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Original Article

Postcopulatory consequences of female mate choice in a fish with alternative reproductive tactics

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Mate choice plays a well-known role in the evolution of secondary sexual traits important in precopulatory competition. However, few studies have linked mate choice with the evolution of postcopulatory competitive traits. Here, we explore how variation in male mating behaviors and female mate choice influences male investment in reproductive traits that enhance sperm competition, a form of postcopulatory male–male competition. By combining ecological and physiological data from wild plainfin midshipman (*Porichthys notatus*), a marine fish species with 2 alternative reproductive tactics (guarder and sneaker males), we show that female mate choice is associated with uneven sperm competition risk between male reproductive tactics as well as among males using the same reproductive tactic. Larger guarder males attracted more females and experienced higher rates of attempted cuckoldry compared with smaller guarder males. In turn, larger guarder males appear adapted to this increased sperm competition risk, producing faster sperm than smaller guarder males. Sneaker males (the smallest males of all) had faster swimming sperm, with larger sperm midpieces and smaller sperm heads than did guarder males. These results suggest that female choice can amplify the selection gradient acting on males both between and within reproductive tactics.

Key words: **male quality, reproductive investment, sexual selection, sperm competition, sperm phenotype, teleosts, toadfishes.**

INTRODUCTION

Female mate choice is firmly established as a force shaping the evolution of male traits important in precopulatory sexual selection (Darwin 1871; Andersson 1994). Yet little attention has been devoted to examining how female choice influences the strength of postcopulatory sexual selection (Wong and Candolin 2005; Andersson and Simmons 2006). Female mate choice is hypothesized to drive the evolution of divergent male phenotypes or alternative reproductive tactics (ARTs, Taborsky 1994, 1998; Alonzo and Warner 2000; Alonzo 2008). In species with ARTs, mating males within a single population use divergent behaviors to secure fertilizations. Large guarder males often build nests, defend territories, and court females, whereas small sneaker males attempt to surreptitiously participate in matings between larger guarder males and females, and thereby "steal" fertilizations (Taborsky 1994, 1998). These alternative male mating behaviors can evolve if strong female preference for high-quality males leaves few reproductive options available other than sneaking for smaller, less competitive males (Taborsky 1994, 1998; Alonzo and Warner 2000). Once established, the postcopulatory consequences of ARTs are clear: sneaker males release sperm only in the presence of another male, and hence experience a greater risk of sperm competition compared with the guarder males that are not necessarily cuckolded at every mating (Parker 1990; Taborsky 1994, 1998). This natural dichotomy in sperm competition risk makes species with ARTs convenient models to study evolutionary responses to sperm

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competition (Taborsky 1994, 1998; Simmons 2001; Montgomerie and Fitzpatrick 2009; Simmons and Fitzpatrick 2012).

The specific impact of female mating behavior on a males' risk of sperm competition has rarely been investigated (but see Reichard et al. 2007; Alonzo and Pizzari 2013; Requena and Alonzo 2014). Recent theoretical models suggest that female remating interval, patterns of sperm use, and sperm storage duration can influence the evolution of male ejaculate allocation strategies (Alonzo and Pizzari 2013; Requena and Alonzo 2014). However, female mate choice also has the potential to influence the degree of postcopulatory competition among guarder males by, for example, leading cuckolding males to preferentially parasitize the males who possess traits strongly preferred by females, such as the loudest song, brightest colors, largest body size, or highest quality territories (Waltz 1982; Reichard et al. 2007). Therefore, female mate choice can generate 2 effects that have important consequences for postcopulatory sexual selection. First, strong female preferences for high-quality males put the selective pressures in place for the evolution of sneaker males with more competitive ejaculates in comparison with conventional or guarder males, in line with classic sperm competition theory (Parker 1990). Second, if sneaker males preferentially parasitize those guarder males that are favored by females, either by releasing sperm when a preferred male releases sperm in externally fertilizing species or by mating with females before or after they have mated with a preferred male in internally fertilizing species, then variance in sperm competition risk should also occur *within* a male reproductive tactic (i.e., among guarder males), leading to a positive correlation between sperm competition risk and guarder male quality.

To explore the effect of female choice on sperm competition dynamics, we combined field ecological surveys of nests with physiological data on male reproductive traits of the plainfin midshipman (*Porichthys notatus*). Midshipman fish are an ideal model system to study sperm competition, as males exhibit 2 distinct reproductive tactics that have been extensively characterized (Brantley and Bass 1994; Lee and Bass 2004). In this Pacific North American marine fish, which breeds under rocks in the intertidal zone from April to August (Arora 1948), there are 2 types of males. Large guarder males, called Type I males, build and defend nests under rocks where they acoustically court females with a low frequency "hum" produced by rapidly contracting sonic muscles causing vibrations of their swim bladders (Ibara et al. 1983; Brantley et al. 1993). Small sneaker males, called Type II males, do not court females or defend a nest but instead steal fertilizations by releasing sperm in, at the periphery, or just outside of the guarding male nests (Brantley and Bass 1994). Gravid females are attracted by, and can discriminate among, the acoustic signals produced by the guarder males, as can other guarder and sneaker males (McKibben and Bass 1998).

Here, we examine how variance in sperm competition risk within and between tactics covaries with ejaculate traits of plainfin midshipman. To do so, we first performed a broad population survey to determine whether, and identify which, guarder males are preferred by female and preferentially parasitized by cuckolding males. We then compared male investment in reproductive tissue and in sperm morphology and performance between the 2 male ARTs to test the prediction from classic sperm competition theory (Parker 1990) that sneaker males invest more in ejaculates in response to their increased sperm competition risk than do guarding males. Finally, because our population survey revealed that the largest guarder males were subject to a greater risk of sperm competition, we also tested the prediction that the most preferred, largest guarder would exhibit more competitive ejaculates in response to the increased sperm competition risk they encounter. Our results both confirm classic sperm competition theory and point to previously unappreciated variance in within-tactic sperm competition risk that is driven by differential female mate choice for high-quality guarding males.

