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Original Article

Impacts of direct and indirect paternity cues on paternal care in a singing toadfish

Aneesh P.H. Bose, Henry H. Kou, and Sigal Balshine
Aquatic Behavioural Ecology Laboratory, Department of Psychology, Neuroscience, and Behaviour, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada

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Effort spent on raising unrelated offspring can be costly and wasteful, and parents are expected to reduce their level of investment when they have low or uncertain relatedness to the young under their care. Although the relationship between parental certainty and parental investment is theoretically well established, empirical support has been mixed. Here, we report on a series of lab and field experiments that test whether paternal investment is reduced as paternity decreases in the plainfin midshipman fish (*Porichthys notatus*), a species of toadfish with male-only care. We explored what cues plainfin midshipman males use to assess their paternity. We show that a nest takeover, in which a male displaces another male from a nest, can be a reliable indirect cue of paternity information and leads to a drop in offspring survival. We also show that, when presented in isolation, direct cues of reduced offspring relatedness do not result in a decline in offspring survival in midshipman. Our findings help clarify what systems, species, and theoretical assumptions best reveal the link between parental investment and parentage.

Key words: **allocare, nest takeovers, offspring transplants, parentage, parental care, plainfin midshipman.**

INTRODUCTION

Raising offspring is a demanding endeavor and so parents are expected to ensure that their parental efforts are not misdirected (Alonzo and Klug 2012). When parentage is low or uncertain, a caregiver is expected to reduce parental investment and preserve resources for more certain reproduction in the future (Alonzo and Klug 2012). Theory suggests that parental investment will fluctuate in response to variation in certainty of parentage but only when the following 3 conditions are satisfied: 1) parental care is costly such that investment into current offspring diminishes the ability to invest in future reproduction, 2) relatedness to the offspring varies between reproductive bouts, and 3) caregivers have access to cues that reliably predict their relatedness to the offspring (Westneat and Sherman 1993). When studying how patterns of parentage relate to parental effort, it is important to carefully consider these 3 conditions in order to determine whether a relationship can be expected. Many of the empirical studies conducted to date on this topic have been correlational in design and have revealed mixed results with some studies revealing a positive relationship between parentage and parental care (e.g., Sheldon and Ellegren 1998; Hunt and Simmons 2002; Neff 2003; Apicella and Marlowe 2007) where others have uncovered no relationship (e.g., Peterson et al. 2001; Östlund-Nilsson 2002; Härdling et al. 2007; Svensson and Kvarnemo 2007) or even found a negative relationship (Alonzo and Heckman 2010). This inconsistency may, in part, be the result of various study systems not meeting the above 3 criteria, not properly accounting for confounding variables, or not using the appropriate proxies for parental investment (Kempenaers and Sheldon 1997; Sheldon 2002; Alonzo 2010). Hence, there is currently a research need to better determine and characterize the relationship between certainty of parentage and parental investment and to identify the particular recognition mechanisms used during such parentage assessments across species (Alonzo and Klug 2012).

Recognition mechanisms employed by parents to assess their relatedness to offspring are generally categorized into the use of direct cues versus indirect cues (Sherman and Neff 2003). Direct, or phenotypic, cues are those that emanate from the offspring themselves, such as how an offspring looks or smells, and these cues are often compared for similarity with the parent (e.g., via self-referent phenotype matching, Hauber and Sherman 2001). The use of direct cues is known to occur in numerous taxa, including mammals (e.g., Belding's ground squirrels, *Urocitellus beldingi*, Mateo 2010), birds (e.g., brown-headed cowbirds, *Molothrus ater*, Hauber et al. 2000), arthropods (e.g., ladybirds, *Adalia bipunctata*, Agarwala and Dixon 1993), and fishes (e.g., bluegill sunfish, *Lepomis macrochirus*, Neff and Sherman 2003, 2005). In contrast, indirect cues are those that originate from the individual's ecological or social environment (Hauber and Sherman 2001). For example, a parent may use the presence of sexual competitors in the vicinity during mating, or during their mate's fertile period, as a cue of reduced parental certainty (Waldman 1987; Sherman and Neff 2003). The use of

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Address correspondence to A.P.H. Bose. E-mail: boseap@mcmaster.ca.

such indirect cues is also taxonomically widespread (e.g., dunnocks, *Prunella modularis*, Davies et al. 1992; wolf spider, *Pardosa milvina*, Anthony 2003; silversides, *Telmatherina sarasinorum*, Gray et al. 2007; poison-dart frogs, *Oophaga pumilio*, Stynoski 2009). Interestingly, Alonzo and Heckman (2010) documented a counterintuitive case in the ocellated wrasse, *Symphodus ocellatus*, wherein the degree of paternal care actually increased with risk of sperm competition. The influence of a particular cue on parental investment should depend on several factors, including the cue's reliability in predicting parentage and the costliness of losing parentage (Neff and Sherman 2002). Thus, it is informative to assess multiple potential cues, direct and indirect, within a single system in order to identify which affect parental behaviors and which do not.

