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Differences in potential reproductive rates of male and female seahorses related to courtship roles

HEATHER D. MASONJONES & SARA M. LEWIS

Department of Biology, Tufts University

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Dwarf seahorses, *Hippocampus zosterae* (Syngnathidae), are distinguished by extreme morphological specialization for paternal care, the formation of monogamous pair bonds and mating repeatedly over the course of a breeding season. To determine the potential reproductive rates of male and female dwarf seahorses, we measured (1) the maximum number of offspring produced per breeding cycle when sexually receptive mates were unlimited, and (2) the relative time each sex was unavailable for mating ('time out'). We paired sexually isolated males and females with sexually receptive partners and observed them from the day of introduction through to copulation, to determine the length of time it takes each sex to prepare to mate. We conducted additional experiments to determine the length of gestation, which when added to the time needed to prepare to mate and copulate gives an estimate of total reproductive cycle duration, T . We estimated potential reproductive rate by dividing the mean number of offspring produced per breeding cycle by the duration of the breeding cycle (T). We estimated reproductive 'time out' by identifying the period of time males and females were physiologically capable of mating ('time in', S) and subtracting time S from time T . When provided with sexually receptive partners, females took 2 days longer than males to complete courtship and copulation, but neither males nor females remated during gestation. Therefore, males could potentially produce 17% more offspring than females over the course of one breeding season. Females had reproductive 'times out' 1.2 times longer than did males, as they were only capable of mating during the 4 h directly preceding copulation. Thus, *H. zosterae* males have higher potential reproductive rates and shorter reproductive 'times out' compared with *H. zosterae* females. These results and previous work indicating that seahorses display traditional courtship roles support the prediction that the sex having the higher potential reproductive rate, or equivalently, the shorter 'time out', will compete more intensely for access to the opposite sex.

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Sexual selection arises from differential reproductive success based on intersexual choice and intrasexual competition (Darwin 1871; Anderson 1994). In most species, females are more selective of their mates and males compete for access to females (Gwynne 1991; Andersson & Iwasa 1996), a pattern hypothesized to reflect the relative parental investments per offspring made by males and females (Williams 1966; Trivers 1972, 1985). Because females always invest more in their gametes than males and in many species also provide most of the parental care (Clutton-Brock 1991), relative parental investment theory accurately predicts the prevalent courtship roles of

male mate competition and female mate choice. However, when male parental investment per offspring exceeds that of females, then relative parental investment theory predicts males should be more selective of their mates and females should compete for access to males (Trivers 1972).

Relative parental investment can be difficult to measure experimentally (Knapton 1984; Clutton-Brock & Vincent 1991). Potential reproductive rate, defined as the 'maximum number of independent offspring that parents can produce per unit time', has been invoked as an alternative predictor of courtship roles, particularly for species with paternal care (Clutton-Brock 1991; Clutton-Brock & Vincent 1991; Clutton-Brock & Parker 1992). More recently, Kvarnemo (1994) suggested a useful distinction between the 'realized' and 'potential' reproductive rates

Correspondence: H. D. Masonjones, Campus Box 2237, Department of Biological Sciences, Amherst College, Amherst, MA 01002-5000, U.S.A. (email: hmasonjones@amherst.edu). S. M. Lewis is at the Department of Biology, Tufts University, Medford, MA 02155, U.S.A.

of males and females. In her definition, potential reproductive rate can be determined by measuring the maximum number of offspring produced after providing each sex in a population with unlimited, sexually receptive partners. Experimentally this has proven to be a useful concept in determining courtship roles. Male bushcrickets, *Requena verticalis*, when provided with ample food, have a higher potential reproductive rate than females and display low selectivity when interacting with females (Kvarnemo & Simmons 1998). When food is scarce, however, males have a lower potential reproductive rate than females and reject females at a higher rate than when resources are abundant.

Additional theoretical work has suggested that courtship roles are determined by a combination of the adult sex ratio, the relative parental investment and the relative time involved in reproductive tasks ('times out') versus the amount of time each sex is available to mate ('times in'; Parker & Simmons 1996). If the adult sex ratio is equal, then courtship roles should be determined by the relative time sexes are unavailable for mating ('time out'), independent of the number of offspring they produce during that time (Clutton-Brock & Parker 1992; Parker & Simmons 1996; Kvarnemo & Anhesjö 1996; Wiklund et al. 1998). Using this method, potential reproductive rate can be estimated by comparing the relative 'time out' from mating in males and females. In species with predominantly female parental care, females would have the greatest 'time out' from mating opportunities and thus are predicted to be the least competitive sex.

Males that care for their offspring are relatively uncommon across most taxonomic groups except for fish, where paternal care occurs in over 60% of the species that display any care (Ridley 1978; Blumer 1979, 1982). Although male care of offspring is the dominant parental care pattern in fish, little evidence exists to determine whether these males actually invest more per offspring than females, and thus complete estimates of parental investment have proven difficult. In addition, most male fish that care for their offspring also compete intensely for mates, with females being highly selective of males (Turner 1986; Clutton-Brock 1991). Thus, fish provide a good model system to evaluate the value of estimates of potential reproductive rate in predicting courtship roles, because they display male care of offspring, estimates of parental investment are difficult and males are the predominant competitors for mates.

