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High degree of paternity loss in a species with alternative reproductive tactics

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Abstract In many mating systems, males adopt alternative reproductive tactics (ARTs) to maximize reproductive success. In fishes, guarding males often invest more energy into courtship, defense, and paternal care, whereas cuckolding males forego such costs and steal fertilizations by releasing their sperm in the nest of a guarding male. These two tactics have been documented in the plainfin midshipman fish (*Porichthys notatus*), yet the relative reproductive success of the guarding and cuckolding male tactics remains unknown. In this study, we used microsatellite markers to determine the level of paternity of the guarding type I males. We explored how paternity varied with male phenotype and across the breeding season. Our results revealed the lowest documented levels of paternity in a species with obligate paternal care. Although paternity remained consistently low, it did increase as the breeding season progressed. Male body size did not significantly predict paternity. The low paternity in this species may be explained, in part, by aspects of their reproductive ecology including the duration of parental care period, limited nest availability and competition for nests, as well as the occurrence of nest takeovers. Overall, our findings contribute to the understanding of the ultimate factors underlying ARTs in this species and highlight the importance of investigating reproductive success across the entire breeding season.

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Introduction

Alternative reproductive tactics (ARTs) occur when there are two or more discontinuous reproductive phenotypes within a population, each attempting to maximize reproductive success through alternative means (Taborksy et al. 2008). One male morph, sometimes called guarding, bourgeois, parental, territorial, or type I, is typically associated with showy displays used to court females and deter rivals. The alternative male morph instead exploits the guarding male's investment in courtship by gaining reproductive success in sneaky or coercive ways. Such alternative male morphs are often called parasitic, sneakers, cuckolders, or type II males. Although many studies have described the existence of ARTs across a wide variety of taxa, relatively few studies have used molecular techniques to quantify the reproductive success of the male morphs.

In the last few decades, the application of molecular techniques to behavioral research has revolutionized our understanding of mating systems and the fitness consequences of mating behavior. These techniques have been applied to quantify individual reproductive success, and the reproductive success associated with the alternative male tactics (e.g., Shuster 1989; Brockmann et al. 2000; Neff 2001; Lank et al. 2002). Among fishes with obligate male care, the mean paternity of guarding males is typically greater than 80 % (for reviews, see Avise et al. 2002; Coleman and Jones 2011). Møller and Thornhill (1998) predicted that the degree of male parental care should be negatively correlated with extra-pair paternity, such that low paternity is only expected in species where male care is not required or important for offspring survival (also see Møller 2000). This hypothesis was developed based on avian mating systems, where paternal care is typically a depreciable resource; that is, there are greater costs imposed on the parent for each additional offspring in the nest (Altmann et al. 1977; Clutton-Brock 1991). Although paternal care in fishes is often thought to be nondepreciable, parental investment can increase in relation to brood size (Gross 1980; Coleman et al. 1985; Coleman and Fischer 1991). Thus, the Møller and Thornhill (1998) hypothesis should still hold for many fish mating systems, yet several fish species with obligate male care have low paternity (e.g., Bessert et al. 2007; Neff and Clare 2008; Alonzo and Heckman 2010). For example, Alonzo and Heckman (2010) found that paternity in the ocellated wrasse (*Symphodus ocellatus*) was around 72 % and they argued that their data did not lend support for the Møller and Thornhill (1998) hypothesis. Instead, other aspects of an animal's mating system and ecology might also be important in governing parental care patterns, including the opportunity for remating, the temporal distribution and density of females, and resource availability (Emlen and Oring 1977; Westneat and Sherman 1992; Avise et al. 2002).

Among the studies that have investigated the paternity of male ARTs, only a handful have incorporated temporal variation in reproductive success (Sefc et al. 2009; Alonzo and Heckman 2010; see also Coleman and Jones 2011). Temporal variation in reproductive success has long been recognized as an important factor influencing mating system dynamics (e.g., Schultz 1993; Verhulst et al. 1995; Oliveira et al. 1999) as well as an important factor to consider when calculating the relative reproductive success of the ARTs (e.g., Neff and Clare 2008). Importantly, measures of cuckoldry rates calculated at one time point during the reproductive season may not accurately reflect the relative reproductive success of each tactic overall (Neff and Clare 2008; Sefc et al. 2009). As proposed by Neff and Clare (2008), temporal variation in paternity could arise from (1) variation in the number of spawning males and females across the season, (2) variation in male body condition and the ability to effectively defend against cuckolders, and (3) variation in the number of reproductively active cuckolders across the season. In this study, we determined the reproductive success of the "type I" male tactic, and how this varied across a protracted breeding season, in a species with ARTs, the plainfin midshipman (*Porichthys notatus*).

