



Original Article

Comparing population level sexual selection in a species with alternative reproductive tactics

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The description of a species' mating patterns is often based on observations from a single exemplar population; however, environmental variation can lead to variation in mating patterns and to differences in the strength of sexual selection among populations. In this study, we explored how resource distribution across a species' range affects competition and the strength of sexual selection in a northern and southern population of plainfin midshipman (*Porichthys notatus*), a species with 2 male reproductive tactics. Male plainfin midshipman can be guarders that compete for nest sites and court females, or sneakers that attempt to steal fertilizations from the guarder males during spawning. Males from the north population grow larger, suggesting that there might be more competition among males in the north. However, we found that the variance in body size and in nest availability were similar between populations, suggesting instead a similar degree of male-male competition. We found no significant population differences in reproductive success (north: 517 ± 50 eggs/nest \pm SE; south: 412 ± 68 eggs/nest \pm SE), paternity (north: 52%; south: 58% for the guarding male), or tactic frequencies (north: 88% guarders; south: 91% guarders). There was a marginally steeper Bateman gradient in the south population but no difference at 8 other measures of the strength of sexual selection between the 2 populations. Thus, despite a wide geographic distance, our results show remarkable conservation of mating patterns between the north and south populations of this benthic toadfish.

Key words: cuckoldry, mating competition, nest availability, paternity, plainfin midshipman.

INTRODUCTION

The strength of sexual selection is shaped by both social and environmental factors, or “ecological constraints” (Emlen and Oring 1977; Andersson 1994; Shuster and Wade 2003). Often, studies that look at the strength of selection and the mating behaviors do so in a single exemplar population and extrapolate the results for the species in general. Yet, it is known that temporal and spatial variation in ecological factors exist within and across populations and this can lead to variation in mating behavior and in the strength of sexual selection (e.g., Davies 1985; Endler and Houde 1995; Kasumovic et al. 2008; Reichard et al. 2008; Candolin and Vlieger 2013). For example, a comprehensive study looking at 5 low predation and 5 high predation populations of guppy (*Poecilia reticulata*) found a significantly higher frequency of multiply sired broods in the higher predation populations, likely a result of more coercive copulations by males (Kelly et al. 1999). Mobley and Jones (2007) compared 2 populations of the sex-role reversed dusky pipefish

(*Syngnathus floridae*) and found that males from the larger population had higher rates of multiple mating and greater reproductive success. The authors argued that these differences were driven by variation in population densities. However, additional studies on both guppy (Neff et al. 2008) and dusky pipefish (Mobley and Jones 2009) have not supported these findings and instead found no clear link between mating patterns and predation intensity or population density, respectively. Certainly, more cross-population studies of mating patterns are needed to clarify and provide better understanding of how ecological factors influence sexual selection and mating systems.

Precisely how to measure sexual selection remains an ongoing debate (e.g., Mills et al. 2007; Klug et al. 2010a; Krakauer et al. 2011; Fritzsche and Booksmythe 2013). Several metrics have been proposed and are thought to capture the potential or the actual strength of sexual selection. An early popular choice for assessing the potential strength of sexual selection was the operational sex ratio (OSR; Emlen and Oring 1977). The OSR is based on calculating the ratio of reproductively active males to females in the population at a given time; individuals of the more common sex,

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especially when populations become highly skewed, are expected to experience intense mating competition and thus stronger sexual selection (Emlen and Oring 1977; Clutton-Brock and Parker 1992; Kvarnemo and Ahnesjö 1996). Additional metrics developed to measure the maximum strength of selection are based on Bateman's 3 principles (Bateman 1948; Trivers 1972; Arnold 1986; Arnold and Duvall 1994). The sex experiencing stronger sexual selection is expected to have 1) greater variance in reproductive success (number of offspring), 2) greater variance in mating success (number of mates), and 3) a steeper regression slope relating reproductive success to mating success (Wade 1979; Wade and Arnold 1980; Arnold 1986; Arnold and Duvall 1994).

From Bateman's first 2 principles, the opportunity for selection (I) and the opportunity for sexual selection (I_s) metrics can be calculated, respectively, and these metrics capture the maximum potential of selection (Crow 1958; Wade 1979; Wade and Arnold 1980; Arnold and Wade 1984a,b; Shuster and Wade 2003; Jones 2009). Bateman's third principle, the linear relationship between reproductive success and mating success, is now often called Bateman's gradient (β_{ss} ; Arnold and Duvall 1994; Andersson and Iwasa 1996). A steeper β_{ss} indicates a greater potential for sexual selection to occur (Jones 2009). The maximum standardized sexual selection differential (s'_{max}) is calculated as the product of the β_{ss} and the square root of the opportunity for sexual selection ($\beta_{ss} * \sqrt{I_s}$) and is used to investigate selection generated by differential mating success (Jones 2009). Another metric used to measure sexual selection is the selection gradient (β'), which is a trait-based measure that quantifies the degree of sexual selection on a particular phenotypic trait (Lande and Arnold 1983). Although debate continues about which metric to use, the emerging consensus is that the best metric of sexual selection will depend on the research question (e.g., actual selection on a trait versus potential selection across sexes or populations), and that multiple measures should be employed whenever possible (see Fritzsche and Booksmythe 2013).