In seahorses (genus *Hippocampus*), males show a high degree of anatomical specialization for paternal care (Herald 1959; J. P. Boisseau, unpublished data), but remain the predominant competitors for mates (Vincent et al. 1992; *H. fuscus*: Vincent 1994a; *H. zosterae*: H. D. Masonjones, unpublished data). Males incubate developing embryos within a sealed abdominal pouch following egg deposition and fertilization (Fiedler 1954; Linton & Soloff 1964; J. P. Boisseau, unpublished data). The brood pouch epithelial lining is highly vascularized and may provide developing embryos with gas exchange, osmoregulation and waste removal during their 10–30-day gestation (J. P. Boisseau, unpublished data). Female seahorses continuously mature ovarian follicles (Selman

et al. 1991), suggesting they may be physiologically able to mate more often than observed in field populations (Vincent 1990; Vincent & Sadler 1995).

Potential reproductive rates of seahorse males and females may be useful in predicting and explaining patterns of mate competition (Clutton-Brock & Vincent 1991; Vincent 1994b). Seahorse species studied to date display complete sexual monogamy over the course of a breeding season, both in the laboratory (*H. fuscus*, Vincent 1994a, b, 1995; *H. zosterae*, Masonjones & Lewis 1996) and in the field (*H. whitei*, Vincent & Sadler 1995; *H. angustus*, Jones et al. 1998). Recent laboratory studies by Vincent (1994b) have suggested that among unpaired *H. fuscus* seahorses, male potential mating rate exceeds that of females, because *H. fuscus* males can prepare to mate faster than females, are sexually receptive longer than females and neither males nor females remate during the gestation period of the male.

The purpose of the present study was to estimate the potential reproductive rates of male and female dwarf seahorses, *H. zosterae*, using two approaches. First, we measured the number of offspring produced per reproductive time when sexually receptive mates were unlimited (as suggested by Berglund et al. 1989; Clutton-Brock 1991; Clutton-Brock & Vincent 1991; Clutton-Brock & Parker 1992; Kvarnemo 1994). Following the methods of Vincent (1994b), we determined the length of time it takes for each sex to court and mate by pairing nonreceptive males and females with sexually receptive partners and then measuring the time until copulation. We estimated potential reproductive rate for males and for females by dividing the mean number of offspring produced per breeding cycle by the time it took each sex to prepare to mate plus the mean gestation duration (breeding cycle duration, time T ; Parker & Simmons 1996) to obtain the potential number of offspring produced per unit time. We used the mean number of offspring instead of the maximum number of offspring, according to the suggestions of Parker & Simmons (1996).

Second, we determined the reproductive 'time out' for each sex, measured as the time needed to process an event of mating and reproduction when sexually receptive mates were unlimited (Parker & Simmons 1996). We compared the time it took each sex to prepare to mate plus the mean gestation duration to obtain a measure of 'time out' for males and for females. 'Time in' was defined as the period of time when individuals were physiologically capable of mating with any individual of the opposite sex (Clutton-Brock & Parker 1992) and was not directly equivalent to courtship.

We used time measurements from the first breeding cycle of a newly established pair, because to use potential reproductive rates to predict courtship roles accurately, individuals must be evaluated at a time when potential mate competition or mate choice can occur. In seahorses, genetic evidence indicates that they do not copulate outside the pair bond (Jones et al. 1998) and rarely interact with individuals other than their partner (Vincent & Sadler 1995). Thus, the only time that individuals might compete for mates is during the establishment of a pair bond, either at the beginning of the

breeding season or when a mate is lost. Both approaches were used in the calculation of potential reproductive rates of seahorses, because each has theoretical or experimental strengths and weaknesses, which are discussed.

METHODS

Study Animal

Hippocampus zosterae occur in shallow seagrass beds from the Gulf of Mexico east to the Bahamas, Bermuda and Cuba (Ginsberg 1937; Böhkle & Chaplin 1966). Adult size ranges from 16 to 38 mm, measured as the linear distance from the top of the coronet to the end of the tail (Strawn 1958). The monogamous mating system of *H. fuscus* and *H. whitei* (Vincent 1995) is also observed in laboratory studies of *H. zosterae*, with a male and female remaining together and mating repeatedly over the course of the breeding season (H. D. Masonjones, unpublished data). In the laboratory, females transfer one entire clutch of eggs to a single male ($\bar{X} \pm \text{SE} = 12.5 \pm 1.32$ eggs, $N=14$), and 5–25 ($\bar{X} \pm \text{SE} = 11.4 \pm 1.58$, $N=18$) fully independent young are born after approximately 12 days of gestation within the male brood pouch (Masonjones 1997). Pairs usually remate within 4–20 h of the male's releasing young. Brood sizes in males that copulate in the field ($\bar{X} \pm \text{SE} = 17.3 \pm 1.31$, $N=24$) are larger than those of laboratory-mated males (independent t test: $t_{40}=2.89$; $P<0.01$). However, offspring conceived and born in the laboratory are about 40% larger than offspring conceived in the field ($t_{44} = -7.74$, $P<0.0005$), indicating that there may be tradeoffs in the more predictable environment of the laboratory (Masonjones 1997).

