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Indirect cue of paternity uncertainty does not affect nest site selection or parental care in a Pacific toadfish

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Abstract

Parents are expected to reduce offspring investment when confronted with reliable cues of compromised parentage, yet establishing which cues are reliable is an empirical challenge. Presenting a potential cuckolder to a breeding male is often used in experiments as an indirect cue of paternity loss. However, determining the reliability and hence the utility of this cue is an important but often-overlooked research step. Furthermore, cues of compromised parentage are typically manipulated only during the narrow time window(s) when copulations take place, and so we currently have a poor understanding of whether these cues also convey useful information at other critical timepoints in the reproductive cycle, such as during nest site selection. Here, we present a series of field and laboratory studies using a paternal care giving toadfish, the plainfin midshipman fish (*Porichthys notatus*) to address these questions. We tested whether the presence of a potential cuckolder near a potential nesting site reduces the odds that males will choose to nest at that site, or reduces the amount of care they provide for offspring. Overall, we found no clear effect of cuckolder presence on the likelihood that a male would occupy nor abandon a nesting site, nor on the amount of paternal care provided. The presentation of a single sneaker male may have been too weak a signal of cuckoldry to elicit a response from guarder males. Alternatively, a single sneaker male may not represent a severe enough threat to paternity to warrant a response. We highlight the importance of considering the diverse range of natural history and ecological factors that underlie paternity cue utility across different model organisms.

Significance statement

Breeding decisions, such as which nesting sites to occupy or how much to invest into offspring, may be affected by external cues of parentage loss (e.g. cuckoldry) or risk thereof. Here, we tested whether the presence of a cuckolder male in the breeding environment affects male nest site selection and paternal care. Despite being a commonly used putative indirect cue of sperm competition and paternity loss, we found no clear evidence that the presence of a single sneaker male affected these breeding decisions. Our results underscore the importance of first establishing the utility of various cues, which involves considering the ecological context from which the cues arise, before using them to assess the mechanisms underlying animal decision-making.

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Introduction

Theory indicates that parents should reduce parental investment when relatedness to the young they are caring for is low (Westneat and Sherman 1993; Alonzo and Klug 2012), but how can parents 'know' that their parentage has been compromised? One way that parents make assessments about their parentage is through the use of cues, which come in two forms: direct and indirect cues (Neff and Sherman 2003; Sherman and Neff 2003). Direct cues originate from offspring themselves. Examples of direct cues include the physical appearances, vocalizations, or odours of the offspring, which

offer parents information about their degree of relatedness to these offspring. Indirect cues on the other hand, emanate from the environment or the social context during a mating event, for example the mere presence of sperm competitors at the nest or their intrusions during a mating bout. Ultimately, whether and how a parent will respond to a particular cue depends on the cue's reliability for predicting parentage relative to the parent's evolved predispositions (i.e. other sources of information available to an individual regarding a decision, which may be learned or innate, sensu Neff and Sherman 2002). While the prediction that parents should reduce their investment into offspring when confronted with signs of compromised parentage is well-grounded in theory, the empirical evidence remains equivocal (Kempenaers and Sheldon 1997; Sheldon 2002; Alonzo 2010; but see Griffin et al. 2013). For empiricists seeking to test the relationship between parentage (or perceived parentage) and parental care, it is useful to first identify cues that reliably influence parental behaviours. Although examining the utility of a cue is an important and necessary research step, it is also often overlooked or ignored experimentally. This can be problematic when researchers accidentally choose to manipulate and present an unreliable cue rather than a reliable one as this could lead to negative and misleading results.

One possibly reliable indirect cue of paternity loss is the presence of cuckolders in the nesting environment. Indeed, the sperm competition literature suggests that the presence of rival males can affect ejaculate competitive traits (Crean and Marshall 2008; Immler et al. 2010; Parker and Pizzari 2010). Rival male presence has been linked to reductions in some proxies of paternal investment by care giving males in bluegill sunfish (*Lepomis macrochirus*, Neff 2003) and in scissortail sergeant (*Abudefduf sexfasciatus*, Manica 2004), but not in common goby (*Pomatoschistus microps*, Svensson et al. 1998) nor in sand goby (*Pomatoschistus minutus*, Svensson and Kvarnemo 2007). Overall, empirical support for the reduction in paternal investment due to the presence of potential cuckolders in the environment appears to vary from species to species. Due to this ambiguity, further research is clearly still needed to better assess how and when cuckolder presence acts as an indirect cue that alters paternal behaviour.