The plainfin midshipman fish (*Porichtys notatus*) represents an excellent model system in which to examine how cues of relatedness might influence parental behavior. Plainfin midshipman satisfy 2 of the required conditions identified by theoretical models for parentage to influence parental investment. First, parental care in *P. notatus* is extremely costly. Males provide sole paternal care for offspring over a 3–4-month-long breeding season, and this care incurs a high physiological cost severely draining paternal energy reserves and body condition (Sisneros et al. 2009; Bose, McClelland, et al. 2015). Furthermore, caring for non-kin offspring imposes an additional cost because these offspring take up valuable space in an already space-limited nest (DeMartini 1991). Non-kin offspring within a nest therefore restrict a male's opportunity for additional or future reproduction. Second, males of this species compete intensely for reproduction (Brantley and Bass 1994; Lee and Bass 2004; Bose, Cogliati, et al. 2014) leading to highly variable levels of paternity among broods in the wild (range 0–100%, Cogliati, Neff, et al. 2013). The third condition of whether or not males have access to reliable cues of paternity loss has yet to be tested. In this study, we aimed to uncover whether nesting plainfin midshipman males have access to reliable cues of paternity loss and specifically tested whether guarder males use direct (i.e., offspring) cues and/or indirect (i.e., environmental and social) cues to inform their paternity assessments over a brood of offspring.

METHODS

Study species

The plainfin midshipman is a marine toadfish native to the western coast of North America (Arora 1948; Miller and Lea 1972; Walker and Rosenblatt 1988). Male plainfin midshipman fish are found as one of 2 well-characterized alternative reproductive morphs (Brantley and Bass 1994; Lee and Bass 2004). Guarder males (also called Type I males) build nests within the intertidal zone by excavating nesting cavities beneath intertidal rocks. From their nests, each guarder male produces a low-frequency, long-duration acoustic signal in order to attract gravid females (Ibara et al. 1983; Brantley and Bass 1994). Females adhere their eggs to the underside of the rock, the roof of the nest (Arora 1948). Guarder males are polygynous, acquiring eggs from numerous females over the breeding season, and will simultaneously care for several cohorts of offspring at different stages of development. Eggs develop for approximately 30 days and then hatch. The hatched offspring remain adhered to the roof of the nest, absorbing a large yolk sac and develop for another ~30 days before reaching independence and will finally swim freely and leave the nest (Arora 1948; Cogliati, Neff, et al. 2013). Intense competition among guarder males for adequate nesting sites leads to high frequencies of nest takeover early in the breeding season (Cogliati, Neff, et al. 2013; Bose, Cogliati, et al. 2014). In contrast to guarder males, sneaker males (also called Type II males) do not physically compete for nests nor do they construct nests or acoustically court females. Instead, they use sneaking and satellite spawning tactics to steal fertilizations away from guarder males effectively parasitizing the guarder male's courtship and parental investment (Brantley and Bass 1994; Lee and Bass 2004).

Experiment 1: manipulation of direct cues via offspring transplants

Between 14 and 17 May 2014, 91 artificial nests were constructed using concrete tiles (12″ × 12″, 929.0 cm^2) placed within the intertidal zone of a private beach located in Dabob Bay of the Hood Canal, Washington (47°76′N, 122°86′W). Such tiles serve well as artificial nests and are easier to lift and inspect than are the nests found beneath natural rocks. Guarder males readily dig nesting cavities beneath these tiles, from where they will acoustically court females, spawn, and care for offspring. Females adhere their embryos on the roof of the nest (the underside of the tile). The tile nests were checked again on 12 June 2014, and we found that 76 of them contained both a guarder male and developing offspring. The males and their broods were digitally photographed with a ruler (for later measurement of the male standard length and quantification of the brood size using the software ImageJ [v1.48]). Care giving males were each given a unique dorsal fin mark with injectable elastomer (Northwest Marine Technology, Inc.) for future identification. These 76 nests were then randomly assigned to either a control group ($N = 37$) or to a transplant group ($N = 39$). No differences in standard length of males (t-test, $t = 1.35$, df = 65.6, $P = 0.18$, mean standard length ± [standard error] SE = 23.1 ± 0.3, range: 16.5–28.9 cm) or in initial brood sizes (t-test, $t = 0.38$, df = 72.6, $P = 0.71$, mean brood size ± SE = 1234 ± 71 embryos, range: 94–2983 embryos) were observed between the males and nests assigned to either the control or transplant treatment groups prior to the manipulation.

Nests in the transplant group were swapped with one another, controlling as closely as possible for brood size and offspring developmental stage between the swapped tiles. Only broods in which all offspring were still eggs (prehatch stages of development) were used in these transplants. Note, eggs take 30 days to hatch and hatched embryos remain adhered to the nest ceiling for an additional 30 days absorbing the large yolk sac before leaving the nests. The swapped tiles were always spatially distant (>5 m apart) from one another in the intertidal zone, making it highly unlikely that males within the transplant group could have fathered the brood of offspring on the transplanted tile they received. To control for disturbance, the tiles in the control group were lifted, rotated 180°, and then placed back on the nest cavity with their original brood intact (Figure 1a). Thus, the males in the transplant group each received an entire foreign brood, whereas males in the control group each received their own brood.

