

Post-copulatory sexual selection and sexual conflict in the evolution of male pregnancy

Kimberly A. Paczolt¹ & Adam G. Jones¹

Male pregnancy in seahorses, pipefishes and sea dragons (family Syngnathidae) represents a striking reproductive adaptation that has shaped the evolution of behaviour and morphology in this group of fishes^{1–4}. In many syngnathid species, males brood their offspring in a specialized pouch, which presumably evolved to facilitate male parental care^{5,6}. However, an unexplored possibility is that brood pouch evolution was partly shaped by parent–offspring or sexual conflict, processes that would result in trade-offs between current and future pregnancies. Here we report a controlled breeding experiment using the sexually dimorphic Gulf pipefish, *Syngnathus scovelli*, to test for post-copulatory sexual selection within broods and for trade-offs between successive male pregnancies as functions of female attractiveness. Offspring survivorship within a pregnancy was affected by the size of a male's mate, the number of eggs transferred and the male's sexual responsiveness. Significantly, we also found that embryo survivorship in a current pregnancy was negatively related to survivorship in the prior pregnancy, clearly demonstrating fitness trade-offs between broods. Overall, our data indicate that post-copulatory sexual selection and sexual conflict occur in Gulf pipefishes. The conflict seems to be mediated by a strategy of cryptic choice in which males increase rates of offspring abortion in pregnancies from unattractive mothers to retain resources for future reproductive opportunities. Hence, the male brood pouch of syngnathid fishes, which nurtures offspring^{7–9}, also seems to have an important role as an arbiter of conflict between the sexes.

Male pregnancy, a phenomenon unique to seahorses and their relatives, is facilitated by an elaborate brood pouch into which a female deposits eggs during mating^{10,11}. Far from being a passive bag for offspring (Fig. 1), the male's pouch provides aeration, protection, osmoregulation and nutrition to the developing offspring during a pregnancy that can last several weeks^{7–9,12–14}. The brood pouch is usually viewed as a structure whose main role is to nurture offspring. Recent work has established that nutrients move both from father to offspring^{7–9} and from offspring to father¹⁵ during the pregnancy, raising the hitherto unexplored possibility that the brood pouch may have a role in modulating post-copulatory sexual selection and sexual conflict^{16–18}. Such conflict could occur as a consequence of either males withholding (or taking) resources from some broods to save resources for future pregnancies^{19,20} or females transferring substances during mating that stimulate males to invest more resources in the current pregnancy^{21,22}. Whether sexual conflict is driven by male- or female-mediated effects, the demonstration of a role for the brood pouch in such processes would precipitate a major shift in thought regarding adaptive mechanisms responsible for the evolution of male pregnancy and parent–offspring interactions in this group of fishes.

We focus here on post-copulatory processes in the sexually dimorphic, sex-role-reversed Gulf pipefish, which has the highest

documented opportunity for sexual selection in females of any taxon²³. In nature, males normally mate with a single female per pregnancy^{23,24}, whereas the most successful females can mate with several males, resulting in a limited supply of receptive males. Previous studies of post-copulatory processes in a related pipefish (*Syngnathus typhle*) show that larger eggs experience higher survivorship, but fail to identify male-mediated effects or address between-pregnancy trade-offs^{25,26}. Gulf pipefishes provide an excellent system in which to investigate such trade-offs while eliminating potential complications from multiple mating within a pregnancy.

Our experimental design involved mating each focal male with a single female, allowing the male to carry his first brood to term, mating the male with a second female (Fig. 1) and then monitoring embryo mortality in the second brood. We photographed each brood immediately after egg transfer, near the pregnancy midpoint and just before parturition (Fig. 1a, b). Embryos failing to develop can be distinguished from viable embryos by their diminished size and colour at day seven of the pregnancy (which typically lasts 12–14 days), so we measured brood reduction by dividing the number of surviving embryos by the number of eggs initially received. Offspring survivorship is defined as one minus brood reduction. We controlled the size of the females with which males mated to ensure that males sometimes mated with females that differed from one another in size and sometimes mated with females similar to one another in size. Most of the analyses presented here are based on the second brood for each male

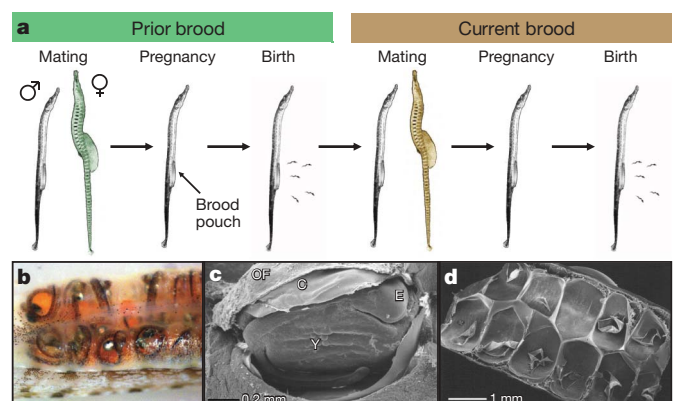


Figure 1 | Experimental design and brood pouch morphology. **a**, Each focal male mated with a single female for each of two successive pregnancies. **b**, We documented the development of offspring during each pregnancy by photographing the brood through the transparent pouch. **c**, Scanning electron micrograph illustrating the close connection between father and offspring; OF, outer pouch flap; C, chorion; E, embryonic tissue; Y, yolk. **d**, A honeycomb of paternal tissue is present in the brood pouch when the embryos are removed at about the half-way point of a pregnancy. (Images, N. Ratterman and C. Partridge.)

¹Department of Biology, 3258 TAMU, Texas A&M University, College Station, Texas 77845, USA.

($N = 22$), which we refer to as the ‘current brood’ (Fig. 1). The purpose of the first brood (which we call the ‘prior brood’) was to establish a known mating history for each male. First, we addressed whether or not males practice pre-copulatory mate choice; second, we quantified post-copulatory sexual selection by examining relationships between offspring survivorship and female size; third, we examined factors affecting offspring survivorship within broods, with a particular emphasis on trade-offs between broods; and, finally, we used an individual-based model to investigate the circumstances under which a brood reduction strategy would be adaptive in nature.

With respect to pre-copulatory choice, our results indicate that males prefer to mate with larger females. Each mating trial was a no-choice preference experiment, and we found that males’ reluctance to mate was significantly shorter for larger females (Fig. 2a), a result that was even more pronounced when we considered female size relative to male size (Fig. 2b). Thus, pre-copulatory mate choice favours larger females, and the pattern is very strong, as might be expected for a highly sexually dimorphic species such as Gulf pipefishes²³.

Our second goal was to investigate the nature of post-copulatory sexual selection in Gulf pipefishes. Our results indicated that pregnancies showed substantial variation with respect both to the number of eggs transferred per copulation (range, 4–42; mean, 22.5; variance, 89.8) and to survivorship of eggs in the brood pouch (range, 0–1; mean, 0.71; variance, 0.12), setting the stage for post-copulatory sexual selection. If post-copulatory sexual selection occurs in pipefishes, then one very important question is whether it reinforces or opposes pre-copulatory sexual selection^{27,28}. Other studies of post-copulatory sexual selection in a wide range of taxa have provided mixed results on this issue²⁷. Our results show that males prefer to mate with larger females (who also tend to have more pronounced secondary sexual characters) and that post-copulatory sexual selection acts in the same direction. We observed a strong positive correlation between the number of eggs transferred and female size (Fig. 2c), and saw the same pattern for offspring survivorship (Fig. 2d). In short, larger females transferred more eggs per mating and the resulting embryos experienced a greater probability of surviving to parturition. Thus, post-copulatory sexual selection reinforces pre-copulatory sexual selection in Gulf pipefishes.