In this study, we investigated population differences in the strength of sexual selection and explored how differences in ecological factors influence the degree of male competition and mating patterns in a species with alternative reproductive tactics (ARTs), the plainfin midshipman (*Porichthys notatus*). ARTs refer to the occurrence of 2 or more discontinuous reproductive phenotypes, with each phenotype maximizing their reproductive fitness through alternative means (Gross 1996; Taborsky et al. 2008). The strength of sexual selection likely influences the frequency of alternative tactics in a given population. However, relatively few studies have investigated the strength of sexual selection and how it influences mating patterns in species with ARTs (e.g., Fleming and Gross 1994; Mills and Reynolds 2003; Simmons et al. 2004; Pomfret and Knell 2008), and fewer have done so in species with ARTs across ecologically distinct environments (Tomkins and Brown 2004; Reichard et al. 2008; Munguía-Steyer et al. 2012; Candolin and Vlieger 2013).

In plainfin midshipman, males adopt 1 of 2 distinct reproductive tactics called guarder (also known as type I) males and sneaker (also known as type II) males (Brantley and Bass 1994). Guarder males aggressively compete for limited nest sites, which are excavated areas under rocks in the intertidal zone. The largest guarder males typically win the largest rock/nest sites and, by acoustically courting spawning females, will also gain the most eggs (Ibara et al. 1983; DeMartini 1988; Bass 1992; Brantley et al. 1993). In contrast, sneaker males do not guard nests or court females, but

instead, steal fertilizations from guarder males by either sneaking into the nest when a female is present, or by fanning sperm in from the nest periphery (Brantley and Bass 1994). Additionally, guarder males can be behaviorally flexible, and will sometimes adopt a cuckolding behavior and move to a nest of another guarder male (Lee and Bass 2004). This behavior occurs particularly when nesting sites are severely limited and these cuckolding guarder males can fertilize between 11% and 24% of the offspring within a nest (Cogliati et al. 2014).

Plainfin midshipman are distributed along the Pacific coast of North America, from Sitka, Alaska to Baja California, Mexico (Hubbs 1920; Arora 1948; Miller and Lea 1972). However, the distribution of this species appears to be discontinuous along the Oregon coast, with a northern population stretching from Oregon to Alaska and a southern population stretching from Oregon to Mexico (Warner and Case 1980; Thompson and Tsuji 1989; Harper and Case 1999). Guarder males from the south (California) are much smaller (Brantley and Bass 1994) than guarder males from the north (Washington; Lee and Bass 2004; Sisneros et al. 2009). Because large male body size is an important trait in competitive interactions and strongly influences reproductive success in this species (DeMartini 1988), we propose that there will be more competition and stronger sexual selection in the north than in the south population.

One of the most important resources for a male midshipman is the nest (DeMartini 1988). Nest availability should strongly influence the degree of mating competition experienced by males and greater variation in nest availability should select for more exaggerated male competitive traits and a higher frequency of male alternative tactics. Because males are larger in the north, we predicted that competition among guarding males over nest availability would be greater in the north than the south population and that we would observe more variance among guarding males in body size and reproductive success in the north population. Another way that male midshipman can compete with one another is via nest takeovers and cuckoldry by both guarder and sneaker males (Lee and Bass 2004). Therefore, we also predicted that we would see more of these competitive behaviors resulting in more takeovers, cuckolders, and an overall lower paternity for guarder males in the north population. Finally, we predicted that, as a result of more competition among males, all measures of sexual selection would be higher in the north compared with the south including the OSR, opportunity for selection (I), opportunity for sexual selection (I_s), β_{ss} , maximum standardized sexual selection differential (s'_{max}), and trait based selection gradient for male size (β').

METHODS

Field sampling

From May 10th to July 14th, 2010, and May 29th to July 21st, 2011, we located and sampled a total of 374 plainfin midshipman nests during low tide in the intertidal zone on 6 rocky beaches in British Columbia, Canada (3 sites in Ladysmith Inlet: 49°01'N, 123°83'W; 1 site in Mill Bay: 48°63'N, 123°53'W; and 1 site in Stuart Channel: 49°04'N, 123°75'W, Figure 1a; 1 site in Crescent Beach: 49°04'N, 122°88'W, Figure 1b), 1 beach in Washington, USA (Seal Rock: 47.71°N, 122.89°W, Figure 1c), and 4 beaches in California, USA (all sites in Tomales Bay: 38°15'N, 122°90'W, Figure 1d). Based on the clear population divergence described in Warner and Case (1980), we designated nests on British Columbia

