The relationship between sex roles, sex ratios, and sexual dimorphisms in the mating system of the gulf pipefish, *Syngnathus scovelli*.

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Oh, look at your fish!

-Professor Alexander Aggasiz

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Abstract

This study was the first to use the gulf pipefish, *Syngnathus scovelli*, as an experimental animal in a laboratory setting. As such, it establishes guidelines for the husbandry of the species. I found the gulf pipefish to be a sex role reversed species, where females competed more intensely than did males for access to mates because males limited female reproductive success. As a result, females were more aggressive than males and possessed extensive secondary sexual characteristics (ornaments), which were used in intrasexual competition and during courtship. The ornaments were found to be reliable indicators of fitness because starved or sick individuals could not express them. This study also provides supporting evidence that the extent of sex role reversal and mating system of the gulf pipefish is highly dependant on the operational sex ratio, the ratio of sexually mature females to sexually mature males. I observed significant differences between the levels of aggression displayed by both males and females in male- and female-biased tanks, showing the importance of environmental factors on the behaviors of a species. In addition, both females and males were able to mate with multiple partners under certain conditions. Therefore, I classified the gulf pipefish as polygynandrous, contrary to previous studies.

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Introduction

Seahorses have long captured the human imagination with their unusual shape and extreme morphological specialization for parental care (Aristotle, 350 BCE). Male seahorses brood their young in a pouch, and although this is unlike traditional male roles, their sex roles are now beginning to be understood (Vincent, 1994; Masonjones & Lewis, 1996, 2000). However, we know very little about related members of their family, the pipefishes. Although the shape of the pipefish is not as unique or commercial as that of the seahorse, it is still a species that should be studied because, as in seahorses, the fact that males brood the eggs in a pouch presents very interesting implications on the theories of sex roles and mating systems. Furthermore, pipefishes present a new challenge to current theories because they exhibit striking sexual dimorphisms, which are not found in seahorses. This raises several questions about the sex roles in pipefish. Does exclusive male parental care automatically reverse courtship behaviors and sex roles? Are typical "male" characteristics such as aggression, bright coloration, larger size, and eagerness to mate retained when he broods the embryos, or does the female acquire these characteristics? How is the mating system related to secondary sexual characteristics? This study sets out to find answers to these questions by investigating the morphological and behavioral features of a little-known, but very common species of pipefish, the gulf pipefish *Syngnathus scovelli*. In order to understand the sex differences, secondary sexual characteristics, and the mating system of the gulf pipefish, we must first become acquainted with basic biological theories that help to explain these traits in other animals.

Sex differences

At its most fundamental level, the difference between the sexes is that the female produces large, protein-rich, stationary eggs, while the male produces cheap, mobile sperm. This disparity has led to the evolution of tremendous differences between the sexes: morphological differences such as a male peacock's huge tail, and behavioral differences such as those exhibited by humans that has led authors to say "Men are from Mars, Women are from Venus" (Gray, 1992). The differences in physiologies and behaviors originate from the attempt by each sex to maximize its own reproductive success. Charles Darwin (1859) referred to this occurrence as sexual selection, "a struggle between the individuals of one sex, generally the males, for the possession of the other sex." Darwin argued that sexual selection leads to the evolution of ornaments and weapons, such as the peacock's tail or a moose's antlers, which help an individual compete against rivals and gain a mate. Because these extravagant characteristics seem to also reduce survival, they cannot be explained simply through natural selection.

Darwin (1871) noted that the males of most species have secondary sexual characteristics, while the females have organs that protect or nourish the offspring. Although by Darwin's time there was clear evidence that males actively compete and fight for the possession of females, he was the first to observe that females were not passive members in the mate-selection process. In fact, Darwin argued that "the females, supposing that their mental capacity sufficed for the exertion of a choice, could select one out of several males." Females, therefore, by selecting their mates, are also selecting for those ornaments or weapons in males that they find attractive, and so are partly responsible for the evolution of secondary sexual characteristics present in males.

However, he failed to explain why females choose their mates and why males compete against each other for the possession of females.

This problem was partly resolved by Bateman (1948), who tried to discover the fundamental cause of intra-masculine competition. To do this, Bateman bred *Drosophila* and looked at the reproductive success of both males and females. He found that mating success varied more for males than for females: while 4 percent of females did not reproduce, 21 percent of males did not. Therefore, if some males failed to mate, others must have mated excessively. As a result, he found there was greater competition for the possession of mates between males than between females. Bateman argued that males must therefore be under greater sexual selection pressure than females. In addition, male fertility increased with the number of mates they had, which was not the case for females. This suggested that males should be more aggressive than females in seeking additional mates. Bateman also found that the fertility of females was limited by their costly egg production. In the males, fertility was not limited by sperm production but instead by the number of mates available to him, "consequently there is competition between male gametes for the fertilization of the female gametes…With intra-masculine selection males will be expected to show polygamous tendencies, whereas in females there would be selection in favour of obtaining only one mate after which they would become relatively indifferent." Accordingly, males have evolved to be aggressive and compete for mates while females have the luxury of being choosy.

Williams (1966) later explained that a male's greater willingness to copulate is understandable because of his smaller physiological sacrifice in reproduction: sperm is cheap while eggs are not. He argued that it is highly adaptive for a female to be reluctant to mate, as mating means a greater sacrifice to her. Therefore, a female reluctant to mate

is an evolutionary adaptation by which she can determine the right moment to assume the burden of parenting offspring. Males, in turn, have developed ornaments and other secondary sexual dimorphisms to attempt to successfully coax a female to mate with him.

However, it is incorrect to assume that all sexual dimorphisms are caused by sexual selection. Dimorphisms may also be a result of increased fecundity or ecological causation (Darwin, 1871; Hedrick & Temeles, 1989). For example, female satyrid butterflies are larger than males because larger females are able to lay more eggs, and thus have greater fecundity (Wiklund & Karlsson, 1988). The sexes have also been known to differ in their feeding apparatus to reduce ecological competition. Female hummingbirds, although smaller than males, have larger and more curved beaks than males (Temeles, Pan, Brennan, & Horwitt, 2000). Female beaks are better at feeding on one flower, while male beaks are better at feeding at a different flower, and this reduces feeding competition between the sexes. Lastly, sexual dimorphisms can be a result of sexual selection in which males or females develop ornaments, weapons, or body size differences to increase its chance of mating with the opposite sex.