METHODS

Field locations

Sexually mature guarder and sneaker plainfin midshipman males were sampled from intertidal nests during low tides in the intertidal zone along Vancouver Island and the Vancouver mainland in British Columbia, Canada. Data were collected over 3 field seasons (2006–2008) from 5 field sites—Bamfield Inlet (June 2006, 2007; 48°81′N, 125°14′W), Nanoose Bay (June 2007; 49°26′N, 124°18′W), Mill Bay (June 2007; 48°63′N, 123°53′W), Ladysmith Inlet (June 2007; May and June 2008; 49°01′N, 123°83′W), and Crescent Beach (May 2008; 49°04′N, 122°88′W). Henceforth, field sites are specified as BI^{06}, BI^{07}, NB^{07}, MB^{07}, LI^{07}, LI^{08}, and CrB^{08} for males sampled from Bamfield Inlet, Nanoose Bay, Mill Bay, Ladysmith Inlet, and Crescent Beach, respectively. Superscripts associated with each field site indicate the year of data collection, with 06, 07, and 08 indicating samples collected in 2006, 2007, and 2008, respectively. To avoid observer bias, all samples were examined by a single researcher who was blind to the male's reproductive tactic, collection year, and field site.

Nest dynamics and cues of male quality and sperm competition risk

We performed population censuses of intertidal nests from LI^{08} throughout the breeding season to assess how sperm competition risk is distributed among males, which males are preferred by females, and how sperm competition risk and female preference change over time. In May (early in the breeding season) and June (later in the breeding season) 2008, we monitored 169 independent intertidal nests ($n_{\mathrm{May}} = 82$, $n_{\mathrm{June}} = 87$). Each nest was sampled once to provide a "snapshot" of reproductive dynamics at 2 time points in the breeding season. Nests were located by inspecting the underside of rocks exposed during low tides for the presence of a guarding male. In each nest, the presence/absence of eggs was recorded. In addition, whenever a nest was found, all fish (guarder and sneaker males and females) present on the nest were sexed, measured for standard length (cm) and body mass (g), marked with a unique dorsal fin clip, and returned to the nest. In nests containing 2 or more guarder males, we identify the nest-holding male as the fish positioned centrally under the egg clutch, and other males (henceforth referred to as "cuckolding guarder males") were identified by their "sneaking" position on the periphery of the nest with their heads positioned away from the nest and their genitalia pointed deeper in the nest, and oriented toward the eggs (see Lee and Bass 2004; Cogliati et al. 2013, 2014 for a more detailed description of guarder males that employ cuckolding behavior). We used chi-square tests to compare between early and later in the breeding season, the proportion of guarder males with eggs in their nests, and the number of females and sneaker males present on nests. Generalized linear models (GLMs) with binomial error distribution with logit link function were used to determine if a guarder male's body size influenced his ability to attract females by analyzing the effect of guarding male body mass on the presence/absence of eggs in nests. Two-tailed Student's *t*-tests were used to examine if guarder males with ≥2 females were larger than guarder males with only 1 female present on their nest. Wilcoxon signed-rank tests were used to assess if guarder males with cuckolding guarder males in their nests were larger than average (this was based on the mean body mass of guarder males in the population). To test if there was an association between female and sneaker presence on nests, we used a Fisher's exact test.

Clutch sizes and tactic-specific investment

Body mass was measured to the nearest 0.1 g in 34 guarder males from intertidal nests from BI^{07}, NB^{07}, MB^{07}, and LI^{07} (n = 8, 7, 15, and 4, respectively). A digital photograph was taken of each of these guarder males' nests and used to quantify brood size. Clear orange-colored eggs or eggs with discernible developing embryos were classified as living eggs. White eggs and eggs without yolks were classed as dead eggs, whereas egg casings or the presence of only the adhesive disk (used to attach eggs to the rocky nest ceiling) were classified as missing eggs (Sopinka et al. 2012; Cogliati et al. 2013). To estimate the strength of female choice for a particular nest/male, the relationship between total brood size (including live, dead, and missing eggs) and guarder male body size was assessed using a linear mixed model (LMM) including sampling beach as a random factor.

To compare reproductive investment between tactics, 19 guarder and 16 sneaker males were collected during low tides from intertidal nests at BI^{06}, BI^{07}, and CrB^{08} (n = 9, 17, and 9, respectively) and transported to the laboratory in 10–13 °C aerated seawater. In the laboratory, fish were given a lethal dose of anesthetic (MS-222 or Benzocaine), measured for standard length (from the tip of the snout to the end of the last vertebra) to the nearest mm, weighed to the nearest 0.1 g, and their testes, accessory glands, and sonic muscle organ were removed and weighed to the nearest 0.01 g. To compare absolute investment in reproductive organs, we used LMMs that included sampling site and year added as random factors. When assessing if males adopting different tactics invested differentially in reproductive investment, we used LMMs with somatic mass (i.e., body mass − organ mass) added as a covariate in models including sampling site and year added as random factors to the model. The sample size was reduced in models assessing investment in accessory gland, as this reproductive organ was not measured from the males collected from BI^{06}.