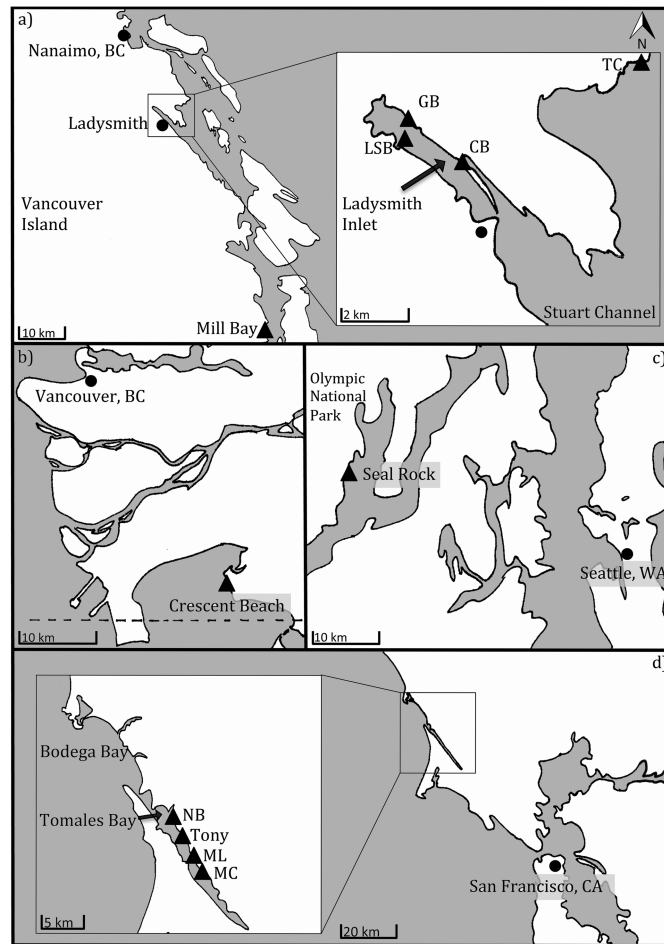


Figure 1

Location of study sites (▲) along the Pacific coast of North America, with reference cities labeled (●). (a) Shows 3 study sites in Ladysmith Inlet, 1 in Stuart Channel, and 1 at Mill Bay on the East coast of Vancouver Island, south of Nanaimo, British Columbia. (b) Shows the study site at Crescent Beach on the West coast of mainland British Columbia, south of Vancouver. (c) Shows the Seal Rock study site in Puget Sound, south of Strait of Juan de Fuca. This study site is located east of Olympic National Park and west of Seattle, Washington. (d) Shows the 4 study sites in Tomales Bay located on the West coast of California, north of San Francisco. Study site labels are referenced in [Supplementary Table S1](#). Scales shown at bottom left are specific to each location. Water shaded in gray.

and Washington beaches as our north population ($N = 310$ nests), and the nests on California beaches ($N = 64$ nests) as our south population.

At the different beaches, we sampled by laying down a 50 m transect and sampling all nests in a 2.5 m \times 2.5 m grid, every 10 m along this transect. In total, we sampled 163 quadrants over 2 years in the north population and 21 quadrants in 1 year in the south population. Within each quadrant, we checked all rocks and classified them as either active nests (occupied), potential nests (unoccupied but suitable; i.e., any rock with a relatively flat underside that was greater in size than the mean guarder male length in that population; see [Supplementary Material](#) for further details), or unsuitable as a nest (too embedded or too small). We marked quadrants and each nest in the quadrants with plastic pegs, and also marked the guarder males with a unique elastomer injection (see [Cogliati et al. 2013](#)). We returned to the same quadrants in subsequent low tides over the course of the breeding season (~ every 2–4 weeks). When we re-sampled a quadrant, we recorded whether or not a nest was still in use and recorded whenever a previously unused rock (a potential nest) had become an active nest. We always checked whether existing nests had a new resident

guarder male or the same resident guarder male as the previous sampling date and marked any new resident guarder males that we found in the nest.

At each nest, we sexed all fish based on the shape of the urogenital papilla and overall body coloration and identified males as guarder or sneaker based on their body size ([Brantley and Bass 1994](#)). Sometimes more than 1 guarding male was found in a nest and we further distinguished between actively guarding and cuckolding guarder males based on their position in the nest; guarder males that are the nest owners are usually the largest and most centrally placed in the nest ([Lee and Bass 2004](#); KC and SB pers. obs.) whereas cuckolders are smaller and remain on the periphery. Such field-based classifications to identify sex and reproductive type have been verified in extensive studies and further confirmed through dissections ([Bass and Marchaterre 1989](#); [Bass and Anderson 1991](#); [Brantley and Bass 1994](#); [Cogliati et al.](#), unpublished data).

For each fish in each nest, we measured its standard length (SL in cm) and collected a small caudal fin clip for genetic analyses. To estimate reproductive success, we digitally photographed the embryos in each nest and, because midshipman eggs are laid in a monolayer, we later enumerated the eggs from the photos using

ImageJ software (Schneider et al. 2012). We used egg number as one measure of reproductive success and used paternity as a second measure. To calculate paternity, we collected and preserved 40–50 offspring from each cohort (i.e., a distinct group of offspring at the same developmental stage within the nest; see Cogliati et al. 2013) from a subset of nests ($N = 47$ in BC and $N = 20$ in CA). After sampling, we returned all adults to their nest and repositioned all rocks in their original location.

Genetic and paternity analyses

To investigate the population structure in plainfin midshipman, we collected microsatellite data for our north population ($N = 222$ adults sampled across all sites in BC), and from our south population from ($N = 92$ adults sampled across all sites in CA). Two previous studies have shown that fish from across all the study sites in British Columbia represent a single panmictic population (Suk et al. 2009; Cogliati et al. 2013). Based on microsatellite data from both adult males and females, we investigated whether individuals from the predefined north and south spatial populations represent distinct genetic populations using *structure* (v2.3) software (Pritchard et al. 2000). *Structure* assigns individuals to populations and infers the structure of populations using a clustering method (Pritchard et al. 2000). We performed 5 independent simulations for each value of K (number of populations assumed) from 1 to 6, for a total of 30 simulations. The parameters of each simulation included a “burn length” of 50 000 followed by 100 000 Markov chain Monte Carlo (MCMC) repetitions after burning, an admixture ancestry model without using prior sampling location (site), and independent allele frequencies. We checked that the length of the MCMC procedure was adequate for convergence based on similar likelihood and α values. To identify the number of possible populations in our study, we inputted the output files into *Structure Harvester* for analyses (Dent and vonHoldt 2012). In addition, we calculated a measure of genetic differentiation (F_{ST}) between the north and south adult samples using GenAlEx (v6.5b3; Peakall and Smouse 2006, 2012).

