The operational sex ratio and density influence spatial relationships between breeding pipefish

T. Aronsen,† K.B. Mobley,‡ A. Berglund,§ J. Sundin,∥ A.M. Billing,★ and G. Rosenqvist†,‡
†Centre for Biodiversity Dynamics, Department of Biology, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway, ‡Department of Evolutionary Ecology, Max Planck Institute for Evolutionary Biology, 24306 Plön, Germany, §Department of Ecology and Genetics/Animal Ecology, Uppsala University, Norbyv. 18d, SE-752 36 Uppsala, Sweden, and ★University of Gotland, SE-621 67 Visby, Sweden

INTRODUCTION

Sexual selection is a process that arises from unequal and nonrandom mating success, and this causes the evolution of a host of morphological and behavioral traits expressed by sexually reproducing organisms (Darwin 1871; Andersson 1994). Although we have made progress in understanding the causes and consequences of sexual selection, there are still many aspects that are not well understood. One such gap in our knowledge is how and to what degree demographic processes affect behavioral interactions within and between the sexes. Demographic factors such as the sex ratio and population density may be particularly relevant in regulating mating behaviors as individuals may switch behavioral strategies under various inter- and intrasexual competitive scenarios (Jirotkul 1999a; Mills and Reynolds 2003; Kokko and Rankin 2006).

The operational sex ratio (OSR), that is, the ratio of sexually receptive males to receptive females (Emlen and Oring 1977) is a main predictor of the intensity and direction of mating competition (e.g., Kvarnemo and Ahnesjö 1996). As the OSR becomes biased toward one sex, competition for mates should increase within that sex as mating opportunities with the sex in demand becomes rare (Emlen and Oring 1977; Kvarnemo and Ahnesjö 1996). As a result of this increase in competition, individuals may respond by altering their behaviors in response to the change in the competitive background. For example, male guppies (Poecilia reticulata) switch from courtship behavior to aggressive interactions with other males as the OSR becomes increasingly male biased (Jirotkul 1999a). Studies have also shown that differences in the OSR can influence mating system organization and the strength and direction of
sexual selection (e.g., Prohl 2002; Mills and Reynolds 2003; Jones et al. 2004, 2005; Clark and Grant 2010; Weir et al. 2011).

Although the OSR clearly influences mate competition, it is less known how other demographic factors, such as population density, affect the behaviors expressed by individuals within a population (Kvarnemo 1995; Jirokulu 1999a; Mobley and Jones 2007, 2009). Theoretical work by Kokko and Rankin (2006) suggests that competition for partners, and thus reproductive skew, will be correlated with density due to the increased opportunity for dominant individuals to monopolize matings at higher densities. Therefore, it follows that sexual selection should increase with increasing density. Some studies have found the predicted increase in intrasexual competitive behaviors (Smith 2007) and sexual selection with population density (Zeh 1987; Tomkins and Brown 2004). However, several studies have found the opposite: sexual selection decreased at high densities when high competition prevented the monopolization of mates by a few individuals (McLain 1992; Mills and Reynolds 2003; Knell and Pomfret 2008). Moreover, other studies show no clear relationship between density and mate competition (e.g., Head et al. 2008; de Jong et al. 2009), suggesting that in some species density might not play a uniform role in determining the level of competition for mates.

The OSR and population density are not necessarily independent and have the potential to interact and influence sexual selection through the mate encounter rate. Assuming a constant density, if the OSR is biased, then one sex will have increased encounters with potential mates, whereas the other sex will suffer a decrease in mating opportunities. However, an increase in population density will increase mate encounters for both sexes (Kokko and Rankin 2006). If both the OSR and population density fluctuate in time and/or space, one can envision a multitude of scenarios where both the direction and intensity of mate competition may vary depending on the mate encounter rate. One method to tease apart the relative influences of density and sex ratio is to manipulate the mate encounter rate by altering the sex ratio of breeding adults and the area in which adults can interact. This method is preferable to simply varying the numbers of individuals within the same breeding area because it manipulates the encounter rate but not the overall numbers of interacting individuals.