We obtained *H. zosterae* from a commercial fish supplier (Aqualand Pet Center, Miami, Florida). Subjects used in this study were collected under Environmental Protection Agency license 94093 near Key Largo, Florida, from January through to September. We maintained the fish in small (five to eight fish) same-sexed groups for 10 days to 8 weeks before trials. Fish were isolated in an attempt to standardize reproductive status, by allowing males to give birth and females to reach the end of their postmating refractory period. Fish were kept in 57-litre aquaria prior to and during experiments. Tanks were maintained at 25–28°C on a 13:11 h light:dark cycle with a salinity range of 26–33‰, and two plastic seagrass plants were supplied for attachment sites. Fish were fed each day immediately after observation with recently hatched *Artemia* brine shrimp and supplemented every other day with Selcon (American Marine), a food additive containing highly unsaturated fatty acids.

Courtship Behaviour Patterns

Four discrete phases of courtship in paired dwarf seahorses occurring over 3 days have been previously described (Masonjones & Lewis 1996). Previous observations have indicated that all seahorse courtship occurs in the morning, except on the day of copulation (Vincent

1994a; Masonjones & Lewis 1996). Phase 1 of courtship occurs 1–2 days before the day of copulation. Courtship phases 2–4 occur on the day of copulation. During phase 2 of courtship, females first display pointing, defined as when a female raises her head upward towards the water surface to form an oblique angle with the main body axis and then lowers it again to a horizontal position. This behaviour is a reliable indicator of a female's readiness to mate (indicating sexual receptivity) and the beginning of the day of copulation. Pumping is shown exclusively by males, and consists of males opening their brood pouch and repeatedly flexing the tail in a motion similar to that displayed during the release of young. This behaviour is most often observed for the first time in pairs during or immediately after phase 2 of courtship, becomes more frequent up to copulation, and then is not observed again until the birth of young. Phase 2 of courtship is usually followed by a latency period of 23–220 min (median 111 min), during which females apparently undergo the last stages of egg maturation and egg hydration (Vincent 1990; H. D. Masonjones, personal observation). In phase 3 of courtship, males begin to display pointing in response to female pointing. Phase 4 of courtship is characterized by the male and female repeatedly rising, during which the fish release their respective holdfasts and rise into the water column facing one another. In a copulatory rise, the female genital papilla is placed inside the male brood pouch opening, followed by egg transfer. Eggs are fertilized as they are transferred to the pouch, by the release of sperm from the cloacal opening of the male just above the pouch opening (Fiedler 1954).

Latency to Mate Trials

We determined the latency for each sex to prepare to mate in newly introduced pairs of *H. zosterae*. Previous behavioural studies of seahorses have indicated that there is at least a 2-day courtship period before mating in newly introduced pairs (Vincent 1994a, b; Masonjones & Lewis 1996). However, in *H. zosterae*, in subsequent reproductive cycles of the same pair there is only a delay of 4–20 h between birth and subsequent copulation (H. D. Masonjones, unpublished data). We used the longer mating latency in this study to determine the potential reproductive rates in *H. zosterae* because it is during this period when the potential exists for mate competition or mate choice to occur. Among paired-bonded males and females, when placed with sexually receptive, but unfamiliar partners, mating will not occur until the end of gestation in the previous pair bond, even in females, unless under highly specific circumstances (Vincent 1994b; H. D. Masonjones, unpublished data).

This experiment included two treatments: (1) sexually receptive females with sexually isolated males ($N=18$); and (2) sexually receptive males with sexually isolated females ($N=16$). We prepared sexually receptive fish using two techniques, similar to those used in Vincent (1994b). In the first method ($N=4$ successful trials), we prepared sexually receptive females by placing two females into a tank with one male. We then observed them continuously during the first 3 h after tank lights came on

(defined as dawn) on each day before copulation, and on the day of copulation, until one of the females mated with the male. Under these conditions, often both females would court with the male and, on the day of copulation, both females would display pointing, indicating their readiness to mate. Once the male and one of the females had mated, we removed the pair, leaving the unmated but sexually receptive female in the tank. We prepared sexually receptive males ($N=6$ successful trials) by starting with two males and one female, observing them through early courtship and copulation of one male and the female. We considered the unmated male ready to mate if he displayed pumping, either alone or in response to the female's points, and engaged in courtship with the female on the day that she displayed pointing.

In the second technique, we prepared sexually receptive males and females ($N=5$ successful female trials; $N=9$ successful male trials) simultaneously. One male and one female were placed together and observed each morning through their first bout of courtship. On the day of courtship when the female displayed pointing and the male pumping, pairs were watched continuously through the second and third phases of courtship. At the first indication that pairs were about to rise together (indicating the beginning of phase 4 of courtship), we separated the male and female by sliding an opaque barrier down through the centre of the tank creating two completely separate compartments, one containing the male and one containing the female.