Sperm morphology

We collected 13 guarder and 13 sneaker males from BI^{07} and CrB^{08} (n = 17 and 9, respectively), which were used to assess tactic-specific investment (described above). Males were given a lethal dose of anesthetic and their testes removed, weighed, and split open with a scalpel. To assess sperm morphology, ~2 µL sperm from each male was placed in a 1.5-mL microcentrifuge tube, diluted with 1 mL seawater, and fixed by adding 0.5 mL buffered formalin. Sperm were examined with a light microscope under ×1000 magnification and a digital photograph was taken of ~10 spermatozoa per male using a mounted PixeLINK Megapixel PL-A662 digital camera (PixeLINK, Ottawa, Ontario, Canada). Midshipman have biflagellate (2-tailed) sperm (Stanley 1965). Because no difference was detected in the 2 flagellum lengths from a single sperm (repeated measures analysis of variance [Anova], $F_{1,480} = 4.91$, $P = 0.30$), the average length of the 2 flagella was used in all subsequent analyses. The sperm head length (HL, which houses haploid genetic material), midpiece length (ML, thought to reflect sperm adenosine triphosphate (ATP) production, Cardullo and Baltz 1991), flagellum length (FL, which provides the propulsive force, Gomendio and Roldan 1991), and total sperm length (TL = HL + ML + FL) were measured (to the nearest 0.1 µm) from digital images using NIH ImageJ software (v. 1.37) by tracing a freehand line over each sperm component using an Intuos graphic table (Wacom Co. Ltd., Japan). For each spermatozoa, we calculated the head length to flagellum length ratio (HL:FL), which reflects the relationship between sperm drag and propulsion and is hypothesized, based on biomechanical modeling, to be a more robust predictor of sperm swimming speed than measures of the individual sperm components (Humphries et al. 2008, but see Simpson et al. 2014) and used these to calculate an average HL:FL value for each male. Sperm components were compared between male reproductive tactics using LMMs that included sampling site as random factors to the model.

Sperm swimming speed

Sperm swimming speed was compared between and within male reproductive tactics from 15 guarding and 11 sneaking males collected from nests from BI^{06} and BI^{07} (n = 17 and 9, respectively). To assess sperm swimming speed, a drop of milt (sperm and seminal plasma) from the split testes was placed in a 2.0-mL microcentrifuge tube and diluted by rapidly adding 0.5 mL seawater to activate sperm. A 60-µL subsample of the sperm/water mixture was placed on a prefocused 1-mm deep-welled slide with a coverslip covering half of the depression as quickly as possible. Video recordings of sperm swimming speed began at the moment sperm were diluted with seawater, prior to viewing sperm under the microscope (Fitzpatrick et al. 2009a, 2009b). Sperm swimming speed was captured at 60 frames/s under ×200 magnification with a PixeLINK Megapixel PL-A662 digital video camera mounted on a Leica DME light microscope (Leica Microsystems Inc., Buffalo, NY). Images were recorded using PixeLINK PL-A600 Series Camera Software (v. 3.1).

Sperm swimming speed of each male was assessed from digital videos at 7 different time periods: 10, 30, 60, 120, 300, 600, and 900 s post-activation using the NIH ImageJ software manual tracking function (v. 1.37, available at http://rsb.info.nih.gov/ij/), where spermatozoa were manually tracked at each time period by following the center of the sperm's head for 74.6 ± 0.77 frames (mean ± standard error [SE]). Using this method, ~10 spermatozoa were recorded at each time period. We used the manual tracking function to determine sperm swimming speed rather than the more commonly used computer-assisted sperm analysis (CASA) approach because the videos captured for this study had many multiple overlapping sperm tracks that prevented accurate analysis using CASA. However, we have previously demonstrated in midshipman that sperm swimming speed values obtained by tracking ~10 sperm/time period using manual tracking are tightly correlated with values obtained using CASA (correlation coefficients range r = 0.74–0.82; Fitzpatrick et al. 2009a).

We tested the prediction that sneaker males would have faster swimming sperm than guarder males by comparing mean sperm swimming speed from each male at each sampling times. We used repeated measures LMMs that included reproductive tactic, time period, and the interaction between reproductive tactic and time as fixed effects and male identity and sampling year were included as random effects in the model. Differences between guarder and sneaker male sperm swimming speeds were assessed at each time period using post hoc linear contrast analyses. We also examined if larger guarder males produced faster swimming and larger sperm when compared with smaller guarder males by performing tactic-specific LMMs including body size as a covariate, time as a fixed effect, and male identity (for models assessing sperm swimming speed) and sampling year as random factors in the models.

General statistical analyses

All statistical analyses described above were performed with JMP (version 9.0, 2010; SAS Institute Inc., Cary, NC). Whenever males from multiple field sites or years were included in an analysis, we used LMMs fitted using a restricted maximum likelihood approach, including field site or year as a random factor in our models to account for possible differences across field sites or year. In all LMMs, continuous data were log transformed to achieve normally distribution. Nonsignificant interaction terms were removed from full models (which included all possible interactions) and reduced models are presented below.

RESULTS

Nest dynamics and cues of male quality and sperm competition risk

Monitoring 82 and 87 intertidal nests in May and June, respectively, revealed that mating and sperm competition dynamics shifted throughout the breeding season. The proportion of guarder males with eggs in their nests more than tripled from early to late in the breeding season; in May, eggs were found in 25.6% (n = 21) of nests surveyed, compared with June where eggs were found in 83.9% (n = 73) of the nests surveyed ($\chi^2 = 58.12$, $P < 0.001$). Female presence did not differ across the breeding season, with females in 19.5% (n = 16) of nests in May and 11.5% (n = 10) of nests in June ($\chi^2 = 2.08$, $P = 0.15$). In May, 13.4% (n = 11) of nests contained a cuckolding guarder male compared with only one such male found in nest in June, making cuckolding by guarder males 13 times more likely early in the breeding season compared with later in the breeding season ($\chi^2 = 9.63$, $P = 0.002$). Sneaker males were marginally more common on nests later in the breeding season, being found on 1.2% (n = 1) of nests in May versus 6.9% (n = 6) of nests in June ($\chi^2 = 3.43$, $P = 0.06$).