The plainfin midshipman is a nocturnally active deepwater marine fish distributed along the Pacific Coast of North America (Hubbs 1920; Arora 1948; Miller and Lea 1972). It is characterized by two distinct reproductive tactics known as guarding type I and cuckolder "type II" males (Brantley and Bass 1994). At the onset of the breeding season in early spring, reproductive adults undergo a large-scale vertical migration from deep waters (>200 m) to the spawning grounds in calm rocky shores in the intertidal zone (Arora 1948; Miller and Lea 1972). Type I males aggressively compete for limited nest sites, and the largest males typically win the largest nest sites (DeMartini 1988). Larger males and larger nests attract more females (DeMartini 1988) and also attract more type II males (Lee and Bass 2004). Type I males acoustically court females (Ibara et al. 1983; Bass 1992; Brantley et al. 1993), who produce only a single clutch of fewer than 200 eggs (each 5–7 mm in diameter) per year, which are released into a single nest (DeMartini 1990). Type I males will remain in the nest and continue to court and spawn with additional females until the nest is filled with multiple "age cohorts" that typically are at different developmental phases (Arora 1948; DeMartini 1988; Brantley and Bass 1994). Midshipman fishes have direct development, where embryos transition into juveniles with no major morphological remodeling or a distinct larval phase (Balon 1999). The offspring developmental phases include embryos (pre-hatching), free embryos (post-hatching), and juveniles (first oral feeding, but may still be absorbing their yolk sacs, Crane 1981; Balon 1999). Offspring "ages" can be inferred by current developmental status. It takes about 60 days for embryos to develop into free-swimming juveniles, and because there are multiple cohorts acquired across the breeding season, type I males may be caring for offspring for up to 4 months (Arora 1948; KC, personal observation). Paternal care involves fanning and brushing the eggs and hatched embryos, to help keep them clean and deliver fresh water and oxygen (Arora 1948). Because larger broods typically require more fanning behavior (Coleman and Fischer 1991), this aspect of parental care in midshipman is likely depreciable. Type II males, on the other hand, do not guard nests or court females. Instead, they steal fertilizations from type I males by either sneaking into the nest when a female is present or fanning sperm into the nest from the periphery. Interestingly, type I males are behaviorally plastic and may adopt cuckolding behaviors, particularly when nesting sites are severely limited (Lee and Bass 2004).

Although alternative reproductive tactics have been well described in plainfin midshipman (Brantley and Bass 1994), the genetic reproductive success of each tactic has never before been examined. The aim of this study was to determine the level of paternity assigned to the nest guarding type I males and, in turn, the relative rate of cuckoldry in this species. In addition, given the lengthy breeding and parental care period and the presence of multiple age cohorts within a nest, we explored how male phenotype and the timing in the breeding season influenced paternity. Specifically, we postulated that if male condition deteriorated as the breeding season progressed, then paternity should decrease due to a decrease in males' abilities to effectively defend their nest against cuckoldry.

Methods

Field collections

Between May 5 and June 5, 2008, May 18 and May 27, 2009, and May 10 and July 14, 2010, we located and sampled plainfin midshipman nests during low tide in the intertidal zone on three rocky beaches in British Columbia (Ladysmith Inlet: 49°01′N, 123°83′W and Mill Bay: 48°63′N, 123°53′W on the eastern coast of Vancouver Island, and Crescent Beach: 49°04′N, 122°88′W in South Surrey). At each nest, we measured total length (TL in centimeter), standard length (SL in centimeter), and body mass (in grams) of each fish present and sexed each fish (based on the shape of the urogenital papilla and overall body coloration). Using mass and length values, we calculated a body condition index using the residuals from the regression of log (mass) against log (length) (Fechhelm et al. 1995; Blackwell et al. 2000). We digitally photographed each nest so that we could later quantify the offspring in terms of number, size, and developmental phase (embryo, free embryo, juvenile). We collected a small amount of fin tissue from each adult for genetic analyses and preserved the tissue in 95 % ethanol. In addition, for a subset of nests, we collected and preserved 40–50 offspring from each cohort present within the nest that had suitable embryonic development for genetic analyses (2008, *N*=10; 2009, *N*=13; 2010, *N*=24). Here, we define a “cohort” as a group of offspring laid by one or more females in a nest in relative synchrony such that they are developing together, but distinct from other cohorts in the same nest based on age and the timing of egg laying. Thus, cohorts in the nests may be from multiple offspring developmental phases. Embryos were suitable for genetic analyses once they passed the blastodermic cap stage, when the neural tube was visible through the egg envelope. After sampling the adults and the offspring, we returned the adults to the nest and carefully replaced all rocks to their original position.