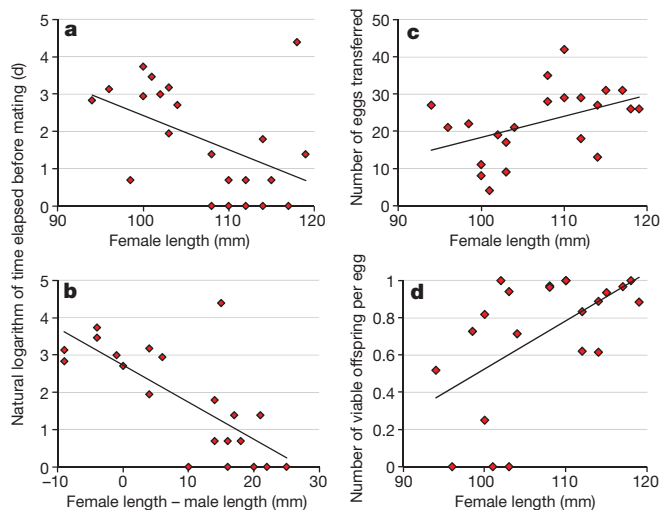


Figure 2 | Evidence for pre-copulatory mate choice and post-copulatory sexual selection by male Gulf pipefishes. **a**, We observed a strong negative correlation between a female’s total length and the time that elapsed before mating took place for male–female pairs (linear regression: $N = 22$, $r^2 = 0.23$, $P = 0.025$). **b**, The pattern was more pronounced when we took into account the female’s length relative to the male’s length ($N = 22$, $r^2 = 0.53$, $P < 0.001$). **c**, We observed a significantly positive relationship between the female’s total length and the number of eggs transferred to the male’s pouch ($N = 22$, $r^2 = 0.20$, $P = 0.038$). **d**, Males also experienced higher levels of offspring survivorship when they mated with larger as opposed to smaller females ($N = 22$, $r^2 = 0.56$, $P = 0.007$).

To ascertain the extent to which interbrood trade-offs are involved in Gulf pipefish reproduction, we examined brood reduction in light of a male’s mating history. If sexual conflict occurs, we expect to see trade-offs in subsequent broods. We addressed this hypothesis by using a stepwise regression and path analysis to study the effects of current and prior brood characteristics on offspring survivorship in the current brood (see Methods for statistical details). Our main result with respect to post-copulatory sexual selection and sexual conflict is that current offspring survivorship is dependent on characteristics of the prior brood (Fig. 3). In particular, current offspring survivorship is negatively correlated with prior female size and prior brood size, suggesting that valuable (and energetically expensive) previous broods hinder a male’s ability to invest in current broods (Fig. 3). Similarly, current offspring survivorship is negatively correlated with prior offspring survivorship (Fig. 3), suggesting that males are capable of reducing investment in some broods to save resources for future reproduction and that investment in a valuable brood decreases survivorship in subsequent broods.

In terms of the post-copulatory process, the simplest explanation for our results is that broods with larger mothers are energetically more costly for males. These costs could be manifested in two ways: either males are selected to invest more resources in embryos from large, attractive mothers as an adaptive strategy of cryptic male choice, or larger females (or their offspring) have evolved a mechanism that induces greater reproductive investment by males (see Supplementary Information for additional data bearing on the second possibility). Overall, the hypothesis of cryptic male choice is most consistent with our data, because all of our observations can be explained by discrimination against smaller females at every phase of pre- and post-copulatory sexual selection. Moreover, the mechanism would be relatively simple, as males could reduce the rate at which resources are transferred from them to the broods from less attractive females, increasing competition among siblings and reducing offspring survivorship.

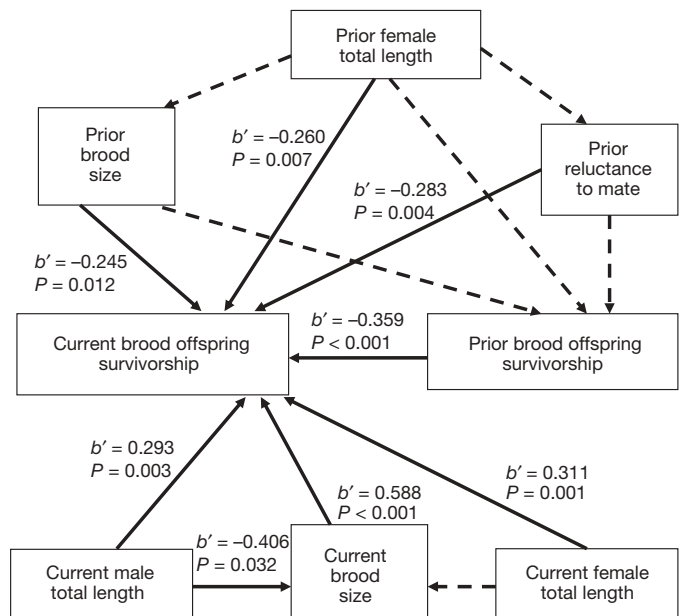


Figure 3 | Results of a path analysis showing the effects of variables from the prior and current broods on current offspring survivorship. Solid arrows represent significant partial regressions; dashed arrows show relationships that were included in the analysis but were not statistically significant. For each significant relationship, we label the corresponding solid arrow with the partial regression coefficient, b' , and the P value. The key result illustrated in this figure is that the survival of embryos in the current brood depends on features of the previous brood; in particular, current offspring survivorship was negatively correlated with prior offspring survivorship and prior female length.

This conclusion is further bolstered by recent observations in a related pipefish, *S. typhle*, which show that amino acids originating in the eggs pass from the embryos through the male's brood pouch to be incorporated into his liver and muscle tissue¹⁵. Thus, male pipefishes possess a mechanism to take resources from their broods, and the male-mediated strategy of brood reduction that our data illustrate is an example of sexual conflict, analogous to infanticide or filial cannibalism¹⁸. Males are partly, or in some cases completely, aborting the offspring from smaller females, at the expense of the reproductive interests (and Darwinian fitness) of those females. This strategy appears to be beneficial to the males, as their future broods, which should have larger mothers on average, have enhanced offspring survivorship. This clash of reproductive interests of the sexes is the crux of sexual conflict¹⁸, so our results raise the hypothesis that females should be selected to somehow resist this male strategy.