Indirect paternity cues are thought to be most reliable when they occur concurrently with a mating event (e.g. for external fertilisers) or within a female's fertile window (e.g. for internal fertilisers). However, to date, little research attention has been given to whether indirect cues can also affect behaviours at other critical timepoints in the reproductive cycle outside of the actual mating event. For example, during nest site selection, potential cuckolders or rival males sighted near prospective mating sites could inform males about the risk or intensity of sperm competition that they are likely to experience at these sites. Males may therefore be less likely to select such sites for the constructing of nests and/or rearing of young. Nest site

selection is known to be influenced by a range of factors including the risk of nest predation (Siberian jay, *Perisoreus infaustus*, Eggers et al. 2006), the presence of other care giving individuals in the vicinity (collared flycatchers, *Ficedula albicollis*, Pärt and Doligez 2003), and the abiotic nesting conditions (plainfin midshipman fish, *Porichthys notatus*, Bose et al. 2019a). The presence of potential sperm competitors around a prospective nest could reduce the benefit to cost ratio of nesting at a particular location and hence might reduce the likelihood of particular sites being chosen or increase the chances that these sites will eventually be abandoned. These possibilities have rarely been studied explicitly.

Here, we tested whether the presentation of a single potential cuckolder male, i.e. a putative indirect cue of paternity loss, might affect males at two different stages in their reproductive cycle, during nest site selection and during brood tending. We predicted that if the presence of a cuckolder male at a nest site diminishes its perceived value, then such sites would be less likely to be occupied and/or would be more likely to be abandoned. We also predicted that if the indirect cue of paternity loss (the presence of a cuckolder male) occurred concurrently with a spawning event, then males would reduce the amount of parental care given to the resulting brood of eggs (a proxy for paternal investment). To test these predictions, we conducted one field experiment and two lab experiments using a fish with paternal care, the plainfin midshipman fish.

Methods

Study species

The plainfin midshipman is a marine fish found along the western coast of North America from Alaska to California (Miller and Lea 1972; Walker and Rosenblatt 1988). Plainfin midshipman provide an excellent system in which to test our predictions because of their prolonged and energetically costly parental care (Sisneros et al. 2009; Cogliati et al. 2013; Bose et al. 2014, 2015) and the occurrence of cuckolding males in the breeding environment (Cogliati et al. 2014a). At the onset of the breeding season, in late April, reproductive plainfin midshipman migrate from depths of ~300 m to breed in the shallow waters of the rocky intertidal zone (Arora 1948; Miller and Lea 1972; Warner and Case 1980; Sisneros et al. 2004). Many large males (mean ± SE standard length = 20.4 ± 0.5 cm, Fitzpatrick et al. 2015) build nests in the intertidal zone by excavating nesting cavities beneath large rocks from where they attract gravid females and care for young. Females lay a monolayer of eggs during spawning on the nest roof (i.e. the underside of the rock) and then return to sea, leaving the parental care to the guarding males who remain for two or more months tending to young (Arora 1948; Cogliati et al.

2013). However, not all males court females, establish nests, or care for young; some males of this species fall into a well-characterised alternative reproductive tactic, termed 'sneaker' males (Brantley and Bass 1994; Lee and Bass 2004; Cogliati et al. 2014b). In contrast to 'guarder' males, sneaker males are much smaller (mean ± SE standard length = 10.6 ± 0.3 cm, Fitzpatrick et al. 2015) and rely on sneak and satellite spawning tactics to fertilise eggs in the nests of guarder males, parasitising the considerable parental effort of the guarding males (Brantley and Bass 1994; Lee and Bass 2004). Field census data suggest that sneaker males make up approximately 10% of the male breeding population in the intertidal zone (Brantley and Bass 1994; Cogliati et al. 2014a). However, it is currently unclear whether interactions between guarder and sneaker males in the wild are rare enough for the presentation of a sneaker male to be viewed as a reliable indirect cue of paternity loss.

Furthermore, field experiments suggest that guarder males cannot perceive *direct* cues of relatedness, as they continue to care for related or unrelated young alike in transplant experiments (Bose et al. 2016). However, plainfin midshipman guarder males do use an *indirect* cue of relatedness to inform their caring decisions; when a male takes over a nest from another male, he will be highly cannibalistic towards the offspring inherited from the previous male nest holder (Bose et al. 2016, 2019b). It is currently unknown whether guarder males use the presence of sneaker males around their nests to inform their decisions on nest choice and parental care. To test this question, we conducted three related experiments.

Experimental methods and results

Experiment 1: Effects of sneaker male cues on nest selection

Methods

From May 1 to 6, 2015, and June 3–5, 2015, we constructed 60 artificial nests by placing flagstone slate tiles along the intertidal zone at Ladysmith Inlet, British Columbia, Canada (49° 01′ N, 123° 83′ W). Midshipman guarder males readily and quickly use these tiles as nesting sites, burrowing beneath them and digging out nesting cavities (Demartini 1988, 1991; Bose et al. 2016). The flagstone slate tiles were made of a natural stone material and thus varied in size (mean ± SD surface area = 695 ± 190 cm², range = 410.7–1187.5 cm²), but the areas created on the underside of the tiles closely matched the sizes of naturally occurring available nests at our field sites (mean ± SD natural nest size = 582 ± 349 cm², Bose et al. 2018). Each artificial nest was randomly assigned to either a control ($N = 30$ nests) or treatment group ($N = 30$ nests, and

nest size did not differ between the groups, t test, $t = 0.87$, df = 58, $P = 0.39$).