We used the paternity estimates for guarder males produced in Cogliati et al. (2013) for our north population (based on 1713 offspring from 47 nests in BC) and, following the same protocols, calculated a paternity estimate for the guarder males from our south population (based on 531 offspring in 21 nests in CA). Briefly, we extracted DNA from fin clips and embryos and genotyped individuals using 6 microsatellite loci developed for plainfin midshipman (Suk et al. 2009; Cogliati et al. 2013). We used the *two-sex paternity* model (Neff et al. 2000a,b; Neff 2001) to calculate a paternity value for each cohort present in the nest of a given male. In some cases, we had very low paternity estimates (<10% paternity), which we considered to be a result of nest takeovers (see Cogliati et al. 2013). We used COLONY (v2.0) to determine the number of cuckolders that sired offspring in each cohort, the number of offspring that each cuckolder sired, and the number of genetic mates (females) that mated with the nest guarder male (Wang 2004; Jones and Wang 2010).

Selection metrics

As suggested in the recent review by Fritzsche and Booksmythe (2013), we used multiple measures to compare the strength of sexual selection between the north and south populations. First, we determined the OSR at each site based on the total number of reproductive guarder males and spawning females observed at each

site. Females are often found in the nests of guarder males during low tides, and spawning takes several hours to complete (Brantley and Bass 1994). Aside from embryos, only reproductive adult fish are found in the nesting intertidal zone during the breeding season; juveniles are never at the nests, but instead found in offshore eelgrass beds (Bass 1992). Although the OSR does not directly measure the strength of selection, we include it here to describe the potential level of competition among individuals at each site within each population. Second, following Klug et al. (2010b), we calculated the opportunities for selection (I) based on nesting success (I_{nest} : guarder males were classified a nest owner (1) or not (0)), fertility of mates (I_{eggs} : number of eggs received), and paternity (I_{pat}). The variables measuring the opportunity for selection are calculated by dividing the site variance in these 3 measures of reproductive success by the site mean reproductive success squared (Crow 1958; Wade 1979). Third, we calculated the opportunity for sexual selection (I_s) using the site variance in mating success (number of genetic mates; Barreto and Avise 2010) divided by the site mean mating success squared (Wade 1979; Wade and Arnold 1980). Fourth, we calculated the β_{ss} as the least squares regression slope of reproductive success (using paternity) on mating success for each site (Arnold and Duvall 1994; Andersson and Iwasa 1996). Both mating success and reproductive success measures were divided by their site mean to produce measures of relative fitness with transformed means equal to 1 (see Jones et al. 2004). As suggested by Jones (2009), we also calculated the maximum standardized sexual selection differential (s'_{max}) as $\beta_{ss} * I_s$ to investigate selection generated by differential mating success. Finally, because male size is an important trait for reproductive success in plainfin midshipman (DeMartini 1988), we calculated the selection gradient (β') as the slope of the regression of reproductive success (using egg number β'_{egg} and paternity β'_{pat}) on body size (SL) for each site. We first log transformed SL and standardized this variable within site to have a mean of 0 and a variance of 1 (Jones et al. 2004).

Statistical analyses

We performed all analyses using R version 3.0.3 (R Core Team 2014). For the ecological variable of interest, we calculated nest availability as the proportion of potential nests that were occupied per quadrant (total number of occupied nests divided by total number of potential nests). We summarized nest availability first by quadrant (to account for re-sampling) then by summarizing the mean, standard deviation (SD; absolute variance), and coefficient of variation (CV; relative variance) by site. We similarly summarized the mean, SD, and CV of guarder male, sneaker male, and female body size (SL) by site.

We used several measures to compare mating patterns including egg number, tactic frequency, paternity, takeovers, and cuckoldry. For tactic frequency, we identified males based on their true morphological tactic (guarder vs. sneaker) as well as on their behavioral tactic (regardless of morphology, whether males were guarding vs. cuckolding) and we calculated the proportion of males that were either true guarders or behaviorally guarding, respectively. We included this second test because cuckolding behavior is not limited to sneaker males (Lee and Bass 2004; Cogliati et al. 2014), thus the intensity of selection may affect the degree of cuckoldry behaviors observed by guarder males. We calculated paternity estimates as the proportion of offspring related to the guarding male from all 4 sites in the south population and from 5 of 7 sites in the north population. To assess the frequency of takeovers, we

calculated the proportion of nests where no takeovers appeared to occur in any cohort. Based on data from COLONY, we evaluated cuckoldry by determining the number of actual sires and calculated the number of effective sires for each cohort following Neff et al. (2008). The number of effective sires is a measure of reproductive skew among males, and the number of effective sires will equal the number of actual sires when all males contribute equally in terms of offspring number. Briefly, we calculated the number of effective sires as $1/\sum(rs_i/\text{brood size})^2$, where rs_i is the number of offspring assigned to sire i , and brood size is the total number of offspring analyzed in the cohort. We summarized paternity and both measures of cuckoldry at the nest level first to account for multiple cohorts within a nest, then, along with the other measures of mating patterns we summarized the mean, SD, and CV for each of these variables by site. Finally, we calculated all measures of sexual selection at the level of individual.

To compare each variable across populations, we ran linear models with population (north and south) as the predictor variable. These models are equivalent to 1-way ANOVAs or pooled t -tests; however, rather than assuming normality of the residuals, we used a permutation testing procedure (lmp, as implemented in the lmpPerm package for R, version 1.1–2) to estimate the P -values for each term in the model. Because we are most interested in the among-population comparison and because the basic experimental design is nested (i.e., the effects of interest vary at the among-site, not the within-site level), the simplest approach was to aggregate the data to the level of site rather than applying mixed models (Murtaugh 2007). Indeed, since among-site variability is likely to dominate the overall ecological pattern, the linear model with permutation approach may be preferred over more sophisticated generalized linear mixed models that make stronger assumptions about the distribution of among-site variation. Although we were most interested in population level differences, we also investigated the possible fine scale ecological differences (nest availability) at each site using 2 additional linear permutation models for each variable. The models included either nest availability instead of population or both nest availability and population as predictors. Because our focus is on population differences, we discuss the results of these models only when significant. Specifically for the β_{ss} , in addition to the aforementioned analyses, we compared the slopes for all sites using an ANCOVA as is traditionally done when comparing the slopes of regressions between groups. Unless otherwise stated, the means presented in the results for the north and south are calculated using the site means as opposed to pooling data across all sites.