If males are capable of manipulating female broods, as our data suggest, then a key question is whether or not such a strategy is likely to be adaptive in natural populations. Intuitively, the best strategy for males would be to mate with large, attractive females every pregnancy. However, several lines of evidence suggest that this strategy may be impossible. For example, the average potential reproductive rates of females are only about twice those of males²⁹ and pipefish sex ratios fluctuate dramatically in nature, often showing a large excess of males³⁰. Thus, attractive females prepared to mate often will be in short supply. Moreover, results from our individual-based model show that under a wide range of circumstances, males are expected to benefit from the cryptic choice strategy observed in our experiments, especially when large females prepared to mate are in short supply (Supplementary Information). The fitness of the cryptic choice strategy increases when the preference threshold is high, as might be expected for a strongly sexually selected species such as the Gulf pipefish, and when the sex ratio is biased towards an excess of males, which is a common occurrence in nature³⁰. In addition, the model predicts that males are reluctant to mate with small females, as we observed. Males that are too eager to mate with small females suffer fitness costs by missing opportunities to mate with females above the preference threshold. Overall, the results of the model indicate that the cryptic choice strategy implied by our empirical results should be adaptive in natural populations of pipefishes.

The results of our study are significant at two levels. First, post-copulatory sexual selection has been virtually unexamined in sex-role-reversed taxa. If cryptic choice is a general mechanism of sexual selection, then we should expect it to evolve in sex-role-reversed species as well as in the more commonly studied species with conventional sex roles. Our results show that such a mechanism has evolved in Gulf pipefishes, despite the fact that this species has effective mate choice before mating, implying that cryptic choice is indeed a process of fundamental significance. Second, our results bear on the adaptive significance of the brood pouch. On casual inspection, the pouch seems to be a structure that evolved to nurture and provide for offspring, and it does serve this function. However, it also may grant the male better control over reproduction. Males seem to be able to adaptively affect their investment in broods as a function of the value of the pregnancy. Perhaps they simply invest fewer resources in broods originating from smaller females (resulting in competition among siblings within the pouch for resources), but males also have the ability to exploit the reproductive contributions of some females by actively absorbing embryos from less valuable broods¹⁵. Thus, the brood pouch serves a more complicated purpose in pipefish reproduction than previously believed, certainly providing parental care but also participating in a conflict between the sexes.

METHODS SUMMARY

We collected Gulf pipefishes from seagrass meadows near Port Aransas, Texas, maintained them in 9.5-l salt-water tanks and fed them *Artemia* nauplii twice daily. Each experimental male mated twice in the lab, producing two successive pregnancies with one mate per pregnancy. For each mating event, the male was randomly assigned to mate with either a large (length, 108–122 mm) or small

(length, 93–106 mm) female. We inspected male brood pouches daily. The brood pouches of pregnant males were photographed under a dissecting microscope on days one, seven and 11 after mating. On these days, embryos were counted and undeveloped eggs were noted. We calculated brood reduction by dividing the number of undeveloped eggs on day seven by the total number of eggs transferred by the female. Offspring survivorship was calculated as one minus brood reduction. Males were paired with their second mate within 24 hours after parturition of the first brood, and this brood also was photographed and scored on days one, seven and 11 after mating. At the end of each trial, we measured male and female body size (total length, snout–vent length, depth and weight). Our statistical analyses focused on the second brood for each male. We examined how both prior and current brood (and mate) characteristics affected mating latency, brood size and offspring survivorship.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 13 October 2009; accepted 22 January 2010.

- Berglund, A., Rosenqvist, G. & Svensson, I. Mate choice, fecundity and sexual dimorphism in two pipefish species (Syngnathidae). *Behav. Ecol. Sociobiol.* **19**, 301–307 (1986).
- Vincent, A., Ahnesjö, I., Berglund, A. & Rosenqvist, G. Pipefishes and seahorses—are they all sex-role reversed? *Trends Ecol. Evol.* **7**, 237–241 (1992).
- Jones, A. G., Rosenqvist, G., Berglund, A., Arnold, S. J. & Avise, J. C. The Bateman gradient and the cause of sexual selection in a sex-role-reversed pipefish. *Proc. R. Soc. Lond. B* **267**, 677–680 (2000).
- Wilson, A. B., Ahnesjö, I., Vincent, A. C. J. & Meyer, A. The dynamics of male brooding, mating patterns, and sex roles in pipefishes and seahorses (family Syngnathidae). *Evolution* **57**, 1374–1386 (2003).
- Harlin-Cognato, A., Hoffman, E. A. & Jones, A. G. Gene co-option without duplication during the evolution of a male pregnancy gene in pipefish. *Proc. Natl Acad. Sci. USA* **103**, 19407–19412 (2006).
- Stölting, K. N. & Wilson, A. B. Male pregnancy in seahorses and pipefish: beyond the mammalian model. *Bioessays* **29**, 884–896 (2007).
- Berglund, A., Rosenqvist, G. & Svensson, I. Reversed sex roles and parental energy investment in zygotes of two pipefish (Syngnathidae) species. *Mar. Ecol. Prog. Ser.* **29**, 209–215 (1986).
- Ripley, J. L. & Foran, C. M. Differential parental nutrient allocation in two congeneric pipefish species (Syngnathidae: *Syngnathus* spp.). *J. Exp. Biol.* **209**, 1112–1121 (2006).
- Ripley, J. L. & Foran, C. M. Direct evidence for embryonic uptake of paternally-derived nutrients in two pipefishes (Syngnathidae: *Syngnathus* spp.). *J. Comp. Physiol. B* **179**, 325–333 (2009).
- Dawson, C. E. *Indo-Pacific Pipefishes* (Gulf Coast Research Lab, 1985).
- Herald, E. S. From pipefish to seahorse—a study of phylogenetic relationships. *Proc. Calif. Acad. Sci.* **29**, 465–473 (1959).
- Partridge, C., Shardo, J. & Boettcher, A. Osmoregulatory role of the brood pouch in the euryhaline Gulf pipefish, *Syngnathus scovelli*. *Comp. Biochem. Physiol. A* **147**, 556–561 (2007).
- Ripley, J. L. Osmoregulatory role of the paternal brood pouch for two *Syngnathus* species. *Comp. Biochem. Physiol. A* **154**, 98–104 (2009).
- Berglund, A. & Rosenqvist, G. Sex role reversal in pipefish. *Adv. Stud. Behav.* **32**, 131–167 (2003).
- Sagebakken, G., Ahnesjö, I., Mobley, K. B., Braga Gonçalves, I. & Kvarnemo, C. Brooding fathers, not siblings, take up nutrients from embryos. *Proc. R. Soc. Lond. B*. doi:10.1098/rspb.2009.1767.
- Trivers, R. L. in *Sexual Selection and the Descent of Man* (ed. Campbell, B.) 136–179 (Aldine, 1972).
- Parker, G. A., Royle, N. J. & Hartley, I. R. Intrafamilial conflict and parental investment: a synthesis. *Phil. Trans. R. Soc. Lond. B* **357**, 295–307 (2002).
- Arnqvist, G. & Rowe, L. *Sexual Conflict* (Princeton Univ. Press, 2005).
- Burley, N. The differential allocation hypothesis—an experimental test. *Am. Nat.* **132**, 611–628 (1988).
- Sheldon, B. C. Differential allocation: tests, mechanisms and implications. *Trends Ecol. Evol.* **15**, 397–402 (2000).
- Wolfner, M. F. The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. *Heredity* **88**, 85–93 (2002).
- Zeh, D. W. & Zeh, J. A. Reproductive mode and speciation: the viviparity-driven conflict hypothesis. *Bioessays* **22**, 938–946 (2000).
- Jones, A. G., Walker, D. & Avise, J. C. Genetic evidence for extreme polyandry and extraordinary sex-role reversal in a pipefish. *Proc. R. Soc. Lond. B* **268**, 2531–2535 (2001).
- Jones, A. G. & Avise, J. C. Microsatellite analysis of maternity and the mating system in the Gulf pipefish *Syngnathus scovelli*, a species with male pregnancy and sex-role reversal. *Mol. Ecol.* **6**, 203–213 (1997).
- Ahnesjö, I. Apparent resource competition among embryos in the brood pouch of a male pipefish. *Behav. Ecol. Sociobiol.* **38**, 167–172 (1996).
- Partridge, C. *et al.* The effect of perceived female parasite load on post-copulatory male choice in a sex-role-reversed pipefish. *Behav. Ecol. Sociobiol.* **63**, 345–354 (2009).