As we have seen, sexual selection pressures may give rise to ornaments or weapons in males as a result of intra-sexual competition. However, it is still unclear why females prefer males with such extravagant traits. Many secondary sexual traits cannot be explained by natural selection because they do not enhance survival, in fact, they seem to do the opposite. Therefore, we need to ask why females still prefer males with colorful traits if these seem to make the individual less fit. Why should peahens prefer male peacocks with large, colorful tails, if these tails make males more conspicuous to predators and hinder their movement?

Fisher (1929) attempted to answer this question. He recognized the importance of mate choice and noticed that reproductive success is not only measured by how many offspring an animal leaves, but also by the quality of those offspring. Therefore, it is extremely important to be able to choose a partner of high quality. Fisher saw the inherent problem with the presence of secondary sexual characters—they seem to make a male less fit than those without secondary sexual characters. Why, then, would females prefer the less-fit male? He explained that female preference could lead to extravagant secondary sexual characters in males when there is also an initial advantage for the trait. For example, female birds might be initially attracted to the large wings of a male because large wings make him fly better. After some time, however, male birds might evolve wings that are so large that they actually hinder flight. However, females will continue to choose these large-winged males because their offspring will also have large wings and will therefore be considered more attractive by other females. This creates the potential for a "runaway process," in which female preference and male ornaments develop exponentially. The process will continue until checked by a great diminishing reproductive advantage of those ornamented males. In a sense, Fisher considered extravagant secondary sexual characteristics as merely "war paint" that are used to scare other males away or attract females, but which have no intrinsic value.

Handicap Principle

Fisher's idea of the runaway process was accepted for many years as the logical explanation for extravagant ornaments that males acquire through sexual selection. However, Amotz Zahavi (1975) presented powerful arguments against Fisher's runaway model, and offered an alternate explanation for the observed phenomenon. Initially,

Zahavi noticed that Fisher's model could not explain why the same wasteful ornaments that attract females also deter rival males. If the ornaments do not signify quality, and they are merely war paint, then males should not be deterred by ornamented individuals. Fisher himself noticed this weakness and proposed that with time, rival males should stop reacting to meaningless war paint.

Zahavi's solution to the enigma was the handicap principle. He began with the assumption that ornaments and weapons are signals that communicate messages. In order for these signals to be reliable, they must impose a cost on the signaler. This cost must be great enough so that faking the signal is unprofitable for a "cheater," and so only those individuals that convey truthful information will accept to expend the cost of the signal. Furthermore, these signals must have differential costs, so that they are more easily expressed in high-quality than in low-quality individuals. Consequently, all animal signals should be costly and therefore reliable—from chemical signals to predator-prey interactions and human communication. These signals are commonplace because they benefit both the signaler and the receiver, much as a non-zero-sum game may benefit both players.

The handicap principle is useful in explaining the extravagance of the peacock's tail, and why females prefer mates with such ornaments. The huge tail is costly because it is truly a handicap: it is a physical burden, it requires ample nutrients to keep it healthy, and it increases conspicuousness to predators and reduces mobility. In a sense, a mature, colorful male is an individual that has survived a test of his fitness. Therefore, precisely because the tail is costly, only well-fitted males can afford to carry such a luxury. Males who are not fit cannot afford to have large tails, and therefore large tails can be considered reliable indicators of fitness. Ornaments such as these are an easy and reliable

way for females to pick the fittest males among many in a population. Females that use these signals in choosing a mate will select better mates and have more successful offspring. Accordingly, female choice for large, beautiful tails will spread through the population.

In addition to ornaments and weapons, the handicap principle is useful in explaining the complex courtship rituals that most animals follow. Courtship behaviors are the communication that animals use to show their quality to a potential mate. Signalers show off their skills by engaging in courtship movements that are elaborate and difficult to perform. For example, some male birds hang upside-down on a branch while spreading their wings (Zahavi, 1991). Hanging upside-down does not make the movements more noticeable, but it makes them considerably harder to perform. Male bowerbirds spend considerable time building bowers of twigs on the ground that have no purpose other than to serve as the stage on which they perform courtship displays. Other birds sing incredible songs for lengthy periods to attract females. These songs are physically demanding and they expose the bird to predators. Courtship behaviors, precisely because they are costly, express the message that the individual who is performing them is fit and willing to invest in mating since it is putting forth great effort in the mating ritual while foregoing feeding or resting.

Many of these complex courtship behaviors have become ritualized in order to reveal small differences between individuals (Zahavi & Zahavi, 1997). In humans, for example, in order to determine whom the fastest athlete is, runners line up together at the start line and begin running at the same time, ending after a certain length. This is done to compare small differences in the speeds of the runners. Similarly, animals use rituals to compare small differences in qualities of their potential mates. The handicaps come

from advertising defects as well as their virtues. Seahorses, for example, have a highly ritualized courtship which last three days, and is characterized by several behaviors such as quivering, pointing, and rising (Masonjones & Lewis, 1996). This courtship is ritualized to differentiate between the quality of potential mates. Behaviors such as these endure as long as watchers gain information that is beneficial to them, and as long as the investment is worthwhile to the signaler but unworthy to a cheater (Zahavi & Zahavi, 1997).

Zahavi's theory was rejected from the start. His proposition that ornaments and weapons were reliable signs of quality did not begin to gain empirical support until 10 years after his first publication. Presently, the handicap principle has been repeatedly tested and supported in several different species and for several different traits. These include tail length in barn swallows (Møller & de Lope, 1994), saliva secretion in scorpionflies (Kurtz & Sauer, 1999), calling songs in crickets (Ryder & Siva-Jothy, 2000), and male eye span in stalked-eyed flies (David, Bjorksten, Fowler & Pomiankowski, 2000). For example, in the male barn swallows, *Hirundo rustica,* tail length, a sexually-selected trait which is preferred by females, is also a handicap (Møller & de Lope, 1994). When researchers artificially elongated the tails of male birds, survival decreased; when they shortened the tails, survival increased. They found that males with elongated tails captured smaller, less profitable food, while males with shortened tails captured larger, more profitable food. This supported the argument that the secondary sexual character is costly. In this case, the large tail was a handicap because it increased the cost of flight and decreased foraging success, it impaired the immune system because energy was reallocated from immune defense to locomotion, and it increased the risk of predation. Møller and de Lope also found that naturally long-

tailed males were better able to survive with elongated tails than naturally short-tailed males, suggesting that there was a also differential cost of the signal.