RESULTS

Genetic divergence

Structure indicated that there were 2 distinct genetic populations, with 93% and 96% of individuals aligning with their predefined north and south collection regions, respectively. The 2 populations (individuals pooled across sites within populations) showed a moderate level of genetic differentiation ($F_{ST} = 0.07 \pm 0.02$; mean \pm SE), with an estimated 8.7 ± 5.5 (mean \pm SE) effective migrants, or individuals, moving between populations based on 222 adults in BC and 92 adults in CA. Therefore, for the paternity analyses (see below), we calculated separate population level allele frequencies for our microsatellite loci using the adult genotypes from that population.

Ecological differences

The north and south populations did not significantly differ in terms of nest availability (lmp: estimate = 0.03, $F_{1,8} = 0.34$, $P = 0.58$; mean proportion \pm SD for north: 0.41 ± 0.25 ; south: 0.36 ± 0.21). The absolute variance (SD: estimate = 0.02, $F_{1,8} = 1.32$, $P = 0.28$) and the relative variance in nest availability per site also did not vary between populations (CV: estimate = 0.05, $F_{1,8} = 0.42$, $P = 0.54$).

Variance in body size

As expected based on previous studies, guarder males were significantly larger in the north compared with the south (lmp: estimate = 2.40, $F_{1,9} = 22.15$, $P = 0.001$; Table 1, Figure 2a). However, the variation in body size across guarder males was no greater in the north than the variance in guarder body size observed in the south either in absolute or relative terms (SD = estimate = 0.37, $F_{1,9} = 3.22$, $P = 0.11$, Figure 2b; CV: estimate = 0.002, $F_{1,9} = 0.02$, $P = 0.88$, Figure 2c). Females and sneaker males were also larger in the north compared with the south (females: estimate = 2.21, $F_{1,9} = 26.04$, $P = 0.0006$; sneaker males: estimate = 1.38, $F_{1,6} = 5.37$, $P = 0.06$; Table 1).

Variance in reproductive success

The average number of eggs per nest in the north was 517 ± 444 (mean \pm SD; range 0–3193 across sites) compared with 412 ± 415 eggs per nest in the south (mean \pm SD; range 0–2032 across sites). Mean egg number (lmp: estimate = 47.61, $F_{1,9} = 1.29$, $P = 0.29$), the SD of egg number (estimate = 18.78, $F_{1,9} = 0.14$, $P = 0.71$), nor the CV of egg number (estimate = 0.13, $F_{1,9} = 1.13$, $P = 0.31$) significantly differed between populations.

Variance in paternity and mating patterns

On average, guarder males in the north sired $52\% \pm 0.04$ young/nest (mean \pm SE; $N = 74$, range 0–100%) and $58\% \pm 0.07$ of the young/nest (mean \pm SE; $N = 27$, range 0–100%) in the south (data pooled across sites within population). Males in the north and south populations did not significantly differ in their mean paternity (lmp: estimate = 0.07, $F_{1,7} = 0.55$, $P = 0.48$), the SD of paternity (estimate = 0.04, $F_{1,7} = 1.46$, $P = 0.26$), nor the CV of paternity (estimate = 0.07, $F_{1,7} = 0.74$, $P = 0.42$).

We considered the very low paternity (<10%) for some guarder males as likely takeover events. Using this criterion, we detected takeovers in 23% of nests in the north, and in 24% of nests in the south. The mean frequency of takeovers did not significantly differ between populations (estimate = 0.04, $F_{1,7} = 0.52$, $P = 0.49$) nor

Table 1
Standard lengths (mean \pm SE and range) for plainfin midshipman (*Porichthys notatus*) by sex and population

	North			South		
	Mean (cm) \pm SE	N	Range (cm)	Mean (cm) \pm SE	N	Range (cm)
Guarder males	20.5 \pm 0.15	500	10.4–29.5	15.6 \pm 0.28	68	10.7–20.9
Sneaker males	12.3 \pm 0.32	34	9.2–16.6	10.1 \pm 0.29	14 ^a	7.4–11.4
Females	16.6 \pm 0.24	126	10.0–21.9	12.5 \pm 0.22	16	11.3–13.9

^aEight sneaker males were added for body size measurements only and not included in analyses because they were collected outside of our quadrants.

did the SD nor CV of takeover frequency (SD: estimate = 0.09, $F_{1,7} = 2.07$, $P = 0.19$; CV: estimate = 0.15, $F_{1,7} = 1.21$, $P = 0.31$).

Known cuckoldry occurred in 78% of cohorts in the north and 67% of cohorts in the south. In the north, we detected 2.60 ± 1.07 actual sires (mean \pm SD; range 1–5) and 1.82 ± 0.70 effective sires (range 1–4.0) per cohort. In the south, we detected 2.34 ± 1.25 actual sires (mean \pm SD; range 1–5) and 1.78 ± 0.80 effective sires (range 1–4.3) per cohort. The populations did not significantly differ either in the mean number of actual sires (estimate = 0.13, $F_{1,7} = 0.64$, $P = 0.45$) or the mean number of effective sires (estimate = 0.02, $F_{1,7} = 0.02$, $P = 0.90$). Furthermore, the patterns of cuckoldry between populations did not differ in SD (actual sires: estimate = 0.09, $F_{1,7} = 0.77$, $P = 0.41$; effective sires: estimate = 0.05, $F_{1,7} = 0.33$, $P = 0.58$) nor in CV (actual sires: estimate = 0.06, $F_{1,7} = 2.31$, $P = 0.17$; effective sires: estimate = 0.02, $F_{1,7} = 0.28$, $P = 0.61$).