27. Andersson, M. & Simmons, L. W. Sexual selection and mate choice. *Trends Ecol. Evol.* **21**, 296–302 (2006).
28. Hunt, J., Breuker, C. J., Sadowski, J. A. & Moore, A. J. Male-male competition, female mate choice and their interaction: determining total sexual selection. *J. Evol. Biol.* **22**, 13–26 (2009).
29. Scobell, S. K., Fudickar, A. M. & Knapp, R. Potential reproductive rate of a sex-role reversed pipefish over several bouts of mating. *Anim. Behav.* **78**, 747–753 (2009).
30. Brown, J. D. *A Comparative Life History Study of Four Species of Pipefishes (Family Syngnathidae) in Florida*. PhD thesis, Univ. Florida (1972).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements This work was supported by grant IOS-0455927 from the US National Science Foundation. We thank S. Scobell and R. Carter for help with animal husbandry. We are also grateful to S. Arnold, A. Berglund, M. Giresi, N. Ratterman, E. Rose, G. Rosenthal, C. Small and D. Zeh for comments on the manuscript.

Author Contributions K.A.P. performed the experiments. A.G.J. and K.A.P. designed the experiments, analysed the data and wrote the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to K.A.P. (kpaczolt@mail.bio.tamu.edu).

METHODS

Animal collection and maintenance. We collected Gulf pipefishes near Port Aransas, Texas, on three occasions between June and August 2007, transported them to the Texas A&M University campus in College Station and housed them in a flow-through system until they were used in the experiment. Collection date had no significant effect on brood reduction, brood size or time spent in the lab (Tukey–Kramer HSD test; $\alpha \leq 0.05$). Most males were pregnant at the time of collection, so we housed them in groups until they gave birth. Males usually were added into the mating experiment less than 24 hours after releasing their field-conceived brood. Females were housed in female-specific tanks until they were used in the mating experiment.

Mating experiment. We housed males in 9.5-l aquaria equipped with sponge filters and artificial seagrass, and fed them *Artemia* nauplii twice daily. Each male was maintained in the same tank for the duration of the trial, and tanks were thoroughly cleaned between trials. Males were randomly assigned to mate with either a large (length, 108–122 mm) or small (length, 93–106 mm) female. The reproductive status of females was assessed by the presence of the female secondary sexual ornament, which appears as silvery-blue lateral bars on the trunk.

We presented each male with a randomly assigned female of the appropriate size class immediately upon addition to the experiment. Male brood pouches were visually inspected daily. Of the 48 males that started the experiment, 34 accepted eggs from their first mate. The remaining 14 males were excluded on the basis of the following criteria: the death of either individual in the pair, mating outside the intended pair or a failure to mate before the experiment end date (4 November 2007). Two more males were excluded because they died before the brood emerged. Broods were monitored during development (see below). We maintained males and females together until the male was moved into a brooding chamber 11 days after mating. On the day the fry emerged, the female was killed by administration of an overdose of tricaine methanesulphonate (MS222), photographed and preserved in formalin solution. We made measurements of female total length, snout–vent length, weight, standard depth²⁹, maximum depth and dorsal fin area. Up to five eggs were removed from each female, photographed and measured for diameter.

The 30 males still in the experiment after the first pregnancy were each presented with a second mate within 48 hours of giving birth. Males were again randomly assigned to either a large or a small female, and the pairs were monitored as previously described. No females were re-used during the experiment, so the second mates were independent of the first mates. Twenty-three of the 30 males accepted eggs from the second mate and 22 males survived to maturity of the brood. The remaining seven males, which did not mate, were excluded from the experiment. On the day of parturition of the second brood, both the male and female in each pair were killed using MS222, photographed and preserved in formalin. Females were measured as previously described. We also made measurements of male total

length, snout–vent length, weight and standard depth at this time. At the end of the experiment, male total length ranged from 87 to 105 mm. Male and female total lengths were not correlated in this experiment ($N = 22$, $r^2 = 0.13$, $P = 0.107$).

Brood monitoring. On days one, seven and 11 after mating, we counted embryos and assessed the progress of development. The male was transferred to a small amount of water and examined under a dissecting microscope. Embryos are visible through the brood pouch epithelium and can be counted by eye. All broods were counted at least twice to verify accuracy. Unusual-looking eggs were noted, and eggs that showed no obvious development (in comparison with other embryos in the brood) counted towards brood reduction. Undeveloped eggs often shrank and became more transparent during the brooding period. We produced a time series of brood development by photographing the broods on these days using a digital camera (Zeiss AxioCam MRc 5) attached to a stereo microscope (Zeiss Stemi 2000C).

Analysis. We calculated brood reduction by dividing the number of undeveloped eggs on day seven by the total number of eggs transferred by the female. Offspring survivorship was calculated as one minus brood reduction. The photographic time series of the brood was consulted if a question arose as to the fate of a particular egg.

Because of the large number of variables measured in this study, we used stepwise regression as an exploratory tool to evaluate which variables should be included in a path analysis. The response variable was current brood reduction and all included variables had individual P values of less than 0.05 in the stepwise regression analysis. On the basis of these results, we kept variables describing adult total length, reluctance to mate, brood size and prior brood reduction. All variables were transformed to fit a normal distribution before statistical analysis. Univariate and stepwise regressions were performed in JMP 7.

In our path analysis, all variables retained from the stepwise regression analysis were connected to our focal variable, namely current offspring survivorship. Other connections were included to represent all likely biological interactions between the measured variables. We estimated partial regression coefficients, standardized to 1 s.d., by using the maximum-likelihood procedure implemented in the computer program AMOS 5.0.1. The path analysis model resulted in a χ^2 value of 13.05, leading to a P value of 0.522, which implies that the hypothesis of a perfect fit of the model cannot be rejected. This model explained 84% of the variance in current offspring survivorship. The model fit had a root mean squared error of approximation of <0.001 , which is less than the value, 0.05, expected for a model with excellent fit³¹. Overall, the measures of fit for the path analysis suggest that the estimated model is an accurate representation of our data.

31. Steiger, J. H. & Lind, J. C. Statistically based tests for the number of common factors. *Proc. Ann. Spring Meeting Psychometric Soc.* (1980).