A transparent acrylic tube (15 cm in length, 5 cm in diameter) was positioned beneath each tile inside the nesting cavity. Every tube was perforated with 20, 1-cm diameter drilled holes and was capped at both ends with mesh to permit water flow and olfactory cue transfer. Nests assigned to the treatment group received a single small sneaker male placed into the tubes, while the nests assigned to the control group simply contained empty tubes (Fig. 1a). Sneaker males (average ± SD standard length = 14.4 ± 1.3 cm) were identified based on their external morphology (they are small and similar in appearance to females, see Brantley and Bass 1994) and were collected by hand from naturally occurring nests at the same field site either on the same day or on the day prior to being used in this experiment. All sneaker males used here were sexually mature. We conducted this experiment in two iterations, once in May and again in June, and each time we monitored the tiles daily for up to 5 days. Different nests were used in either iteration. On each day, we checked for evidence of nest occupation: a guarder male sitting in the nest cavity, the presence of a brood of eggs, or both. Any of these signs would indicate nest uptake and occupation.

Statistical analyses

All analyses in this study were conducted in R (v. 3.5.2, R Development Core Team 2018). We tested whether treatment nests (with sneaker males) were less likely to be occupied or if they took longer to be occupied than control nests (empty). To do so, we fit an interval-censored Cox proportional hazards survival model to our nest occupation data. We included experimental group (i.e. control versus treatment), nest size (cm²), and experimental round (i.e. first versus second) as predictor variables ('icenReg' R package, Anderson-Bergman 2017). We also tested whether the proportion of days that nests were occupied by a guarder male differed between the treatment and control nests. We fit a generalised linear model (GLM) assuming a binomial error distribution and included experimental group, nest size, and experimental round as predictor variables.

Results

There was no clear difference in how quickly guarder males occupied treatment nests containing sneaker males versus control nests containing empty tubes (Cox proportional hazards survival analysis, est. ± SE = 0.15 ± 0.35, z = 0.43, $N = 60$, $P = 0.68$, Fig. 1b). Nest size did not correlate with how quickly the nests were occupied (est. ± SE = 0.002 ± 0.001, z = 1.61, $P = 0.11$), although nests were occupied more quickly late in the season during the second round of the experiment (est. ± SE = 1.01 ± 0.38, z = 2.67, $P = 0.008$). The presence of

Fig. 1 **a** An illustration of the artificial flagstone slate tile nests placed in the intertidal zone for experiment 1 (not drawn to scale). Control nests contained an empty perforated acrylic tube, while treatment nests contained a tube housing a sneaker male. **b** Kaplan-Meier survival curves indicating the probability for a nest type to still be vacant at a given time point. Hollow triangles indicate right-censored data points, indicating that the nests were still vacant when we stopped monitoring them
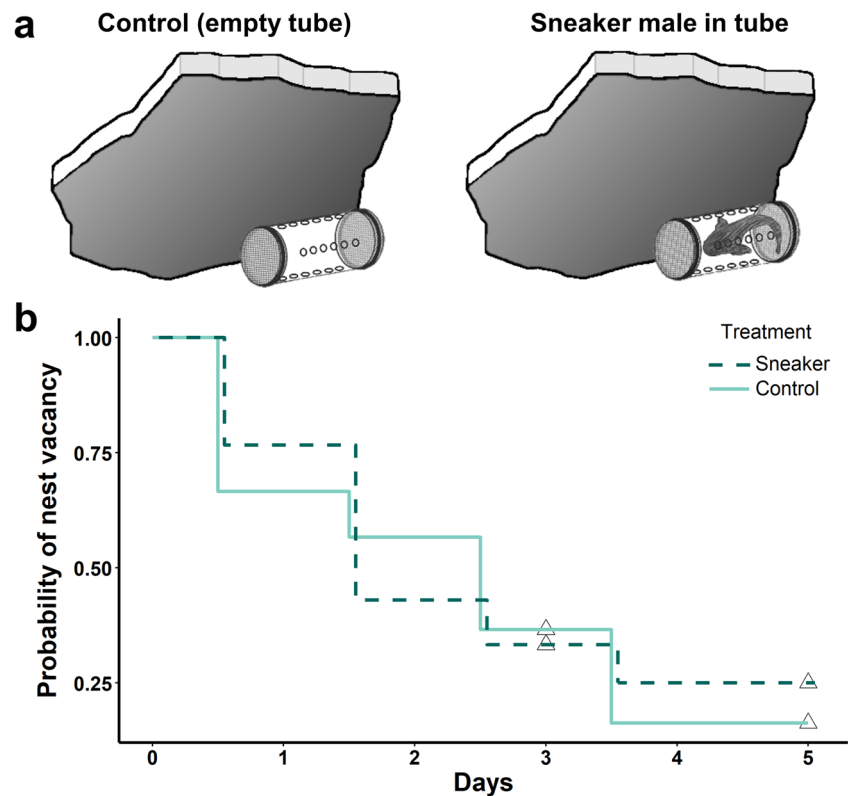
a sneaker male did not reduce the proportion of days that a nest was occupied by a guarder male (GLM, est. ± SE = − 0.23 ± 0.28, z = − 0.84, N = 60, P = 0.40) but smaller nests were occupied for more days than larger nests (est. ± SE = − 0.002 ± 0.001, z = − 1.99, N = 60, P = 0.047).