Once a fish was sexually receptive, we added a sexually isolated fish of the opposite sex to the tank. We then observed experimental pairs continuously for the next 2 h, and subsequently checked them at 1-h intervals for courtship behaviours and indications of mating until the lights went off. Indications of mating included an increase in pouch volume coupled with a change in pouch colour from neutral to light orange in males, and a decrease in abdomen girth coupled with a change in colour from light orange to neutral in females (Vincent & Sadler 1995). Both indicate a transfer of orange-red eggs from the female to the pouch of the male. Each morning thereafter we observed pairs through their first courtship bout, until the appearance of pointing in females and pumping in males. On the day that these behaviours were noted, we observed pairs continuously until copulation.

The two techniques used to prepare sexually receptive fish appeared to cause similar disruptions to the tank environment. It is possible there were slight differences in courtship phase, because one method paired sexually receptive fish with unprepared fish at the beginning of phase 4 of courtship, while the other method paired them at the end. However, this difference is unlikely to affect estimates of latency to mate, because phase 4 is quite short ($\bar{X} \pm \text{SE} = 41.5 \pm 3.9$ min; Masonjones & Lewis 1996).

We measured wet masses for all fish by blotting the fish dry and weighing them to the nearest 0.01 mg in sea water in a 2-ml plastic vial. Experimental fish ranged from 80 to 212 mg wet mass, with no mass difference between males and females used in trials ($\bar{X} \pm \text{SE}$: females = 134.0 ± 4.1 mg; males: 141.7 ± 3.8 mg; t test: $t_{66} = 1.36$, NS). Fish that had been sexually isolated were size-

matched to sexually receptive fish, with no more than a 30-mg mass difference, because dwarf seahorses will often reject mates that differ greatly in size from themselves (Masonjones 1997).

We observed 18 isolated male/receptive female trials and 16 isolated female/receptive male trials daily from the day of introduction until copulation took place, which in 15 and nine trials, respectively, resulted in successful copulations. Trials that did not end in copulation were omitted from further analyses. We recorded duration (in hours) from pair introduction to copulation, as well as the length of time each fish was sexually isolated prior to introduction, and its field collection date.

Female and Male Remating Interval

To determine whether females can potentially remate before the end of their previous mating partner's gestation period, we housed females with an additional sexually receptive male ($N=9$). Two males and either one ($N=6$) or two ($N=3$) females all of similar size were kept in 38-litre aquaria at 28°C and allowed to interact freely. One additional trial consisting of one male and two females was included in the analysis because the male aborted the clutch of eggs and was ready to mate before the end of a normal gestation period. We observed tanks each morning for courtship behaviours and recorded the date, time and identities of fish involved in all copulations and births. In addition, for each male, we recorded the duration from copulation to birth and for each male and female, the duration from copulation to subsequent copulations. Male seahorses have not been observed to mate multiply and will not accept eggs from a female until the previous brood is born (Fiedler 1954; Vincent 1994b; Masonjones 1997).

Calculation of Potential Reproductive Rate and Reproductive 'Time Out'

Potential reproductive rate

Because seahorses are monogamous, fish presented with new mates do not remate until the end of their previous mates' gestation period (Vincent 1994a). To overcome this limitation, we estimated potential reproductive rate by determining the time it took for males and females to prepare to mate and then combining this with the difference in number of offspring produced over the course of the breeding season. To obtain the breeding cycle duration (time T , Fig. 1), we added the time (in hours) from pair introduction to mating for sexually isolated males and females (mating latency) to the average gestation length in the laboratory at 26°C, 12.3 days (289.7 h; seven pairs). We calculated the potential reproductive rate per breeding cycle (number of offspring per hour) by dividing the mean number of offspring per brood for field-mated males ($\bar{X} \pm \text{SE} = 17.3 \pm 1.31$) by the cycle duration (T) for both males and females. We estimated the difference in potential reproductive rate between males and females over the breeding season by determining how many broods males and females could