Guarder male body mass predicted whether or not males had eggs in their nests both early (GLM, $\beta = -1.58$, $\chi^2 = 5.58$, degrees of freedom [df] = 1, $n = 82$, $P = 0.02$, Figure 1a) and later ($\beta = -2.18$, $\chi^2 = 3.81$, df = 1, $n = 45$, $P = 0.05$) in the breeding season, suggesting that females preferentially visit nests of larger males throughout the breeding season. Of the guarder males sampled with females in their nest, those with multiple females (n = 6) were significantly larger than those with only 1 female (n = 10) in their nest (t-test, $t = 2.73$, df = 14, $P = 0.02$, Figure 1b). Guarder males found with cuckolding guarder males in their nest (mean male body mass ± SE: 186.4 ± 26.2 g) tended to be larger than average nest-holding males (mean average male body mass ± SE: 131.4 ± 5.9 g; Wilcoxon signed-rank test, $H = 9.5$, $n = 6$, $P = 0.06$: the sample size is reduced for this analysis as body mass data were not collected for all guarder males). Guarder males employing the cuckolding strategy (mean male body mass ± SE: 79.0 ± 12.9 g) were significantly smaller than average nest-holding males ($H = 17.0$, $n = 8$, $P = 0.02$). Sneaker males appeared to target nests with females as we detected a significant association between the presence of sneaker males and females on nests (Fisher's exact test: $P < 0.001$).

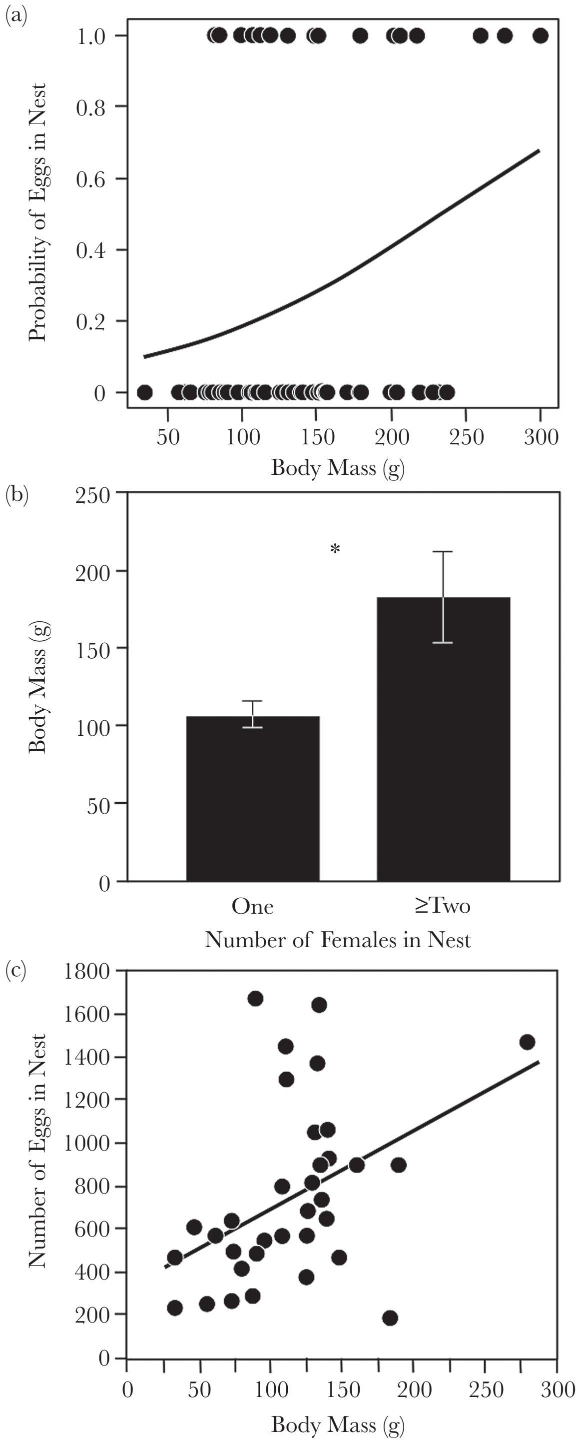

Figure 1
Female choice for larger guarder males in plainfin midshipman. (a) Body mass of guarder males predicts the probability that a male will have eggs present in their nest; larger males are more likely to have eggs in their nest. Results presented in this figure are from the population survey from early in the breeding season. However, we found qualitatively similar effects later in the breeding season. (b) Mean ± SE body mass of guarder males found with 1 versus 2 or more females in their nests. Again these results were obtained from the population survey. * indicates significant ($P < 0.05$) differences between groups. (c) The relationship between the body mass of guarder males and the number of eggs males they have in their nest. Egg number was used a proxy measure for female preference for particular guarder males.