In 2010, we also estimated the duration of male parental care and nest tenure by labeling 131 nests with a marked plastic tent peg positioned by the nest and returned to these nests in subsequent low tides. In 61 of these 131 nests, we tagged the type I male in the dorsal fin tissue between fin rays with a unique four-mark code of nontoxic injectable Elastomer (Northwest Marine Technology, Inc., WA, USA). On return to the labeled nests, we took morphological measurements from all fish present, collected fin clips from all nontagged fish, rephotographed the nests, and collected a sample of any embryos (new cohort) that were not previously present in these nests. Additionally, we determined if the original type I male was still the nest owner or not based on the unique code. The majority of the nests included in our genetic analyses in 2010 were marked and monitored over the season (17 of 24 nests); however, seven were located for the first time late in the breeding season and therefore only sampled once.

Embryonic development classification

Because we collected across the breeding season, cohorts were at different development phases. We therefore used a back-calculation to determine a Julian date of egg laying for all cohorts in each nest. We developed this back-calculation based on the cohort’s current development state and the Julian date of collection. During both sampling and DNA extractions, we observed key embryonic development characteristics (Balon 1999). From these characteristics, we developed a classification scheme that placed each sample into one of nine distinct categories based on the degree of embryonic development. We tested the reliability of our classification assignment (interobserver reliability was 87 %). Four of 30 samples were inconsistently classified among observers, but in those cases, the discrepancy involved one (of three) observers classifying the sample either one category before or after the classification designated by the other observers. In addition, we used photographs of broods from nests that were sampled multiple times during the breeding season to ground-truth our technique and to determine the numbers of days between development categories represented in the nests. For each cohort in our analyses, we determined the developmental category and then subtracted the back-calculated number of days for that developmental category from the sample date to arrive at the date of egg laying.

Genetic analyses

In total, we extracted DNA from 222 adults and 1,713 offspring (approximately 25 offspring per cohort per nest) and genotyped these individuals using six microsatellite loci developed from plainfin midshipman (Pon22, Pon23, Pon25, Pon30, Pon32, and Pon47; see Suk et al. 2009 for primer sequences). We carried out PCR amplifications on T1 Thermocycler (Whatman-Biometra) using fluorescent-dyed forward primers (Sigma-Genosys, Woodland, TX), following the protocol outlined in Suk et al. (2009), and conducted fragment analyses on a capillary sequencer (CEQ 8000, Beckman Coulter; Western University). Microsatellite alleles for each locus were scored for each individual based on characteristic peaks.

Using adult male and female genotypes, we investigated whether individuals from our three different sampling sites were from distinct genetic populations using *structure* (v2.3) software (Pritchard et al. 2000). Structure indicated a single panmictic population (data not shown, consistent with results described in Suk et al. 2009). Therefore, we calculated population-level allele frequencies for each locus using all adult genotypes.

Paternity analyses

We calculated the paternity estimate for each type I male using the genotypes of the male and offspring (offspring were required to show at least three representative loci for inclusion in the analysis), the allele frequencies of the breeding population, and the *two-sex paternity* model developed in Neff et al. (2000a, b) and Neff (2001). The two-sex paternity model calculates the proportion of the offspring that is compatible with the putative father (ng_{dad}), the expected proportion of offspring that are compatible with the putative father by chance (NG_{dad}), an estimated paternity measure expressed as a proportion, and a 95 % confidence interval (CI). Males with multiple cohorts present in their nest had an additional model constructed to obtain individual paternity estimates for each cohort.

In addition to calculating paternity, we used COLONY (v2.0) software to calculate the number of females that were genetically represented in the sample of offspring that also belonged to the type I guarding male found in the nest (Wang 2004; Jones and Wang 2010). We had very low paternity estimates in some cases (<10 %), which we considered to be a possible result of nest takeovers. For those cohorts, we used COLONY to infer the likely male genotype of the previous nest owner, as well as the number of possible females represented, based on best family grouping assigned by the program (Wang 2004; Jones and Wang 2010). COLONY is unable to differentiate male from female genotypes. Using the distribution of maternity values within a cohort, paternity values were typically equal to or greater than the maternity values (in 48 of 62 cohorts). In addition, there were 3 out of 62 cohorts where only one female was represented (100 % maternity), while there were 11 cohorts out of 62 where the male was the sole father for that cohort (100 % paternity). Therefore, we chose the most common genotype represented in COLONY as that of the previous type I male nest owner. The inferred genotype of the previous nest owner was then used in the two-sex paternity model to obtain a new paternity estimate with confidence intervals. We followed Jones et al. (2010) and used the two-sex paternity model rather than COLONY to calculate the parentage in all cases so that we could use the calculated confidence intervals in further analyses. Also, the two-sex paternity model is especially useful in species with nest-holding males and suspected cuckoldry (Jones et al. 2010).