We visited these nests 3-, 14-, and 28-day postmanipulation. On each visit, we recorded the presence or absence of the marked guarder male and took additional digital photographs of the brood to quantify the number of offspring remaining. Note, the challenging time restrictions of working within a low tidal schedule meant that we did not always manage to visit every nest at every time point leading to slight variation in sample sizes between time points.

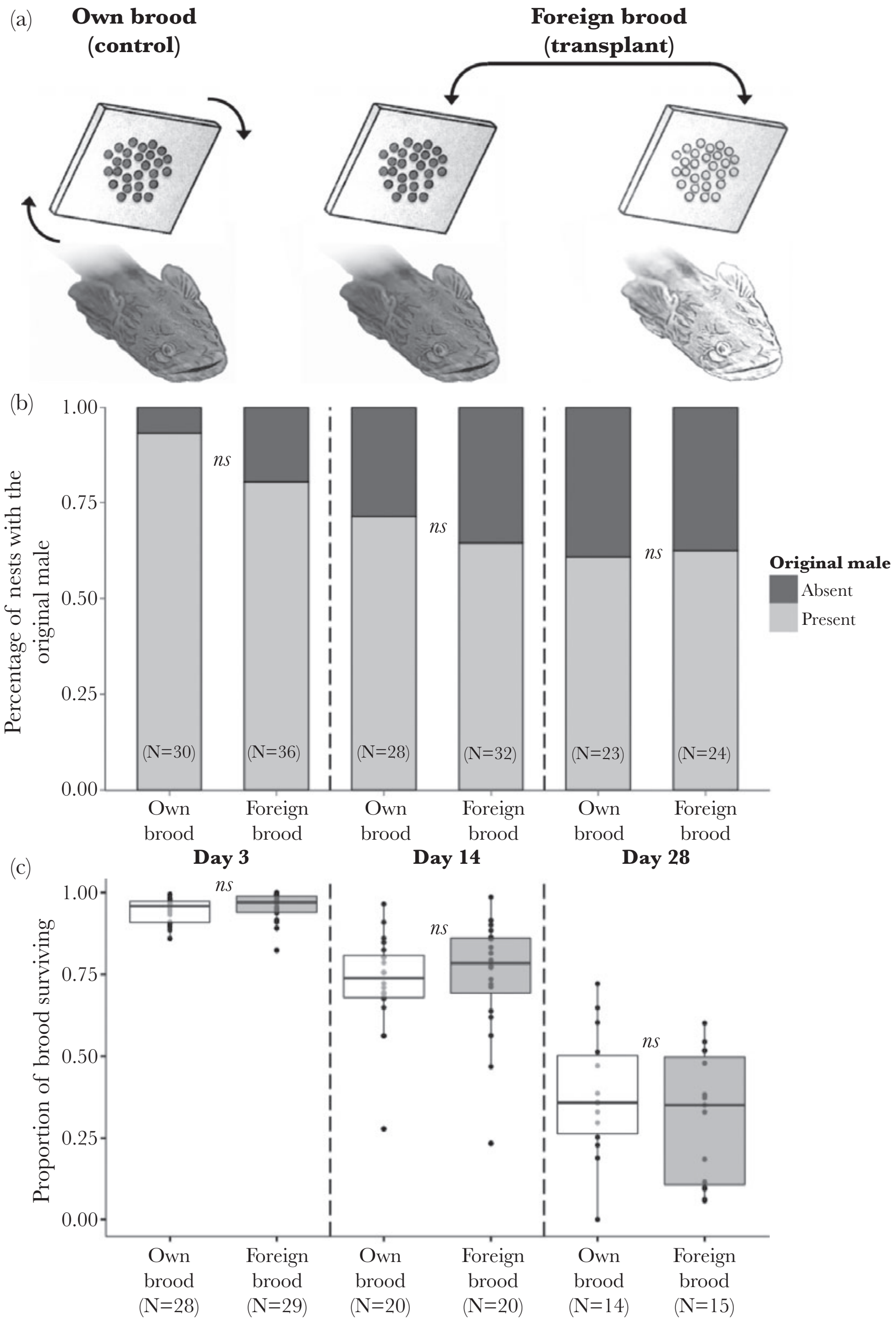

Figure 1

(a) A schematic of the design of Experiment 1. Tiles with offspring were transplanted between the nests of caregiving males (foreign brood), or lifted, rotated, and returned to the original caregiver (own brood). (b) The presence or absence of the original guarder male between treatment conditions, on days 3, 14, and 28. The dark bars refer to cases where the original guarding male was absent (presumed to have abandoned), and the light bars refer to cases where the original guarding male remained with the nest. (c) Proportion of the offspring surviving under the care of an alloparent (transplanted foreign brood) or the care of the original parent (own brood) at 3-, 14-, and 28-day postmanipulation.

