

Monogamous pair bonds and mate switching in the Western Australian seahorse *Hippocampus subelongatus*

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Abstract

Apparently monogamous animals often prove, upon genetic inspection, to mate polygamously. Seahorse males provide care in a brood pouch. An earlier genetic study of the Western Australian seahorse demonstrated that males mate with only one female for each particular brood. Here we investigate whether males remain monogamous in sequential pregnancies during a breeding season. In a natural population we tagged males and sampled young from two successive broods of 14 males. Microsatellite analyses of parentage revealed that eight males re-mated with the same female, and six with a new female. Thus, in this first study to document long-term genetic monogamy in a seahorse, we show that switches of mates still occur. Polygynous males moved greater distances between broods, and tended to have longer interbrood intervals, than monogamous males, suggesting substantial costs associated with the breaking of pair bonds which may explain the high degree of social monogamy in this fish genus.

Introduction

Sexual selection on males and females is greatly influenced by the relationship between the number of mating partners and the reproductive success of males and females (Andersson, 1994; Cunningham & Birkhead, 1998). Higher variance in reproductive success may indicate a greater potential for sexual selection (Bateman, 1948; Arnold & Duvall, 1994). In socially monogamous species, the variance in reproductive success for both sexes is often assumed to be low, and therefore sexual selection is taken to be weak, but not necessarily absent (Kirkpatrick *et al.*, 1990). However, the genetic mating system is more germane than the social mating system to theory pertaining to the strength of sexual selection.

Until recently, genetic mating systems of natural populations were empirically inaccessible, but the introduction of molecular genetic techniques has provided important insights into the interpretation of behavioural data, enabling us to know the actual reproductive outcomes of observed social interactions (Avice, 1994; Ketterson *et al.*, 1998; Coltman *et al.*, 1999). Such techniques have become indispensable tools in the study of parentage in nature, particularly of birds. Among the avian species previously believed to be monogamous, the vast majority are genetically polygamous (Birkhead & Møller, 1992; Avice, 1994). However, there are some examples of true monogamy. For example, in Leach's storm-petrel (Mauck *et al.*, 1995), the common loon (Piper *et al.*, 1997), and the black vulture (Decker *et al.*, 1993) monogamy has been demonstrated using DNA fingerprinting. In addition molecular techniques have been used to confirm genetic monogamy in some non-avian taxa such as the California mouse (Ribble, 1991), Kirk's dik-dik (Brotherton *et al.*, 1997), and the Western Australian seahorse (Jones *et al.*, 1998).

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In seahorses, the male provides all postzygotic parental care by carrying the eggs on his body. Exclusive paternal brooding is a shared feature across the fish family Syngnathidae, in which care ranges from the eggs simply being attached to the skin in the genera *Entelurus* and *Nerophis*, to protection and osmo-regulation in the highly specialized brood pouches of *Hippocampus* and *Syngnathus* (Dawson, 1985). Despite ubiquitous male pregnancy in seahorses and pipefishes, mating patterns and sex roles differ greatly among species. For example, the pipefishes *N. ophidion* and *Syngnathus scovelli* are polyandrous, whereas polygyny is the dominant mating system for *S. typhle* and *S. floridae* (Berglund *et al.*, 1988; Vincent *et al.*, 1992; Jones & Avise, 1997a, b; Jones *et al.*, 1999). In addition, monogamy has been observed in the pipefish genus *Corythoichthys* (Gronell, 1984) and possibly also in *Hippichthys* (Watanabe *et al.*, 1997). In contrast to the mating system diversity observed in pipefishes, seahorses invariably have been reported to exhibit social monogamy. The formation of pair bonds has been documented by behavioural observations of *H. fuscus* (Vincent, 1995), *H. whitei* (Vincent & Sadler, 1995), and several other species listed in Vincent *et al.* (1992) and Lourie *et al.* (1999). In *H. fuscus* (Vincent, 1995), *H. whitei* (Vincent & Sadler, 1995) and *H. zosterae* (Masonjones & Lewis, 1996), pairs perform daily greetings throughout the breeding season. Each morning they twist their tails and nod their heads to one another, behaviours that recur in their courtship displays.

