposing the females was identical to that used for the baseline courtship assay, with male zigzag displays quantified over a 10-min exposure period. To obtain a precise estimate of each male's propensity to court that is

minimally biased by differences in stimulus females, we tested each male twice, using a different female each time presented one after another (female standard length:  $58 \pm 5$  mm [ $\bar{x} \pm$  SD]; mass:  $2.7 \pm 0.7$  g), with a 5-min break in between. As with females that were used for the baseline assays, each female was used only once, with no difference in female standard length (single-factor ANOVA:  $F_{2,145} = 1.09$ ,  $p = 0.340$ ) or mass (single-factor ANOVA:  $F_{2,145} = 1.38$ ,  $p = 0.256$ ) between treatments.

### Statistical Analysis

To investigate whether males in each of the three treatment groups differed in their motivation to court following the manipulation of their parental status, we used R (R Core Team 2016) to analyse the number of zigzag dances performed by males during these post-manipulation courtship assays with generalised linear mixed models (GLMM(s)). We used fixed effects for treatment assignment ('egg-tending', 'eggs-removed' or 'courtship-only'), and, as the courtship of individual males was tested twice following manipulation of parental status, we included male ID as a random effect in our models (Crawley 2005). Additionally, to control for variation in the courtship vigour of males that was unrelated to treatment, a measure of male performance in the baseline courtship assay was also used as a covariate. Specifically, here we took the natural logarithm of the sum 1 + the number of zigzag dances observed in the baseline courtship assay for each male and then standardised the result for our covariate measure. The log transform was chosen here to maintain direct proportionality between the number of zigzag dances observed in the baseline assay and the number of zigzag dances predicted by the model. However, as some males did not engage in zigzag dances in the baseline courtship assay, it was necessary to add one to each baseline assay zigzag dance count as a pragmatic solution to potential undefined values, which does not require estimation of additional parameters.

We initially modelled the data using a Poisson GLMM (with log link function). However, as this proved to be overdispersed, a negative binomial GLMM was employed (which also used a log link function). In the light of the controversy over how best to conduct hypothesis testing for GLMMs using Wald tests or likelihood ratio tests under a frequentist framework, we fitted the model using a Bayesian approach (Bolker et al. 2009). Here, the posterior distributions of the model parameters were estimated

using Markov Chain Monte Carlo simulation via the R interface to JAGS (version 3.4.0) (Plummer 2003) in the R2jags package (version 0.04-03) (Su & Yajima 2014). Every 100th value from three Markov chains of 1 000 000 iterations in length was used, with the first 100 000 iterations of each chain discarded as burn-in. The chains were visually assessed for convergence, and potential scale reduction factor values were all less than 1.002 (Brooks & Gelman 1998). Following Zuur et al. (2012), vague priors were used for all parameters, with priors for the mean and fixed effects all being normal distributions centred on 0 with variance 100 000, and the prior for the standard deviation of the male ID effect was a uniform distribution from 1 to 10 000. The negative binomial dispersion parameter prior was a uniform distribution from 0.5 to 5. Inferences were drawn from 95% credibility intervals from the Bayesian analysis, where credibility intervals that do not overlap zero are considered as significant. We also note that fitting the same model using frequentist methods (via the R package glmmADMB (version 0.8.1); Fournier et al. 2012; Skaug et al. 2015) and testing null hypotheses using Wald Z tests (with significant level of  $\alpha = 0.05$ ) produced qualitatively similar results which we do not present here.

### Results

Males in the 'eggs-removed' treatment engaged in significantly fewer zigzag dances after manipulation of their parental status than those in the 'egg-tending' (Table 1, Fig. 2) and 'courtship-only' treatments (Table 1, Fig. 2). By contrast, there was no significant difference in the number of zigzag dances between the 'egg-tending' and 'courtship-only' males (Table 1 and Fig. 2). The covariate, which was based on the number of zigzag dances performed in the baseline courtship assay, was also significant (Table 1), with more vigorously courting males in the baseline assay also courting more after manipulation of their parental status (Table 1).