As some nests were abandoned or taken over by a new male after our manipulations, we compared the proportion of original guarder males still within their nests between the control and transplant groups. To do this, we conducted a generalized linear model (GLM) at each time point, specifying a binomial error distribution suitable for binary response data. Treatment condition (own brood, foreign brood), guarder male standard length (centimeter), and initial brood size (embryo count) were all included as predictor variables in the models. We then focused only on the nests that retained a guarder male, and compared the proportions of offspring still surviving from the original brood at each time point between the control and transplant groups. To do this, we performed a GLM at each time point, specifying a quasibinomial error distribution (accounting for overdispersion, Kabacoff 2011) suitable for proportion data. Parameters for treatment condition (own brood, foreign brood), guarder male standard length (centimeter), and initial brood size (embryo count) were included in the models.

Experiment 2: brood recognition via direct cues

In 55 April 2015, artificial nests were constructed using concrete tiles as described above in Experiment 1 within the intertidal zone of Ladysmith Inlet, British Columbia, Canada (49°01′N, 123°83′W). These nests were monitored every other day between 24 April 2015 and 27 June 2015 for the presence of a guarder male and for broods of embryos. Of these, a total of 52 guarder males and their tiles (with embryos adhered to these tiles) were transported to the Pacific Biological Station in Nanaimo, British Columbia, Canada. Each tile was digitally photographed so that brood size could be quantified. Each male was housed individually in a 300-L outdoor fiberglass holding tank, lined with a sand substrate and fitted with a flow-through system that supplied temperature controlled (18 ± 1 °C) filtered seawater. Each male was provided with 2 artificial nests placed 30 cm apart, identical in size (361.0 cm^2) and in layout (rectangular with one 5×6 cm entrance, Figure 2a). Each artificial nest was constructed from 4 bricks and a square concrete garden tile. The males in tanks swam around actively, and even sang nocturnally suggesting that the captive fish still engage in natural breeding behaviors (e.g., Brantley and Bass 1994; Bose A, personal observation). Males were initially placed in a start chamber for an acclimation period of 30 min before being given 24 h to choose between the 2 artificial nests within their tanks.

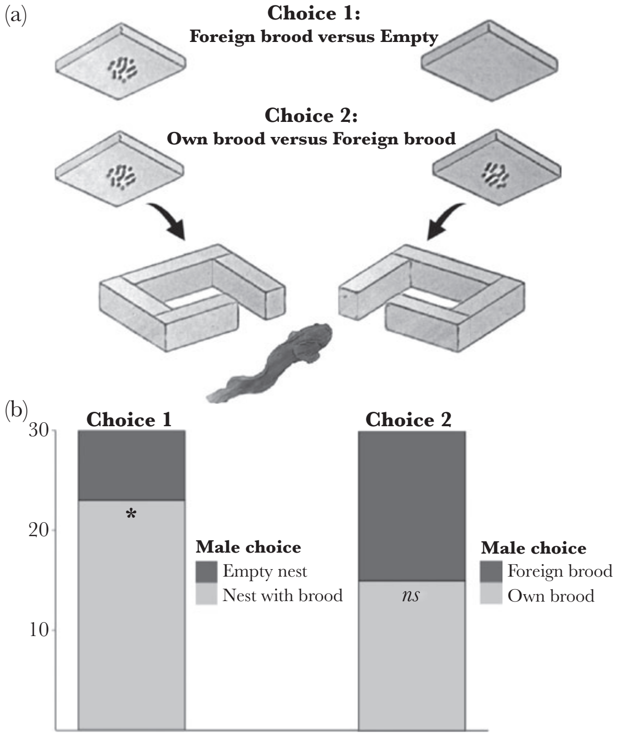

Figure 2
(a) A sketch of the design of Experiment 2 (not drawn to scale). Guarder males were given a choice between 2 artificial nests. In Choice 1, males were simultaneously presented with 2 nests, one that contained another male's offspring and a second nest without any offspring. In Choice 2, males were presented with 2 nests, one that contained another male's offspring and one that contained their own offspring. (b) Number of males choosing each nest type when given the choice.

Choice 1: nest with embryos versus empty nest

Thirty-eight fish were used to test whether males preferentially choose nests that already contained embryos over a nest that did not. For this test, one nest contained a brood of embryos (not belonging to the focal male, mean brood size ± SE = 461.0 ± 79.9 embryos), whereas the other nest contained no embryos (Figure 2a). Embryos at both prehatch and posthatch stages of development were offered to these males over the course of the experiment. The side of the tank where the brood-bearing tile was positioned in relation to the empty tile was alternated each trial.