Statistical analyses

For all statistical analyses, we arcsine square root transformed the paternity estimates. Paternity estimates calculated in the two-sex paternity model have individual 95 % CI indicating varying levels of confidence for each estimate. Therefore, we weighted all paternity data by their confidence using log (1/CI + 1). We checked data for normality and then ran one-way ANOVAs to test for the effects of location (sampling beach) and collection year (2008–2010) and controlled for these effects in further analyses when these factors were significant.

To investigate how paternity varied on a temporal scale, we constructed a linear mixed-effects model with random slopes fit by restricted maximum likelihood (REML) using transformed paternity as our response variable and the back-calculated Julian date of egg laying as our predictor variable. We included nest ID as a random factor (to account for males with multiple paternity estimates), and the random interaction between Julian date and nest ID was also incorporated into the model. Similarly, we constructed additional linear mixed-effects models fit by REML using male phenotypic characteristics as predictor variables in place of Julian date of egg laying, keeping nest ID as a random factor.

Results

Paternity analyses

In total, we calculated paternity for 74 different cohorts within 47 separate nests. Some nests (N=30) had only one cohort and therefore only one associated paternity estimate, while other nests (N=17) had up to four cohorts and paternity estimates (two cohorts: N=8, three cohorts: N=8, four cohorts: N=1) (Table 1). Using all 74 paternity estimates calculated from all cohorts, the average paternity for type I males was 52 %±0.04 (mean ± SE; N=74, range 0–100 %). There were no differences in paternity based on sampling location (ANOVA: $F_{2,71}$= 0.34, p=0.71) or year of collection (ANOVA: $F_{2,71}$=0.86, p=0.43), so neither factor was included in further analyses. On average and within a cohort, males mated with 3.3±0.2 females (mean±SE; N=65, range 1–5). In general, we observed a decrease in paternity as the number of females that mated with the male increased ($F_{4,60}$=2.74, p=0.04; Fig. 1); however, post hoc comparisons revealed no significant difference in average paternity between males that mated with one, two, three, four, or five females (Tukey HSD: all p>0.12). The average NG_{dad} value (1-exclusion probability) calculated from our samples was 0.14±0.009 (mean ± SE, range 0.01–0.33), indicating that on average and within this population, 86 % (1–0.14) of all individuals could be excluded as a potential father by chance alone. There were no significant differences in the paternity values estimated using the

Table 1 Summary of paternity analyses from nests with multiple cohorts in 2010 in plainfin midshipman (*P. notatus*)

Nest	Cohort[a]	Date[b]	$N_{\text{offspring}}$	NG_{dad}[c]	ng_{dad}[d]	Pat (%)	95 % CI	Females[e]
1	1	May 31	28	0.30	0.36 (10)	8	1–45	1
1	2	June 14	25	0.30	0.84 (21)	77	29–91	2
1	3	June 28	24	0.31	0.33 (8)	3	0–44	1
2	1	June 5	24	0.11	0.79 (19)	77	48–90	3
2	2	June 12	24	0.11	0.63 (15)	58	25–77	4
2	3	July 3	25	0.11	0.92 (23)	91	69–97	1
3	1	May 10	25	0.13	0.00 (0)	0	0–13	0
3	2	May 18	24	0.14	0.75 (18)	71	41–86	5
3	3	June 10	24	0.15	0.88 (21)	85	60–95	3
4	1	May 18	24	0.14	0.75 (18)	71	39–86	5
4	2	May 25	25	0.14	0.60 (15)	54	20–74	3
4	3	June 10	24	0.15	0.79 (19)	76	45–89	2
5	1	May 9	26	0.17	0.85 (22)	81	54–92	2
5	2	May 17	24	0.17	0.58 (14)	50	15–71	3
5	3	June 24	26	0.17	0.38 (10)	26	3–51	2
6	1	May 23	25	0.21	0.56 (14)	44	8–67	5
6	2	May 30	30	0.21	0.50 (15)	36	5–60	5
6	3	June 21	29	0.21	0.90 (26)	87	60–95	3
7	1	May 9	23	0.12	0.83 (19)	80	54–92	4
7	2	May 17	24	0.15	0.42 (10)	31	5–57	5
8	1	May 12	25	0.12	0.00 (0)	0	0–13	0
8	2	June 3	25	0.12	0.84 (21)	82	56–93	2
8	3	June 10	24	0.12	0.75 (18)	72	41–86	3
8	4	June 24	24	0.12	0.92 (22)	90	68–97	4
9	1	May 29	25	0.05	0.76 (19)	75	52–88	4
9	2	June 18	25	0.05	0.48 (12)	46	23–65	5
10	1	May 11	48	0.06	0.65 (31)	62	44–75	5
10	2	May 29	25	0.07	0.80 (20)	79	57–91	4
11	1	May 21	25	0.11	0.68 (17)	64	37–81	4
11	2	July 2	25	0.15	0.48 (12)	39	10–62	5
12	1	May 23	25	0.05	0.20 (5)	15	2–37	4
12	2	July 6	25	0.05	0.44 (11)	41	19–61	2
13	1	June 3	23	0.03	0.00 (0)	0	0–14	0
13	2	June 24	25	0.03	0.80 (20)	79	59–91	3
14	1	May 7	25	0.11	0.00 (0)	0	0–13	0
14	2	June 3	22	0.11	0.95 (21)	95	74–99	2
14	3	June 10	25	0.12	0.92 (23)	91	69–97	3
15	1	May 14	24	0.25	0.42 (10)	22	2–53	2
15	2	June 5	25	0.23	0.64 (16)	53	12–75	4
15	3	June 18	25	0.22	0.40 (10)	23	2–52	3
16	1	June 7	25	0.08	0.48 (12)	43	17–64	3
16	2	June 14	24	0.08	0.38 (9)	32	8–55	1
17	1	June 20	25	0.11	0.04 (1)	0	0–18	0
17	2	June 28	25	0.12	0.92 (23)	91	70–97	4