However, not all signals need to have an energetic cost in order to be reliable. Amplifiers, through their design, help receivers assess preexisting signals, although they are not in themselves indicators of quality. For example, the dark border that outlines a butterfly's wing is not costly, but it makes damage to the edges seem more apparent to potential mates (Zahavi & Zahavi, 1997). An amplifier cannot make the bearer seem bigger, but it will make its size easier to ascertain. The cost, in a sense, comes from amplifying the defects as well as the virtues. Like other reliable signals, amplifier costs are differential because it is less costly for fit individuals to amplify their flaws (since they have fewer of them) than it is for less-fit individuals. Taylor, Hasson, and Clark (2000) tested whether a black patch found on the abdomen of spiders is a reliable signal of the spider's fitness. They found that the black patch amplifies the physical condition of the spiders. This helps rivals and potential mates accurately assess the fitness of the individual by seeing how the abdomen bulges around the patch (more bulging reflects the spider has been feeding well). However, although the patch was not energetically expensive to produce, it accurately amplified the physical condition of the spider, and thus they concluded that it serves as a reliable amplifier of the spider's condition.

Mating systems

The relationship between secondary sexual characteristics and mating systems was first described by Darwin (1871). He noticed that polygamous males generally have the most extensive ornaments and weapons, and proposed that it was because they spend much of their energy in attracting mates. He gave several examples in which

polygamous males have striking secondary sexual characteristics such as in gorillas, lions, and peacocks. However, he observed some cases in which a monogamous species became temporarily or permanently polygamous due to a change in its environment. Darwin made little attempt to explain his observations.

Emlen and Oring (1977) finally established a clear relationship between ecology, sexual selection, and the mating systems of animals. Mating systems of animals, they explained, vary from complete monogamy to polygamy. In a monogamous mating system, the same pairs mate with each other during one or more breeding seasons. Polygamy, on the other hand, occurs when one of the sexes has access to more than one mate. This includes polygyny, when males have multiple partners, polyandry when females have multiple partners, or polygynandry, when both males and females are able to have multiple partners.

Emlen and Oring (1977) explained, as Darwin (1871) observed, that sexual selection is generally slight in monogamous species while it is intense in highly polygamous societies. The differing intensities of sexual selection result from the ability of a portion of the population to control the access of others to potential mates. Monogamy, they explained, occurs only when there is the economic defensibility of a mate, and when neither sex has the opportunity to monopolize additional members of the opposite sex. Polygamy, on the other hand, occurs when there is the economic monopolizability of several mates. Polygamy depends on the degree to which multiple mates, or resources used to gain multiple mates, are defendable.

To further analyze the cause of the different mating systems, Emlen and Oring (1977) coined the term operational sex ratio (OSR) to refer to the "ratio of fertilizable females to sexually active males at any given time." They noticed that with a greater

imbalance of the OSR, there is greater variance in reproductive success among members of the limited sex, and in turn, there is a greater degree of polygamy in the mating system of the species. In addition, they suggested that the environment can have severe effects on the mating system of a species. They observed that ecological constraints impose limits on the degree to which sexual selection can operate. Mating systems are not rigid and stable; rather they are greatly influenced by ecological variables. Like Darwin (1871), Emlen and Oring noticed that shifts from monogamy to facultative polygamy might occur due to changing environmental conditions.

Davies and Lundberg (1984) tested this theory by studying how food distribution influences the mating system in dunnocks. Food distribution was found to have an effect on female ranges: dense food patches allowed females to have smaller ranges. This, in turn, made it easier for males to monopolize several females and shift the mating system towards polygyny. Sparse food patches, on the other hand, promoted females to have larger ranges, and were therefore more difficult to be monopolized by one male. This shifted the mating system towards polyandry.

Parental investment and potential rates of reproduction

Another factor that may play a role in defining the mating systems is parental investment in offspring. Robert Trivers (1972) defined parental investment as "any investment by the parent in an individual offspring that increases the offspring's chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring." He believed the operation of sexual selection is controlled by the relative investment of males and females onto their offspring, and not by the OSR as

proposed by Emlen and Oring (1977). Trivers argued that the sex that invests least in their offspring will compete against each other to breed with members of the opposite sex, since individuals of the sex investing less will gain more by having additional copulations. Because males usually invest very little in their offspring, intrasexual competition among males will be more prevalent than among females. When investment by both sexes is equal, however, then sexual selection should operate similarly in males and females, and the mating system should be monogamous. On the other hand, when male parental investment exceeds that of the female, females should compete among themselves for mates while males should then become the choosy sex.

In contrast to Trivers's (1972) prediction of sexual selection due to increased parental investment, Clutton-Brock and Vincent (1991) argued that it is not only easier, but more accurate to use the potential rate of reproduction of males and females to predict which sex will compete more intensely for mates. The sex with the highest potential rate of reproduction, "the maximum number of independent offspring that parents can produce per unit time," should be the sex that competes more intensely for mates. Clutton-Brock and Vincent looked at the maximum rate of reproduction of 29 species in which males were responsible for parental care. As predicted by Trivers (1972), females in these species should compete for mates because they invest less in each offspring. However, in contrast to Triver's predictions, Clutton-Brock and Vincent observed that in all but two species, males still were the predominant competitors for mates. They also noticed that in all but two species, males also had higher rates of reproduction than females, thus supporting their hypothesis of the importance of the potential rate of reproduction. For one of their exceptions, seahorses, new research suggests that males actually have a higher potential rate of reproduction than females, thus agreeing with

their initial predictions (Masonjones & Lewis, 2000). The other exception was *Rhea americana*, in which females have a higher reproductive potential during the entire breeding season, but during production and mating, males can accept eggs faster than females can produce them, so the operational sex ratio is male-biased.