Tactic frequencies

The mean proportion of each male tactic type (guarder vs. sneaker) did not significantly differ between populations (Imp:

estimate = 0.0008, $F_{1,9} = 0.001$, $P = 0.97$). In both populations, 94% of males were guarders. When we looked strictly at the behavioral types (guarding vs. cuckolding), the mean proportion of guarding males sampled was also not significantly different between populations (estimate = 0.02, $F_{1,9} = 0.44$, $P = 0.53$); 88% of males in the north and 91% of males in the south were nest guarding. For both measures of tactic frequency, populations did not significantly differ in SD (tactic type: estimate = 0.05, $F_{1,9} = 1.23$, $P = 0.30$; behavioral type: estimate = 0.06, $F_{1,9} = 1.83$, $P = 0.21$) or in CV (tactic type: estimate = 0.04, $F_{1,9} = 0.54$, $P = 0.48$; behavioral type: estimate = 0.07, $F_{1,9} = 1.22$, $P = 0.30$).

Sexual selection across populations

Table 2 summarizes selection metrics by population (see Supplementary Material for the summaries of selection metrics by site). The observed OSRs were strongly male biased at each site but not different between the north and south populations (Imp: estimate = 0.009, $F_{1,9} = 0.12$, $P = 0.74$). In the north, 92% of guarder males had a nest while in the south, 97% of guarder males acquired

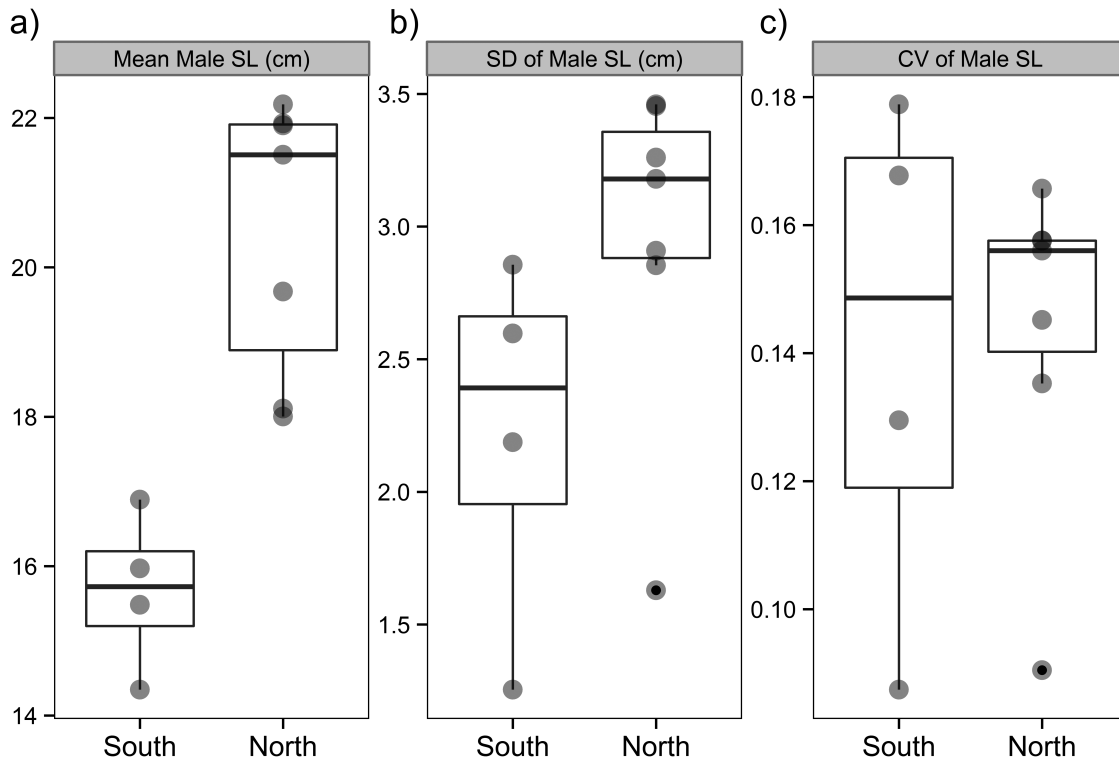


Figure 2 Standard length differences between north and south populations of plainfin midshipman (*Porichthys notatus*) as measured by (a) mean (cm), (b) SD (cm), and (c) CV. Large gray dots show individual site level values and the black dot indicates an outlier.

Table 2 Population comparisons of sexual selection metrics calculated for plainfin midshipman (*Porichthys notatus*)

	OSR (M:F)	Nesting success ^a	Mating success ^b	I_{nest}	I_{egg}	I_{pat}	I_s	β_{ss_pat}	δ'_{max}	β'_{pat}	β'_{egg}
North	5.5:1	0.92	3.03	0.098	0.777	0.278	0.207	0.34	0.03	0.02	0.28
South	8:1	0.97	2.88	0.035	1.551	0.426	0.258	1.05	0.07	-0.25	0.41

Population means for each variable are based on site level summaries. Statistics are defined in the text.

^aBased on whether a guarder male obtained a nest (scored as a 1) or did not acquire his own nest when sampled (scored as a 0).

^bMating success refers to the number of genetic mates per cohort, determined by COLONY.