[a] The different age cohorts collected for each nest, shown in chronological order from the oldest to youngest offspring

[b] The back-calculated date on which the eggs were likely laid in the nest (see text for details), all in year 2010

[c] The expected proportion of offspring compatible with the father by chance alone; analog of the exclusion probability, calculated according to Neff et al. (2000a,b)

[d] The proportion of offspring (and number) that was compatible with the putative father calculated according to Neff et al. (2000a, b)

[e] The number of females that sired the compatible offspring of the putative father, determined using COLONY

two-sex paternity model versus those generated by COLONY (paired *t* test: $t_{86}=1.13$, $p=0.26$), and, as expected, the paternity values from each of these programs were highly correlated ($R^2=0.72$, $p<0.0001$).

Temporal and phenotypic patterns of paternity

Paternity increased across the breeding season (Julian date $F_{1,29.39}=5.59$, $p=0.02$). To determine if this seasonal variation

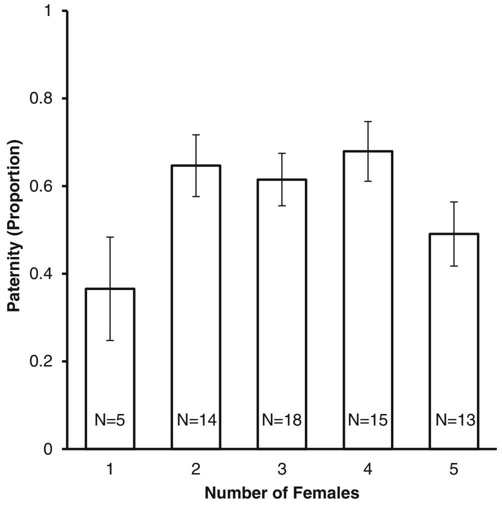

Fig. 1 Paternity in plainfin midshipman (*P. notatus*) as a function of the number of females that mated with the nest owner per cohort. Statistics were performed on weighted transformed data, but for clarity, we present the raw data here. *Bars* represent mean values ± 1 SE

in paternity may have arisen from variation in total brood size, we included brood size as a predictor variable in our original model and found no effect ($F_{1,39.08}$=0.48, p=0.49). Likewise, male size, mass, and body condition, based on the residuals of body mass and body length, were not significant predictors of paternity when these were included in the linear mixed-effects model (standard length: $F_{1,32.04}$=0.02, p=0.90; body mass: $F_{1,19.82}$=0.91, p=0.35; mass by length residuals: $F_{1,50.71}$= 0.71, p=0.40). Taken together, these results indicate that paternity increased later in the season regardless of brood size, male size, and male body condition.

Nest takeovers and loss of paternity

Of the 47 nests sampled in our study, 11 had paternity estimates of less than 10 % for at least one cohort (Table 2). When we compared paternity based on multiple cohorts sampled and controlling for the random effect of nest, these low values of paternity were found significantly more often in the oldest or most developed offspring ($F_{3,13}$=39.98, p<0.0001; Fig. 2). Similarly, when we controlled for multiple cohorts collected from some of the nests using the linear mixed-effects model, we found that the low paternity values occurred significantly more often earlier in the breeding season ($F_{1,20.73}$=11.82, p=0.003). Previous changes in male resident status may have contributed to these low values of paternity. Looking at the 131 nests that we monitored in 2010, we found that 45 % were no longer in use or were abandoned after our initial sampling, 34 % of the resampled nests were occupied by new type I males, while the original type I male remained in only 21 % of the resampled nests. Among the 34 % of nests where a new male had been observed, the new takeover males were not significantly larger (matched pairs—length: $t_{1,31}$=1.45, p=0.16; mass: $t_{1,23}$=1.52, p=0.14) or in better condition (residuals matched pairs: $t_{1,23}$=0.33, p=0.74) when compared to previous, now missing, nest owners. Furthermore, males that remained on the nest throughout the season were not larger or in better condition than either males who lost or abandoned their nests or males that took over nests (all body measures taken: $F_{2,79}$<1.65, p>0.20).