Clutch sizes and tactic-specific investment

Guarder male body mass was positively correlated with the total number of eggs found in the nest (LMM, $F_{1,29.66} = 16.73$, $P < 0.001$, Figure 1c). Guarder and sneaker males had dramatically different reproductive investment patterns (Table 1). Guarder males were significantly heavier ($F_{1,31.28} = 339.1$, $P < 0.001$) and longer ($F_{1,31.23} = 326.7$, $P < 0.001$) than sneaker males: guarder males weighed almost 8 times more and were almost twice as long as sneaker males (body mass; guarder male: 121.91 ± 11.03 g, sneaker male: 15.30 ± 1.09 g and body length; guarder male: 20.44 ± 0.54 cm, sneaker male: 10.60 ± 0.30 cm). Despite the massive difference in body size between male tactics, the absolute mass (i.e., not correcting for body mass) of testes did not differ between sneaker and guarder males (guarder male: 1.50 ± 0.21 g; sneaker male: 1.19 ± 0.19 g; $F_{1,31.59} = 1.53$, $P = 0.22$). Absolute accessory gland mass was greater in guarder (0.63 ± 0.16 g) compared with sneaker males (0.10 ± 0.02 g, $F_{1,31.59} = 17.24$, $P < 0.001$). After correcting for the massive difference in body mass between guarder and sneaker males, sneaker males had significantly heavier testes mass and accessory gland mass than did guarder males (Table 1, Figure 2a,b). In contrast to the pattern with gonads, guarder males had sonic muscle of larger absolute mass (i.e., not correcting for body mass) compared with sneaker males (guarder male: 3.37 ± 0.33 g; sneaker male: 0.21 ± 0.02 g; $F_{1,31.09} = 600.9$, $P < 0.001$) and larger relative mass than did sneaker males even after body mass corrections were applied (Table 1, Figure 2c).

Between-tactic and within-tactic comparisons of ejaculate traits

To test the classic sperm competition theory prediction that sneaker males invest more in their ejaculates than guarder males, we compared sperm morphology and performance between guarder and sneaking males. A comparison of the midshipman's biflagellate sperm (Figure 3a) between male reproductive tactics revealed that guarder males produced sperm with longer heads ($F_{1,23.04} = 15.22$, $P < 0.001$, Figure 3b), whereas sneaker males produced sperm with longer midpieces ($F_{1,23.02} = 10.69$, $P = 0.003$, Figure 3c). No tactic-related differences were detected in flagella length ($F_{1,23.27} = 0.83$, $P = 0.37$, Figure 3d) or in overall total sperm length ($F_{1,23.04} = 0.80$, $P = 0.38$) between male tactics. The head length to flagellum length ratio (HL:FL) was greater in guarder than sneaker males ($F_{1,23.19} = 5.08$, $P = 0.03$, Figure 3e). Sperm swimming speed declined over time across all males, but the rate of decline differed between guarder and sneaker males (repeated measures Anova: time effect: $F_{6,124.8} = 35.12$, $P < 0.0001$; tactic effect: $F_{1,74.13} = 0.007$, $P = 0.98$; tactic × time: $F_{6,124.8} = 2.28$, $P = 0.04$; Figure 4a). Post hoc linear contrast analyses revealed that sperm from sneaker males swam significantly faster than the sperm from guarder males at 60 s ($F_{1,71.91} = 4.02$, $P = 0.049$) and at 120 s ($F_{1,70.21} = 4.94$, $P = 0.03$) post-activation. Sneaker male sperm also

Table 1

Comparisons of morphological traits between guarder and sneaker plainfin midshipman males.

Trait	*n*	Predictor	*F*	*P*
Testes mass	35	Body mass	$8.40_{1,30.31}$	**0.01**
		Tactic	$5.64_{1,29.84}$	**0.02**
Accessory gland mass	26[a]	Body mass	$54.83_{1,22.9}$	**<0.001**
		Tactic	$18.43_{1,22.89}$	**<0.001**
Sonic muscle organ mass	35	Body mass	$53.75_{1,29.45}$	**<0.001**
		Tactic	$16.60_{1,29.47}$	**<0.001**
		Tactic × body mass	$4.46_{1,29.74}$	**0.04**

Tactic-specific investment in testes, accessory gland, and sonic muscle organ mass were examined using linear mixed effects models (see Methods for details). Significant relationships are presented in bold text.

[a] Indicates cases where sample size was reduced as a result of no accessory glands samples being collected in the 2006 field season.