Choice 2: nest with own embryos versus nest with foreign embryos

Thirty fish were used to test whether males preferred and/or could distinguish between their own familiar embryos versus foreign unfamiliar embryos. For this test, one nest in the male's tank contained that guarder male's original brood from the field and the other nest in the tank contained a brood of foreign embryos. The 2 nests were matched as best as possible for brood size (paired *t*-test after ln-transformation, $t = 1.1$, degrees of freedom [df] = 29, $P = 0.28$, mean brood size ± SE = 305.3 ± 29.1 embryos, range: 42–938 embryos) and were matched for stage of embryo development (Figure 2a). Embryos at both prehatch and posthatch stages of development were offered to males over the course of the experiment. The positions of the tiles within the tanks were alternated for each trial.

For both choice tests, we recorded in which nest each male resided after 24 h before digitally photographing the broods once again. Males were measured for standard length (centimeter; to the nearest 0.1 cm) and total body mass (gram; to the nearest 0.2 g). Twenty-two fish participated in both choice trials counterbalancing for order.

We tested whether guarder males were more likely to choose nests that already contained offspring as opposed to empty nests, using a binary logistic GLM specifying a binomial error distribution, including parameters for the developmental stage of the brood (prehatch, posthatch), guarder male standard length (mean-centered, centimeter), and brood size (mean-centered, embryo count).

Next, we tested whether guarder males were more likely to choose a nest that contained their own embryos versus a nest that contained unfamiliar foreign embryos, using a binary logistic GLM specifying a binomial error distribution, including parameters for the developmental stage of the brood (prehatch, posthatch), guarder male standard length (mean-centered, centimeter), and relative brood size (mean-centered, difference in embryo counts). Lastly, we compared rates of offspring mortality between the 2 broods (own vs. foreign) using a Wilcoxon signed rank test ("MASS" package, Venables and Ripley 2002) to test whether the unfamiliar foreign broods suffered higher mortality (suggestive of embryo cannibalism) than did the familiar broods over the 24-h choice period.

Experiment 3: benefits of paternal care versus allopaternal care versus no care

On 16 and 17 May 2015, a total of 60 concrete tiles (12″ × 12″, 929.0 cm^2) were placed within the intertidal zone of the private beach in Dabob Bay, Washington. These artificial nests were monitored daily until they were occupied by a guarder (Type I) male and had received embryos. Within 2 days of setting out the tiles, every nest had been taken up by a guarding male, and it took on average

1 further day (range 1–5 days) for these males to acquire broods of eggs. The broods were digitally photographed for later quantification of embryo number and to provide estimates of embryo survival. These males and nests were then randomly assigned to one of 3 experimental treatments: a "paternal care" group, an "allopaternal care" group, and a "no care" treatment group (Figure 3a). In the paternal care treatment, the original males were left in their nests to continue caring for the brood. In the allopaternal care treatment, original males were removed from their nests and new males were permitted to take up these nests (this nearly always occurred within 24 h of removing the original male, range 1–2 days). On nest takeover, the embryos were digitally photographed again to accurately quantify the starting brood size under allopaternal care. Each caregiving male in the paternal and allopaternal care treatment groups were also given a unique dorsal fin mark as described above for future identification. Lastly, in the no care treatment, original males were removed from the nest, but no new males were permitted to take up the nest. In order to hinder any further nest takeovers by a new male, *all* nests were covered with plastic mesh (mesh size ~1″ × 1″). We returned to check these nests, lifting the plastic mesh, 14- and 28-day postmanipulation to verify the presence of the marked guarder male (or in the case of no care nests, to verify that a male was still absent). We also took a digital photograph of each brood for later quantification of changes to embryo number and development. At the beginning of the experiment, there were no differences in standard length of the male care givers (t-test, t = 1.73, df = 19.0, P = 0.10, mean standard length ± SE = 19.1 ± 0.6 cm, range: 14.9–23.6 cm) or initial brood sizes (Anova, $F_{2,36}$ = 2.85, P = 0.07, mean brood size ± SE = 535.1 ± 62.2 embryos, range: 15–1320 embryos) between treatment groups.

We performed a GLM at each time point, specifying a quasibinomial error distribution (accounting for overdispersion, Kabacoff 2011) on the proportion of offspring still surviving from the original brood. At the 14-day time point, the model included parameters for treatment condition (paternal care, allopaternal care, no care), and initial brood size (embryo count). To test for an effect of male body size, this model was run again excluding the "no care" condition and including a parameter for male standard length (centimeter). At the 28-day time point, offspring survival was compared between treatment conditions, and parameters for male standard length (centimeter) and initial brood size (embryo count) were also included in the model.

Ethical note

Plainfin midshipman fish are neither threatened nor endangered (Collette et al. 2010). All animal collections and handling were in accordance with the Canadian Department of Fisheries and Oceans rules. Fish were collected and studied in British Columbia, Canada, on scientific license XR 121 2014 and XR 81 2015 and in Washington, on Washington State scientific collections permit 14-147. All procedures were approved by the McMaster University Animal Research Ethics Board (AUP 13-12-52), DFO's Animal Care Committee (AUP 13-12-52), and the University of Washington Institutional Animal Care and Use Committee (AUP 4079-06) and are in line with the guidelines set by the Canadian Council on Animal Care (CCAC).