Seahorses and pipefishes

As we have seen, in traditional sex roles males possess secondary sexual characteristics and compete against each other for females during courtship, while females have the privilege of choosing their mate. In addition, if there is any parental care after fertilization, it is most often provided by the females (Clutton-Brock & Vincent, 1991). But what would happen to courtship behaviors in a species where males provide all parental care? This occurs most noticeably in the Syngnathidae family, where male seahorses and pipefishes brood the embryos in a pouch or on their body, and provide them with nourishment, gas exchange, waste removal, and protection against predation and the environment (Quast & Howe, 1980; Berglund, Rosenqvist & Svensson, 1986b; Masonjones, 1997). These fish are already parentally sex-role reversed, since the males provide all parental care. However, they also have the possibility of being courtship sex-role reversed, where females compete more intensely than do males for access to mates. In this case, females should be more modified by sexual selection because intrasexual competition will be stronger between females than between males (Vincent, Ahnesjö, Berglund, & Rosenqvist, 1992).

The family Syngnathidae, of the order Gasterosteiformes, includes both pipefishes and seahorses (McEachran & Fechhelm 1998). These fish have an armored body composed of dermal plates arranged in rings, a small mouth located at the end of a snout,

and they lack teeth. Male pipefish and seahorses are highly specialized to carry the

embryos in a pouch or glued to their bodies. Pipefishes are slender and elongate, while

seahorses have a prehensile tail. Both feed on zooplankton, which are sucked into their

mouths. They are found worldwide in tropical to temperate climates mostly in marine

water, although a few species occur in freshwater. There are about 215 species and 52

genera in this family.

Brief history of research

Seahorses and pipefishes have long been subjects of interest for biologists,

beginning with Aristotle in 350 BCE. The first modern article about syngnathids

appeared in1867, as the Reverend Samuel Lockwood published a paper in the American

Naturalist called "The sea-horse and its young." In it, he gives an account of his

experience with keeping three gravid seahorses he had received from a fisherman. He

described the process by which the male emitted the young from his pouch, and his failed

attempt at keeping the young seahorses alive. Darwin (1871) was also attracted to these

creatures, amazed by the fact that females lay the eggs in the "marsupial sacks" of males.

A few years later, Gudger (1905) wrote an article titled "The breeding habits and

the segmentation of the egg of the pipefish, *Syphostoma floridæ*" (now known as

Syngnathus floridae). In addition to giving a thorough literature review of the species,

Gudger was the first to document courtship and mating behaviors in pipefish:

> The act of copulation is preceded by a very curious "liebesspiel." The two
> fish swim around in the aquarium with their bodies in nearly vertical positions,
> but with the head and shoulder region sharply bent forward like the letter f. Then
> they swim slowly past each other, their bodies touching and the male being
> perhaps more demonstrative. Just before the actual transfer, the male becomes
> violently excited and demonstrative, shakes his head and anterior body-parts in a
> corkscrew fashion and with his snout caresses the female on the belly. The
> female responds to this but does not become so excited. This is repeated several
> times, the fishes becoming more excited each time they touch each other.

Presently, quick as a flash, the sexual embrace takes place and then the fishes separate to begin again in a few minutes.

This embrace consists in the fishes intertwining their bodies like two capital letter S's, the one reversed on the other, thus bringing them face to face. Thus they hold their bodies together while the eggs pass from the oviduct into the pouch…The anal papilla, or the protruding oviduct of the female, is, at the moment of contact of their bodies, thrust into the buttonhole-shaped opening at the anterior end of the marsupium. Some eggs, in number a dozen or more, now pass into the pouch and are presumably fertilized at this moment. (Gudger, 1905)

Williams (1966) prematurely assumed that sex roles should be reversed in all syngnathids. He suggested that male pipefishes and seahorses would contribute more energy in the young or undergo greater risks during pregnancy than females. Therefore, he expected females to show traditional male characteristics such as aggressiveness and promiscuity, while males would show a reluctance to mate. However, Williams never performed any experiments to test his hypotheses.

Mating systems in pipefishes

The mating system of syngnathids has been studied in a handful of species. Each species differs from the others in several aspects: coloration, size, ornamentation, and dimorphisms. Accordingly, each species differs from the others in its mating system, and each offers a different solution to the inherent problems that arise from sexual differences (Bateman, 1948). All of the seahorses studied to date are sexually monomorphic and follow a monogamous mating system (Vincent *et al.*, 1992; Vincent & Sadler, 1995; Masonjones & Lewis, 1996). Monomorphic pipefishes also follow a monogamous mating system, while sexually dimorphic species follow a polygamous mating system (Vincent et al., 1992). Since very little is known about the mating system of the gulf pipefish, it is necessary to have a thorough understanding of how other species interact with each other, and use this as a guideline in the investigation of the gulf pipefish.

However, we must remember that the mating system of a species is not a fixed trait, but that it is able to shift due to ecological and physical factors that affect the reproductive rate of males and females (Emlen & Oring, 1977; Vincent *et al.,* 1992).

Corythoichthys intestinalis is one of the monomorphic pipefish that follows a monogamous mating system (Gronell, 1984). Field studies indicate that courtship in *C. intestinalis* is highly ritualized, consisting of several distinct patterns of movement. Every morning at sunrise, the females leave their sleeping sites and travel to the spawning sites, which are near male sleeping areas. After arriving, the male and female follow a set routine of movements that includes parallel swimming, arching, crossing, rising, and entwining. These movements end with spawning, when the female places all of her eggs at once in the pouch of a single male. Both male and female *C. intestinalis* were highly involved in the courtship process, even though the male provides all parental care. Unlike 'traditional' courtship in which the females play a passive role, both sexes of *C. intestinalis* are actively involved in courtship, suggesting that the roles have not been completely reversed, although females still show some slight "masculinization."

