Mate choice, operational sex ratio, and social promiscuity in a wild population of the long-snouted seahorse Hippocampus guttulatus

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Mate competition and mate choice are not mutually exclusive behaviors. Both behaviors may drive sexual selection in one or both sexes of a population. One of several factors affecting which behavior is exhibited by which sex is the operational sex ratio (OSR) in the study population. The present study combines behavioral observations in the field with controlled experiments in aquaria to investigate social interactions and mate choice in both male and female long-snouted seahorses Hippocampus guttulatus in the context of the population OSR. Compared with the more readily studied pipefishes, data on OSR and mate choice in seahorses are scarce in the published literature. Our field data provide novel evidence of social promiscuity, size-assortative mating, and an OSR that varies from being unbiased early and midseason to male biased at the end of the breeding season. Our mate choice experiments revealed intersexual differences in mate preference with males significantly preferring larger females to familiar ones. Taken together, our field and experimental results suggest that mate choice rather than intrasexual competition could drive sexual selection in seahorses. Key words: assortative mating, reproductive behavior, sexual selection, Syngnathidae. [Behav Ecol 20:160–164 (2009)]

The ratio of sexually active males to sexually active females in a population (operational sex ratio [OSR]) may influence the evolution of mating systems by creating a skew in the reproductive success among members of one sex (Emlen and Oring 1977). Parental investment required for the care of developing embryos or offspring may also contribute to mating system evolution (Trivers 1972), just as sexual selection may in turn influence patterns of parental investment (Kokko and Jennions 2003). Thus, monogamy can be favored if potential mates are scarce or dispersed and/or if the OSR is close to unity (Emlen and Oring 1977). Compared with polygamous mating systems where one sex can increase its reproductive rate by mating with as many partners as possible regardless of their quality, monogamy usually favors mate choice by both sexes. Such choice can be based on a wide variety of characteristics of a potential mate including dominance, body size, or ornamentation (Bateson 1983), but familiarity can also affect mating decisions (e.g., Zajitschek et al. 2006). Male choice based on familiarity may play an important role in, for example, reducing inbreeding (Hain and Neff 2007). In some monogamous species, where both partners invest substantially in maintaining pair bonds, familiarity may increase social/sexual behaviors (e.g., Vincent 1995).

In the family Syngnathidae (including pipefish and seahorses), parental investment is both substantial and unusual in that males, rather than females, carry and care for the brood. In some pipefish species, there is a reversal of conventional sex roles whereby females compete more intensely and males are choosier (Jones et al. 2000). Studies on mate choice in Syngnathus typhle, for example, have shown that ornamented females are attractive to males but that dominance in competition between females can override ornamental attractiveness and drive male mate choice (Berglund and Roseqvist 2001). Importantly, mutual mate choice studies in this species showed that female S. typhle are also choosy and that their choice can affect offspring quality (Sandvik et al. 2000; Berglund et al. 2005). In addition, female quality, as reflected by body size, was shown to influence multiple matings by male (Jones et al. 2000).

Male seahorses (Hippocampus) also provide paternal care in specialized brood pouches, but contrary to the closely related pipefishes, sex-role reversal is not thought to occur in this genus. In Hippocampus fuscus, males compete for mates (Vincent 1994), and female Hippocampus zosterae may have lower potential reproductive rates (PRRs) than males (Masonjones and Lewis 2000), both suggesting no sex-role reversal. Recently, however, sexual selection was shown to act strongly on females in a natural population of the Western Australian seahorse (Hippocampus subelongatus), characterized by female-biased adult sex ratios (ASRs), and this suggested that sex-role reversal may occur in this population (Kvarnemo et al. 2007).

Seahorses have been reported to be socially monogamous (Vincent and Sadler 1995; Jones et al. 1998; Kvarnemo et al. 2000, 2007), even if opportunities for polygamy exist (Hippocampus whitei, Vincent et al. 2004, but see Wilson and Martin-Smith 2007). However, recent parentage studies have revealed that male H. subelongatus were involved in mate switching (Kvarnemo et al. 2000, 2007), perhaps to mate with “better”, larger females (Kvarnemo et al. 2000). Current knowledge of
mate choice and mate switching in seahorses is, however, very limited compared with the closely related pipefishes.