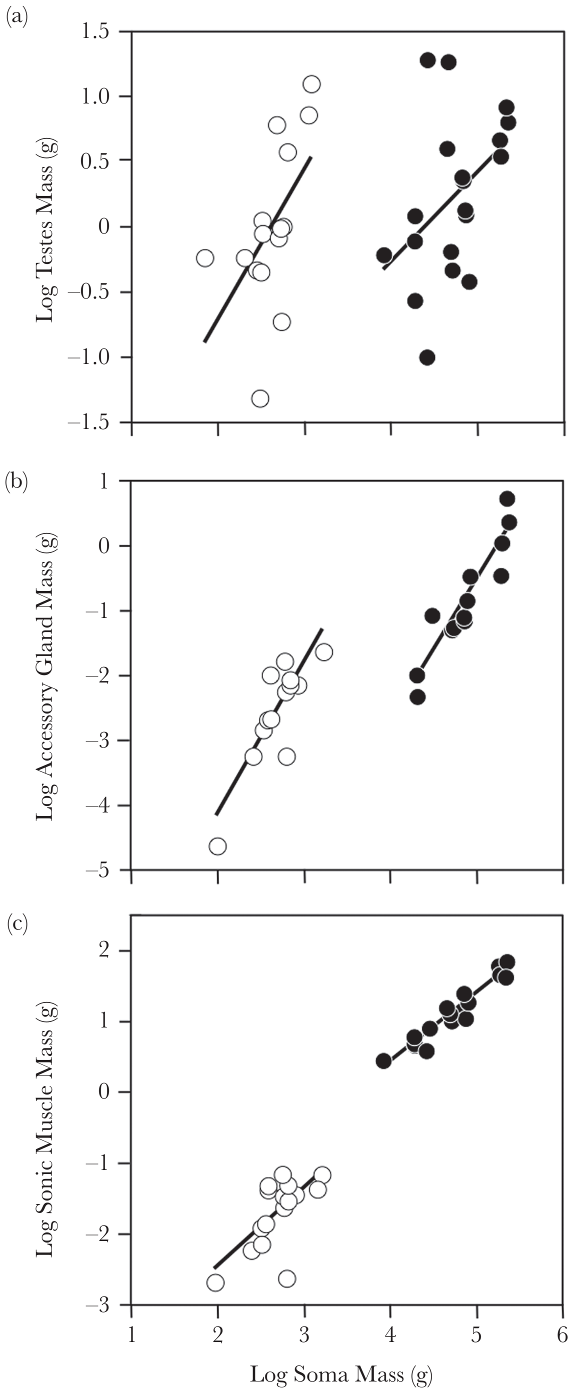

Figure 2

Tactic-specific reproductive organ investment in plainfin midshipman. The relationship between soma mass (body mass − organ mass) and (a) testes mass, (b) accessory gland mass, and (c) sonic muscle mass for guarder (filled circles) and sneaker (open circles) males.

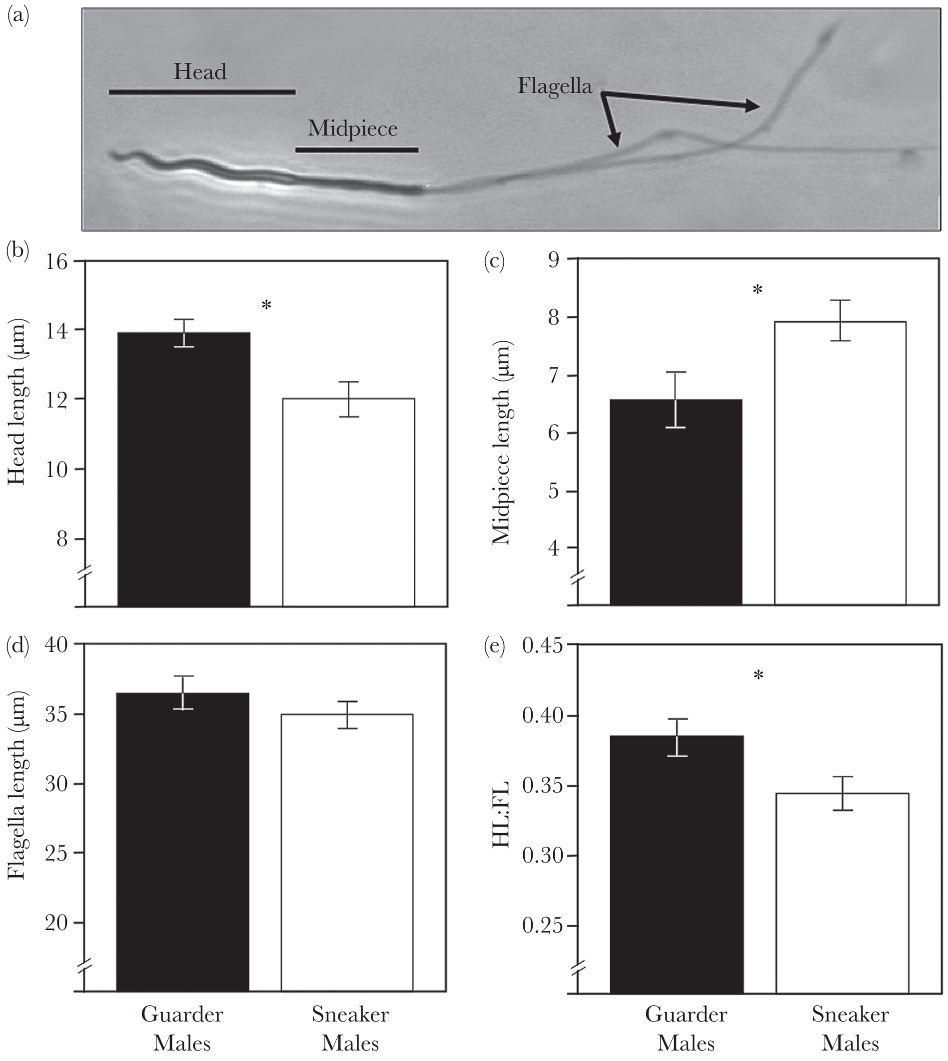

Figure 3

Comparison of sperm morphology between guarder and sneaker plainfin midshipman males. (a) A representative midshipman spermatozoon magnified at ×1000. Head, midpiece, and biflagellate (2-tailed) flagella are labeled. Mean ± SE (µm) of (b) sperm head lengths, (c) midpiece lengths, (d) flagella lengths, and (e) head length to flagellum length ratios (HL:FL) in guarder and sneaker males. * indicates significant ($P < 0.05$) differences between male tactics.

tended to swim faster at 30 s ($F_{1,70.21} = 3.50$, $P = 0.06$) and 300 s ($F_{1,75.53} = 3.11$, $P = 0.08$) post-activation.