Shuster and Wade (2003) and Shuster (2009) introduced a new framework for measuring sexual selection through the spatial (or temporal) distribution of males and females (see also Wade 1995). Their approach is based on the microspatial distributions of males or females measured by mean crowding (Lloyd 1967). Mean crowding \( \bar{m} \) measures the number of competitors the average individual experiences (Lloyd 1967; Shuster and Wade 2003). It has been suggested that the use of mean crowding can provide a utilitarian method to measure the strength of sexual selection in the absence of information on the genetic mating system (Wade 1995; Shuster and Wade 2003).

In this study, we investigate whether there is a relationship between the spatial distribution and mating competition of breeding pipefish. The spatial mean crowding of each sex should capture the strength of intrasexual competition via the spatial aggregation within the sexes. In addition, how the sexes relate to each other spatially (i.e., the spatial association between the sexes) can also reveal intersexual interactions such as courtship and mate sampling behaviors. Further, if there is a link between the spatial distributions and mating competition, then measures of spatial relationships should respond to manipulations of the competitive environment. Thus, it follows that measures of inter- and intrasexual competition should respond to changes in the OSR, which is an important predictor of mating competition (Emlen and Oring 1977; Kvarnemo and Abunejo 1996; Forsgren et al. 2004). Finally, in species without fixed territories or strong aggressive behaviors, the spatial distribution may provide a useful tool to reveal subtle behavioral interactions at the population level.

The broad-nosed pipefish (Syngnathus typhle) is particularly suited to investigate the effects of OSR and density on the behavioral interactions within and between the sexes. Both the OSR and population density fluctuate over the breeding season in natural populations (Vincent et al. 1994; Mobley KB, Jones AG, unpublished data). The species is sex-role reversed such that the strength of sexual selection is typically stronger in females than in males, primarily due to male pregnancy. The long pregnancy of the male causes females to have a higher potential reproductive rate than males, as females can produce twice as many eggs than needed to fill a male during the same period of time (Berglund et al. 1989). Males can receive eggs from several females, and females can deposit eggs in several males; thus, the genetic mating system can be described as polygynandrous (Berglund et al. 1988; Jones et al. 1999).

Adult broad-nosed pipefish do not establish breeding territories or form long-term pair bonds (Vincent et al. 1994, 1995). Female pipefish competes for access to males and display a temporary ornament used both to attract males and to deter females (Berglund and Rosenqvist 2001, 2009). This competition can either be indirect, with larger females suppressing the reproduction of smaller females (Berglund 1991), or direct, with larger females chasing smaller females off from potential mates (Vincent et al. 1994). Experimental evidence shows that males can court as well as compete over mates, but to a lesser extent than females (Berglund et al. 2005).

Several experimental studies have shown that S. typhle changes mating behavior in response to changes in the OSR. Male pipefish are more choosy if they have a choice between many partners (Berglund 1995), and males may switch tactics from being choosy (i.e., maximizing mate quality) to mating quickly (i.e., reducing the risk of failing to mate) when the OSR becomes more male biased (Berglund 1994). In the wild, males tend to be more clumped early in the mating period when the OSR is even or sometimes male biased, perhaps because males aggregate around courting females (Vincent et al. 1994). Females have also been observed in nature to display in groups with other females, presumably to attract mates (Vincent et al. 1994, 1995).

The aim of this study is to test whether the intensity of mating competition is related to the spatial distribution. We investigated the spatial relationship of adult S. typhle using experimental breeding populations with 2 adult sex ratios (male biased or female biased) and 2 population densities (high or low) in a balanced factorial design. We investigated the spatial intersexual competitive relationships using mean crowding \( \bar{m} \), Lloyd 1967; Shuster and Wade 2003), which is a measure of how many competitors of the same sex an individual experiences. If mean crowding responds in a predicted way to changes in OSR and density, then mean crowding might provide a useful tool in the study of behavioral interactions and sexual selection. We also explored male-female interactions using the index of association \( \Lambda \), which is a measure of how well 2 spatial distributions (in our case males and females) correlate (Perry et al. 1999). In addition, we recorded the number of copulations in each treatment to investigate how differences in sex ratio and density affect the number of copulations.