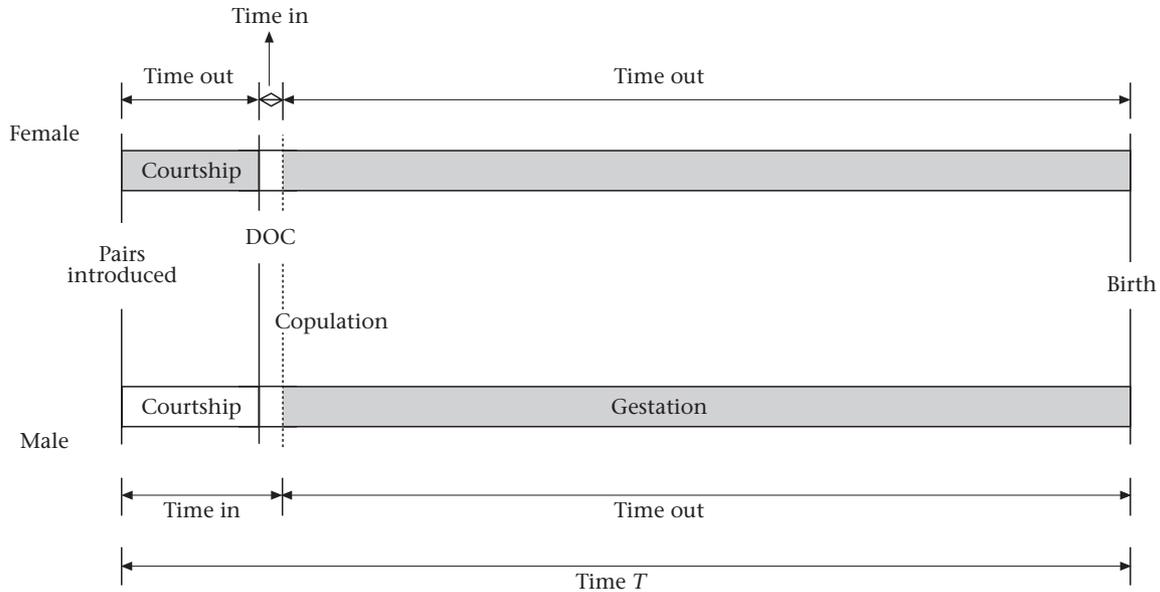


Figure 1. Calculation of ‘times in’ (□) or ‘times out’ (□) for paired *H. zosteræ* females and males in their first courtship interaction and subsequent mating (schematic after Parker & Simmons 1996). Major behavioural milestones are indicated, including pair introduction, early courtship, courtship on the day of copulation (DOC), copulation (dashed line), gestation and birth of offspring. Breeding cycle duration (T) is indicated by the line at the bottom.

produce over their approximately 200-day breeding season (March–October; Strawn 1958), if the number of sexually receptive mates were unlimited.

Reproductive ‘time out’

Clutton-Brock & Parker (1992) define reproductive ‘time in’ (S) as the time when ‘an adult is capable of mating if it has access to a receptive partner of the opposite sex’. Time in includes activities such as active mate searching, waiting for a mate and guarding females that are not yet sexually receptive. Time out (G) is everything else, including gamete preparation, courtship, copulation and parental care (Clutton-Brock & Parker 1992; Parker & Simmons 1996). A single reproductive event (T), is the sum of all reproductive time ($G+S$).

In the current experimental system, the time spent mate searching is zero (field data indicate that mate search time ranges from 1 to 30 days for *H. whitei*; Vincent & Sadler 1995), and the time spent waiting for mating is most equivalent to the courtship interactions on the day of copulation (for males and females: $\bar{X} \pm SE = 3.9 \pm 0.4$ h at 25.5°C; Masonjones & Lewis 1996). Thus ‘time in’ (S) would be about 4 h for both males and females. This is the only ‘time in’ period for females because females are not capable of mating at any other time during courtship. ‘Time out’ for males and females can be calculated as the mean time to prepare to mate plus the mean gestation length. However, because the latency to mate measurement includes the sexually receptive period of males and females (beginning with the first display of pointing by females and ending with copulation), a more appropriate calculation of male and female ‘time out’ would subtract the time spent in courtship on the day of copulation ($S=4$ h, $G=T-S$).

Statistical Analysis

Because differences may exist in the time it takes for fish to prepare to mate across the breeding season, we determined mating latency as a function of gender and collection date, using an extension of the Kruskal–Wallis test modified for two-factor analysis (Zar 1996). We categorized collection date into early season (fish collected during January–April), and late season (fish collected during May–September). We estimated the relationship between mating latency and duration of sexual isolation using a linear regression analysis. We determined differences between remating latencies and gestation lengths using Student’s t test when the assumptions of a normal distribution and equal variances were met, and with Mann–Whitney U tests when they were not.

RESULTS

Male and Female Latency to Mate

When paired with an opposite-sexed, sexually receptive fish, sexually isolated males showed a significantly shorter latency to mate than sexually isolated females (males: $\bar{X} \pm SE = 7.4 \pm 1.1$ h, $N=15$; females: $\bar{X} \pm SE = 55.6 \pm 3.8$ h, $N=9$; Kruskal–Wallis test: $H_1=15.75$, $P < 0.001$; Fig. 2). Thus it took sexually isolated females an average of 2 days longer than sexually isolated males to prepare to mate with a sexually receptive mate. However, there was no effect of collection date on mating latency ($H_1=1.51$, NS) and no interaction between gender and collection date ($H_1=0.270$, NS).