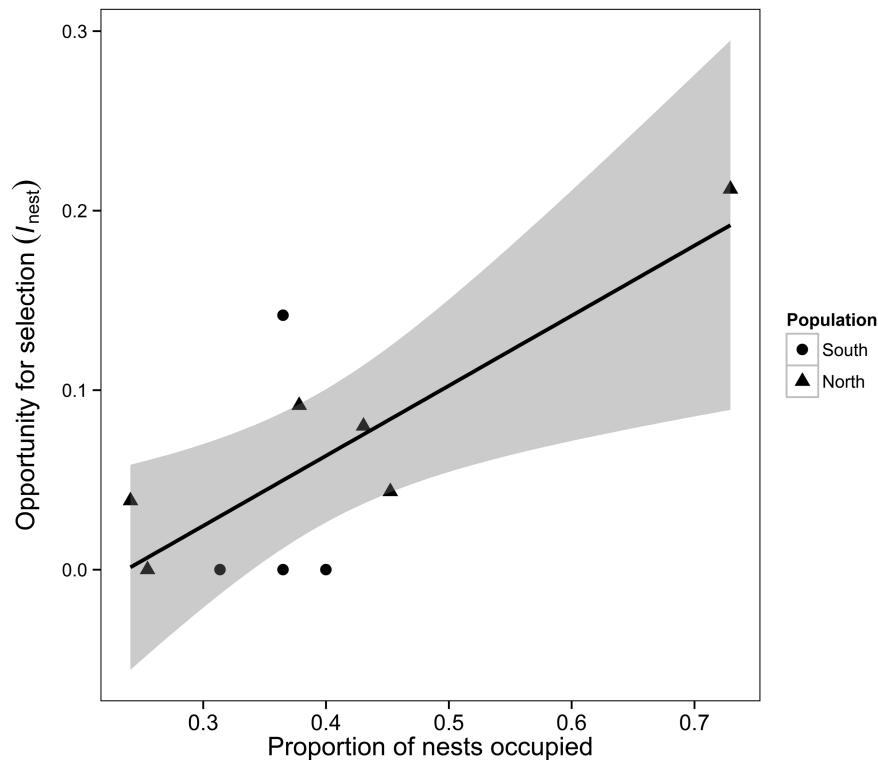


Figure 3

Relationship between the opportunity for selection based on nesting success (I_{nest}) and the proportion of possible nests that are occupied by guarding male plainfin midshipman (*Parichthys notatus*). Study sites from the north population are represented by (\blacktriangle) while study sites from the south population are represented by (\bullet). Linear regression is shown with 95% confidence intervals shaded in gray.

a nest; this did not result in a difference in the opportunity for selection (I_{nest} ; estimate = 0.03, $F_{1,9} = 1.50$, $P = 0.25$). When nest availability was included in the linear permutation model, we found a higher opportunity for selection at sites where more of the nests were occupied (estimate = 0.39, $F_{1,8} = 9.94$, $P = 0.01$; Figure 3). In the north, 87% of the nesting males had received eggs, while in the south 84% of nesting males received eggs. The populations did not significantly differ in the opportunity for selection based on egg counts, a measure of reproductive success (I_{egg} ; estimate = 0.39, $F_{1,9} = 1.81$, $P = 0.22$), or based on guarder male paternity (I_{pat} ; estimate = 0.07, $F_{1,7} = 0.65$, $P = 0.45$). The opportunity for sexual selection (I_s) based on mating success did not significantly differ between populations (estimate = 0.03, $F_{1,7} = 0.64$, $P = 0.45$). The standardized Bateman gradients (based on relative fitness using male paternity on relative mating success) were not significantly different across all sites (ANCOVA: $F_8 = 0.76$, $P = 0.64$). When we ran the linear permutation model using the Bateman gradient regression slope from each site to investigate population differences, we found that the β_{ss} was steeper in the south population (Imp: estimate = 0.36, $F_{1,7} = 4.79$, $P = 0.06$; Figure 4a). The maximum standardized sexual selection differential (s'_{max}) was also marginally higher in the south population (estimate = 0.02, $F_{1,7} = 3.70$, $P = 0.10$; Figure 4b). The selection gradients (based on the regression of relative reproductive success on guarding male body size (SL, using either egg number or paternity) did not differ between populations (β'_{egg} : estimate = 0.08, $F_{1,9} = 1.00$, $p = 0.34$; β'_{pat} : estimate = 0.13, $F_{1,7} = 1.25$, $P = 0.30$). However, at 5 of 7 sites in the north, egg number and body size were positively correlated while none of the sites from the south had slopes that were significantly different than zero (see Supplementary Table S1).

DISCUSSION

Our results confirm previous claims that there are 2 genetically distinct plainfin midshipman populations along the Pacific Northwest, with a moderate level of genetic differentiation (Warner and Case 1980; Thompson and Tsuji 1989; Harper and Case 1999). When we investigated the ecology of these 2 populations, we found that the proportion of available nests did not differ. Thus, the competitive environment experienced by guarder males across sites and between populations is likely similar. We confirmed that male body size is greater in the north; however, the absolute and relative variance in body size among guarder males did not differ between populations. No differences in mating patterns between populations were detected: there were no significant population differences in male reproductive success (as measured by the number of eggs per nest or by paternity), in the frequency of nest takeovers, patterns of cuckoldry, or observed tactic frequencies (either the developmental tactics—guarder vs. sneaker, or behavioral tactics—guarding vs. cuckolding). The Bateman gradient was greater in the south, suggesting that paternity may be more strongly tied to mating success in the south than in the north, but no other measure of sexual selection differed between populations.