Yet, there may be opportunities for extra-pair copulation in seahorses. Although males spend most of their time in relatively small home ranges, females move over considerably larger areas. Thus, home ranges of both males and females frequently overlap with several individuals of both sexes. This pattern of home range use, observed in *H. whitei* (Vincent & Sadler, 1995) and *H. subelongatus*, means that an individual is often seen in the presence of members of the opposite sex other than its primary social mate (Kvarnemo and Moore, unpublished data).

In contrast to some species of pipefish, which are sex role reversed with respect to the direction of sexual selection (Berglund *et al.*, 1986, 1988), behavioural studies of several seahorse species suggest that males are the predominant competitors for mates (reviewed in Vincent *et al.*, 1992; also Vincent, 1994a; Masonjones & Lewis, 1996; Moore, unpublished results). Vincent (1994b) ascribed this to a male biased operational sex ratio among unmated individuals. However, it may also suggest that the potential reproductive rate of males could be higher than that of females. A recent experimental study of *H. zosterae* supports the latter hypothesis by showing both a higher potential reproductive rate of males than of females, and a lower paternal than maternal energy expenditure (Masonjones, 1997; Masonjones & Lewis, 2000). Thus, males may be prone

to sequential polygyny, particularly if that behaviour would reduce the interbrood interval.

The present study focuses on determining whether the genetic mating system of seahorses conforms to expectations based on observed social monogamy in the genus. An earlier genetic study of the recently renamed Western Australian seahorse *H. subelongatus* (previously *H. angustus*; Lourie *et al.*, 1999) demonstrated that males receive eggs from only one female for each particular brood (Jones *et al.*, 1998). Our goal was to investigate whether males of this species are monogamous between broods, as has been suggested by behavioural observations of related species (Vincent, 1995; Vincent & Sadler, 1995; Masonjones & Lewis, 1996). The approach was to sample tagged males repeatedly from a natural population and to deduce the maternity of sequential broods using microsatellite-based markers.

Materials and methods

Our main study site, about 45 km south of Perth, was monitored by SCUBA in 1998 from the end of January (well after the *H. subelongatus* breeding season had started) to the end of March (when the breeding season ended). These seahorses occurred near 22 pylons of a jetty, at a maximum depth of about 5 m, and around 46 stumps (0.1–2.0 m high) of a demolished jetty, in about 10 m of water. Other than the pylons and stumps in the study area, there were very few sites with habitat suitable for seahorses within 200 m in any direction. Two additional males were sampled from a small population near a jetty in the saline part of Swan River, at about 4 m depth. The mean temperatures were 22.9 °C at the main site (range 21–24 °C) and 23.3 °C at the river site (range 23–24 °C).

Pregnant males were collected by SCUBA diving and brought to the jetty for tagging, body measurements and sampling of broods. Tagging was accomplished using miniature tags of waterproof copy paper, with printed numbers sealed with super glue, attached to a thin nylon string tied loosely around the males' necks. Mobility and behaviour were not evidently affected by the tags. Measurements of head length (coronet to tip of the snout), and of the head-vent length (coronet to vent), were taken using callipers, and a ruler was used for the tail length (vent to tail tip). For brood samples, we used a capillary tube (whose sharp edges had been reduced using a Bunsen burner flame) attached to a small bulb. Pregnant males have a strong muscle with which they close the pouch. We carefully inserted the tube into the brood pouch and removed approximately 15 embryos per brood. From an earlier study, we know that a brood can contain as many as 720 offspring (mean value 458 young) (Jones *et al.*, 1998). Although a sample of 15 embryos is only a small fraction of all offspring in a brood, such samples were sufficient for our purposes,

because all offspring in a brood share the same mother (Jones *et al.*, 1998).

We recorded at which pylon or stump each male was collected. Males were returned to their original location immediately after sampling. Estimates of interbrood intervals (i.e. days between parturition of first and second brood) were based on the developmental state of the embryos at sampling, in combination with observations from the field of each male's state of pregnancy. During each dive, we recorded the position and reproductive status of any tagged males encountered. Invariably, the broods and offspring of studied males developed normally after sampling. After the sampling of a male's second brood, a small (<2 mm²) portion of the posterior edge of his dorsal fin was clipped for genetic analysis. We took fin samples only from males whose successive broods were sampled. The cut was sterilized by iodine solution. Fin clips and sampled embryos were stored and transported in a buffer containing 20% DMSO, 250 mM EDTA (pH 7.5), and saturated NaCl.