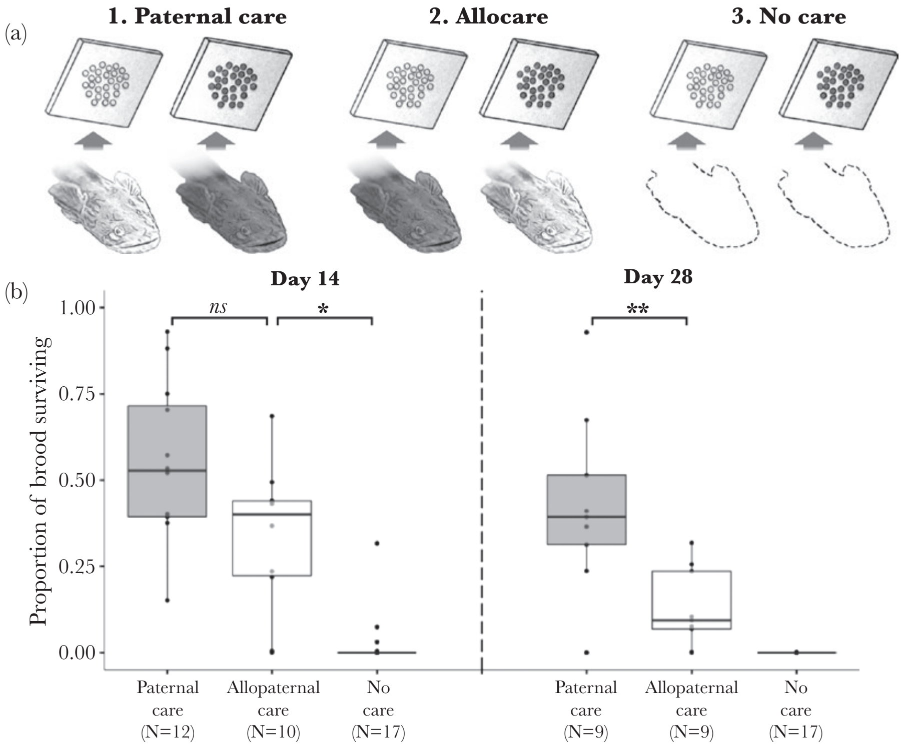

Figure 3
(a) A schematic of the design of Experiment 3. Offspring were either cared by the original male (paternal care), adopted by a new male (allopaternal care), or not cared by any male (no care). Nests under all conditions were covered with mesh barriers to hinder changes in nest ownership postmanipulation. Note the difference in embryo-to-male coloration between the paternal care and allopaternal care treatments (specific coloration patterns on the males are for illustration purposes only). (b) Proportion of the brood surviving under paternal care, allopaternal care, and no care at 14- and 28-day postmanipulation. Note that at day 28, the no care condition was not included in the analysis because offspring survival had dropped to zero with no variance.

RESULTS

Experiment 1: do males adjust parental care in response to direct cues of reduced paternity?

No, when we manipulated direct cues of paternity via offspring transplants, guarder males receiving transplanted foreign embryos were not more likely to abandon than males caring for their own embryos (GLM, day 3: $z = -1.1$, df = 62, $P = 0.29$; day 14: $z = -0.17$, df = 55, $P = 0.86$; day 28: $z = 0.19$, df = 43, $P = 0.85$; Figure 1b). Initial brood size did not significantly predict brood abandonment at any time point (GLM, day 3: $z = -0.32$, df = 62, $P = 0.75$; day 14: $z = 0.73$, df = 55, $P = 0.46$; day 28: $z = 1.3$, df = 43, $P = 0.18$) and neither did male body size (GLM, day 3: $z = 1.7$, df = 62, $P = 0.10$; day 14: $z = 0.94$, df = 55, $P = 0.35$; day 28: $z = 0.09$, df = 43, $P = 0.93$).

Males receiving transplanted foreign embryos successfully reared similar numbers of offspring compared with males who continued to care for their own offspring (GLM, day 3: $t = 1.03$, df = 53, $P = 0.31$; day 14: $t = 1.19$, df = 36, $P = 0.24$; day 28: $t = -0.60$, df = 25, $P = 0.55$; Figure 1c). Initial brood size did not significantly predict brood mortality at any time point (GLM, day 3: $t = 1.69$, df = 53, $P = 0.10$; day 14: $t = 1.69$, df = 36, $P = 0.06$; day 28: $t = 0.98$, df = 25, $P = 0.34$). Male body size had no detectable influence on offspring survival at any time point (GLM, day 3: $t = -0.91$, df = 53, $P = 0.37$; day 14: $t = 1.0$, df = 36, $P = 0.30$; day 28: $t = 1.34$, df = 25, $P = 0.19$). It should be noted that although the offspring on these tiles had matured by day 28, the offspring had not yet reached the free-swimming stage of nest independence. This suggests that offspring disappearances were due to mortality rather than having matured and left the nest on their own. Interestingly, when a caregiving male was absent from a nest, it was common for the nest cavity to either have filled in completely with sediment or to be occupied by several species of crab (*Cancer gracilis*, *Hemigrapsus oregonensis*, *Hemigrapsus nudus*, *Pagurus* spp.).