Duration of time fish were kept in the laboratory prior to experiments may also influence their latency to mate. For both males and females, the median time in the

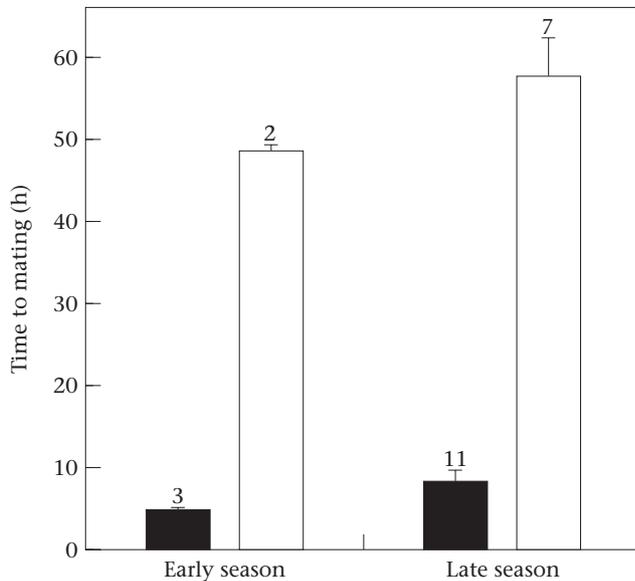


Figure 2. Mean \pm SE mating latency (time from placement of sexually isolated fish with sexually receptive fish through to copulation) for *H. zosteræ* males (■) and females (□) collected and assayed early in the season (January–April) as compared to those collected later in the season (May–September). Sample sizes are given above each bar.

laboratory and in sexual isolation was 4 weeks, but as the length of time in the laboratory increased, males took significantly longer to mate (linear regression: $r^2=0.569$, $F_{1,12}=15.87$, $P<0.005$); whereas, no effect was found for females ($r^2=0.113$, $F_{1,7}=0.895$, NS).

Remating Intervals

The length of time from copulation to birth in males was 10.6 ± 0.49 days ($N=7$) at 28°C . For both males and females, the time from copulation to subsequent copulation was 11.8 ± 0.51 days ($N=10$), and the time from birth to the next copulation was 17.5 ± 4.4 h (range 7.5–30 h, $N=7$). In the seven trials in which a male and female successfully copulated and the male gave birth, the female was never observed to copulate with the other male, and none of the other males became pregnant during the gestation of the first male. In two additional trials, the male and female either failed to copulate successfully (eggs were dropped outside of the male's pouch), or they successfully copulated but the male aborted the clutch within 5 days. In neither of these trials did the male or female remate until after the original male would have given birth (one mated 14 days, and the other 12 days, after the first attempted copulation). In one remaining trial, a pair successfully copulated, then the male died 7 days later, and that female copulated with the other male 12 days after the initial copulation.

Potential Reproductive Rate

To calculate potential reproductive rates of males and females given unlimited availability of sexually receptive

mates, we added mean mating latency for each sex to the mean gestation period to yield the breeding cycle duration for each sex (Table 1). Because mating latency of sexually isolated females exceeded that of sexually isolated males, female cycle duration was 48 h longer than that of males. Because of strict monogamy, males and females produce the same number of offspring per cycle, but males produced 1.2 times more offspring per cycle hour because of their shortened cycles. When extrapolated over the entire breeding season, males could potentially produce 17% more offspring than females, if they mated with a sexually receptive female at the end of each cycle.

Relative Reproductive 'Time Out'

For both males and females, the mean mating latency (interval from pair introduction to copulation) contains both a reproductive 'time in' component and a reproductive 'time out' component. The period of sexual receptivity in both sexes is best estimated by the period of courtship on the day of copulation from the time the female first displays pointing through to copulation. Therefore, to determine overall reproductive 'time out', we subtracted the period of sexual receptivity (S , $\bar{X} \pm \text{SE} = 3.9 \pm 0.4$ h for both sexes) from mating latency (males: $\bar{X} \pm \text{SE} = 7.4 \pm 1.1$ h, $N=15$; females: $\bar{X} \pm \text{SE} = 55.6 \pm 3.8$ h, $N=9$), and added this value to the gestation length ($\bar{X} \pm \text{SE} = 289.7 \pm 12.3$ h). This yielded a mean reproductive 'time out' of 341.4 h (G_f) for females and 293.2 h (G_m) for males.

DISCUSSION

In this study, latency to mate was longer for female *H. zosteræ* than for males; females required longer to prepare an egg clutch to transfer to the male than males required to get ready to accept an egg clutch when both were provided with sexually receptive mates. Sexual differences in latency to mate were even more pronounced early in the breeding season, with males preparing to mate in about half the time it took them later in the breeding season (females showed no change over the breeding season). In addition, females were not observed to remate during the gestation of their partner, despite being offered sexually receptive males.