Gronell noticed that every morning, partners that had previously mated come together to perform daily "greetings" that involve the first few movements of the courtship displays. Greetings occur every day, and individuals only greet the partners that they had mated with at their original spawning site. This greeting behavior occurred year-round, even outside of the breeding season. The same pairs were also seen greeting each other during different mating seasons, indicating a monogamous mating system and long-term mate fidelity. Gronell suggested that daily greetings in pipefish may maintain contact between spawnings and to ensure that the fish are synchronized in the process. Additionally, they help inform of a missing partner, and they reinforce mate fidelity

because the greeting takes place during a short time after sunrise, and it would be difficult for an individual to greet more than one partner sequentially. Gronell noticed that the potential reproductive rate for both sexes was equal, which can explain the monogamous mating system. There was no evidence of polygamous matings or of mate displacement even though the ratios of males to females varied widely during the observed breeding seasons.

Other species of pipefish follow a polygamous mating system. Two of the most studied pipefishes in this group are *Nerophis ophidion* and *Syngnathus typhle* (Figure 1). The first species, *N. ophidion*, is a sexually dimorphic pipefish species in which females have permanent sexual coloring, possess skin folds, and are larger than males (Berglund *et al.*, 1986a). Females of this species are more active than males in courtship. Males receive all of their eggs from a single female, and brood embryos openly on their
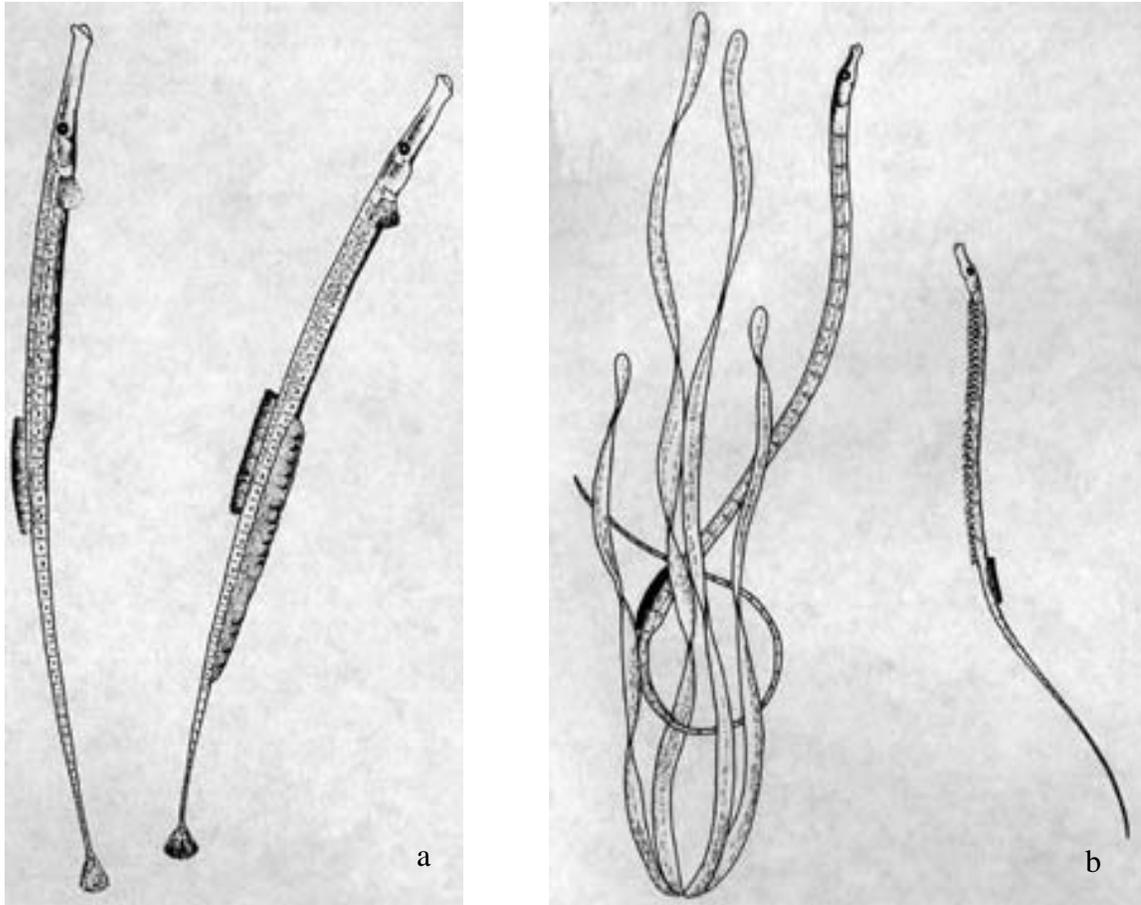
Figure 1. a) Female *S. typhle* (left) and brooding male
               b) Female *N. ophidion* (right) and brooding male

abdomen. Females give an entire clutch to one male, but mate with several males during

the course of a male pregnancy (sequentially polygamous). The second species, *S.*

typhle, is sexually dimorphic, although not to the same extent as *N. ophidion*. Males and

females are phenotypically similar, except during intrasexual competition and courtship,

when females develop a temporary ornament (Berglund et al., 1997; Bernet et al., 1998).

Both sexes are equally involved in the courtship process. Males receive eggs from more

than one female, and females deposit eggs in several males (simultaneously polygamous).

We might have expected *S. typhle*, the species that is highly polygamous, to be the most

dimorphic, while *N. ophidion* to be the less dimorphic species (Darwin 1859, Williams 1966), though this is clearly not the case.

In *N. ophidion,* female fecundity is positively related to amount of sexual coloring, body length, and body weight (Berglund *et al*., 1986a). Sexual coloring is considered a reliable sign of quality, since it would be difficult for an unfit female to produce large areas of color, as these may reflect actual physical condition (Rosenqvist, 1990). There was no correlation in males between weight and number of embryos brooded, probably because they can essentially accommodate on their bodies all the eggs that a female may produce. Males prefer to mate with females that have the most sexual coloration. Therefore, in *N. ophidion*, larger female size is due to both increased fecundity (natural selection) and male preference (sexual selection). Since there is little selection for male size, this results in the observed sexual dimorphism.