Experiment 2: do males prefer nests with embryos and do they prefer their own embryos?

Yes, males were more likely to take up residence in nests containing embryos (23 males chose nests with embryos, whereas 7 males chose empty nests, Figure 2b, GLM, intercept: $z = -2.52$, $P = 0.012$). Of the 38 males used in this trial, 30 were found to have chosen a nest after 24 h. Male choice of nest was also not influenced by the developmental stage of the offspring (GLM, $z = 1.60$, $P = 0.11$), by male size (GLM, $z = -1.05$, $P = 0.29$), or by the size of the brood (GLM, $z = 0.53$, $P = 0.59$). Although males expressed a strong preference for embryo-containing nests over empty nests, they were not more likely to choose their own brood over a foreign brood (15 males chose their own broods, whereas 15 males chose foreign broods, Figure 2b, GLM, intercept: $z = -0.56$, $P = 0.58$). All 30 males used in this second trial were found to have chosen a nest after 24 h. Developmental stage of the offspring did not affect nest choice (GLM, $z = 0.78$, $P = 0.43$) nor did male size (GLM, $z = 1.58$, $P = 0.11$). However, although we attempted to size match to the best of our abilities, males did prefer the larger of the 2 broods (i.e., relative brood size = size of familiar brood − size of unfamiliar brood, GLM, $z = -2.44$, $P = 0.02$). Lastly, after 24 h, the broods had suffered on average 6.2 ± 1.5% mortality (i.e., offspring disappearance). However, the proportions of offspring found to be missing from own familiar broods did not differ significantly from unfamiliar foreign broods (Wilcoxon signed rank test, $V = 224$, $N = 30$, $P = 0.90$).

Experiment 3: is allopaternal care as effective as paternal care?

No, offspring survival was lower under allopaternal care than under paternal care. Although offspring survival declined in all nests over the care period, approximately 22.6% fewer offspring survived to day 14 in nests under allopaternal care compared with nests under paternal care. Although this difference was statistically significant in the model that ignored male standard length (i.e., the model including the "no care" condition, GLM, $t = 2.4$, df = 38, $P = 0.022$), it was no longer significant when male standard length was included (i.e., the model excluding the "no care" condition, GLM, $t = 1.48$, df = 21, $P = 0.16$; Figure 3b). By day 28, approximately 31.5% fewer offspring survived under allopaternal care than under paternal care. This difference was statistically significant (GLM, $t = 2.45$, df = 17, $P = 0.03$; Figure 3b). When no care was provided, survival dropped dramatically to a mere 4 ± 2% (mean ± SE) by day 14, which was significantly lower than the offspring survival recorded under allopaternal care at that time point (GLM, $t = -4.05$, df = 35, $P = 0.0003$). By day 28, offspring survival under no care had dropped to 0 ± 0% (mean ± SE). Initial brood size did not predict offspring survival at either time point ($P > 0.08$). Again, by day 28, the surviving offspring in these nests had not yet reached the stage of nest independence, suggesting that any offspring disappearances were due to mortality rather than fully developed offspring leaving the nest. In the absence of a caregiver, the nest cavities had often completely filled in with sediment.

DISCUSSION

Our study shows that a manipulation of an indirect cue of paternity can influence offspring survival, whereas a manipulation of a direct cue does not have such an effect. We show that male midshipman fish do not appear to use direct cues on which to solely base their parental investment decisions. Males showed no obvious preference for their own broods in either controlled choice tests in captivity or in transplant experiments in the field. Following a nest takeover, however, offspring survival declined in comparison with nests still under the care of the original parent. We also show that the presence of a caregiving guarder male is crucial for the survival of plainfin midshipman embryos. A key role of the male guarding a brood is to actively maintain the nesting cavity and to defend the brood against egg predators.

Why are direct cues not used for offspring recognition?

Plainfin midshipman guarder males do not appear to recognize their own offspring based on our manipulations of direct cues alone. Although guarding males were more likely to choose a nest that already contained embryos over an empty nest, when they were offered a choice between their own brood versus a foreign brood, males did not preferentially choose their own broods. Moreover, male abandonment rates and the survival of offspring did not differ between foreign transplanted offspring and control offspring, which remained with their original caregiver. The lack of response to direct offspring cues might mean that 1) plainfin midshipman males *cannot* identify their own offspring based on direct offspring cues alone or 2) plainfin midshipman males *can* detect their own offspring via direct offspring cues, but do not act on these cues. The choice not to act on a detected cue may occur when alternate sources of information about parentage, which we did not manipulate, are more reliable (see "evolved predispositions" in Neff and Sherman 2002). For example, if the probability of being cuckolded decreases over the breeding season, then males might rely on cues of paternity only early in the season when paternity is naturally more variable, and would not rely on these cues (i.e., have a predisposition to ignore these cues) later on when paternity is more certain. Cuckoldry and competition in midshipman fish are indeed more common early in the breeding season (Cogliati, Neff, et al. 2013; Bose, Cogliati, et al. 2014). However, all our experimental manipulations were conducted in the early season. Therefore, guarder males at this time would be expected to be least certain about their paternity and to be most attentive to potential cues of paternity loss.