One unusually dense population (1.5/m² of substrate; Curtis and Vincent 2006) of wild long-snouted seahorses Hippocampus guttulatus provided us with a unique opportunity to assess social and sexual interactions. The aim of this study was to characterize social interactions and calculate OSR in a wild population of this species and to assess, for the first time, the effects of familiarity and body size on subsequent mate choice in both males and females in a free-living population.

MATERIALS AND METHODS

In situ reproductive behavior

Individually tagged adult seahorses were observed by SCUBA divers on a 1.69-m² focal site in the Ria Formosa lagoon, Portugal (39°59′ N, 7°51′ W; Curtis and Vincent 2006). Social interactions and reproductive status were observed 3–5 times per week, 1–2 h after sunrise during 1–5 months of the 2001–2004 breeding seasons, which generally last from March to October. Observers swam at least 1 m above the substrate and recorded individuals engaged in greeting, courtship, mating, or aggression. Greetings were characterized by body brightening and at least 2 of the 4 common ritualistic behaviors exhibited by mutually responsive seahorses (Vincent and Sadler 1995): quivering side by side, entwining tails, twirling around a holdfast together, and promenading (swimming together, usually with tails entwined). Courtship, which indicates readiness to mate (Vincent 1994; Masonjones and Lewis 2000), was identified by greeting behaviors coupled with pointing (female lifting head toward surface) and pumping (male contracting pouch) or rising (pair swimming vertically). Mating was identified by rising and transfer of eggs. We also recorded displays that involved a male or female brightening, approaching, and quivering next to, entwining tails with, or following an unresponsive conspecific. Aggression was assumed when individuals engaged in tail wrestling, snapping (rapid snout strikes), or chasing.

The degree of social promiscuity was investigated (using data from 2001 to 2002) by recording the number of partners that tagged individuals greeted or courted during each breeding season. For this analysis, we assumed that untagged partners were always the same individual (to make a conservative estimate). In order to assess the degree of size-assortative mating, the trunk length of all tagged individuals was measured in situ with a ruler from the top of the coronet to the last trunk ring. The measurement was taken as a straight line to the nearest millimeter while holding the head at a right angle to the trunk (as in Curtis and Vincent 2006). In addition, observers recorded the reproductive status (following Curtis 2007) of all males (full pouch and empty pouch) and females (presence or absence of hydrated eggs) encountered during each dive so that OSR could be calculated. Male pouches were visually or manually inspected to determine whether or not they were full, whereas female trunk girth was assessed visually and used as an indicator of reproductive status as in Vincent and Sadler (1995); females whose trunks were bulging between trunk rings (occasionally with visible eggs) were assumed to have hydrated eggs (Curtis 2007) and be ready to mate.

Ex situ mate choice trials

Seahorses were collected on several mornings in September to October 2005 by SCUBA diving on the focal site. Familiar pairs were identified as males and females engaged in social interactions or within 1 m of each other (the average home range size for this species at this site is 20 m²; Curtis and Vincent 2006) and were collected and kept together in aquaria. No brooding males were collected. Haphazardly sampled individuals were captured from another area approximately 2 km SSW of the focal site. Trunk length of each collected seahorse was measured at the aquaculture facility to the nearest millimeter as described above. One fish from each familiar pair was used as the focal animal, whereas the other fish from the pair and all haphazardly sampled individuals were used as object fish.

Paired and single fish were held in tanks with individual compartments at IPIMAR’s aquaculture facilities (adjacent to the focal site). Seahorses were fed Artemia sp. once a day ad libitum after the test trials. Trials were undertaken in 5 sets of 4 days each (1 day for animal collection followed by 3 days of trials). We studied the mate choices of 19 focal females and 18 focal males who chose mates from a total of 88 object fish; focal fish were never used as object fish. All fish were returned to the collection site on completion of the trials.