### Discussion

We did not find evidence of any difference in the courtship levels of males that were tending eggs and males that had been given the opportunity to court a female but not acquire eggs. This finding was surprising as we had expected male sticklebacks to elevate their courtship effort when they had obtained an initial clutch of eggs, as it is likely to be more cost-effective for males to rear multiple clutches

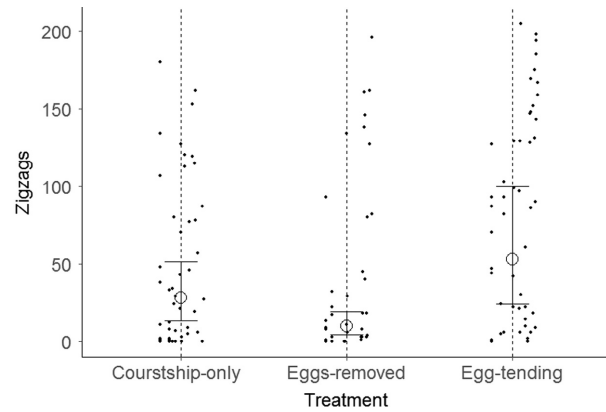
**Table 1:** Parameter estimates from a negative binomial GLMM of male zigzag dances performed after parental status manipulation. For the effects of male parental status treatments, estimated treatment–treatment differences are presented, as these are the quantities about which we wish to draw inferences.  $\beta$  represents fixed effect coefficients,  $\sigma$  represents the estimated value of standard deviations and  $\alpha$  represents the dispersion parameter where the variance of a negative binomial distribution with mean  $\mu$  is  $\mu + \alpha\mu^2$ .

		$\beta$	95% Credibility interval	Incidence rate ratio
Fixed effects	Courtship-only – Eggs-removed	1.052	0.082–2.065	2.86
	Egg-tending – Eggs-removed	1.675	0.678–2.712	5.34
	Egg-tending – Courtship-only <sup>a</sup>	0.623	–0.366–1.607	1.86
	Baseline courtship assay covariate	0.911	0.499–1.332	2.49
		$\sigma$	95% Credibility interval	Incidence rate ratio
Random effects	Male ID	1.469	1.043–1.920	4.35
		$\alpha$	95% Credibility Interval	
Negative binomial dispersion parameter		1.034	0.733–1.546	

<sup>a</sup>Note that this table presents estimates for all pairwise comparisons of treatment groups. Thus, although typically parameter estimate tables only show differences of treatments to a reference group here, we also estimate one additional parameter, the difference between egg-tending and courtship-only males. This parameter is not independent from the estimates of the difference between courtship-only and eggs-removed males and the difference between egg-tending and eggs-removed males but is shown as we are interested in the difference between all treatment groups.

simultaneously (van Iersel 1953; Perrone & Zaret 1979; Lazarus & Inglis 1986; Smith & Wootton 1995a; Manica & Johnstone 2004; cf. Jamieson & Colgan 1989). Indeed, male sticklebacks are more inclined to abort the care of young when there are fewer eggs in their nest (van den Assem 1967; but see: Mehlis et al. 2009, 2010), suggesting that the per capita cost of rearing offspring is greater for smaller clutches. So, why did we not find any difference in the courtship effort of egg-tending males and males that did not receive eggs?

The most likely explanation is that egg-tending males, as well as those that had not spawned, both stood to benefit by courting females at similar levels. In this respect, we exclude the possibility that unspawned males were simply courting at comparable levels to egg-tenders because the former had been



**Fig. 2:** Plot showing predictions from a negative binomial GLMM of the number of zigzag dances performed by male sticklebacks after manipulation of parental (open circles) and corresponding raw observed counts of zigzag dances performed by males in courtship assays (closed circles). Error bars represent 95% credibility intervals of the predicted values. Note that predicted values shown here represent the number of zigzag dances that males in each of the three treatment groups would be expected to engage in conditional on having an average courtship propensity as measured in a baseline courtship assay. Of course in the actual baseline courtship assay, individual males varied in their propensity to court females. Accordingly, on this graph, we have displaced the points showing the raw observed data (closed circles) on the horizontal axis to depict the variable propensity of males to court in the baseline assay. Those points displaced to the right of the hashed centreline for their treatment represent observed values from males that courted more vigorously in the baseline assay, whilst those points displaced to the left represent observed values from males that had less vigorous courtship in the baseline assay. Specifically, the extent of displacement is proportional to the baseline courtship assay covariate score for each male (see methods for calculation details). It should be noted that each male is represented by two data points on the above plot, as each male's courtship was assayed twice following manipulation of parental status.