Our population survey (see Results for details) demonstrated that the largest guarder males in the population were subject to increased sperm competition risk (relative to smaller guarder males). Therefore, we next examined the relationship between male body size and sperm swimming speed and morphology in each tactic separately. Our a priori hypothesis was that larger guarder males produce faster swimming sperm compared with smaller guarder males, whereas body size would not be associated with sperm swimming speed in sneaker males, who experience sperm competition at all matings regardless of their body size. As predicted, we did detect a positive relationship between guarder male body size and sperm swimming speed (body mass: $F_{1,13.23} = 4.25$, $P = 0.03$; time effect: $F_{6,76.56} = 25.63$, $P < 0.0001$, Figure 4b, 1-tailed tests based on a priori prediction). In contrast, sperm swimming speed was not related with body mass among sneaker males (body mass: $F_{1,8.68} = 0.02$, $P = 0.89$; time effect: $F_{6,48.88} = 16.54$, $P < 0.0001$, Figure 4b). When assessing sperm morphology, there was no relationship between guarder males body size and any sperm measurement (head: $F_{1,10} = 0.15$, $P = 0.70$; flagellum: $F_{1,10} = 0.16$, $P = 0.70$; or total length: $F_{1,10} = 0.50$, $P = 0.50$; HL:FL: $F_{1,10} = 0.44$, $P = 0.52$). Contrary to our expectations, larger guarder males had sperm with smaller midpieces ($F_{1,10} = 23.49$, $P < 0.001$). No relationships were detected between sneaker male body mass and sperm morphology (head: $F_{1,10} = 3.35$, $P = 0.10$; midpiece: $F_{1,10} = 89$, $P = 0.37$; flagellum: $F_{1,10} = 0.35$, $P = 0.57$; total length: $F_{1,10} = 0.15$, $P = 0.71$; HL:FL: $F_{1,10} = 2.51$, $P = 0.14$).

DISCUSSION

When strong female preferences lead to skewed reproductive success for high-quality males, low-quality males can be left with few reproductive options other than to attempt sneak fertilization (Alonzo 2008). Yet little evidence exists to support the idea that female mate choice directly influences sperm competition dynamics. Here, we explored this idea using a combination of field ecological surveys of midshipman nests and physiological data of male reproductive traits. We show that female midshipman preferentially visit nests of larger guarder males, who have nests with more eggs

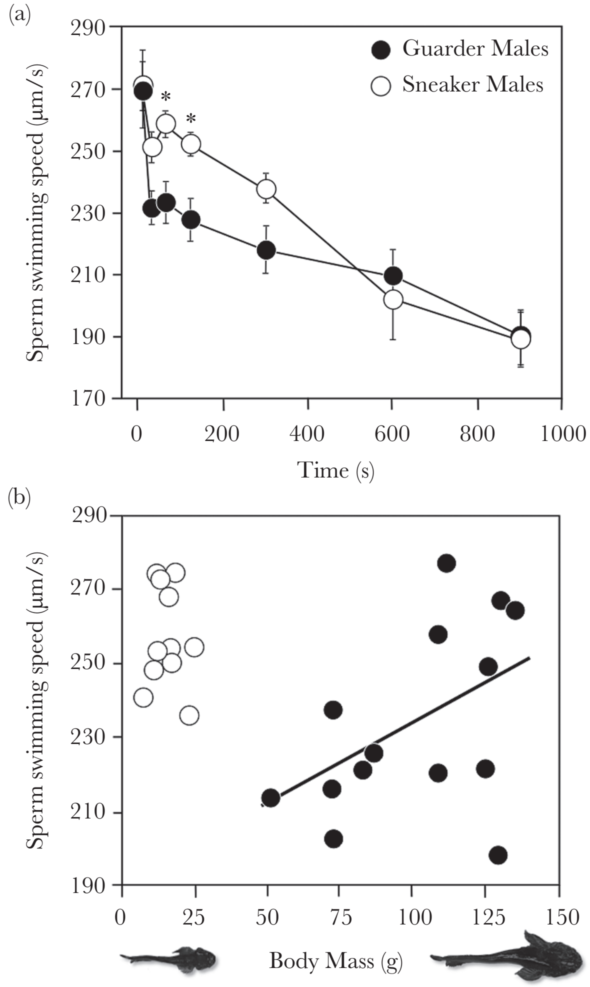

Figure 4

Sperm swimming speeds and male ARTs in plainfin midshipman. (a) Mean sperm swimming speed (±SE) among males at different times points post-activation for guarder (filled circles) and sneaker (open circles) males. * indicates significant ($P < 0.05$) differences between male tactics at this time point. (b) A tactic comparison between guarder males (filled circles) and sneaker males (open circles) of sperm swimming speed against male body size. Statistical analyses were performed using repeated measures Anova but for clarity we have shown data from a single time period (60 s post-activation). A best-fit line has been fitted through the guarder male data. To illustrate the difference in body size between male reproductive tactics, an image of a 24 g sneaking male and a 134 g guarding male taken at the same scale are presented on the left and right side, respectively, of the x axis.

and are more likely to have multiple females in their nests compared with smaller guarder males. Female preference for larger guarder males was associated with an increased risk of sperm competition, as nests of larger guarder males were also more likely to be visited by cuckolders (also see Brantley and Bass 1994; Lee and Bass 2004 for similar patterns in other midshipman populations). We show that the patterns of cuckoldry were dynamic across the breeding season: cuckolding guarder males were more common early in the breeding season, whereas sneaker males appeared to be more common later in the breeding season (but see Bose et al. 2014 who found that there were more sneaker males early in the season). Throughout the breeding season, the nests of the larger guarder males act as a hub where females and both types of cuckolders (sneaker and cuckolding guarder males) congregate. This coalescence of nest preference by females and cuckolding males has important implications for sperm competition risk within and across tactics.