Supplementary Analysis

Mating system simulation methods. To investigate the fitness consequences of post-mating brood reduction, we developed an individual-based, Monte Carlo model of Gulf pipefish mating patterns. The simulation focused on a single breeding season, during which we compared two reproductive strategies by simulating a population with equal proportions of males employing each strategy. In the pre-mating choice strategy, males chose to mate only with females above a preference threshold, which is a parameter of the model. In the cryptic choice strategy, males used the strategy observed in the present study (see below for the exact implementation of the strategy in the model). We applied this model to determine the conditions under which the cryptic choice strategy would confer higher fitness than a strict pre-mating choice strategy. Even though the model, like all evolutionary models, is an oversimplification in some ways, it does produce some general insights regarding the likely adaptive significance of our empirical observations.

Many important mating system parameters have already been measured in natural populations of Gulf pipefish, permitting us to restrict attention to a relevant parameter space. In Gulf pipefish populations inhabiting the northern Gulf of Mexico, breeding activity seems to peak in the spring and summer months, possibly continuing throughout the year¹, so we assumed a breeding season length of 250 days (the exact length of the breeding season is not important, provided it is lengthy). The gestation period for a typical male pregnancy is approximately 12 days at 26-28 °C, so we assigned males a 12 day time out after each mating event. Thus, each Gulf pipefish male has the potential to produce 20 or more broods per year, further strengthening the argument that tradeoffs among broods might be important in this species. Females have about twice the reproductive rate of males on average², so we assigned females a six day timeout after mating. Female total length and number of eggs transferred per mating

were drawn from a bivariate normal distribution based on data from the present study. Thus, number of eggs transferred and female length were positively correlated ($r = 0.33$), resulting in larger females having higher potential reproductive rates on average compared to smaller females, as has been observed in laboratory studies².

In Gulf pipefish, most reproductive activity occurs in the morning within the first few hours of lights on in the lab, so we modelled the mating system on a day-by-day basis. We allowed each male to encounter N_{enc} females per day. If any of the females were above the preference threshold and ready to mate (i.e., not on a time out), the male was assumed to mate with one of these attractive females (chosen at random if several were encountered). In other words, the probability that a male would mate with a receptive female above the threshold was one, regardless of male strategy. If, however, no females above the threshold were encountered, the males using the pre-mating choice strategy would refuse to mate, whereas cryptically choosing males would mate with a receptive female below the threshold, if he encountered one, with a probability of p_s . Thus, pre-mating choice males in the simulation mated only with females above the threshold, whereas cryptic choice males had the potential to mate with a mixture of females above and below the threshold.

If we categorize the females in our study into two classes, large (>106 mm total length) and small (< 106 mm), broods with large mothers experienced an average brood survivorship of 0.96 when the previous brood had a small mother and 0.82 when the previous brood had a large mother. Similarly, broods with small mothers experienced survivorships of 0.56 and 0.44 when the previous broods had small and large mothers, respectively. Thus, having a previous brood with a small mother increased the survivorship of the current brood by about 12 to 14 percent on average in our study. We used these offspring survivorship proportions in our simulation. Therefore, mating with a small female (i.e., a female below the preference threshold) conferred a benefit in

terms of future brood survivorship. However, such a mating also carried costs in terms of a smaller current brood, lower survivorship in that brood, and possible missed reproductive opportunities with large females.

The final key parameter was the adult sex ratio. In Gulf pipefish, the sex ratio fluctuates dramatically during the breeding season, often with a bias toward an excess of females. Hence, we considered sex ratios ranging from about 0.4 to 1.8 females per male. In a 15-month survey of Gulf pipefish near Cedar Key, FL, five of the eleven months with reasonable sample sizes ($N > 30$) showed significant departures from an equal sex ratio¹, with sex ratios of 2.50 (September), 0.47 (June), 0.51 (July), 0.72 (September), and 0.55 (October) females per male. Interestingly, the proportion of males pregnant decreased in the months with the most severely skewed sex ratios. Across the entire study, the average proportion of adult males pregnant was 0.91, but the proportion of pregnant males dropped to 0.87 and 0.79 in June and July¹, suggesting that males may sometimes have difficulty finding attractive females that are ready to mate.

During each run of the simulation, we tallied the number of offspring for each male during the breeding season and compared the average fitnesses of males employing the two strategies under consideration. We simulated 1000 independent breeding seasons under each set of parameter values and calculated means across these replicates to obtain precise estimates of the relative fitnesses of the two male strategies. These simulations lead to some clear conclusions regarding the potential for the cryptic choice strategy to be adaptive in natural Gulf pipefish populations.

Simulation model results. Our results show that conditions under which the cryptic choice strategy would be adaptive probably arise frequently in natural populations of Gulf pipefish. The benefit from the cryptic choice strategy is dependent on the social

setting for mate choice, however, so the results of the model predict that males should facultatively use the cryptic choice strategy when attractive females become difficult to find.

The first important result of the model is that large, preferred females ready to mate will sometimes be difficult for males to find. We addressed this issue by tallying the average number of mates per male in a population in which males used only the pre-mating choice strategy. The availability of females above the threshold is strongly dependent on the preference threshold and the sex ratio. Given our mating season length of 250 days and pregnancy length of 12 days, males had the potential to have 20 pregnancies per breeding season. However, the results from the model show that males rarely were able to fulfil their reproductive potential by mating with only preferred females (Figure S1). For example, with an equal sex ratio and a preference threshold equal to the mean female size, males carried an average of 16.6 and 17.7 pregnancies when they encountered 5 or 25 females per day, respectively (Figure S1, yellow diamonds). With higher preference thresholds or male-biased sex ratios, these figures dropped dramatically (Figure S1), suggesting that males may often find themselves in situations in which attractive females are hard to find (Figure S1).

The conclusion that males will sometimes have difficulty finding attractive females ready to mate is bolstered by several important observations. First, in addition to temporal variation, pipefish show spatial variation in sex ratios³, so a male may sometimes find himself in a local breeding aggregation with a dearth of attractive females. Second, compared to most other fishes pipefish are poor swimmers, so each male probably has access to only a small proportion of the females in the population at any given time. Our simulation assumes the best case for males, in that they sample an

independent set of females each day, but males in nature that find themselves near egg-depleted, attractive females may be unable to move sufficient distances to sample very many additional females, especially in seagrass meadows broken by patches of sandy substrate. Third, in a species like the Gulf pipefish, characterized by very strong sexual selection on females, the preference threshold is likely well above the mean. It is difficult to determine a precise threshold from existing data. Indeed, a fixed threshold is a modelling convenience, and in natural populations mating decisions probably are much more complex. Nevertheless, existing data suggest that males may prefer females that are considerably larger than average. For example, in the present study, the large females used for the second brood ranged from 108 to 119 mm in total length and small females ranged from 94 mm to 104 mm. Offspring survivorship was much lower for embryos with small mothers compared to those with large mothers, and the distributions of survivorship displayed surprisingly little overlap between the two classes of females. Our large females were a full standard deviation above the field mean in June (mean = 96.5 mm, S.D. = 9.89), just before the start of our experiment. Some growth may have occurred during the summer months while we conducted our experiment, but the largest females contributing to our current brood (119 mm) were similar in size to the largest females collected in June (120 mm), suggesting that the distribution of female sizes did not change dramatically. In addition, a field-based parentage study in the Gulf pipefish found that mated females were about a standard deviation above the mean female length on average⁴. Finally, the idea that the most attractive females could become egg depleted is consistent with the sexual selection literature. In species with strong sexual selection acting on males, the most attractive males often find themselves sperm depleted⁵⁻⁷. This situation is compounded for female pipefish, because they have to

make a substantial energetic investment in every gamete⁸. Overall, we can conclude that Gulf pipefish males sometimes face situations in which the available attractive females have insufficient eggs to fill all of the empty brood pouches.