Average paternity excluding nest takeovers

Considering cohorts with a likely takeover, average paternity for the previous type I male calculated using COLONY and the two-sex paternity model was 66 %±0.06 (mean ± SE; N=12, range 35–100 %). When we excluded potential takeover events (where paternity estimates were less than 10 %) and included the estimates of the previous putative type I male, average paternity for type I males rose from 52 to 63 %±0.03 (mean±SE; N=74, range 15–100 %). Also, egg laying date no longer significantly predicted paternity when we included these paternity estimates of the previous nest owners in place of the very low paternity values in our original linear mixed-effects model ($F_{1,22.20}$= 1.32, p=0.26). That is, our initial pattern for seasonal variation in paternity appeared to be driven by these very low paternity estimates and likely takeovers occurring early in the season.

Discussion

Our study documents the lowest reported levels of paternity in a vertebrate with male-only care; on average, only 52 % of the young in plainfin midshipman nests could be assigned to the adult type I male guarding them. Additionally, paternity remained consistently low even after accounting for likely nest takeovers by type I males. In two reviews of paternity in fishes, Avise et al. (2002) and Coleman and Jones (2011) showed that in species with alternative male tactics, the typical paternity for the guarding male tactic was generally around 80 % or higher. A number of authors have argued that paternity cannot be any lower in species where male care is required and important for offspring survival (Møller and Thornhill 1998; Møller 2000). This argument, however, does not hold for plainfin midshipman where paternal care is obligate and, at least to some degree, depreciable.

There are several important ecological and reproductive differences between plainfin midshipman and other species with published paternity estimates that may explain why our observed levels of paternity are relatively low. First, the costs of missed foraging opportunities associated with parental care vary across species. Ocellated wrasse (72 % mean paternity to

Table 2 Summary of paternity analyses from nests with suspected takeovers in plainfin midshipman (*P. notatus*)

Nest	Cohort[a]	Male[b]	Date[c]	$N_{\text{offspring}}$	NG_{dad}[d]	ng_{dad}[e]	Pat (%)	95 % CI	Females[f]
1	1	1	May 28, 2008	12	0.08	0.08 (1)	1	0–34	0
1	1	Inferred 1	May 28, 2008	12	0.01	0.58 (7)	58	31–80	2
2	1	2	May 19, 2008	24	0.16	0.08 (2)	0	0–22	0
2	1	Inferred 2	May 19, 2008	24	0.35	0.88 (21)	81	28–93	2
3	1	3	May 20, 2009	12	0.09	0.17 (2)	8	1–43	2
3	1	Inferred 3	May 20, 2009	12	0.03	0.75 (9)	74	44–91	4
4	1	4	May 20, 2009	19	0.09	0.00 (0)	0	0–17	0
4	1	Inferred 4	May 20, 2009	19	0.02	0.47 (9)	46	25–68	3
5	1	5	May 20, 2009	15	0.19	0.13 (2)	0	0–34	2
5	1	Inferred 5	May 20, 2009	15	0.09	0.47 (7)	42	14–68	2
6	1	6	June 3, 2010	23	0.03	0.00 (0)	0	0–14	0
6	1	Inferred 6	June 3, 2010	23	0.06	0.96 (22)	95	77–99	2
6	2	6	June 24, 2010	25	0.03	0.80 (20)	79	59–91	3
7	1	7	May 12, 2010	25	0.12	0.00 (0)	0	0–13	0
7	1	Inferred 7	May 12, 2010	23	0.02	0.64 (16)	63	43–79	2
7	2	7	June 3, 2010	25	0.12	0.84 (21)	82	56–93	2
7	3	7	June 10, 2010	24	0.12	0.75 (18)	72	41–86	3
7	4	7	June 24, 2010	24	0.12	0.92 (22)	90	68–97	4
8	1	8	May 31, 2010	28	0.30	0.36 (10)	8	1–45	1
8	1	Inferred 8a	May 31, 2010	28	0.06	0.39 (11)	36	14–56	3
8	2	8	June 14, 2010	25	0.30	0.84 (21)	77	29–91	2
8	3	8	June 28, 2010	24	0.31	0.33 (8)	3	0–44	1
8	3	Inferred 8b	June 28, 2010	24	0.09	0.58 (14)	54	24–74	1
9	1	9	May 7, 2010	25	0.11	0.00 (0)	0	0–13	0
9	1	Inferred 9	May 7, 2010	25	0.03	0.80 (20)	79	69–97	4
9	2	9	June 3, 2010	22	0.11	0.95 (21)	95	74–99	2
9	3	9	June 10, 2010	25	0.12	0.92 (23)	91	69–97	3
10	1	10	May 10, 2010	25	0.13	0.00 (0)	0	0–13	0
10	1	Inferred 10	May 10, 2010	25	0.11	1.00(25)	100	85–10	2
10	2	10	May 18, 2010	24	0.14	0.75 (18)	71	41–86	5
10	3	10	June 10, 2010	24	0.15	0.88 (21)	85	60–95	3
11	1	11	June 20, 2010	25	0.11	0.04 (1)	0	0–18	0
11	1	Inferred 11	June 20, 2010	25	0.07	0.68 (17)	65	43–82	3
11	2	11	June 28, 2010	25	0.12	0.92 (23)	91	70–97	4