Experiment 2: Effects of sneaker male cues on nest abandonment

Method

Between April and June 2017, we collected adult plainfin midshipman fish from Ladysmith Inlet and transported them in aerated bins to the Outdoor Aquatic Unit at the University of Victoria. Guarder males, sneaker males, and females were differentiated from one another based on body size, colouration, and inspection of their urogenital papillae. The fish were housed in sex- and tactic-specific outdoor 400 L fibreglass tanks, lined with a gravel substrate and fitted with a recirculating 13 °C seawater system. Fish were held at a maximum density of 8 fish per tank and every fish was provided with its own brick shelter.

We used 87 guarder males in this experiment. Fish were transferred from the holding stock tanks to individual experimental 290 L aquaria, lined with a gravel substrate and holding 13 °C recirculating seawater. Every tank contained two identical breeding shelters positioned on opposite sides of the tank. Shelter walls and roofs were made of standard bricks

and 620 cm^2 ceramic tiles, respectively (Bose et al. 2018). Both shelters contained an empty transparent acrylic tube (as described above in experiment 1). Guarder males were given 3 days to establish residency in one of the two shelters (henceforth, 'nests'). Once the male had taken up a nest (this occurred for 74 of the 87 guarder males), a gravid female was added to his tank. Tanks with a guarder male and a gravid female were randomly assigned to a treatment (sneaker, N = 33) or to a control (no sneaker, N = 41) group. At this time, in treatment tanks, a sneaker male was added to the acrylic tube in the guarder males' chosen nests, while in the control tanks the tubes added to the guarder males' nests remained empty (Fig. 2a). To control for disturbance, the empty tubes in the control tanks were also temporarily removed and replaced. All tanks were monitored daily for the next 3 days to check whether guarder males abandoned their original nest following the manipulations and also to check whether spawning had occurred. If after 3 days no spawning had occurred, we removed the female and replaced her with a new gravid female, and did this one more time if necessary. If no spawning occurred across the (potentially) 9-day period, then the guarder male, the female, and the tubes were removed and a new trial was started with an entirely new batch of fish.

Statistical analyses

One of the 74 trials had to be omitted from the analyses because the sneaker male escaped from the acrylic tube housing.
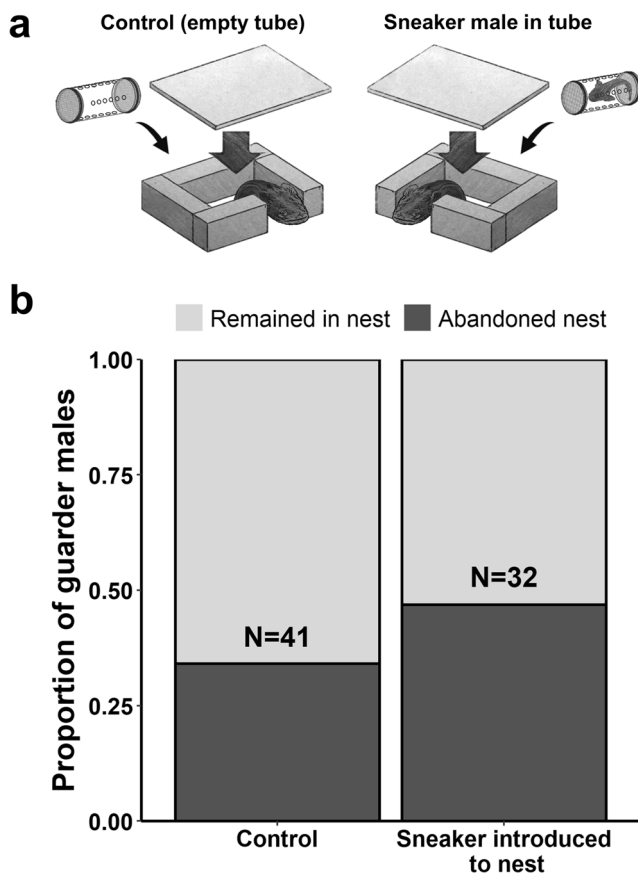
Fig. 2 **a** A depiction of the setup used in experiment 2. Illustration shows the addition of either an empty acrylic tube (control) or a sneaker male in a tube (treatment) to the nests selected by guarder males (not drawn to scale). **b** Proportion of trials from experiment 2 where the guarder male abandoned his chosen nest (dark grey) across treatments