denied the opportunity to spawn (Chiswell et al. 2014). This is because earlier studies have shown that male sticklebacks prevented from spawning maintain consistent courtship levels with subsequent females (van Iersel 1953; Dziewczynski & Forrette 2015). It is important to realise, however, that courtship decisions are not only influenced by benefits. The cost of attracting additional mating opportunities can also be important. For males that have already spawned, both courtship (e.g. Kotiaho et al. 1998; Mitchell et al. 2008) and the act of mating itself (e.g. Telford & Webb 1998; Franklin et al. 2012) can be energetically demanding, which may impinge on the ability of males to provide high-quality care to their eggs. Indeed, male sticklebacks that engage in vigorous courtship are less successful at caring for their young, potentially as a result of energetic depletion (von

Hippel 2000). Young stickleback eggs do not appear to require extensive fanning until they are several days old (van Iersel 1953; Reebbs et al. 1984; Smith & Wootton 1995b; Hopkins et al. 2011), thus making it unlikely that there is a substantial temporal trade-off between the need to fan and the need to court away from the nest. However, intensely courting males may risk exposing existing eggs to a heightened risk of predation (Sargent 1982 and references within) or egg stealing from rival conspecifics (Jamieson & Colgan 1992). Courting can also jeopardise an individual's own survival (Moodie 1972; Whoriskey & Fitzgerald 1985; Magnhagen 1991; Sih 1994; Candolin 1997; Candolin & Voigt 1998; but see Gwynne 1989), a risk that could be particularly costly for males that have dependent offspring. Therefore, it is possible that such costs may also constrain the courtship effort of egg-tending males. In this regard, the possibility that females prefer to spawn in nests containing eggs could also negate the need for egg-tenders to court more as the presence of eggs *per se* may improve subsequent male mating success (Ridley & Rechten 1981; Belles-Isles et al. 1990; Goldschmidt et al. 1993; but see: Jamieson & Colgan 1989; Jamieson et al. 1992; Jamieson 1994).

Whilst we did not find a difference between the courtship levels of egg-tending males and those that were not permitted to spawn, we did find that previously spawned males that had their eggs experimentally removed subsequently engaged in less courtship compared to males in the other two treatments. Although we cannot discount the possibility that courtship levels of males whose eggs were removed may have been affected by costs associated with spawning (but without the counteracting benefits of courting whilst tending eggs), lower courtship motivation might also be related to their perception of the safety of their nest site. Even though we deliberately disturbed the nests of males in all three of our treatment groups (see methods), if male behaviour is particularly sensitive to the loss of eggs, then it is possible that the act of egg removal may have affected male motivation to court. This is because, if loss of the clutch indicates that a nesting site is vulnerable to predation, then the benefits of acquiring further clutches at that site might be reduced. This pattern could also be reinforced if females avoid spawning in nest sites where eggs have recently disappeared, as has been shown, for example, in scissortail sergeant fish (*Abudefduf sexfasciatus*) (Manica 2010) and sand gobies (*Pomatoschistus minutus*) (Lindström & Kangas 1996).

In summary, our results suggest that egg-tending males are not more motivated to court than unspawned

males. The most obvious explanation for this is that both egg-tenders and unspawned males benefit from future mating success. However, the costs of seeking additional mating for egg-tending males could also be important. Further investigation into the nature of these costs is needed, but it is likely that the increased risk of young being predated or the possibility of parents being unable to care for their young as a result of predation or energetic depletion may be involved. We found the lowest courtship levels among males that had lost their clutch of eggs. This suggests that male sticklebacks are less motivated to court when they perceive their nesting location to be vulnerable to egg predators. More broadly, our findings underscore the importance of considering the impact of parental status on male courtship behaviour.

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