[a] The different age cohorts collected for each nest, shown in chronological order from oldest to youngest offspring

[b] Using genotype data from the type I male found on the nest (number only) or using inferred genotypes obtained from COLONY (inferred number)

[c] The back-calculated date on which the eggs were likely laid in the nest (see text for details)

[d] The expected proportion of offspring compatible with the father by chance alone; analog of the exclusion probability, calculated according to Neff et al. (2000a, b)

[e] The proportion of offspring (and number) that was compatible with the putative father calculated according to Neff et al. (2000a, b)

[f] The number of females that sired the compatible offspring of the putative father, determined using COLONY

guarding males), bluegill sunfish (*Lepomis macrochirus*, 77 % paternity), and pumpkinseed sunfish (*Lepomis gibbosus*, 62 % paternity) all breed repeatedly throughout the season, but males in each of these three species have time between broods to replenish energy stores before they rebuild new nests in subsequent reproductive bouts. The broods that are cared for also develop quickly (3–10 days), and all young undergo development in synchrony (e.g., Neff and Clare 2008; Alonzo and Heckman 2010). Hence, the time between broods provides foraging opportunities that plainfin midshipman male parents do not have. Plainfin midshipman also have a protracted breeding season, with a long embryo development

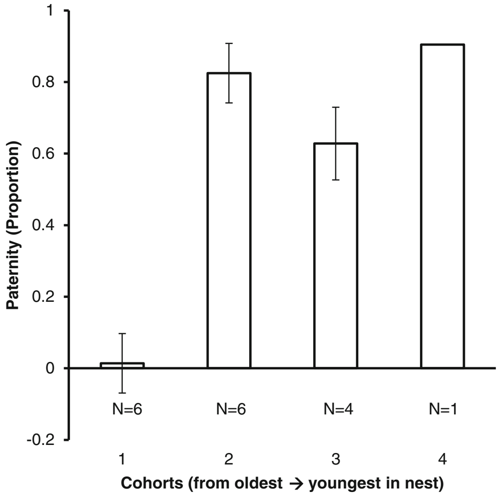

Fig. 2 Proportion of paternity based on cohort in plainfin midshipman (*P. notatus*). Data included nests that had a possible takeover event and multiple cohorts sampled (less than 10 % paternity, *N*=6), from oldest (*1*, laid earlier in season) to youngest (*4*, laid later in season) offspring. Statistics were performed on weighted transformed data controlling for nest, although the raw data are presented. *Bars* represent mean values±1 SE

time (50–65 days), and they have staggered offspring development (Arora 1948; Sisneros et al. 2009; KC, personal observation). Thus, the high cost to care associated with missed foraging opportunities and duration of care in plainfin midshipman may give rise to a greater degree of cuckoldry as a means to avoid the investment in parental care.

Second, nest site availability appears to be more limited in plainfin midshipman compared to these other species with ARTs (DeMartini 1988, 1991). The high male–male competition for nesting sites has probably led to the evolution of the cuckolding tactic (type II males), as well as to the behaviorally plastic cuckoldry behaviors seen by smaller type I males (Lee and Bass 2004; KC, personal observation) and possibly to males tolerating a high degree of paternity loss. This pattern is mirrored by a study of fathead minnows (*Pimephales promelas*), which estimated 95 % paternity to the guarding male when nesting substrate was not limited, but only 79 % when nesting substrate was limited (Bessert et al. 2007). Our two hypotheses (costs associated with missed foraging opportunities and limited nest availability) are not mutually exclusive, and it is possible that the low observed paternity in plainfin midshipman is driven by both factors (Emlen and Oring 1977; Clutton-Brock et al. 1997; Coltman et al. 1999; Bessert et al. 2007).