For the remaining 73 trials, we used a chi square test for equal proportions to test whether males were more likely to abandon their chosen nest when it contained a sneaker male compared with when it remained empty.

Results

Fourteen out of 41 guarder males abandoned their control nests (34%), while 15 out of 32 guarder males abandoned their treatment nests (47%). Overall, guarder males were not more likely to abandon nests containing sneaker males than males who had no sneaker present in their nests (chi square test for equal proportions, $\chi^2 = 0.74$, $N = 73$, $P = 0.39$, Fig. 2b).

Experiment 3: Effects of sneaker male cues on parental care

Methods

Of the 73 trials run in experiment 2, 23 resulted in a spawning. Twelve spawnings took place in control trials, and 11 occurred

in treatment trials (nine spawnings occurred in the same nest as the caged sneaker male, and two occurred in the nest without the caged sneaker male, meaning that the guarder male abandoned his initial nest choice and moved over to the alternative nest without a sneaker). For the purpose of this study, we focused only on the males with eggs in the control trials and those males in the treatment group that spawned in the same nest as the caged sneaker male ($N = 21$ in total).

Upon discovery of eggs, we removed the female and the sneaker male (if any) from the tank and photographed the eggs to quantify brood size (using ImageJ, v.1.45, National Institutes of Health, Bethesda, MD, U.S.A., http://rsbweb. nih.gov/ij/). Broods had on average ($\pm$ SD) 89.9 ± 45.9 eggs and brood size did not differ significantly between the control (no sneaker present) and treatment (sneaker present) nests (two sample t test, $t_{19} = 0.40$, $P = 0.69$). On the second and fourth days post-spawning, we placed a small camera (GoPro Hero 5 Session) into the nest and recorded male behaviours during two specific phases. During phase 1, we recorded male behaviours for 30 min based on an ethogram available in Table 1. During phase 2, we introduced an egg predator to the nest, a purple shore crab, *Hemigrapsus nudus*. The crab was loosely tethered to a pole so that it could be placed in the nest (approximately 10 cm from the egg mass) in a standardised way. In phase 2, we recorded all male aggressive behaviours towards the egg predator over a 15-min period. Guarder males aggressed both overtly (e.g. biting or lunging toward the crab) and vocally (emitting distinctive 'grunts' or 'growls' through high-frequency vibrations of their swim bladder, Brantley and Bass 1994). Audio was captured using the cameras' internal microphones. The researcher scoring behaviour remained blind to treatment. In addition, we randomly chose 10 males who had not spawned and also recorded videos of these males over the course of both phases. Thus, we obtained behavioural data for males in three experimental groups: (1) control males guarding eggs that had been spawned with no sneaker male present ($N = 12$), (2) treatment males guarding eggs that had spawned with a sneaker male present ($N = 9$), and (3) non-caring males with no eggs ($N = 10$). Guarder males were on average ($\pm$ SD) 22.9 ± 3.1 cm in standard length and body size did not differ between the three experimental groups (linear model, $F_{2,48} = 1.60$, $P = 0.21$).

Statistical analyses

First, we tested whether guarder male behaviour differed between the recordings on the second and fourth days post-spawning. To do this, we fit a generalised linear mixed effects model (GLMM, 'lme4' R package, Bates et al. 2015) assuming a Poisson error distribution to the count of each behaviour (movement, nest maintenance, physical aggression, and vocal aggression). We included day (categorical variable: day 2 and day 4) as well as experimental group (categorical variable:

Table 1 Ethogram of behaviours scored from videos of guarder males in their nests during study 3. Phase 1 refers to the duration *prior* to the introduction of the egg predator to the nest (which was phase 2). This ethogram was adapted from a more comprehensive video ethogram that can be found in the Supplementary Materials