The next question we addressed with our simulation model concerned the potential adaptive significance of the cryptic choice strategy. We were interested in determining the conditions under which the cryptic choice strategy would confer higher fitness than a strict pre-mating choice strategy, so we simulated a population with equal proportions of males using each of these two strategies. We found that the fitness of the cryptic choice strategy relative to the pre-mating choice strategy depended heavily on the preference threshold, the adult sex ratio, and the probability with which cryptic choice males would mate with females below the threshold.

The effects of the preference threshold and sex ratio are demonstrated in Figure S2. If the preference threshold is low, such that any above-average female is a preferred female, then the cryptic choice strategy confers higher fitness only when the sex ratio is highly skewed, with an excess of males (Figure S2a). Even in this case, however, the cryptic choice strategy confers a substantial increase in fitness of about 10 percent, compared to the pre-mating strategy, when the sex ratio is severely male biased. Provided the males are reluctant to mate with small females ($p_s = 0.05$), the cryptic choice strategy confers a relatively small fitness decrement (< 3 percent) when the sex ratio is even. However, if the preference threshold is higher than the mean female phenotype, then the cryptic choice strategy confers a much greater fitness benefit relative to the pre-mating choice strategy (Figure S2b, c). When the preference threshold is half a standard deviation above the mean and the sex ratio is biased, the cryptic choice strategy can result in more than a 25 percent increase in fitness compared

to the pre-mating choice strategy (Figure S2b), and the benefit increases substantially with even higher preference thresholds (Figure S2c).

One other important result of the model is that it predicts that males should usually be reluctant to mate with females below the preference threshold (Figure S3). Regardless of the preference threshold, males derive the greatest fitness benefit from the cryptic choice strategy when their probability of mating with a female below the threshold is between about 0.01 and 0.2 per day (Figure S3). This result is consistent with our observations that males were reluctant to mate with small females, but eventually did so when given no other options. Finally, we found that the number of females encountered per day affected the fitness of the cryptic choice strategy relative to the pre-mating choice strategy (Figure S4). When the number of females encountered per day is small, males may have a low probability of finding females above the threshold that are ready to mate. Under these circumstances, the cryptic choice strategy confers higher fitness than the pre-mating choice strategy (Figure S4). This result is relevant to pipefish, because pipefish populations show substantial variation in population density³. Males in low density breeding aggregations may benefit substantially from an ability to utilize a cryptic choice strategy.

In summary, this modelling exercise demonstrates that pipefish populations often experience conditions under which the cryptic choice strategy documented by our empirical findings would confer greater fitness than a strict pre-mating choice strategy. In short, the cryptic choice strategy is most likely adaptive when the sex ratio is biased toward an excess of males and the preference threshold is high, as might be expected for a species characterized by strong sexual selection. The model also predicts that males will be reluctant to mate with small females, a result supported by our empirical

observations. The benefit to the males employing the cryptic choice strategy arises from the additional offspring a male obtains from mating with a small female, despite the small brood size and low survivorship, plus an increase in survivorship of the male's next brood.

The fixed threshold model is an oversimplification, but it serves to define the parameter space in which the cryptic choice strategy may be adaptive. In reality, there are additional reasons to believe that a cryptic choice strategy could be beneficial under even a wider variety of circumstances than those investigated in the model. For example, in the model we assume that a male's willingness to mate with females below the threshold is fixed throughout the breeding season. In nature, males may be able to assess the social setting and adjust their mating behaviour accordingly. Our results would lead us to predict that males will be more willing to mate with small females when females are less abundant or most females are small. By adaptively adjusting the mating threshold, the males could reduce the probability of mating with small females when large females are likely to become available, reducing some of the costs associated with the cryptic choice strategy. In addition, the cryptic choice strategy could come into play when males mate with females above the threshold but receive inferior batches of eggs. In principle, an egg-depleted female could transfer a smaller than usual numbers of eggs or eggs of lower than expected quality, and the male may have no way of detecting egg depletion before mating. Moreover, males possess no mechanism to eject eggs from the brood pouch, and the pouch seals shortly after mating, giving the male very little time during which re-mating is possible. A reduction in investment in such low quality broods could allow the male to ameliorate the costs by saving resources for future pregnancies. Finally, even when large females are available,

energetic constraints may prevent males from investing adequate resources to ensure high survivorship and high offspring quality in many sequential broods. The cryptic choice strategy we observed would allow males to regain energetic resources, possibly by absorbing some nutrients from the eggs in his brood pouch, while still producing some high quality offspring during this resource acquisition period.

Egg quality and female-mediated effects. Our model was constructed assuming that male-mediated cryptic choice was the primary cause of brood reduction. The justification for this approach is that only a cryptic choice model explains all of our observations. Other partial explanations for our results could involve low intrinsic viability of eggs from small females or female-mediated effects. However, neither of these hypotheses is entirely consistent with our results, strongly suggesting that cryptic choice is the most likely explanation for our data.

Importantly, we can decisively rule out the view that the brood pouch is simply a passive receptacle in which embryos develop without any important input from the male. Under this view, the reduced survivorship of eggs originating from small females could be explained if small females produce eggs with exceptionally low viability. However, several observations suggest that this explanation is inadequate. First, a male's mating history influences offspring survivorship, a pattern that would be impossible without interactions between the male and offspring, mediated by the placenta-like connection provided by the brood pouch. Second, we intentionally avoided using extremely small females in our experiment, so all but one of our "small" females was above the average female size observed in our field population at the onset of the breeding season (female field data from 12 June 2007: $N = 54$; mean length = 96.5mm, S.D. = 9.89, range 80-120mm; experimental females for current brood: $N = 43$, mean length = 107.3mm, S.D. = 7.6, range = 93-119mm). The average survivorship

of the eggs originating from the smaller females (94-104mm total length) in our experiment was only 49.7 percent, while 89.0 percent of the eggs from larger females survived. It seems unlikely that the females we used, which had probably been reproductively active in the field for quite some time before collection, would have such low intrinsic egg viability. Finally, we found a weak negative correlation between offspring survivorship and the length of offspring at birth ($N = 20$, $r^2 = 0.23$, $p = 0.03$), suggesting that the surviving eggs from females whose broods were reduced resulted in offspring of at least average quality. Hence, the eggs from small females appear not to be of low enough quality to preclude the production of quality offspring. This latter result brings up the possibility that sibling competition could be occurring in the brood pouch. Our results suggest that sibling competition may be occurring at some level, because offspring from broods with high survivorship were slightly smaller at birth than offspring from broods with lower survivorship. However, in a multiple regression of offspring length on brood size, offspring survivorship, and female egg diameter, only offspring survivorship showed a statistically significant association with offspring length ($N = 20$; egg diameter: $p = 0.68$; brood size: $p = 0.43$; offspring survivorship: $p = 0.001$). In addition, a sibling competition model is directly at odds with our observation that larger broods experienced greater offspring survivorship, so sibling competition alone cannot explain our most important results.