In *S. typhle* females, a positive correlation was found between body length, and number and size of eggs transferred (Berglund *et al*., 1986a). Unlike *N. ophidion* males, however, there is also a positive correlation between *S. typhle* male size and clutch size, since brood pouch size restricts the number of eggs a male can carry. Accordingly, females prefer to mate with larger males, and males prefer to mate with larger females. Therefore, sexual dimorphism did not evolve in this species, where both sexes are selecting large partners. However, this still does not explain why *N. ophidion* females have sexual coloring while *S. typhle* do not.

Theoretically, the presence of extensive secondary sexual characteristics in *N. ophidion* females could be explained if males spend a lot of energy in brooding the embryos, and are therefore a very limiting resource for females. Accordingly, male *S. typhle* may spend less energy brooding their embryos, making them less limiting for

females, which may be the reason why females do not have extensive secondary traits. To test this, Berglund *et al.* (1986b) measured the amount of energy that males spend on the embryos in both *N. ophidion* and *S. typhle*. Contrary to their expectations, they found that *S. typhle* males spend considerably more energy brooding their embryos than *N. ophidion*. They also compared the energetic investments of females to males and found that *N. ophidion* females invest more energy per zygote than did *N. ophidion* males, while both male and female *S. typhle* spent about the same amount of energy. Berglund *et al.* concluded that *N. ophidion* males might be limiting the reproductive success of females in some other way, other than by the amount of energy invested in zygotes. They showed that extensive parental investment, as that shown by *N. ophidion* females, cannot be used to predict sex role reversal and reversed sexual dimorphisms, as was assumed by Trivers (1972). However, it is not clear whether Berglund and others accounted for all male costs during pregnancy.

According to Clutton-Brock and Vincent (1991), calculating parental energy investment in offspring is not enough to determine which sex competes for access to mates. As shown above, although female *N. ophidion* make large energetic investments in their offspring, they still show extensive secondary sexual characteristics and compete for mates, which does not support Trivers' (1972) theory that the sex that spends the least energy in the offspring will develop the greatest sexual dimorphism. Similarly, *S. typhle* females and males spend about the same amount of energy in their offspring, yet females still compete for mates. Clutton-Brock and Vincent (1991), on the other hand, suggested using potential reproductive rates as predictors of which sex will compete for mates and show dimorphisms.

Berglund, Rosenqvist, and Svensson (1989) measured the potential reproductive rates of *N. ophidion* and *S. typhle*. They hypothesized that brooding space limitations and the long pregnancy in males limits female reproductive success. *N. ophidion* females and *S. typhle* females were able to produce more eggs per unit time than males could handle: *N. ophidion* females filled an average of 1.8 males, while *S. typhle* females filled an average of 1.9 males. However, since *N. ophidion* females show greater sexual dimorphisms than *S. typhle* females, Berglund *et al.* expected *N. ophidion* females to have a greater surplus of eggs than *S. typhle* females. This turned out not to be the case— egg surplus was equal in both pipefish species. In any case, they concluded that males of both species limit female reproductive success, not by spending more energy on brooding than females spend on eggs, but by being unable to brood a set of embryos before females produce new eggs. The question why *N. ophidion* still shows extensive ornamentation as compared to *S. typhle* remains unanswered (Table 1).

Table 1. Differences in behavioral and morphological characteristics between
N. ophidion and *S. typhle*. From Berglund *et al.* 1989.

Characteristic	*N. ophidion*	*S. typhle*
Sexual size dimorphism	Male < female	No
Sexual color dimorphism	Female bright	No
Sexual shape dimorphism	Female skin folds	No
Sexual behavior dimorphism	Female active	No
Brood pouch	No	Yes
Male size correlated with clutch size	No	Yes
Female clutch spread among several males	No	Yes
Male clutch received from several females	No	Yes
Females prefer larger males	No	Yes
Males prefer larger females	Yes	Yes
Energy investment in offspring	Male << female	Male • female

Ornaments and armaments in pipefishes

Ornaments are those traits that are preferred by the opposite sex, while armaments are those weapons and status badges used in intrasexual competition to repel competitors. The traditional view is that ornaments are only used to attract mates, while armaments are only used to repel competitors. Only recently has it become evident that ornaments are used in aggressive displays as often as they are used in courtship, and that the two processes are actually dependent on each other (Berglund *et al.,* 1996). For example, bright coloration and sexual calls in mammals and birds are used for attracting mates as well as repelling competitors. Berglund *et al*. proposed that secondary sexual characteristics evolved first as armaments used in same-sex competition. Over time, individuals that selected their mates according to those traits were more successful than those that did not, so the armaments evolved into ornaments by mate choice. Armaments and ornaments are honest signals because they are constantly being tested during intrasexual competition. Berglund and others suggested that the skin fold and blue coloration in *N. ophidion* females was one such example of a trait that serves as both armament and ornament.

In *N. ophidion*, when four females were kept together in one tank, only one female developed the skin fold (Rosenqvist 1990). This suggests that a female may be able to dominate others by suppressing the development of skin folds in other females, thereby using their own fold as an armament. Single females that were kept in a tank with males had large skin fold increases, while females kept in isolation had decreases in skin fold size. In addition, males preferred to mate with females with long rather than with short skin folds (even if females were of the same size), showing that the skin fold is also an ornament used to attract mates. Not surprisingly, the best predictor of female

fecundity was found to be skin fold size. Therefore, a female benefits greatly from large size because it increases her fecundity, her social rank, and her attractiveness to males.

As an armament, the skin fold may be the signal that is used to establish a social hierarchy in *N. ophidion* females. The advantage of having skin folds or other signals of dominance has evolved to avoid physical confrontations. Instead of fighting, these signals represent the probability of an individual winning a fight, should a fight take place (Zahavi, 1981). Fighting is usually harmful to all individuals involved: there is the loss of time and energy and risks of injuries or death, which is why most animals use threats instead of fights to resolve conflicts (Zahavi & Zahavi, 1997). The skin fold, a structure that possibly impairs movement and makes the pipefish more vulnerable to attack, is a clear handicap that only a large and fit female is able to maintain.