Behaviour	Description
Movement (scored in phases 1 and 2)	Guarder male repositions itself within the nest with a discrete hop or a short swim.
Nest maintenance (scored in phases 1 and 2)	Guarder male moves substrate within nest using its mouth or fins.
Physical aggression (scored in phase 2)	We scored two forms of physical aggression: Direct attack: guarder male directly bites or rams the egg predator. Visual display: guarder male lunges at the egg predator with mouth open but does not make contact (sometimes the opercula and pectoral fins are flared).
Vocal aggression (scored in phase 2)	We scored two forms of vocal aggression: Grunt-train: guarder male produces a sequence of short-duration (50–200 ms), regular, broadband sound pulses. Growl: guarder male produces a single long-duration (> 1 s) broadband sound emission.

control and sneaker) as predictor variables. For movement and nest maintenance behaviours, we also included phase (categorical: phase 1 or 2) as an additional predictor variable (there were no aggressive behaviours prior to the addition of the crab). Note that the 'non-caring' group was not included here because we only recorded these males on 1 day. We included male identity as a random intercept as well as an observation-level random intercept to account for overdispersion (Harrison 2014). Likelihood ratio tests indicated that the inclusion of day in the models did not significantly improve their fits (all $P > 0.05$) and so we dropped 'day' from any further models and pooled the counts for each behaviour across both days.

Next, we tested whether male behaviours differed across our three experimental groups (control, sneaker, and no-care). For each behaviour, as above, we fit a GLMM assuming a Poisson error distribution. We included experimental group (categorical variable: control, sneaker, no-care) as a predictor variable as well as the log of the cumulative amount of time that each behaviour was scored for as a model offset. We also included phase as a predictor variable for movement and nest maintenance behaviours (as we observed no aggression in phase 1, prior to the addition of the egg predator). Again, we included male identity as a random intercept and an observation-level random intercept to account for overdispersion (Harrison 2014). We tested for pairwise differences using Tukey contrasts ('multcomp' R package, Hothorn et al. 2008).

Results

Guarder males were significantly less active when they had no eggs in their nests compared with control males that were caring for eggs (GLMM, est. $\pm$ SE $= -2.52 \pm 0.84$, z $= -3.00$, $P = 0.008$, Fig. 3a). None of the other pairwise contrasts were significant (all $P > 0.26$). Nest maintenance behaviour did not differ across the experimental groups (GLMM, all contrasts were at least est. $\pm$ SE $= -2.08 \pm 1.49$, z $= -1.40$,

$P = 0.34$, Fig. 3b) but significantly decreased after the addition of the egg predator (est. $\pm$ SE $= -1.93 \pm 0.33$, z $= -5.84$, $P < 0.0001$). Guarder males only performed aggressive behaviours when the egg predator was introduced to their nest. Guarder males engaged in more physical aggression against the egg predator when they had eggs to defend in their nest. However, this effect was only statistically evident between the non-caring males and the control males (GLMM, est. $\pm$ SE $= 2.01 \pm 0.85$, z $= 2.36$, $P = 0.048$, Fig. 3c). Similarly, vocal aggression against the egg predator also increased when eggs were in the nest, and again the difference was only significant between the control and non-caring males (GLMM, est. $\pm$ SE $= 2.34 \pm 0.84$, z $= 2.79$, $P = 0.014$, Fig. 3d).

Discussion

The addition of a sneaker male inside the nesting cavity did not appear to influence the rate at which potential intertidal nesting sites were occupied by guarder males in the field. In the laboratory, sneaker male presence did not affect the likelihood that a guarder male would abandon his chosen nest, nor did the presence of a sneaker male significantly influence the parental behaviours that the guarder males performed. Our study is linked theoretically to previous research that has attempted to experimentally manipulate the perception of paternity by presenting a male with a cue of paternity loss (a cuckolder) and then monitoring changes in parental behaviours (e.g. Svensson et al. 1998; Neff 2003; Manica 2004; Svensson and Kvarnemo 2007). However, as argued earlier, the results from these past studies have been mixed, suggesting that further research is needed into the 'what', 'where', and 'when' for cues to be reliable sources of information for breeding and parental decisions (Neff and Sherman 2002).

Nest possession is highly valued as guarder males typically gain more reproductive success when they manage to spawn and care for young in their own nests than when they cuckold