The other possible explanation for our results, which also would be extremely interesting, is that inter-brood tradeoffs are determined mainly by some sort of conflict driven by female reproductive strategies. Perhaps larger females produce offspring that are more capable of harvesting resources from males or transfer proteins along with the eggs that increase a male's investment in offspring at the expense of future broods, in a manner analogous to *Drosophila* accessory gland proteins. Under this scenario, males would see some of the same tradeoffs that we observed here. However, this model cannot completely explain our data, because while a female-driven reproductive

strategy would predict that a brood from a small female would do poorly on the heels of a brood from a large female, the universally poor performance of broods from small females is most easily explained by a male-mediated strategy (Figure 2d). In addition, if females are driving the pattern, then we would expect the effects to be most severe immediately after a pregnancy with a large mother, diminishing over time in unmated males as they regained resources. However, we found the opposite pattern. Among males with large prior mates, a male's reluctance to mate was negatively correlated with offspring survivorship in the current brood ($N = 11$; $r^2 = 0.53$; $p = 0.011$). This pattern is easily explained under the cryptic choice hypothesis if the males selectively reduced the broods from less attractive females (with whom they are also reluctant to mate). Thus, even though female-mediated effects may play some role, brood reduction clearly is dependent on a male's mating history, and the pattern we observed is most consistent with the hypothesis that pipefish reproduction includes a substantial component of cryptic male choice through differential abortion or allocation of resources.

Supplementary references

1. Brown, J. D. *A Comparative Life History Study of Four Species of Pipefishes (Family Syngnathidae) in Florida* (University of Florida, Ph.D. Dissertation, 1972).
2. Scobell, S. K., Fudickar, A. M. & Knapp, R. Potential reproductive rate of a sex-role reversed pipefish over several bouts of mating. *Anim. Behav.* **78**, 747-753 (2009).
3. Mobley, K. B. & Jones, A. G. Environmental, demographic and genetic mating system variation among five geographically distinct dusky pipefish (*Syngnathus floridae*) populations. *Mol. Ecol.* **18**, 1476-1490 (2009).

4. Jones, A. G., Walker, D. & Avise, J. C. Genetic evidence for extreme polyandry and extraordinary sex-role reversal in a pipefish. *Proc. Roy. Soc. Lond. B* **268**, 2531-2535 (2001).
5. Shapiro, D. Y., Marconato, A. & Yoshikawa, T. Sperm economy in a coral reef fish, *Thalassoma bifasciatum*. *Ecology* **75**, 1334-1344 (1994).
6. Saether, S. A., Fiske, P. & Kalas, J. A. Male mate choice, sexual conflict and strategic allocation of copulations in a lekking bird. *Proc. Roy. Soc. Lond. B* **268**, 2097-2102 (2001).
7. Smith, C., Pateman-Jones, C., Zięba, G., Przybylski, M. & Reichard, M. Sperm depletion as a consequence of increased sperm competition risk in the European bitterling, *Rhodeus amarus*. *Anim. Behav.* **77**, 1227-1233 (2009).
8. Fitzpatrick, S., Berglund, A. & Rosenqvist, G. Ornaments or offspring -- costs to reproductive success restrict sexual selection processes. *Biol. J. Linnean Soc.* **55**, 251-260 (1995).

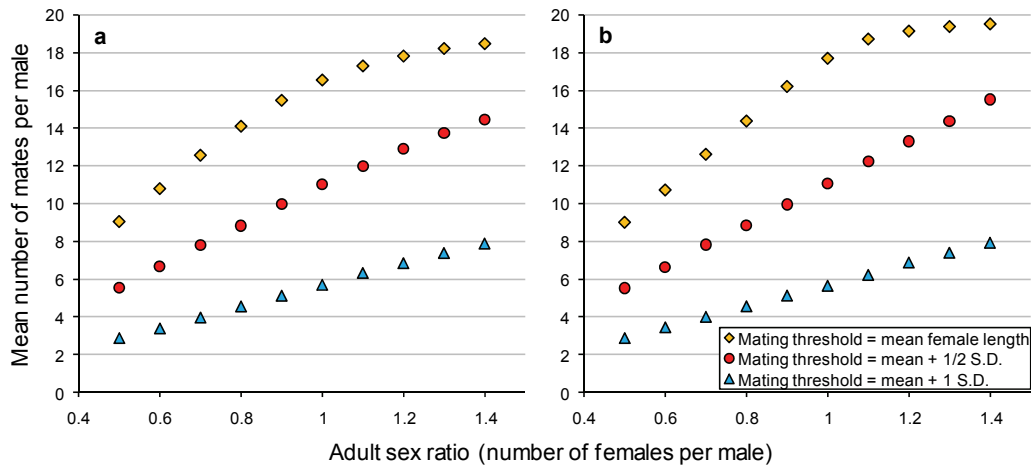


Figure S1. Average number of mates per male when males use a strict pre-mating choice strategy. Under this strategy males will mate only with females above the preference threshold, so this figure indicates the availability of attractive females for a variety of sex ratios and preference thresholds. The left panel, **a**, assumes that males encounter five females per day, whereas the right panel, **b**, assumes an encounter rate of 25 females per day. Each pregnancy lasted 12 days during a breeding season of 250 days, so a male unconstrained by female availability would have 20 mates. Even with a low mating threshold (yellow diamonds), males approached this ideal only when females outnumbered males and males encountered many females per day. As the preference threshold increases, attractive females become even more difficult to find. Since Gulf pipefish are characterized by strong mate choice and often display male-biased sex ratios, this analysis suggests that males often will find themselves in situations in which large, attractive females that are ready to mate will be difficult to find. The primary cause of this situation is that the female potential reproductive rate is only about twice as high as the male potential reproductive rate on average in Gulf pipefish². In this and all other supplementary figures, each data point is a mean based on 1000 replicates, and the error bars for each point are smaller than the symbols.