Based on our previous knowledge of the species, we predict that as males become pregnant and the OSR becomes more female
biased, females should become more crowded as they form competitive groups to court males and male–male crowding should be reduced. This effect should be strongest in the high-density female-biased treatment and lowest in the low-density male-biased treatment. Alternatively, a higher density of competitors could cause individuals to distribute themselves more evenly in response to more intense competition (Shuster and Wade 2003). We also expect males and females to be more associated with each other in the beginning of the experiment when all fish are ready to breed. When males become pregnant, they become less active, usually spending more time resting in the vegetation (Svensson 1988; Vincent et al. 1994) and therefore we expect associations between the sexes to decrease as males start brooding. Finally, we expect that there will be more copulation in the male-biased treatments because little available male brood pouch space will limit the number of copulations in the female-biased treatments. Our density treatment could either increase or decrease the number of copulations. An increase in the encounter rate could result in more copulations as density increases. Alternatively, fewer copulations could result in high-density treatments due to stronger female–female competition in the form of interrupted copulation attempts (Vincent et al. 1994).

METHODS

We collected fish and conducted the experiment from early May until the middle of June in 2008–2010 at the Sven Lovén Centre for Marine Sciences, Kristineberg, Sweden, which is located at the mouth of the Gullmar fjord at the west coast of Sweden (58°15′N, 11°28′E). Fish were collected by trawling shallow eelgrass meadows before the breeding period started (April–May). We kept the fish separated by sex in 225-L plastic barrels (ø 59 cm, height 82 cm) until the fish were ready to breed as judged from fleshy male pouches and round female abdomens. Barrels were supplied with a continuous flow of seawater pumped directly from the Gullmar fjord. Therefore, salinity and temperature followed the natural fluctuations in the study area (salinity 18–20‰, the water temperature during the experiment ranged from 9 to 18 °C, mean 13.1 ± 0.1 °C).

We fed the fish 3 times every day with live and frozen brine shrimp (Artemia sp.) and frozen mysid shrimp. Halogen lamps (emitting light with a color temperature of ~3000 K) and cool white fluorescent tubes (~4000 K) provided light in the 18:6 light/dark cycle typical for the site and season. Barrels were cleaned daily. The same regime of feeding and maintenance were continued in the experimental tanks during the experiment.

In order to investigate the effect of density and sex ratio on the spatial distribution of S. typhle, we established experimental breeding populations at 2 different adult sex ratios, either female or male biased, at 2 different densities, high and low. This produced the following treatments: female biased/low density (F-L), male biased/low density (M-L), female biased/high density (F-H), and male biased/high density (M-H). Female-biased treatments consisted of 20 females and 10 males, and male-biased treatments consisted of 10 females and 20 males (Table 1). We manipulated density by halving the bottom area in the high-density treatments, thereby creating a 2-fold increase in total density while keeping the number of individuals constant across treatments. The experiment was conducted in 3 white fiberglass tanks measuring 202 cm (L) × 113 cm (H) with the water depth of 60 cm. In the 2 low-density treatments, the breeding populations each occupied an entire tank (~2 m²). In the 2 high-density treatments, 1 tank was divided into 2 sealed compartments (to prevent water exchange), such that each breeding population occupied ~1 m². Each experimental enclosure was supplied with a constant flow of seawater and a separate drain. Water flow was adjusted to be ~2 times higher in the low-density treatments compared with the high-density treatments such that the exchange of water was roughly equal across treatments.

A grid was painted at the bottom of each tank with a blue permanent marker. The grid consisted of 50 squares (20 × 20 cm) arranged 5 × 10 in the low-density treatments and 25 equal-sized squares arranged 5 × 5 in the high-density treatments. A tuft of artificial seagrass, consisting of green plastic ribbon weighted down by a stainless steel nut, was placed in the center of each square to provide shelter. Above all 3 tanks, a camera (Sony, Handycam HDR-SR3E) was mounted to constantly record the number of copulations in each treatment. This was only done for 3 of the 7 replicates (2008). A successful copulation was recorded when a male and a female copulated and the male was observed to assume the typical s-shaped posture following copulation and shake the eggs down (Berglund and Rosenqvist 1993). Every second night the cameras were dismounted to transfer video to hard drives. We, therefore, did not have video recordings from every second night. However, few copulations (<10 in total) were observed during night time (when the light was dimmed very low), so we find it unlikely that this would influence our results.