Fig. 3 Guarder male behaviour in experiment 3. Panels **a** and **b** respectively depict the movement and nest maintenance activity performed by the guarder males from each of the three experimental groups. Data shown here are pooled across both days (i.e. days 2 and 4) and both phases of the experiment (i.e. prior to and after the addition of an egg predator to the males' nests). Panels **c** and **d** respectively depict the physical and vocal aggression performed by the guarder male towards the introduced egg predator (i.e. in phase 2 only, but pooled across both days). Error bars indicate ± 1 standard deviation. Significant differences between groups, at $\alpha = 0.05$, are indicated by different lower-case letters
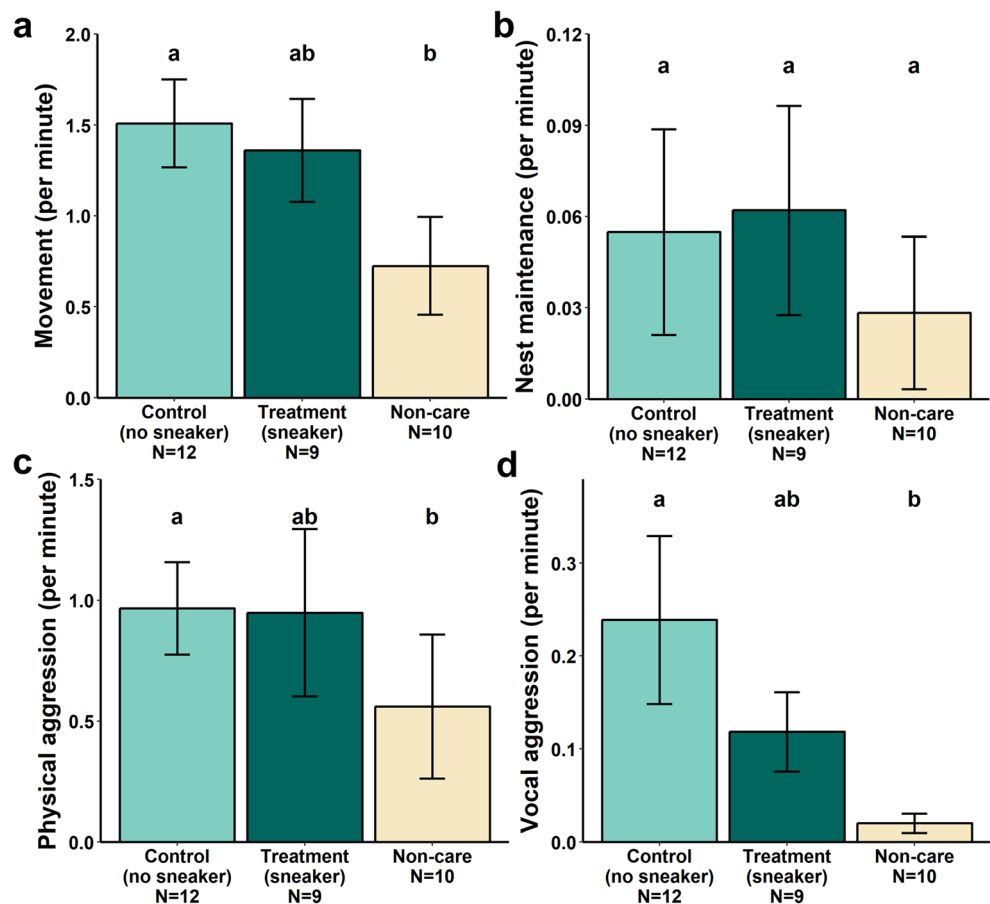
other nesting males in the population (Cogliati et al. 2014b). In the rocky intertidal zone, suitable nesting sites for plainfin midshipman fish are a limited resource (Demartini 1988), which leads to intense competition among guarder males (Cogliati et al. 2013; Bose et al. 2014). The loss of a nest would likely be highly costly, and so guarder males are not expected to give up these limited nests easily. Additionally, when compared with their territorial guarder male counterparts, sneaker males are highly mobile and not bound to a particular nesting site. Therefore, the detection of a single sneaker male near a nest may not represent a reliable source of information about the risk of sperm competition at that particular nest. It is likely that for plainfin midshipman fish, the benefits of nest ownership outweigh any potential costs created by the presence of a single cuckolder male. It should be noted, however, that despite the benefits of nest ownership, we observed a seemingly large percentage of guarder males abandon their nests after our manipulations in experiment 2 (34% in control, 47% in sneaker trials). The guarder males in these lab trials, however, did not have to contend with other territorial males vying for nest space in their tanks, and this could have facilitated higher rates of nest abandonment.

Guarder males were generally more active within their nests when they had eggs present versus when their nests were empty, and this was especially the case for control males, whose broods were spawned in the absence of a sneaker male. Males were also more aggressive, physically and vocally, against the egg predator when they had eggs to care for. However, none of the behaviours we scored (movement, nest maintenance, physical aggression, and vocal aggression) differed statistically between males whose eggs were spawned either in the presence or absence of a sneaker male. Although our analyses may have gained additional power with increased sample sizes, they still suggest that the effect size of our manipulated cue is exceptionally low or non-existent. This implies that a single sneaker male may be an ineffective indirect cue for guarder males when making parental care decisions. Why might this be? First, sneaker males could be a ubiquitous part of the plainfin midshipman fish's breeding environment. After all, cuckoldry and alternative reproductive tactics are exceptionally common in fishes (Mank and Avise 2006; Taborsky 2008). In the plainfin midshipman fish, sneaker males make up an appreciable percentage (~ 10%) of the male breeding population found in the intertidal zone (Brantley and Bass 1994; Cogliati et al. 2014a). Interactions between guarder and sneaker males may be common in the wild, which could reduce the effectiveness of our chosen indirect cue. Second, despite being fairly common, sneaker males only