Consistent with the idea that female choice increases sperm competition, we found that sperm swimming speed increased with male body size across guarder males. When sperm competition risk is elevated (as is the case for the larger guarder males and among sneaker males), males can produce sperm that swim faster than the sperm found in ejaculates produced by guarder males who are not targeted by cuckolding males or by females. Thus, within-tactic variation in sperm swimming speed may represent a previously undetected response to sperm competition in species with ARTs. Consideration of this variance may help resolve puzzles in cases where divergent results have been uncovered for a single species (e.g., bluegill, *Lepomis macrochirus*: Leach and Montgomerie 2000; Burness et al. 2004, 2005; Stoltz and Neff 2006) or where tactic-specific differences in male reproductive investment are not observed (e.g., Munguía-Steyer et al. 2012).

We also demonstrate tactic-specific male reproductive investment that is consistent with predictions from sperm competition theory (Parker 1990; Parker and Ball 2005). Sneaker males (who must always contend with sperm competition) had relatively larger testes than guarder males. These results support earlier descriptions of tactic-specific reproductive investment patterns in a Californian population of this species (Brantley and Bass 1994) and match observations in numerous other species with ARTs (reviewed by Montgomerie and Fitzpatrick 2009). However, we also found that sneaker males invest more in accessory glands compared with guarder males, after controlling for differences in body size. Although the precise role of accessory glands in fertilization success remains unknown in midshipman, a host of studies in insects and mammals have demonstrated the importance of sperm competition in shaping male investment in these glands, and the seminal fluid proteins they produce (Simmons and Fitzpatrick 2012). Moreover, in the grass goby *Zosterisessor ophiocephalus*, a fish species with ARTs, Locatello et al. (2013) recently demonstrated that seminal fluid constituents in the ejaculate of sneaker males negatively impacted sperm performance of guarder males. Consequently, further investigation in midshipman of accessory glands themselves (e.g., Barni et al. 2001), their proteins, and their impact on fitness would help to explain the tactic-specific investment pattern observed in this study.

Compared with all guarder males, sneaker male midshipman produced faster swimming sperm with larger midpieces and smaller heads. As sperm speed predicts fertilization success (Simmons and Fitzpatrick 2012), the faster swimming speeds of sneaker males may help to offset their competitive disadvantage in terms of proximity to spawning females. Sneaker males have been shown to produce faster swimming sperm in a handful of other fish species (e.g., Burness et al. 2004; Fitzpatrick et al. 2007; Locatello et al. 2007). However, tactic-specific variation in sperm swimming speed has more often not been observed (Leach and Montgomerie 2000; Burness et al. 2005; Stoltz and Neff 2006; Marentette et al. 2009; Smith and Ryan 2010). Similarly, there are relatively few demonstrations of tactic-specific differences in sperm morphology (reviewed by Montgomerie and Fitzpatrick 2009), although 1 study also reported longer midpieces (the site of ATP production and the primary source of energy powering sperm movement) in sneaker males versus large courting males of the swordtail fish, *Xiphophorus nigrensis* (Smith and Ryan 2010). Perhaps sneaker male sperm can swim faster due to their larger midpieces and smaller heads (which could reduce drag, Humphries et al. 2008), yet we failed to detect a significant relationship between these sperm component measurements and swimming speed. As we also found that larger guarder males, whose sperm swam faster than smaller guarding males, produced sperm with smaller midpieces, it seems unlikely that there is a straightforward relationship between sperm morphology and performance in midshipman. Whether the unusual biflagellate sperm of midshipman (see Figure 3a) influences the relationship between sperm length and speed in unexpected ways remains unclear. However, exploration of the link between sperm morphology and performance in a species with biflagellate sperm, like midshipman, may yield helpful insights and comparisons with the uniflagellate sperm typically studied.

The impact of female mate choice on the risk sperm competition is likely to be an important and broadly applicable concern when addressing sperm competition in a diverse set of taxa. Female mate choice can create the conditions for increased sperm competition whenever 1) cuckolding males preferentially target high-quality males and/or 2) cuckolding males respond to female behavior when choosing which male to parasitize. Such preferential targeting by cuckolding males of particular males who are most likely to attract females is a widespread observation. For example, in the ruff *Philomachus pugnax*, a bird with alternative male reproductive tactics, cuckolding satellite males time their visit to the lek to coincide with female visits (Widemo 1998). In the peacock blenny *Salaria pavo*, a species where body size predicts male reproductive success (Gonçalves et al. 2002), sneaking males preferentially associate with large males and with males that were previously observed interacting with females (Gonçalves et al. 2003). Preferential targeting of high-quality males by cuckolding males may be particularly common in species where high-quality males routinely generate publically available information in the form of mating calls or other conspicuous displays/traits, which cuckolding males can easily use to focus their sneaking effort. For example, small male house crickets (*Acheta domesticus*) and natterjack toads (*Epidalea calamita*) preferentially move toward acoustic calls from larger males who are preferred by females (Aark 1988; Kiflawi and Gray 2000). Thus, evidence from a wide range of species suggests that female mate choice may generate increased sperm competition risk in preferred males. However, preferred males may not always encounter more sperm competition, as a recent detailed examination of sneaker and territorial harvestmen (*Serracutisoma proximum*) behaviors revealed that males holding larger harems experiencing lower intensities of sperm competition (Muniz et al. 2015). Thus, an important next step will be to examine within-tactic variance in sperm competition risk and assess investment within tactics in males of additional species with ARTs.

In conclusion, in this study, we describe how female mate choice can potentially influence sperm competition risk and shape investment patterns in males between *and* within reproductive tactics. We argue that in general the impact of female behavior needs to be more fully integrated in the study of male postcopulatory competition and male ARTs. This idea may even be applicable to species without reproductive tactics where female mate choice generates predictable differences in the strength of postcopulatory sexual selection. Failure to consider the impact of female choice and within-tactic responses to sperm competition may hamper our ability to understand the selective forces acting on ejaculate trait in species with ARTs.

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