Variation in ecological conditions is known to influence mating patterns across populations. Here, we did not detect any differences across populations in a critical ecological variable for midshipman (the availability of nests), and subsequently, mating patterns between populations did not differ either. Our results are consistent with a number of studies across taxa which have also shown that geographically distinct populations did not differ in their mating patterns (Zane et al. 1999; Jones et al. 2001; Goodisman et al.

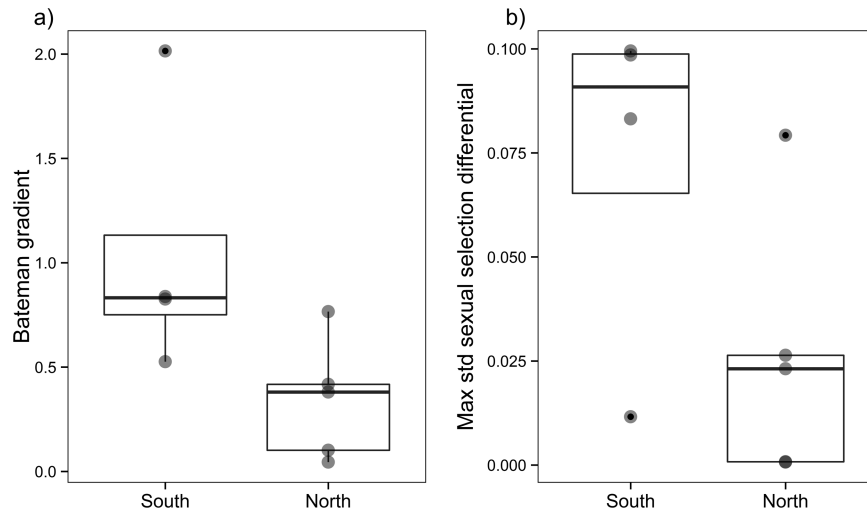


Figure 4

Cross population comparison of sexual selection metrics of the plainfin midshipman (*Porichthys notatus*). (a) Bateman's gradient (β_{ss_pat}), which is the linear relationship between reproductive success and mating success. A steeper gradient (higher value) indicates a greater potential for sexual selection to occur. (b) Maximum standardized sexual selection differential (s_{max}), which is used to investigate selection generated by differential mating success. Large gray dots show individual site level values and the black dot indicates an outlier.

2002; Munguía-Steyer et al. 2012). Although the similarity of mating patterns across populations may be a result of similar ecological conditions, studies have also shown that even populations experiencing different ecological factors may not always experience differences in measures of sexual selection and reproductive success (e.g., Jones et al. 2001; Griffith et al. 2002; Simmons et al. 2004; Singer et al. 2006; Lindstedt et al. 2007). Male size is an important trait for competition and reproductive success in plainfin midshipman (DeMartini 1988), as well as in other species (e.g., Andersson 1994). Our study suggests that the similar *absolute* and *relative* variance of male size between populations could also explain the apparent similarity in mating patterns and sexual selection. The outcomes of competitive interactions are often based on relative differences in fighting characteristics which in turn are related to body size (Parker 1974). Along with similar variation in body size, we also found a similar frequency of alternative tactics across populations, which is consistent with a similar level of sexual selection in the 2 populations. Thus, variation in body size (SD and CV) may be an appropriate metric for assessing differences in competition among populations and may be a key determinant of the strength of sexual selection, at least in plainfin midshipman.

Despite the apparent greater selection in the south, guarder males from the north population were significantly larger than guarder males from the south. Moreover, sneaker males and females were also larger in the north. If not a result of male–male competition, the difference in body size across populations may instead be a result of a physiological adaptation due to natural selection. For example, there are longer low tide events in the north population compared with those in the south leaving fish nesting and mating in the north out of water and cut off from the ocean for a longer duration than fish in the south (Mobile Geographics LLC 2005; Cogliati KM, personal observation). Consequently, fish in the north must endure a greater degree of possible desiccation, hypoxia, and a buildup of waste material in their nest (Craig et al. 2014). Fish respond to hypoxia in a number of ways, including both enhancing the uptake of oxygen present, or limiting metabolic use (e.g., Nilsson 1990; Nikinmaa and Rees 2005; Richards et al. 2009;

Richards 2011). Fish in the north may grow to a larger size in order to increase their gill structure (Sollid et al. 2003, 2005) or to reduce their metabolic rate to reduce oxygen demand (Martinez et al. 2006; Richards et al. 2008; Richards 2009). Further investigation is needed to ascertain the physiological differences between large and small individuals' response to the degree and intensity of hypoxia.

Alternative explanations for the large fish body size in the north includes predation pressure, where fish in the south may be experiencing greater predation intensity resulting in more individuals with a smaller body size at sexual maturity (e.g., Reznick et al. 2001). Additionally, there may be differences in mate preferences, where females in the north may have a stronger preference selecting for larger male body size. Indeed, our results indicate that the majority of sites in the north had positive selection gradients on male size (β'_{egg}), while the ones in the south did not. It would be worthwhile to investigate the degree of predation pressure and differences in female mate choice between the north and south populations. Our study suggests that the larger body sizes of fish in the northern population are not, however, likely a result of an increased male–male competition.

The results of our study show that similar competitive environments as measured through nest availability and similar variance in male size across populations appear to translate into similar mating patterns and sexual selection in plainfin midshipman. Thus, at least for plainfin midshipman, mating patterns appear to be highly conserved across their spatial distribution at sites that experience similar nest availability or similar variance in male size. Additional studies that include sites with greater variation in body size and nest availability or variation in predation pressure may help shed light on the relative importance of these factors in shaping the competitive environment of an organism and mating dynamics. We recommend that cross-population studies continue to be employed whenever possible.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.beheco.oxfordjournals.org/>

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