For the microsatellite assessment, we used three previously characterized seahorse loci (*Han03*, *Han06*, *Han15*; Jones *et al.*, 1998). A simple DNA-extraction protocol was employed (see Jones & Avise, 1997a for details) and polymerase chain reaction (PCR) was performed as described by Jones *et al.* (1998). Polymerase chain reaction fragments were separated on sequencing gels and visualized using a radioactive label and autoradiography. The fathers and approximately 10–15 embryos per clutch were assayed for all three microsatellite loci (Table 1). The maternal genotype for each clutch was determined by subtraction. We estimated allele frequencies using adult genotypes from a previous study (Jones *et al.*, 1998) combined with the genotypes of the 14 males assayed in this study.

Results

In total, 34 males were tagged, of which 17 were seen pregnant at least twice, 15 were seen pregnant once, and another two males remained unmated. Sequential broods from 14 males were sampled successfully, whereas the broods of three other males were sampled prematurely and did not provide useful embryos for genetic analysis. Comparing the 17 males found pregnant twice with the 17 males seen pregnant only once or not at all, no difference was detected in any of the morphological traits measured (*t*-tests, d.f. = 32: head length, *t* = 0.65, *P* = 0.52; head-vent length, *t* = 0.40, *P* = 0.69; tail length, *t* = 0.91, *P* = 0.37).

In all cases, the genotypes of progeny within a clutch were consistent with a single mother, whose genotype was reconstructed unambiguously by subtraction (Table 1). Thus, our results are consistent with the previous finding that males receive eggs from only one female during a pregnancy (Jones *et al.*, 1998). Assuming linkage equilibrium and Hardy–Weinberg proportions,

the expected frequencies of the reconstructed maternal genotypes ranged from 6.1×10^{-8} to 8.7×10^{-5} , so it is extremely unlikely that eggs from two different females that shared the same genotype would appear in our study. Thus, if two clutches had the same maternal genotype, we considered the eggs in both clutches to have originated from the same female. This conclusion is bolstered by the fact that each adult multilocus genotype determined in this and a previous study (Jones *et al.*, 1998) has been unique to a single individual.

Of the 14 males from which two sequential broods were sampled, eight proved by genetic analysis to have re-mated with the same female (Table 1), thus clearly indicating a capacity for sequential monogamy. In addition, two males (males 127 and 143) remained monogamous over three successive broods (Table 1). However, six males had switched mates between successive broods (Table 1). These sequentially polygamous males did not differ from the monogamous males in any morphological measurement (Fig. 1) (*t*-tests: head length, *t* = 0.38, *P* = 0.71; head-vent length, *t* = 0.93, *P* = 0.37; tail length, *t* = 0.67, *P* = 0.51).

Males that mated with the same female in two successive broods stayed within the original home range, whereas males that mated with new partners had moved significantly longer distances between broods (Fig. 2) (Mann–Whitney *U*-test: *U* = 3.0, *P* = 0.006). Furthermore, males that mated with a new partner for the second brood tended to have a longer interbrood interval than those that re-mated with the same female (Fig. 3) (*t*-test: *t* = 1.91, *P* = 0.08). One of the monogamous males was not included in this analysis due to an uncertain estimate of his interbrood interval.

We found one instance in which a divorced female re-mated with a new male. The first mate of male 139 had the same genotype as the second mate of male 131 (Table 1). Male 139 was first found pregnant on pylon 2 of the submerged jetty, and later on a nearby pylon during his second pregnancy. Male 131, on the other hand, was first found pregnant on a far distant pylon. He was later found pregnant with his second brood on pylon 2 and, later in his pregnancy, moved to another nearby pylon. Interestingly, both males were probably near pylon 2 when they received eggs from the shared female. The estimated time between the two successive matings of the female was at the most 20 days.

Discussion

This study of the Western Australian seahorse, *H. subelongatus*, is the first to document successive-brood monogamy in a seahorse using genetic techniques. We successfully sampled the young from two sequential broods of 14 males, eight of which proved to have mated with the same female in both broods, while six had re-mated with a new female. Our data strongly suggest

Table 1 Summary of assayed males. Each row represents the results for a single clutch from a single male pregnancy. Shown are the male ID and clutch number, the number of embryos genetically assayed per clutch, the paternal genotype (assayed directly from a fin clip) and the maternal genotype (reconstructed from the progeny array).