The sexual difference in mating latency translates to a difference in potential reproductive rate of 17% more offspring for unpaired males compared with females over the course of the breeding season. Similarly, a longer female mating latency leads to a reproductive 'time out' 1.2 times that of males. It is not surprising that the results of both methods for estimating potential reproductive rate match; time is the essential element for both. In Parker & Simmons' (1996) model, when the adult sex ratio is equal, the number of offspring produced is unnecessary in the calculation of potential reproductive rate. However, when determining potential reproductive rate experimentally, it may be more tractable to measure for males and females the number of offspring produced

Table 1. Calculation of the potential number of offspring that male and female *H. zosterae* could produce over the breeding season

	Males	Females
Mating latency (h)	7.4±1.1	55.6±3.8
Gestation duration (h)	289.7±12.3	289.7±12.3
Cycle duration (h)	297.1±12.3	345.3±12.9
Cycle duration (days)	12.4±0.51	14.4±0.56
Number of offspring/cycle	17.3±1.3	17.3±1.3
Number of offspring/h	0.058±0.005	0.050±0.004
Number of offspring/day	1.4±0.12	1.2±0.10
Season duration (days, h)	200, 4800	200, 4800
Mean number of cycles per season	16.2	13.9
Mean number of offspring per season	279.5	240.5

The values are $\bar{X} \pm SE$.

and the time required to produce them (time T), when provided with unlimited sexually receptive mates (Kvarnemo 1994). This is because there are two main difficulties in determining 'time out' for males and females. First, recent definitions of 'time in' lack clarity. Parker & Simmons (1996) state that 'time in' is equivalent to time spent 'searching and waiting for matings'. This statement has led to the unfortunate misconception that courtship is included in the calculation of 'time in', as courtship occurs during the period of time when individuals are waiting to mate. When applied to the rather simple case of seahorses, in which the adult sex ratio is equal and there is no collateral investment (males or females investing in offspring with multiple partners), this misconception has led to the inclusion of the longer female mating latency in estimates of 'time in' and the prediction that females should compete for access to males. Earlier work by Clutton-Brock & Parker (1992) defined 'time in' as the time when 'an adult is capable of mating if it has access to a receptive partner of the opposite sex'. We have used this earlier, more descriptive definition of 'time in' because female seahorses are only capable of mating during a relatively short time just before copulation. They probably need the 2-day courtship period to mature eggs; thus this time constitutes female reproductive 'time out'. A second reason that this method for estimating potential reproductive rate is problematic is the difficulty of assessing exactly when individuals are capable of mating.

According to Parker & Simmons (1996), reproductive 'times out' are used to calculate the direction of sexual competition because 'time in' is much more difficult to determine. This is true for seahorses; defining the time a sex is available for searching for mates and waiting for a mating is much more difficult than determining how much time they spend engaged in gamete replenishment or parental tasks. However, what actually may determine the direction of mating competition is the period of reproductive 'time in', or more specifically, the length of time it takes a sex to become sexually receptive and how long they remain sexually receptive (Vincent 1994b). Male *H. zosterae* take a few hours to become fully ready to mate, and remain ready for as long as it takes a female to prepare. If a male drops a clutch before the end of

gestation, he is physically able to remate within a few hours (H. D. Masonjones, unpublished data). Female seahorses take at least 2 days to prepare a clutch of eggs and become sexually receptive. They are only receptive for a few hours, and are essentially incapable of mating before the end of their previous mating partner's gestation period. In addition, females appear to need the daily greeting ritual to stay 'primed' for mating. Females that are removed from their mating partner soon after copulation, kept in isolation and returned as the male is giving birth need the same length of time to prepare to mate (2 days) as an unpaired female at the beginning of the breeding season (H. D. Masonjones, unpublished data). This limited sexual receptivity in female seahorses may be what induces sexual competition among males; access to females ready to mate is extremely limited and females require a long time investment to prepare to mate.

For species without collateral investments in offspring, sperm storage, or extrapair copulations (e.g. New Zealand robin, *Petroica australis*: Ardern et al. 1997; Kirk's dik-dik, *Madoqua kirkii*: Brotherton et al. 1997; common loon, *Gavia immer*: Piper et al. 1997), measuring potential reproductive rate through differences in the potential number of offspring produced may be more straightforward than determining when they are capable of mating. However, species matching these criteria are rare, and for most animals, attributing paternity (and thus correctly estimating the number of offspring males produce) can be difficult, especially in the field. Consequently, to estimate potential reproductive rate, it is necessary to determine reproductive 'time out' experimentally, but the present example from seahorses emphasizes the problem of determining precisely when adults are physiologically capable of mating.

Because *H. zosterae* females need more time to prepare to mate than males, resulting in a lower potential reproductive rate both in terms of offspring production and time away from mate acquisition, females are predicted to be the less competitive sex (Clutton-Brock 1991; Clutton-Brock & Vincent 1991; Clutton-Brock & Parker 1992; Parker & Simmons 1996). Previous work has shown that seahorse males compete more intensely for mates than do seahorse females (Vincent 1994a; H. D. Masonjones &

S. M. Lewis, unpublished data), displaying a greater diversity and frequency of competitive behaviours than females. This is consistent with predictions based on the potential reproductive rates and reproductive 'times out' calculated in the present study for *H. zosterae* males and females. In addition, these results are consistent with Clutton-Brock & Vincent's (1991) suggestion that the sex with the higher potential reproductive rate will compete more intensely for access to mates, because less time spent on reproductive tasks (lower 'time out' value) by one sex translates directly into higher potential reproductive rates. Using estimates of potential reproductive rate based on females' maximum clutch size and male maximum brood size, these authors suggested that the genus *Hippocampus* represented an exception to the rule, with females having higher reproductive rates but males being the more competitive sex. However, more recent work by Vincent (1994b) on *H. fuscus* supports the finding of our study that male potential reproductive rate exceeds that of females, because males prepare to mate faster and are sexually receptive longer than females. As a result, recent studies on seahorses support the original hypothesis of Clutton-Brock & Vincent (1991) that the sex with the higher potential reproductive rate competes more intensely for mates than does the sex with the lower potential reproductive rate.