In addition to the partial paternity loss arising from sneaker males, some guarding males experienced a complete paternity loss in some cohorts within their nest. In fact, 17 % of nests exhibited a complete loss of paternity for at least one cohort in the nest, and another 6 % of nests exhibited nearly complete loss of paternity (less than 10 % paternity) for at least one cohort in the nest. Three scenarios could explain these very low paternity levels in plainfin midshipman: (1) it may be a result of some sterile type I males in our population, (2) it may be a result of highly successful cuckoldry, or (3) it could arise from nest takeovers. A nest takeover is an extreme form of male–male competition where males continue to compete for nest sites even after a male has "won" a nest and previously spawned with one or more females in the nest (see Coleman and Jones 2011). Our examples of very low paternity occurred significantly more often in the oldest offspring in nests when multiple cohorts were present (five of six nests, six of seven cohorts). This pattern lends support for the occurrence of nest takeovers in plainfin midshipman, rather than sterile males or highly successful cuckoldry, both of which would not show temporal variation. Our findings are similar to those of Bessert et al. (2007) where the low paternity values in fathead minnows were always in the oldest cohort, a finding they also used to infer takeovers. Also, when we sampled across the breeding season, we observed a new untagged male in 34 % of our sampled nests. This value is higher than what was generated by our paternity analyses and it likely included both cases of takeover and nest abandonment by type I males. Taken together, these data indicate that nest takeovers occurred in at least 23 % of nests in plainfin midshipman.

Why would new takeover males guard the eggs of a previous competitor? First, takeovers may be necessary when nests are a limited resource such as in plainfin midshipman (DeMartini 1988; DeWoody et al. 2000; Coleman and Jones 2011). Takeover rates differed between substrate-limited (29 %) and substrate-unlimited (5 %) sampling sites in the fathead minnow (Bessert et al. 2007). We found that nest takeovers occurred more often early in the season (83 % of observed takeovers occurred in the first half of the breeding season), likely while males were still competing for these limited nest sites on a particular stretch of beach. As the season progressed, the occurrences of takeovers became less frequent, possibly because the males were more established in their nests and were less likely to desert or to move to a new nest when challenged (sensu Coleman and Gross 1991). Second, takeovers may be particularly beneficial if females have a strong preference for nests with eggs (Rohwer 1978; Ridley and Rechten 1981; Porter et al. 2002). Females may find the presence of eggs attractive because it increases the overall chances that her eggs will survive, largely through the dilution effect (Rohwer 1978; Foster and Treherne 1981). In threespine sticklebacks (*Gasterosteus aculeatus*; Ridley and Rechten 1981) and the sand goby (*Pomatoschistus minutus*; Forsgren et al. 1996), for example, females prefer to lay their eggs in nests that already contain eggs (Dugatkin 1992). Whether or not there is a female preference for eggs in the nest in plainfin midshipman is not yet known.

We found that paternity in plainfin midshipman varied across the breeding season with cuckoldry being more common early in the breeding season. We explored the cause of this variation using the three hypotheses proposed by Neff and Clare (2008). First, as predicted, we found more spawning activity earlier in the breeding season with more eggs being laid early compared to later in the breeding season (a mean of 761 eggs/nest early in the season versus 482 eggs/nest late in the season, KC, unpublished data). Second, while it is possible that males in poor condition are unable to effectively defend their nest against cuckolders, a hypothesis supported by the study in bluegill (Neff and Clare 2008; Cargnelli and Neff 2006), we found no evidence for such relationship in plainfin midshipman: type I male body condition did not vary across the breeding season nor did it correlate with paternity. Through our population monitoring, we observed type II males at equal frequencies across the breeding season (data not shown), refuting the third hypothesis that temporal variation in paternity could be driven by seasonal changes in sneaker number. However, some caution is warranted here because the frequency of type II males observed was low and we did not sample nests outside of low tide events. Finally, an additional possibility that arose from our study to explain seasonal variation in paternity was the occurrence of nest takeover events early in the breeding season. Altogether, our data highlight the importance of investigating reproductive success across the breeding season as opposed to one single time point to ensure accurate population-level paternity estimates.

The results of this study demonstrate low paternity levels in plainfin midshipman that are likely mediated by key aspects of their reproductive ecology, including patterns of parental care and limited resource availability. The comparatively low levels of paternity in plainfin midshipman also demonstrates the need for additional studies that investigate the genetic reproductive success in species with alternative reproductive tactics to further explore the variation in paternity among species and the underlying ecological driving forces. In addition, we have shown that nest takeovers occur and that they contribute to the overall low paternity in this species. Takeovers are likely a result of male–male competition among type I males for limited nest sites. These results collectively demonstrate that direct competition among males can play an important role in determining paternity in a species with alternative reproductive tactics.

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Ethical standards This work conformed to the guidelines outlined by the Canadian Council on Animal Care, and permission to undertake this work was granted by the Department of Fisheries and Oceans, and the First Nations Bands in which sampling occurred.

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

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