One day after collection, the first fish to be tested were introduced into the test tanks (Figure 1), in which water flowed from the object fish to the focal fish, allowing only the focal fish to receive olfactory cues. Before each trial, we placed the object fish in the choice compartments, the focal fish in the middle of the test compartment, and left all fish to acclimate for 1 h. Trials began by lifting the opaque partition separating the focal fish from the object fish. One fish from each pair was the focal animal in 3 separate trials, run over 3 days. For a given trial, the focal fish was presented with 1) two unfamiliar fish of the opposite sex but dissimilar in size (mean difference = 4.5 mm in trunk length), 2) the familiar fish and an unfamiliar fish of the opposite sex of similar size (to the familiar fish; mean difference = 0.2 mm), or 3) the familiar fish and an unfamiliar but larger fish of the opposite sex (mean difference = 4.4 mm). The order of the 3 types of trials and the compartments of the 2 object fish were fully randomized. The position of the focal fish was observed every 20 min for 4 h: 1) positioned in front of and close to one of the 2 object fish or 2) outside the choice region (Figure 1). We considered that the focal fish had made a choice if it was seen in front of one of the object fish at least 7 of the 12 observations (58%; based on Sandvik et al. 2000). Mate choice was analyzed using binomial tests (“no choice” [NC] scores excluded).

RESULTS

In situ reproductive behavior

Overall, 354 interactions were recorded, but only 3 mating events were observed. More than half of the observed interactions (57%) involved at least one untagged seahorse for which trunk length was not obtained. Most interactions were mutually responsive greetings (77.4%) or courtship (5.4%), but 16.4%
were displays, most of which (81%) involved brightened males displaying to unresponsive females. Eleven greeting or courting pairs were interrupted by a second male, and of these interactions, 10 ended with the 2 males snapping, tail wrestling, and/or chasing. Two greeting pairs were interrupted by a second male, and of these interactions, 10 ended with the 2 males snapping, tail wrestling, and/or chasing. Two greeting pairs were interrupted by a second male, and no aggression was observed.

We observed tagged individuals engaged in greetings or courtship 1–9 times within each of the 2001 and 2002 reproductive seasons. Most tagged males (26 out of 29) and females (25 out of 30) that were observed greeting or courting on multiple occasions interacted with at least 2–7 partners during a single reproductive season (assuming that all untagged partners of the same sex were the same individual). However, of these individuals, tagged males greeted or courted significantly more partners ($n = 29$, mean $\pm$ standard deviation [SD] = $3.03 \pm 1.42$) than did tagged females ($n = 30$, mean $\pm$ SD = $2.23 \pm 1.16$) ($t$-test, $t = -2.36$, degrees of freedom [df] = 57, $P = 0.01$).

The sizes of males and females engaged in courtship were significantly correlated (Pearson correlation, $r = 0.63$, $t = 2.94$, df = 13, $P = 0.01$). However, when greetings were also included in the analysis, the correlation was not significant (Pearson correlation, $r = -0.12$, $t = -0.608$, df = 24, $P = 0.55$). In these correlation analyses, only the first of all records for each tagged individual was included.

Although the ASR on the focal site was unbiased over the whole reproductive season (binomial tests: all $P > 0.3$, see also Curtis and Vincent 2006), the OSR was unbiased early and midseason but was significantly male biased late in the breeding season (Figure 2; binomial tests: May, $n$ [number of sampling days] = 13, $P = 0.09$; June, $n = 15$, $P = 0.26$; July, $n = 12$, $P = 0.86$; August, $n = 18$, $P = 0.85$; September, $n = 8$, $P = 0.01$; October, $n = 16$, $P = 0.002$).

**Ex situ mate choice trials**

In trials where the focal fish was presented with 2 unfamiliar individuals of unequal size, males and females chose randomly (Figure 3; males: NC = 11, unfamiliar large [UL] = 5, unfamiliar similar [US] = 2; binomial test, $n = 7$, $P = 0.29$; females: NC = 13, UL = 3, US = 2; $n = 5$, $P = 0.69$). Note, however, that the small sample sizes of choices resulted in very low statistical power to detect a real difference.