account for a small proportion of the paternity losses that males experience in the wild. Cogliati et al. (2013) intensively sampled a breeding population on Vancouver Island, British Columbia, and showed that only 2% of offspring could be definitively assigned to sneaker males. Thus, individual sneaker males may pose only a minor threat to the paternity of guarder males, which may be insufficient for selection to favour adjustments in paternal investment and behaviour. Third, plainfin midshipman sneaker males are morphologically similar to females (Bass and Marchaterre 1989). Such female mimicry likely allows sneaker males to gain proximity to eggs when attempting to sneak-spawn. Female-mimicry could also reduce the reliability of indirect cues if it helps to confuse guarder males as to the true identity of the sneaker male. Nevertheless, plainfin midshipman sneaker males appear unable to entirely evade detection, as guarder males will still respond aggressively towards sneaker males when they are detected in their nests (Brantley and Bass 1994; Lee and Bass 2004). Multiple factors could therefore have worked in combination to diminish the reliability of our indirect cue (i.e. the sneaker male) relative to the guarder males' evolved predispositions (sensu Neff and Sherman 2002). Alternatively, our indirect cue could indeed have reduced the males' perceptions of their paternity, but their behaviours may have remained unchanged if they are willing to tolerate some unrelated eggs in their nests if this helps to attract future females (Jamieson 1995; Kraak 1996). Moving forward, additional indirect cues will need to be tested including the presence of multiple sneaker males as opposed to only one, and the inclusion of chemical cues such as those from sneaker male ejaculates.

Cue reliability has also been a central tenet of sperm competition theory. The sperm competition *intensity* model predicts that if males can estimate their number of competitors (i.e. they have access to a reliable cue of competition intensity), then their investment into ejaculates should decrease with increasing numbers of competitors (Parker et al. 1996). However, if males cannot estimate their number of competitors (i.e. they have no reliable cue of competition intensity), then their ejaculate investment should be shaped by the average number of competitors for their local population (Parker et al. 1996) (i.e. their investment is shaped by an evolved predisposition rather than a cue). Similarly, the sperm competition *risk* model predicts that males should increase their ejaculate investment when they have *detected* a cuckoldry attempt, i.e. when they have a reliable cue (Parker 1990). In the plainfin midshipman system, if guarder males cannot reliably detect sneaker males, or if single sneaker males do not represent a reliable threat to paternity, then guarder males may simply express a level of paternal investment that reflects the average paternity loss for males of their tactic in

their population (see Westneat and Sherman 1993). This emphasizes the importance of having access to reliable cues of information when making investment decisions across numerous reproductive contexts.

Overall, for any given study system, there are numerous factors that dictate whether an indirect cue of paternity loss will elicit a change in behaviour. Failing to detect a significant effect or a change in behaviour when manipulating cues of paternity loss puts empiricists in a tough position. It is possible that their study either truly describes an independent or negligible association between the behaviour (e.g. paternal investment) and what the cue represents (e.g. compromised paternity) or fails to do so because of insufficient statistical power or because an inappropriate or unimportant cue was manipulated. Thus, evolutionary studies of breeding decisions that involve manipulating cues (direct or indirect alike) can only be interpreted when researchers have a full understanding of the cues they use (Neff and Sherman 2002). Ultimately, this understanding can only be achieved by rigorous and systematic experimentation on a range of cues and cue combinations conducted at multiple critical time points in the reproductive cycle. Here, we show that the presentation of a single potential cuckolder male does not elicit a clear response by guarder male plainfin midshipman fish in their parental care and nest site selection behaviours.

Compliance with ethical standards

Conflict of interest   The authors declare that they have no conflicts of interest.

Ethical approval   The plainfin midshipman is a common intertidal species and is not considered threatened or endangered (Collette et al. 2010). This work was done in accordance with Fisheries and Oceans Canada scientific collections permits (XR 812015, XR 582017). The procedures used in this study were approved by the McMaster University Animal Research Ethics Board (AUP 13-12-52) and the University of Victoria Animal Care Committee (AUP 2017-003(1)), and are in line with the guidelines set by the Canadian Council on Animal Care (CCAC) and ASAB/ABS (2018) regarding the treatment of animals in research and teaching. Furthermore, these fish were also used in a number of additional experiments (e.g. Bose et al. 2018; Miller et al. 2019a, b).

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