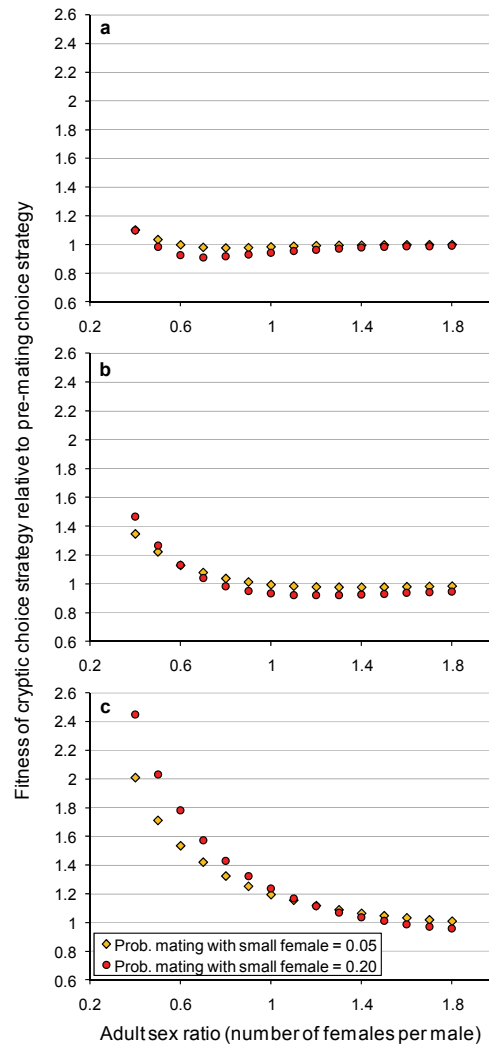


Figure S2. The fitness of the cryptic choice strategy relative to a strict pre-mating choice strategy under various adult sex ratios and preference thresholds. Our measure of relative fitness is based on a population in which half of the males use a pre-mating choice strategy and the other half use a cryptic choice strategy. The average number of offspring for males using the cryptic choice strategy divided by the average number of offspring for males using the pre-mating choice strategy is our measure of relative fitness. Thus, when this value is greater than one, the cryptic choice strategy outcompetes the pre-mating choice strategy. When the preference threshold is low, equal to the mean female phenotype, **a**, the cryptic choice strategy is adaptive only when the sex ratio is strongly skewed toward an excess of females. However, when the threshold is higher, as in **b** (half a phenotypic standard deviation above the mean) or **c** (one phenotypic standard deviation above the mean), the cryptic choice strategy is adaptive over a much wider range of sex ratios. In the Gulf pipefish, the available evidence suggests that the actual preference threshold is somewhere between the scenarios depicted **b** and **c**, so the cryptic choice strategy is probably adaptive under most circumstances when the sex ratio is male-biased. As females become more common, the benefits of the cryptic choice strategy begin to disappear, because attractive females are no longer as difficult to find. The simulations for this figure used a population size of 250 males and the appropriate number of females to achieve the desired sex ratio, and the number of females encountered per day per male was 10.

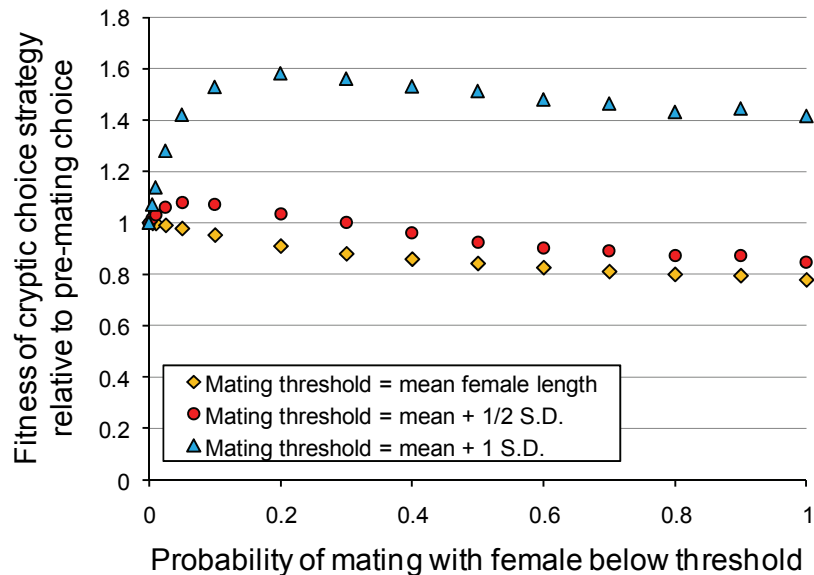


Figure S3. The dependency of the relative fitness of the cryptic choice strategy on the probability of mating with females below the preference threshold. These simulations used a population of 250 males and 175 females to simulate a moderately skewed sex ratio, and an encounter rate of 10 females per day. When the mating threshold is very high (blue triangles), the cryptic choice strategy is adaptive regardless of the male's willingness to mate with small females, because females above the threshold tend to be quite rare. Nevertheless, the peak in fitness for the cryptic choice strategy occurs when the probability of mating with a small female is around 0.2. Similarly, when the threshold is moderate, half a standard deviation above the female phenotypic mean (red circles), the cryptic choice strategy is adaptive only when the males are reluctant to mate with small females, with fitness peaking when the probability of mating with females below the threshold is close to 0.05. When males mate too permissively with small females, they suffer a cost in terms of missed mating opportunities with females above the preference threshold. Finally, if the preference threshold is equal to the mean female phenotype (yellow diamonds), then the cryptic choice strategy is not adaptive under this combination of parameters, but the costs are low as long as the males are reluctant to mate with small females. Thus, the cryptic choice strategy appears to be adaptive under many, but not all, situations that occur in the field. Regardless, males can keep the costs of the strategy low by exhibiting a reluctance to mate with females below the mating threshold.

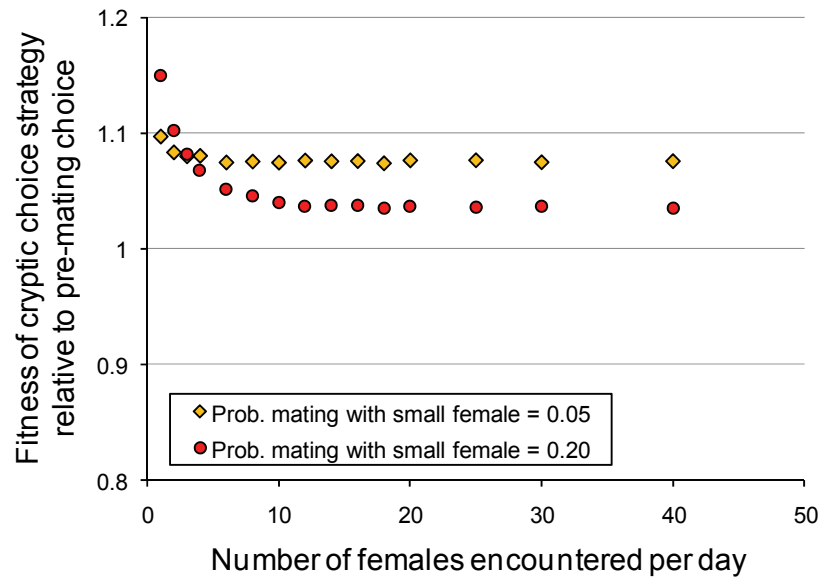


Figure S4. The relative fitness of the cryptic choice strategy as a function of the number of females encountered by each male per day. Under most parameter combinations, the cryptic choice strategy confers a greater fitness benefit when the number of females encountered per day is low. However, this relationship depends upon other parameters, as demonstrated by the interaction between number of females encountered per day and the probability of mating with small females depicted in this figure. This figure is based on simulations assuming a population size of 175 females and 250 males and a preference threshold half a phenotypic standard deviation above the mean. This figure, combined with our other analyses, indicates that the relative benefit of the cryptic choice strategy probably depends on many factors, such as the local abundance of breeding individuals, the population density, the adult sex ratio, and the preference threshold. However, parameter combinations under which the cryptic choice strategy is adaptive appear to be well within the range of parameter values expected based on available empirical data regarding the Gulf pipefish mating system.