Male ID (clutch no.)	Number of embryos assayed	Paternal genotype at locus:			Maternal genotype at locus:		
		<i>Han15</i>	<i>Han03</i>	<i>Han06</i>	<i>Han15</i>	<i>Han03</i>	<i>Han06</i>
Monogamous males:							
117 (1)	13	289/295	248/252	163/233	295/303	206/256	163/187
117 (2)	15				295/303	206/256	163/187
121 (1)	6	257/297	252/254	157/183	297/301	254/258	163/231
121 (2)	10				297/301	254/258	163/231
122 (1)	11	261/309	246/252	163/183	285/303	206/246	163/235
122 (2)	15				285/303	206/246	163/235
127 (1)	15	261/281	256/256	163/235	257/299	248/256	183/183
127 (2)	15				257/299	248/256	183/183
127 (3)	12				257/299	248/256	183/183
128 (1)	11	285/293	206/256	205/235	271/329	252/274	163/237
128 (2)	15				271/329	252/274	163/237
137 (1)	10	287/303	206/256	187/187	303/309	248/250	163/233
137 (2)	10				303/309	248/250	163/233
143 (1)	15	297/313	252/254	163/235	289/295	206/254	163/163
143 (2)	15				289/295	206/254	163/163
143 (3)	15				289/295	206/254	163/163
146 (1)	14	273/343	206/206	163/235	303/309	206/238	163/183
146 (2)	15				303/309	206/238	163/183
Males that switched mates:							
130 (1)	15	305/331	250/250	153/237	299/305	206/256	163/237
130 (2)	15				295/309	206/206	183/203
131 (1)	10	285/299	248/254	163/179	295/299	206/248	177/235
131 (2)	10				285/303	248/252	163/163
139 (1)	15	285/307	256/256	163/183	285/303	248/252	163/163
139 (2)	12				303/305	248/264	163/163
147 (1)	14	299/305	206/256	183/183	261/309	252/258	235/237
147 (2)	15				285/297	256/262	183/183
148 (1)	14	271/293	206/256	163/235	277/303	238/252	183/235
148 (2)	15				341/345	206/254	163/237
149 (1)	14	293/343	206/262	163/163	285/303	252/256	163/233
149 (2)	15				261/303	206/254	163/187

that genetic monogamy is a common mating pattern in this species of seahorse, and they convincingly support previous behavioural observations of social monogamy in other seahorse species.

Nevertheless, almost half of the males switched mates between pregnancies. These males moved greater distances between broods, and tended to have longer interbrood intervals, than the males that remained paired to a single female. As there is no reason to believe that the developmental time of the embryos would differ between these males, the difference in interbrood interval probably is a function of the time from parturition to re-mating. These results suggest that breaking up a pair bond may incur a substantial cost for the male in terms of travel distance and reduced reproductive rate while searching for a new mate. Vincent & Sadler (1995)

reported similar results for *H. whitei*. Further, Vincent (1990) proposed that the formation of a new pair increases the interspawning interval of both sexes, due not only to the additional time spent searching for a new mate but also to the fact that a female must hydrate her eggs before spawning (Boisseau, 1967, as cited on p. 16 in Lourie *et al.*, 1999; Vincent, 1994b). In existing pairs of *H. fuscus*, the hydration is synchronized with the brooding cycle of the male, but in newly formed pairs this process takes about 2 days (Vincent, 1994b, 1995).