Our calculations of reproductive 'time in' and 'time out' are based on the sequence of courtship events that occur during the first cycle of courtship, copulation and gestation in dwarf seahorse pairs (Fig. 1), and not on subsequent, more homogeneous reproductive cycles in a given pair over the breeding season. The methods Parker & Simmons (1996) proposed for calculating the direction of sexual selection should be most accurate when considering reproductive cycles during which competition would naturally occur. During the breeding season, there is no competition for mates among paired *H. zosterae* because of the monogamous mating system. Among unpaired fish at the beginning of the breeding season, however, competition among males is likely to be intense because female fecundity increases with body size (Vincent 1990). In addition, because female *H. zosterae* invest more energy in their offspring than males (Masonjones 1997), females probably vary widely in quality, which recent theoretical work suggests may increase male choosiness for high-quality females (Johnstone et al. 1996). Seahorses are a good model system for testing the hypothesis that relative 'times out' predict mating competition, because during the breeding season, no competition is observed and the ratio of male to female reproductive 'times out' is equal. However, competition is observed as pairs are established, and the ratio of 'times out' is skewed towards females.

The behavioural or physiological constraints that may be responsible for the prolonged mating latency of female hippocampids remain unknown. In *Hippocampus*, female ovaries continuously produce mature follicles, of which a fraction are transferred at each mating event (Selman et al. 1991), suggesting that females may be physiologically capable of mating immediately after encountering a sexually receptive male. Females would also be expected

to be capable of remating before the end of the average male gestational cycle. When their previous mating attempt results in dropping eggs before their transfer to a partner, females will mate 2 days earlier than unmanipulated controls when provided with a new male (H. D. Masonjones, unpublished data). This result indicates that females can prepare a clutch earlier than the normal gestation length, but because of the relatively low density of potential unpaired mates in the field (Vincent & Sadler 1995), it is unlikely to have much influence on the present set of calculations. Vincent (1994b) suggests that *H. fuscus* females may need 3 days to mature a clutch of eggs to transfer to a new mate, but that it is possible that females do not mate sooner because the loss of a clutch is so costly. In dwarf seahorses, females invest a substantial amount of energy in each egg (Masonjones 1997) and egg clutches represent between 8 and 29% ($\bar{X} \pm SE = 15.9 \pm 2.2\%$, $N=9$, by dry weight) of a female's body weight. Thus the loss of even a single clutch represents a significant reproductive cost, and this constraint may be responsible for female mating latency. A female may require courtship repeated over 2–3 days to evaluate a male's availability as a mate before hydrating and transferring a clutch of eggs.

Not all syngnathid females require such a long mating latency or have a lower potential reproductive rate than males. In the pipefish *Nerophis ophidion* and *Syngnathus typhle*, males brood embryos on their ventral surface (Fiedler 1954). In both species, precopulatory courtship can be short (less than 1 h: Berglund & Rosenqvist 1993) and mating is promiscuous for both males and females (Berglund et al. 1986, 1988). Male *N. ophidion* and *S. typhle* have both been shown to be more selective of their mates than conspecific females (Rosenqvist 1990; Berglund & Rosenqvist 1993), and evidence exists indicating that female *N. ophidion* compete indirectly for males (Rosenqvist 1990). Thus, these pipefish are exceptions to the 'male care, male competition' pattern in fish, with males caring for offspring but showing less competition for mates and higher mate selectivity than females. However, in both species, females have a higher reproductive rate than males; *N. ophidion* females could fill 1.8 males during the average course of one incubation period, and *S. typhle* females 1.9 males during the same period (Berglund et al. 1989). This difference in the reproductive rates of male and female *S. typhle* increases as the fish age because males grow more slowly than females (Svensson 1988), but female fecundity increases over 2.5 times from small females (1 year old) to large females (2 years old; Berglund & Rosenqvist 1990).

The relative parental investment that males and females contribute per offspring has been suggested to be the primary determinant of the potential reproductive rates of males and females, and ultimately the dominant factor dictating operational sex ratios (Simmons 1992; Anderson 1994). Recent work suggests a strong link between potential reproductive rate, operational sex ratios and courtship roles (Ahnesjö 1995; Balshine-Earn 1996; Kvarnemo 1996), but little direct information exists to determine how parental investment patterns may influence the expression of these other factors (but see

Wiklund et al. 1998). An important research focus in future studies of the factors influencing courtship roles should be to determine relative parental investment and potential reproductive rates of males and females in a number of species to examine their relationship in natural systems.

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