Prior to the experiment, we measured standard body length (tip of snout to tip of caudal peduncle) to the nearest millimeter. We also measured body depth of females to the nearest 0.5 mm with calipers (Table 1). There were no differences in body length or female body depth between treatments (one-way analysis of variance [ANOVA]), female body length: F_{3,416} = 0.12, P = 0.9; male body length: F_{3,415} = 0.03, P = 1.0; female body depth: F_{3,416} = 0.09, P = 1.0; Table 1).

We counted number of females and males in each square of the grid on 6 occasions (first reading at 08:00 h and then every second hour until 18:00 h) each day, except for the first day when we began recording the spatial distribution starting 2 h after the release of the fish into the tanks. Individuals were judged to be within a square if their eyes were in that particular square. Note that even in the high-density treatment, there were more squares available than there were individuals of the most numerous sex (N = 20). Each evening, after the last spatial recording, we collected all males and estimated pouch fullness to the nearest 10% by eye. The semitransparent male brood pouch facilitates visual estimation of pouch fullness (Berghund et al. 1989; Berghund and Rosenqvist 1990). Food was distributed evenly in the tanks to avoid aggregation because of patchy food distribution. The tanks were cleaned daily while the males were removed for brood pouch inspection.

The experiment was replicated 7 times: 3 times each in 2008 and 2009, and once in 2010. The replicates were standardized to end when all males in the female-biased treatments were filled with eggs, or when breeding activity had stopped (few or no more eggs deposited during the last day of the experiment), giving replicate lengths of between 3 and 10 days (mean 6.9 ± 1.0). We have in total 288 recordings of spatial distribution per treatment, except for 1 females-biased high-density treatment where we are missing 6 observations (N = 282).

Calculation of the OSR

We calculated the OSR as the number of males available for mating as a proportion of all adults available for mating (Vincent et al. 1994). We assumed that all females had eggs and were ready to
mate throughout the experiment (Berglund et al. 1989), and all males with open brood pouches with space for eggs were included in our OSR estimates. We knew the exact OSR only after the last observation each day when we inspected the males’ pouches. Therefore, we used the mean between the OSR on the evening of the day before and the evening of the current day as an approximation for the 5 observations per day when pouch fullness was not estimated.

Calculation of spatial relationships

We calculated mean crowding (m*) to assess how aggregated the distribution of individuals was within a sex (Lloyd 1967; Shuster and Wade 2003). Mean crowding was calculated using the following equation:

\[
m^* = m + \left[ \frac{\mu}{m} - 1 \right]
\]

where \(m\) is the mean number of individuals of a particular sex within a square of the grid and \(\mu\) is the variance in the number of individuals of a particular sex per square (Shuster and Wade 2003, p. 44). When the variance is large relative to the mean, then that sex is clumped in space and \(m^*\) is greater than the arithmetic mean density (\(m\)). If the variance is small compared with the mean, then individuals are overdispersed and \(m^*\) will then be smaller than \(m\). When the variance equals the mean, then individuals are randomly dispersed and \(m^*\) equals \(m\).

The index of association (X) between sexes was quantified in each treatment for each sampling occasion using SADIEShell v.1.22 (see Perry 1998; Perry et al. 1999). The SADIE (Spatial Analysis using Distance IndiCes) method measures the overall degree of aggregation in spatially referred data through the “distance to regularity,” which is the minimum number of individual movements needed to convert the spatial distribution in question into an even distribution which is the minimum number of individual movements needed to convert the spatial distribution in question into an even distribution (Perry 1998; Perry et al. 1999). SADIE also identifies the contribution of each sampling unit (square) by assigning a clustering index to each location that quantifies how much that sampling unit contributes to clustering. The method identifies clusters as patches or gaps. A patch is an area with relatively high numbers of individuals close to one another, and a gap is an area of relatively small numbers of individuals close to one another. When \(X > 0\), then clusters and gaps in the 2 data sets coincide, and the sexes are positively associated. When \(X < 0\), then there is a negative correlation regarding where the gaps or clusters are found in the 2 data sets, and the sexes are negatively associated with each other. When \(X = 0\), the sexes are distributed randomly in relation to each other (Perry and Dixon 2002). We analyzed the spatial data (the coordinates of each square and the number of females or males per square) for each sex separately.