In trials where the focal fish could choose between a familiar or an unfamiliar individual of the same size, males and females also chose randomly (Figure 3; males: NC = 13, US = 4, familiar [F] = 1; binomial tests: $n = 5$, $P = 0.22$; females: NC = 11, US = 5, F = 3; $n = 8$, $P = 0.51$).

In the third type of trial, when the focal males made a choice, they chose significantly more often the large unfamiliar female rather than the smaller familiar female (Figure 3; NC = 10, UL = 7, F = 1; binomial tests: $n = 8$, $P = 0.04$). By contrast, female choices were not significantly different from random (NC = 8, UL = 3, F = 7; $n = 10$, $P = 0.23$).

**DISCUSSION**

Our study suggests that male *H. guttulatus* may be choosier and favor larger females despite the male-biased OSR observed in their population of origin during the period of the mate choice trials. In accordance with the prediction of a male-biased OSR, only males were seen engaged in mate competition in the field.

Mate competition and mate choice are not mutually exclusive behaviors (Owens and Thompson 1994; Berglund et al. 2005). Whereas the relationship between OSR and mate competition is relatively simple, the relationship between OSR and mate choice is more multifaceted (Vincent et al. 1992; Owens and Thompson 1994; Kvarnemo and Ahnesjö 1996). In fact, the presence, strength, and direction of mate choice seems to depend not only on OSR and PRR but also on the cost of breeding (Kokko and Monaghan 2001) and variation in mate quality (Parker 1983; Owens and Thompson 1994) between the sexes. The pattern we observed in our study, with males being both more competitive and choosier, could be explained by one or both of these factors.

A difference in variance in mate quality between sexes can affect the direction and intensity of mate choice (e.g., bush-cricket *Kausanaphila narte*, Kvarnemo and Simmons 1999). It is well established that larger females have higher fecundity in fishes (Bagenal 1978). Although reproductive success was related to female but not male size in a wild population of *H. whitei* (Vincent and Giles 2003), a more recent laboratory study of *H. kuda* found that both male and female size affected survival and growth rate of the broods (Dzuba et al. 2006). The reproductive output of *H. guttulatus* is also significantly correlated with male standard length (Curtis 2007), and thus, potentially, both sexes could gain by selecting a larger partner when given the choice. Mutual mate choice studies in the closely related pipefish *S. typhle*, however, found no relationship between the preferred males or females and length or other mate quality variables such as parasite loads or courtship effort (Sandvik et al. 2000; Berglund et al. 2005).

The cost of breeding may also affect the direction and intensity of mate choice (Kokko and Monaghan 2001). In seahorses, one study showed slightly higher PRR in males (Masonjones...
Mates and females greeted or courted multiple partners, even within reproductive seasons. This represents the second record of social promiscuity in seahorses (Wilson and Martin-Smith 2007) and suggests that promiscuity may be more common among seahorses than previously assumed. However, social promiscuity does not automatically translate into genetic polygamy (Wilson and Martin-Smith 2007), and genetic parentage studies of broods will be needed to determine the genetic mating system of this species.

During our study, *H. guttulatus* were observed to court (but not greet) assortatively according to trunk length. This result, combined with male preference for larger females during a choice trial, suggests that greetings may serve a different purpose in *H. guttulatus* than previously thought (Vincent 1995). In fact, greetings could be a mechanism to assess body size or other quality characteristics rather than, or in addition to, maintaining reproductive synchrony.

Data on mate choice in seahorses are scarce in the published literature, compared with the more readily studied pipefishes. As predicted by an OSR that was sometimes male biased, males behaved aggressively on occasion, but they were also choosier and preferred larger females over familiar females. Moreover, when males were presented with 2 unfamiliar females of different size, they also tended to choose the larger female. In addition, observations in the field highlighted the social promiscuity existing in this system (with males being more promiscuous than females) and assortative pairing of courting individuals. These results suggest that sexual selection could act more strongly on females in such a system. More mate choice studies, with a greater number of fish if possible, will need to be conducted in conjunction with parentage testing in order to understand the selective forces driving seahorse mating systems. Studies such as ours, investigating mating systems in “borderline cases” (Kokko and Monaghan 2001), are valuable to provide information on the relationship between OSR and sex roles.

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