In this study, the prediction that seahorse males might be prone to polygyny due to a higher potential reproductive rate of males was not supported. Under this model a male should engage in polygyny only if this behaviour reduces his interbrood interval. The opposite was found here, as the males that mated polygynously

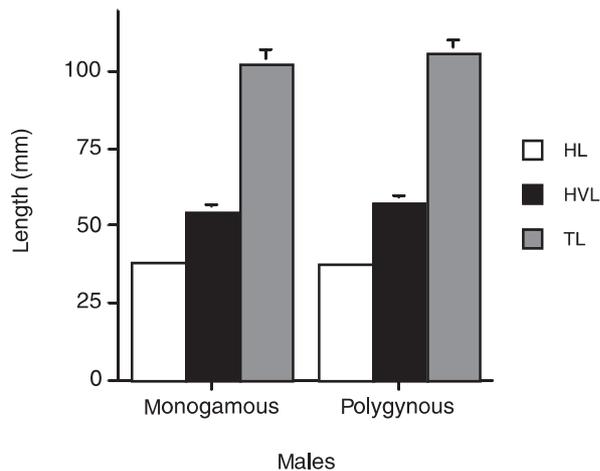


Fig. 1 Body size (mean \pm SE) of seahorse males that mated monogamously and polygamously in two successive broods (HL = head length, HVL = head to vent length, TL = tail length).

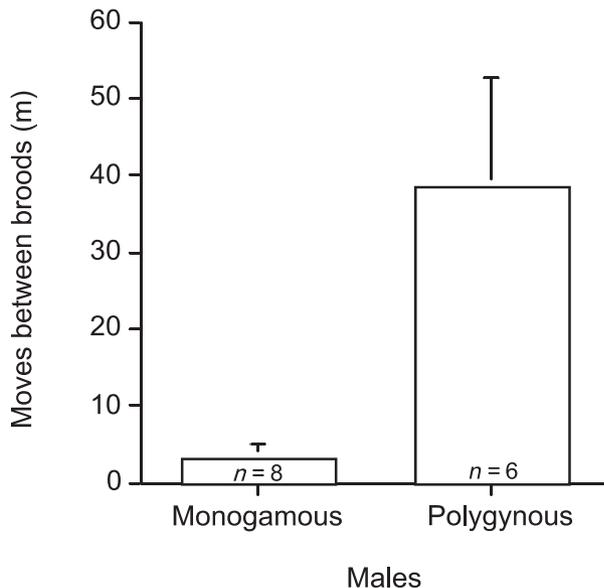


Fig. 2 Distances moved (mean \pm SE) between two successive broods of seahorse males that mated monogamously and polygamously.

between broods tended to increase their interbrood interval. Nevertheless, the possibility remains that these males may still have benefited from switching mates in terms of overall fitness, if the second female was more fecund or showed some other preferred trait that might be naturally or sexually selected.

Of course, mate switching in this seahorse population may result from either male- or female-mediated strategies. Pregnant males were the major focus of this study, so we have fewer data regarding female reproductive behaviours and rates. In the single case for which we

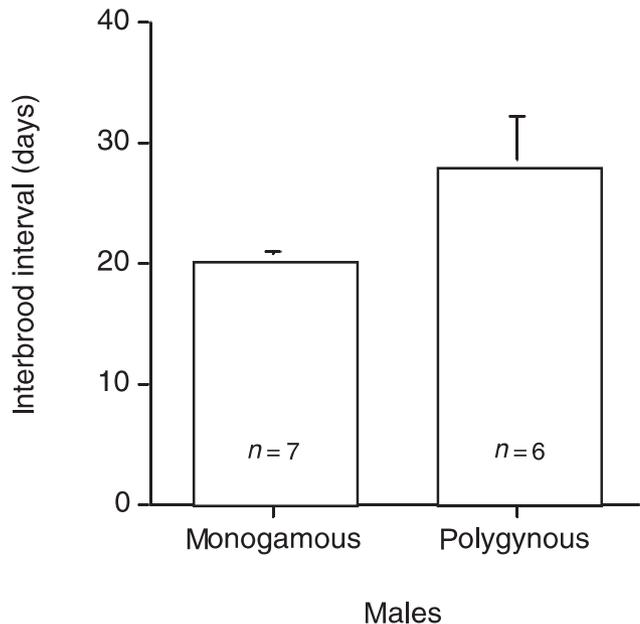


Fig. 3 Interbrood interval (mean \pm SE) of two successive broods of seahorse males that mated monogamously and polygamously.

have data on two successive matings by one female, the interval between broods was at the most 20 days, hinting that the time cost for a female to switch mates might be lower than that for a male. In addition, this particular female appears not to have switched mates to obtain a more fecund partner, as her second mate (131) was smaller than her first (139). One goal of future studies of seahorse mating systems should be to gather additional information on female behaviour and movement patterns together with genetic mating system data.