Statistical analysis

Because the design of the experiment involved replicated populations within each treatment, all analyses were performed with linear mixed-effects models (LMMs) with replicate as a random factor using R v.2.13.1 (R Development Core Team 2011) unless otherwise stated. The response variables were mean crowding (log + 1 transformed) and the index of association. As the 2 adult sex ratio treatments had different numbers of males and females, we investigated whether there were sex differences in mean crowding between the biased sex (females in the female-biased treatment compared with males in the male-biased treatment) and if mean crowding was higher in high-density compared with low-density treatments. Similarly we compared the limiting sex (males in female-biased treatment compared with females in the male-biased treatment) to each other in the 2 different densities. The full model included mean crowding as the response variable and sex and density as fixed factors as well as the interaction between the 2. We also compared the index of association between the 2 adult sex ratios and densities. The full model for the index of association included adult sex ratio, density, and the interaction between the 2 factors.

To investigate whether the OSR influences mean crowding and the index of association, we performed separate analyses on male-biased and female-biased treatments because there was no overlap in the OSR between them. The full model for the mean crowding LMMs included: density and sex as fixed factors, OSR and water temperatures as covariates, and the interactions between density, OSR, and sex. Temperature can affect behavior (Ahnesjö 1993; Kvarnemo 1996, 1998; Silva et al. 2007; Ahnesjö 2008), and changing temperatures during the course of the experiment could affect spatial distribution, so temperature was included as a covariate in the initial models. The full model for the index of association included density as a fixed factor, OSR and water temperatures as covariates, and the interaction between density and OSR.

We used the method of Zuur et al. (2009) for model selection. We compared models with and without interactions and removed nonsignificant (\(P > 0.10\)) fixed effects using log likelihood tests based on maximum likelihood. Only the minimum adequate model is presented in Tables 2 and 3. \(P\) values for the fixed factors in the treatments.
Table 2
Comparisons of mean crowding ($m^x$) between the sexes in the 2 different densities for the biased sex (females under female bias compared with males under male bias) and the same for the limited sex (females under male bias compared with males under female bias)

<table>
<thead>
<tr>
<th>Model</th>
<th>Explanatory variable</th>
<th>Estimate ($\pm$ SE)</th>
<th>t-value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biased sex</td>
<td>Intercept (females, low density)</td>
<td>0.87 (0.04)</td>
<td>22.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Density (high)</td>
<td>0.37 (0.02)</td>
<td>15.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Limiting sex</td>
<td>Intercept (males, low density)</td>
<td>0.48 (0.04)</td>
<td>11.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Sex (females)</td>
<td>0.01 (0.03)</td>
<td>0.43</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Density (high)</td>
<td>0.28 (0.03)</td>
<td>9.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Sex (females)/density (high)</td>
<td>−0.11 (0.04)</td>
<td>−2.59</td>
<td>0.009</td>
</tr>
<tr>
<td>Female biased</td>
<td>Intercept (females, low density)</td>
<td>1.00 (0.05)</td>
<td>21.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Sex (males)</td>
<td>−0.45 (0.02)</td>
<td>−19.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Density (high)</td>
<td>0.19 (0.04)</td>
<td>4.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>OSR</td>
<td>−0.72 (0.23)</td>
<td>−3.16</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Density(high)/OSR**</td>
<td>1.10 (0.29)</td>
<td>3.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male biased</td>
<td>Intercept (females, low density)</td>
<td>1.17 (0.23)</td>
<td>5.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Sex (males)</td>
<td>0.36 (0.03)</td>
<td>11.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Density (high)</td>
<td>0.18 (0.03)</td>
<td>5.84</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>OSR**</td>
<td>−0.61 (0.28)</td>
<td>−2.18</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>−0.03 (0.01)</td>
<td>−2.65</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Sex (males)/density (high)</td>
<td>0.22 (0.04)</td>
<td>4.96</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

We investigated the effect of the OSR on mean crowding separately for the 2 adult sex ratios (ASRs). Parameter estimates of $m^x$ are from LMMs with replicate as a random factor. Mean crowding was log + 1 transformed prior to analysis. All estimates are given as contrasts to the intercept, and only the minimum adequate model is presented.