Berglund, Rosenqvist, and Bernet (1997) studied female *S. typhle* to determine whether their ornaments are honest signals of fitness. Female *S. typhle*, during intrasexual competition or when courting males, develop a temporary ornament which is an amplification of their normal striped pattern. Females that develop high-contrast ornaments copulate sooner, more often, and transfer more eggs than those with low contrast. Furthermore, females that are maintained together in tanks maintain their contrast whereas females that are kept by themselves decrease in contrast. Berglund *et al.* found that the ornament was energetically inexpensive to produce because females with a low-food diet were able to achieve the same contrast levels as those in a high-food diet. However, the ornament could still be costly in other ways by making females more conspicuous, increasing their vulnerability to predators, and likely having costs of social punishment. These costs make the ornament a reliable indicator of quality.

Bernet, Rosenqvist, and Berglund (1998) studied additional costs of the high-contrast ornament in *S. typhle* females. Females were able to develop the ornament when in physical contact with males, and especially when there were other females present. Bernet *et al*. suggested that females use the contrast as an armament to intimidate female rivals, as more-dominant females developed the ornament to greater extents than did less-dominant females. In addition to social costs, the contrast also has costs of increased visibility to predators, as females under the risk of predation displayed the ornaments to lesser extents than those without the risk, thus showing that it can be considered as a reliable indicator of fitness.

Males have been shown to use the high-contrast pattern as a preference for mating, since males do not mate with non-ornamented females. By choosing their mates according to the degree of ornament expression, males reduce their time in a potentially dangerous search for a mate. In addition, since the degree of ornamentation is a sign of dominance, a male that mates with a highly ornamented female will be harassed less by other females while mating. As we have seen, the high contrast serves two functions, it works as an ornament by attracting males, and it works as an armament by repelling females.

Gulf pipefish

As previously mentioned, *S. typhle* are slightly dimorphic yet highly polygamous, while *N. ophidion* are highly dimorphic but are not as polygamous as *S. typhle*. This does not fit Darwin's (1872) prediction that more polygamous species should exhibit greater levels of sexual dimorphisms than less polygamous or monogamous species as a result of more intense sexual selection. In an attempt to understand this paradox, I decided to study the mating system of another highly dimorphic species of pipefish, the gulf pipefish

Syngnathus scovelli (Figure 2). *S. scovelli* is a common pipefish found in shallow grassbeds of the western Atlantic, from as far north as Georgia (Targett, 1984) to southeastern Brazil (Gasparini & Teixeira, 1999), and the Gulf of Mexico (Díaz-Ruiz, Aguirre-León, Pérez-Solis, 2000). Freshwater specimens have also been found in Lake St. John, Louisiana (Whately, 1962), and in reservoirs in Texas (Viola, 1992). The first description of the species, (then known as *Syphostoma scovelli*), was made by Evermann and Kendall in 1896 from specimens taken at Corpus Christi, Texas. Only three unpublished PhD dissertations have attempted to study the life history of the gulf pipefish, primarily through collections and studies of dead samples. Their findings are summarized below.
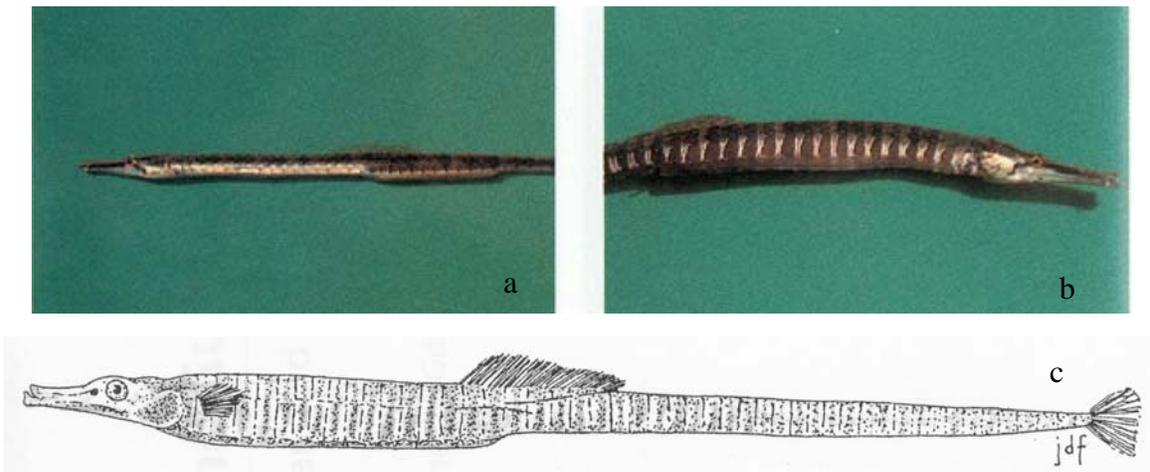
Figure 2. Male (a) and female (b, c) gulf pipefish, *S. scovelli.*

The common name of *S. scovelli*, the gulf pipefish, was suggested by Joseph in his dissertation (1957). It is distinguished from other pipefish species primarily by its extensive sexual dimorphisms, and by its short snout, which is half as long as the head length (Hoese & Moore, 1998). It is a highly dimorphic species with mature females

having very deep trunks and dorsal fins twice the height of those in males of equivalent size (Joseph, 1957). In fact, researchers have reported that "some differences [between the sexes] are so obvious they do not require quantitative treatment" (Brown, 1972). Females have silver V- or Y-shaped marks on each trunk ring. These marks become apparent during the maturation of the ova, and consequently are an accurate indicator of sexual maturity (Joseph 1957, Brown 1972). Male maturity, on the other hand, is determined by the presence of a completely developed brood pouch (Joseph, 1957; Brown, 1972). The maximum size attained by either sex is not significantly different, but average sizes differ widely (Joseph, 1957). Females tend to be larger than males, with average standard lengths of 110.9 mm as opposed to 99.7 mm for males (Brown 1972). Maximum lengths for the gulf pipefish are 130-140 mm: these fish are usually female and are scarce throughout the year (Brown, 1972). Gulf pipefishes vary in color from white, to green, brown, or black (pers. obs.).