In seahorses, individuals of either sex may be predisposed to switch mates as a result of constraints imposed by male pregnancy. Indeed, substantial costs may be associated with monogamy, as fecundity is positively correlated with body size in both males and females (e.g. Vincent, 1990; Masonjones, 1997). If a female has a low fecundity relative to a male, he may only brood with part of his capacity, or *vice versa* for a female if only part of her brood would fit into the male's pouch. In pipefishes, brood reduction has been documented, with males giving birth to fewer young than the number of eggs received at mating (Ahnesjö, 1992), and this seems to be the case in *H. fuscus* as well (Vincent, 1994b). Thus, there would be mutual benefits of mating with large mates for both sexes, and hence size assortative mate choice would be predicted (Johnstone, 1997). Other studies of seahorses have indeed found a pattern in that direction, and more strongly so early in the breeding season (Vincent & Sadler, 1995). Pairs that were formed later in the season were less well matched, possibly as a result of a paucity of available mates at that stage

(Vincent & Sadler, 1995). Hence, if such a pattern is valid for our population as well, individuals switching mating partners from the first to the second brood would be more likely to do this as a 'best of a bad job' strategy, rather than as an attempt to improve their reproductive rate.

A possible explanation for mate switching by either sex is that it is done by necessity rather than choice, for example if an individual's initial mate had for some reason disappeared from the area. We have evidence that one female remained in the area after her divorce, whereas other females may have disappeared from the area due to predation or emigration. We have no data on risk of predation on females, but extrapolating from the male data, the risk of predation seems to be fairly low. All except three tagged males were re-sighted at one time or another and most of them were seen repeatedly. Excluding three males that were tagged during the last week of the study and, thus, were unlikely to be re-sighted, our observations yielded a maximum predation rate of 9.7% over the study period. Thus, predation is unlikely to account for all our cases of split pair bonds.

Emigration of individuals from the area could be an additional reason for seahorses to switch mates. Our general impression from this study and other unpublished observations (Kvarnemo and Moore) is that some new adults do seem to move into the area during the breeding season, indicating that some probably moved out as well. Indeed, three of our tagged males disappeared during the breeding season, but had returned to the area by the time we revisited the site about 4 months later. Females, who tend to have larger home ranges than males, are likely to move between breeding areas as well.

One could argue that the polygynous matings genetically documented were due to altered behaviours caused by human handling. We cannot rule out this possibility completely, but this explanation seems unlikely because the vast majority of males remained in the study area. Furthermore, males sampled twice evidently had re-mated after the first sampling, and, thus, were able to mate despite the annoyance of tag application and progeny removal. Another possibility is that the polygynous matings resulted from disturbances to females caused by our frequent dives. However, this seems even less likely because no females were handled during the study.

For monogamy to evolve and be sustained by natural (or sexual) selection, the benefits of a long-term pair bond must outweigh its costs. A relatively shorter interbrood interval for a monogamous pair compared with a polygamous pair, as documented in this study, could provide such a benefit to monogamy. Other seahorse studies have found that a pair's capacity to produce offspring increases with the time elapsed since pair formation (Vincent, 1994b), suggesting an additional possible advantage to long-term monogamy. In one model, Ranta & Kaitala (1999) investigated the evolution of polygyny vs. monogamy, and concluded that whereas polygyny is an evolutionary stable strategy, monogamy is

expected to replace (or coexist with) polygyny only if the latter entails some fitness decrement. In two passerine species of birds studied in this regard, polygynous males raised considerably fewer nestlings than monogamous males (Ranta & Kaitala, 1999; references therein). The current study of seahorses may provide another example of this phenomenon.

In conclusion, this genetic study of maternity in male broods clearly shows that the Western Australian seahorse often maintains monogamous pair bonds over successive mating episodes. This finding is consistent with, and extends, behavioural studies on related seahorse species that have documented social monogamy with long-term pair bonding. However, our study also revealed that a considerable proportion of males had mated with a new female in the second of the two broods sampled. Thus, in this species, pair bonds were not always as long lasting as previously supposed. These results illustrate how genetic studies can yield unequivocal data on biological parentage and thereby contribute to a deeper understanding of the causes and consequences of mating-system evolution.

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