<table>
<thead>
<tr>
<th>Model</th>
<th>Explanatory variable</th>
<th>Estimate ($\pm$ SE)</th>
<th>t-value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both ASRs</td>
<td>Intercept (female bias)</td>
<td>0.20 (0.02)</td>
<td>9.66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ASR (male bias)</td>
<td>0.05 (0.01)</td>
<td>3.86</td>
<td>0.001</td>
</tr>
<tr>
<td>Female biased</td>
<td>Intercept</td>
<td>0.15 (0.03)</td>
<td>5.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>OSR**</td>
<td>0.47 (0.13)</td>
<td>3.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male biased</td>
<td>Intercept (low density)</td>
<td>0.23 (0.03)</td>
<td>8.83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Density (high)</td>
<td>0.04 (0.02)</td>
<td>2.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Table 3
Comparisons of the index of association ($X$) between the 2 ASRs, and the relationship between the OSR and $X$ separately for the 2 ASRs

<table>
<thead>
<tr>
<th>Model</th>
<th>Explanatory variable</th>
<th>Estimate ($\pm$ SE)</th>
<th>t-value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both ASRs</td>
<td>Intercept (female bias)</td>
<td>0.20 (0.02)</td>
<td>9.66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ASR (male bias)</td>
<td>0.05 (0.01)</td>
<td>3.86</td>
<td>0.001</td>
</tr>
<tr>
<td>Female biased</td>
<td>Intercept</td>
<td>0.15 (0.03)</td>
<td>5.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>OSR**</td>
<td>0.47 (0.13)</td>
<td>3.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male biased</td>
<td>Intercept (low density)</td>
<td>0.23 (0.03)</td>
<td>8.83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Density (high)</td>
<td>0.04 (0.02)</td>
<td>2.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Parameter estimates of $X$ ($\pm$ SE) are from LMMs with replicate as a random effect. All estimates are given as contrasts to the intercept, and only the minimum adequate model is presented.

RESULTS

Densities, the OSR at the start and the end of the experiment, fish length, and body depth (females only) are summarized for each treatment in Table 1. On initiation of the experiment, the OSR decreased and became less female biased in both adult sex ratio treatments. As nearly all males in the female-biased treatment had completely filled brood pouches and were therefore not included in OSR calculations, the OSR approached zero under female bias. In the male-biased treatments, the OSR approached equality (0.5) because many males did not fill their brood pouches and were still able to mate (Table 1 and Figure 1).

Copulations

Fish copulated significantly more in the male-biased than in the female-biased treatments (mean ± SE female-biased low density: 64.33 ± 13.38; female-biased high density: 42.33 ± 10.68; male-biased low density: 154.67 ± 24.13; male-biased high density: 132.00 ± 14.50). We also observed more copulations in the low-density treatment than in the high-density treatment, but this was not significant (2-way Anova—effect of sex ratio treatment: $F_{1,9} = 33.65$, $P < 0.001$; effect of density: $F_{1,9} = 2.57$, $P = 0.14$, $r^2 = 0.80$; Figure 2).

Mean crowding ($m^x$)

Overall, our measure of spatial aggregation within each sex, mean crowding, did not differ between the sexes in excess (females in female-biased and males in male-biased treatments) in the 2 adult sex ratio treatments (Table 2 and Figure 3). However, mean crowding was higher in high-density treatments than in low-density treatments as expected. For the limiting sex (males in female-biased treatments and females in male-biased treatments), there was a...
significant interaction between sex and density. Mean crowding was higher for both the limiting sexes in the high-density treatments, and overall mean crowding was higher for limiting males than for limiting females in the high-density treatments, but not in the low-density treatments (Table 2 and Figure 3).

A significant interaction between the OSR and density indicates that the slope of OSR on mean crowding differs between the 2 densities. In the female-biased low-density treatment, mean crowding increased as the OSR became even more female biased (slope $\pm$ SE = $-0.72 \pm 0.23$). Note that a negative slope indicates an actual increase in mean crowding as the OSR decreases. In the female-biased high-density treatment, however, there was no effect of the OSR on mean crowding (slope $\pm$ SE = $0.32 \pm 0.29$; Table 2 and Figure 3). Because there were fewer males than females in the female-biased treatment, males crowded less than females in both densities (Table 2 and Figure 4). There was no significant effect of
temperature on mean crowding under female bias, and temperature was therefore removed from the final model.