It is also possible, if not likely, that caregivers assess *multiple* cues of paternity rather than just a single cue (Neff and Sherman 2002). For example, direct cues such as offspring odor may only be reliable indicators of paternity loss when they are also accompanied by a congruent indirect cue, such as the presence of a cuckolder in the nest during spawning. We did not control the presence of sneaker males near the experimental nests in this study. Interestingly, if direct cues are unreliable unless they are supplemented by an indirect cue, then parasitic cuckolder males can benefit. Cuckolding males that stealthily and successfully steal fertilizations without being detected, and thus do not elicit a reduction in paternal care by the cuckold, would have their offspring unwittingly raised by another male. This is in line with the observation that the nests of plainfin midshipman fish in the wild display wide variability in mean paternity (Cogliati, Neff, et al. 2013). Average paternity lost to other males has been estimated to be between 26% and 48% across different studies (Cogliati, Neff, et al. 2013; Cogliati, Balshine, et al. 2014). Future studies are now needed to investigate the importance of single cues of paternity in isolation versus multiple cues in combination.

Are indirect cues important for offspring recognition?

Guarder midshipman males do use indirect cues to inform their assessment of paternity over offspring. The act of taking over another male's nest provides a male with a reliable indirect cue of paternity, and so males would be able to follow a simple behavioral rule: "assume that offspring in a newly acquired nest are non-kin." Our observation that allopaternal care following a nest takeover was associated with lowered offspring survival is consistent with take-over males adhering to such a rule. The higher rates of offspring mortality observed under allopaternal care could have been driven by a number of different factors, including cannibalism by the alloparent, deficient fanning and cleaning of the young by the alloparent, and/or a decrease in defense against embryo predation. Several potential egg predators observed around the intertidal zone would have been small enough to still access the nest through the mesh barrier, including *H. oregonensis*, *H. nudus*, and *Pagurus* spp. Consistently, Bose, Cogliati, et al. (2014) recorded a strong likelihood for recent take-over males to have engaged in recent partial-brood cannibalism. Across disparate taxa, adopted offspring often receive less care than own offspring (e.g., African lions, *Panthera leo* L., Bertram 1975; fathead minnows, *Pimephales promelas*, Sargent 1989; Australian social spiders, *Diaea ergandros*, Evans 1998; spottail darters, *Etheostoma squamiceps*, Bandoli 2002).

Interestingly, nest takeovers in our study were not associated with complete termination of the offspring present in the nest. In a recent genetic study, Cogliati, Neff, et al. (2013) found that the oldest offspring in midshipman nests were commonly unrelated to the caregiver. The adoption of non-kin offspring has been documented in many animal taxa including mammals and birds (Riedman 1982), arthropods (e.g., Thomas and Manica 2005; Requena et al. 2013), and fishes (e.g., Rohwer 1978; Porter et al. 2002). Take-over males may be selected to still provide care for non-kin offspring if the alloparent receives a fitness benefit for continuing with care. For example, females may prefer to mate with males that are already caring for offspring (Coleman and Jones 2011). Females may have this preference if the presence of young in a male's nest indicates that he is a high-quality mate or parent, or if laying eggs where other offspring already exist dilutes their predation risk (Kraak 1996). This may explain take-over males' apparent tolerance for some non-kin offspring in their nests. It may also explain why the males in our nest-choice trials rejected empty nests in favor of nests that had eggs, and also preferred to take up nests that had larger broods. However, whether female plainfin midshipman fish display a preference for laying eggs where other eggs already exist still requires explicit testing.

In this study, we used a combination of lab and field studies to manipulate both direct and indirect cues of paternity loss in the nests of breeding guarder plainfin midshipman males. We expected guarder males to reduce parental effort in response to cues of lost paternity loss because 1) plainfin midshipman naturally and commonly experience lost brood paternity due to high rates of nest takeovers and cuckoldry (Brantley and Bass 1994; Cogliati, Neff, et al. 2013; Bose, Cogliati, et al. 2014) and 2) paternal care for non-kin offspring is costly, because care is prolonged and physiologically demanding (Bose, Cogliati, et al. 2014; Bose, McClelland, et al. 2015) and because nest space for eggs is limited (DeMartini 1991). We show that the act of taking over a brood from another male can be a reliable indirect cue of relatedness to that brood and that offspring survival was reduced following such nest takeover events. We also show that direct offspring cues are either undetectable by guarder males or ignored as unreliable sources of paternity information when presented in isolation.

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