By contrast, under male bias, mean crowding increased as the OSR became less male biased in both densities (Table 2 and Figure 5a,b). There was also an interaction between sex and density; mean crowding was higher in high density for both sexes, but the effect of density was stronger for males than females (Table 2 and Figures 3 and 5a,b). Temperature had a negative effect on mean crowding in the male-biased treatment as the fish were less crowded at high temperatures (Table 2).

**Index of association (X)**

We found that our measure of interactions between the sexes (the index of association) was higher under male bias than under female bias, whereas density had no significant effect on the index of association (Table 3 and Figure 6). There was a positive effect of the OSR on the index of association in the female-biased treatments and sexes associated more in the beginning of the experiment, when the males had empty pouches, and less toward the end as the OSR approached zero (Table 3 and Figure 7a). Under male bias, there was no effect of the OSR on the index of association (Table 3 and Figure 7b), but association was higher in the high-density treatment (Table 3 and Figures 5 and 7b). Temperature did not affect the index of association under female or male bias and was removed from the models.

**DISCUSSION**

The purpose of this study was to investigate whether the OSR and density affect the spatial relationships among and between breeding pipefish (*S. typhle*). We used 2 spatial measures (mean crowding, \( m^* \) and the index of association, \( X \)) to investigate how the spatial relationships within and between the sexes changed according to the predicted strength of mating competition. We found that both the OSR and density did influence our measures of spatial relationships, but the spatial relationships within the sexes did not conform to the predicted level of mating competition in all cases.

**Spatial relationships within sexes (mean crowding, \( m^* \))**

The use of mean crowding as a measure of sexual selection was originally developed for species with resource defense polygyny (Wade 1995) and was proposed as a method to measure the opportunity for sexual selection without explicit information about the genetic mating system (Shuster and Wade 2003; Shuster 2009). According to Lloyd (1967) and Shuster and Wade (2003), mean
However, we cannot conclude that mean crowding would increase when the OSR became more even or female biased; this is as expected if mean crowding reflects mating competition. We know from field studies that females will still be eager to compete and mate when males fill up with eggs (Svensson 1988; Vincent et al. 1994, 1995). Several studies in other species have found that intrasexual competition increases as mating opportunities decrease (Kvarnemo et al. 1995; Jirokul 1999a; Grant and Foam 2002; Clark and Grant 2010; Weir et al. 2011). However, in our experiment, males as well as females crowded more as fewer males were available for mating. Furthermore, as for the females, this increase in mean crowding for males was found in both male-biased treatments (low and high density) and in the female-biased low (but not high) density treatment. This finding contradicts our expectation of an opposite response in males from females when more males become unavailable for mating. Earlier experiments have shown that males do compete for mates, but less so than females (Berglund et al. 2005). However, we cannot conclude that an increase in mean crowding for males reflects competition, as the potential for mating competition in males should decrease as fewer males are available for mating in both adult sex ratios.

It is possible that different mechanisms influence crowding in the 2 sexes. For example, receptive males could respond to female crowding by grouping around displaying females (Vincent et al. 1994, 1995), and our results are consistent with this. However, pregnant males, who obviously do not compete for access to females, still crowded more in the female-biased treatments as the OSR approached zero. In the wild, fully pregnant males are less active than females and remain in the eelgrass (Svensson 1988; Vincent et al. 1994). The increase in crowding in males could have occurred because pregnant males were more inactive and in addition these males could choose to occupy certain areas in the tanks that provide a good microclimate with respect to pregnancy (water flow, temperature, etc.) rather than being randomly dispersed. Regardless of the mechanism behind the increased mean crowding in males in relation to the OSR, our results do not support that mean crowding reflects mating competition in males. As expected, the number of copulations was higher in the male-biased than in the female-biased sex ratio because the shortage of male brood pouches limits the possible number of copulations in the latter treatment (Berglund et al. 1989). The OSR in the male-biased treatments was roughly equal at the end of the experiment because there were many males with space left in their brood pouches.

Overall mean crowding did respond in the predicted way to density. In general, mean crowding was higher in the high-density
treatments as expected because there was less space in the high-density treatment. We also found that the effect of OSR on mean crowding under female bias depended on the density. Mean crowding increased as males filled up with eggs in both sexes in the male-biased treatments and in the female-biased low-density treatment; however, this increase in mean crowding was absent for both sexes in the female-biased high-density treatment (no response to more female-biased OSR). In females, it is possible that intense within-sex competition caused by frequent encounter rates may have caused individuals to avoid further crowding at high densities (Shuster and Wade 2003). Mating competition levels off at high densities in the guppy (Jirotkul 1999b) or at very biased OSRs in the Japanese medaka, Oryzias latipes (Clark and Grant 2010). We suggest that the lack of increase in mean crowding in the high-density treatment under female bias can be due to high levels of female–female competition. High female–female competition could also cause males to avoid further crowding if groups of males attract more female attention, but we do not know if female harassment influences male behavior in this species. We also observed less copulation in high-density rather than low-density tanks. Even if this was not a significant result, this pattern was the same in all replicates under female bias. This evidence corroborates our theory that intense female–female competition causes individuals in the high-density tanks to avoid further crowding. Females in this species will frequently interrupt copulations (Vincent et al. 1995; Aronsen T, Mobley KB, Berglund A, Sundin J, Billing A, Rosenqvist G, personal observations). Unlike the female-biased treatments where mean crowding increased only at low density, crowding under male bias increased in low-density as well as in high-density treatment for both sexes. For females under male bias, female–female competition may never reach the same intensity as under female bias, producing increased mean crowding in females under both densities as mating competition increased as more males filled up their pouches. For males, higher density should not affect male–male competition even under male bias, as male–male competition is not as strong as in females (Vincent et al. 1994; Berglund et al. 2003) and in any case competition for mates should decrease as the OSR becomes more even.

Spatial relationships between the sexes (index of association, $X$)

The index of association should capture interactions between the sexes, and our data support our predictions. First, the sexes become less associated with each other when the OSR decreases, but only in the female-biased OSR. As mentioned above, pregnant males will spend more time inactive than nonpregnant males and females (Svensson 1988; Vincent et al. 1994), so it is expected that males and females would associate less strongly in the female-biased OSR.

Second, the index of association was higher under male bias than female bias overall. This result was expected because there are always males available for mating under male bias. The higher association between the sexes indicates that the sexes were interacting with each other (courting/inspecting). Additionally, the index of association was greater in high density rather than low density (as we expected from the higher encounter rates) but only under male bias where there still were males available for mating.

CONCLUSIONS

The spatial distribution of individuals has been proposed as a means to quantify sexual selection (Emlen and Oring 1977; Shuster and Wade 2003). Here we investigated whether 2 methods of measuring spatial distribution reflect mating competition, a major force in the evolution of mating systems. Our study shows that the spatial distribution only partly reflects sexual selection in the form of mating competition. We found that mean crowding behaved according to predictions in relation to increased mating competition (more female-biased OSR) for the most competitive sex. However, mean crowding did not reflect the predicted level of mating competition in males. Our measure of spatial association between the sexes ($X$), behaved in a predicted fashion to the manipulations of density and sex ratio, and even if this measure has not (to the best of our knowledge) been used in studies of animal behavior, it might be a useful measure of between-sex interactions on the population level. In general, we conclude that extensive knowledge about the behavioral ecology of the species is critical to interpret the mechanisms behind spatial patterns. Based on our findings, we recommend caution with the application of measures of spatial distribution in studies of sexual selection because we demonstrate that the spatial distribution of males and females does not necessarily reflect the predicted direction and strength of mating competition in all situations.

FUNDING

This work was funded by the Norwegian Research Council (186163/V40) to G.R., the Swedish Research Council (A.B.), and the Association of European Marine Biological Laboratories (G.R.). K.B.M. was supported by an International Research Fellowship Grant from the National Science Foundation. Catching, handling, and experimentation were done under license Dnr 118-2008 from the Swedish Board of Agriculture.

We would like to thank Elias Berglund, David Hoglund, Hanna Hoglund, and Ronny Hoglund for field assistance and Lisa Cats Myhre, Jisca Huisman, and Grethe Robertson for statistical advice. We would also like to thank Adam Jones, Colette St. Mary, Karin Magnuson, Stephen Shuster, and the Shuster lab for thoughtful discussions, and an anonymous reviewer and Gil Rosenthal for comments.

Handling editor: Gil Rosenthal

REFERENCES


Mobley KB, Jones AG. 2009. Environmental, demographic, and genetic mating system variation among five geographically distinct dusky pipefish (Syngnathus floridus) populations. Mol Ecol. 18:1476–1490.