The gulf pipefish breeds throughout the year over most of its range (Brown, 1972), although in years with more severe winters reproduction can cease for one or two months (Joseph, 1957). The highest levels of reproduction were found to be during the spring and fall seasons (Brown, 1972; Joseph, 1957). There was a greater percentage of pregnant males during the spring and fall, and their brood sizes were larger in these than in other seasons (Joseph, 1957).

Sexual maturity is reached at different sizes for males and for females (Joseph, 1957). Even within sexes, size at which sexual maturity is obtained may differ by populations and by seasons. The smallest males with complete brood pouches were found to be approximately 60 mm in length, although most males reach maturity by the time they attain 90 mm (Brown, 1972). For females, no ovarian development was

evident in those less than 80 mm, and most were mature by the time they reached 110 mm (Joseph, 1957; Brown, 1972). The incubation period lasts 14 or 15 days at 76°F. Newborn pipefish are about 12-15mm in length (Joseph, 1957; Whately, 1969), and grow at an astounding rate. In less than two weeks, they increased in length to 28 mm; within three months, they increased in length five-fold (Whately 1969).

Gulf pipefish are able to reach maturity and reproduce in less than a year, and few survive to their second year of life (Joseph, 1957; Brown, 1972). By studying length-frequency diagrams obtained from monthly samples of a population near Cedar Key, Florida, Joseph (1957) found that there are two complete life cycles during the year. Females born in April and May mature in 6 months and become the breeding stock of October, and few survive the winter months. Likewise, females born in September and October mature over the winter and comprise the main spring breeding stock, but few survive throughout the summer months. This life cycle helps explain the reproductive peaks in the spring and fall.

The sex ratio varies from population to population, and seasonally within a population (Joseph, 1957; Brown, 1972). In the same Cedar Key population, Joseph found that in May and June there were more males than females. In July and August, there was an even sex ratio, and during fall and winter there was an increase in the percentage of females in the population. Two possibilities can explain this difference of sex ratios: differential mortality or a biased sex ratio at birth. Joseph suggested that differential mortality might result from an increased mortality rate among pregnant males. Males predominate during the season of peak reproduction. However, since brooding is physiologically demanding, and since the bright embryos may cause increase in visibility, males could be more subject to predation and death while brooding. This

could cause a gradual reduction in the number of males with an increase in the number of females. However, Joseph admits that this trend could not continue for long without extinction of the population.

Brown (1972), studying the same population several years later, also found that adult females are more abundant than adult males in winter and early spring, when population numbers were low (Figure 3). However, in the summer and early fall, males were more abundant than females, which coincided with the period of greatest abundance of pipefishes. Because male *S. scovelli* reach adulthood at smaller sizes than females, he argued that this may account for their numerical superiority during the summer. At this point many immature females were also collected, frequently exceeding 100 mm in length. Later in the fall, however, these females reached sexual maturity and the sex ratios became nearly equal.

Brown (1972) observed that the digestive track of the gulf pipefish is best suited for a carnivorous diet, as their snout restricts food items to small animals that can be sucked in. The larger part of the diet consists of microcrustaceans, including amphipods, copepods, and small shrimp. Immature fish of less than 70 mm eat mostly copepods, while amphipods are the most frequently eaten item of adults over 100 mm in length.
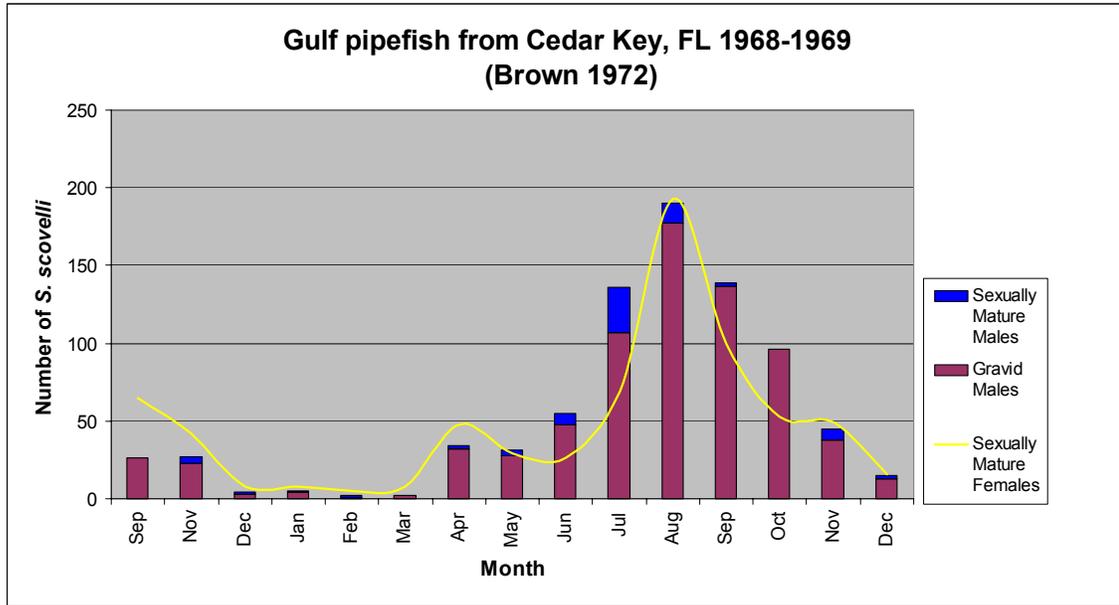
Figure 3. Number of sexually mature males, gravid males, and sexually mature females in a population of pipefish near Cedar Key, FL 1968-1969

However, because of the greater mass of shrimps, they make up most of the volume of adult stomach contents. Brown also found larval pipefishes in the stomachs of adults indicating that cannibalism is present, although it does not constitute a significant portion of the adult's diet.

Both Joseph (1957) and Whately (1969) attempted to keep pipefish in aquaria, and they achieved this to different degrees of success. Although Joseph was only able to maintain fish successfully for a period of one month, he was the first to observe and document courtship and mating in the gulf pipefish. He observed matings on several occasions, but in only one case was the transfer of eggs successful. One of these episodes is quoted below:

> Our attention was first attracted by a large female (approximately 105 mm) swimming in a vertical position and bobbing to the surface in a spiral pathway. The tip of the snout would consistently break the surface. A conspicuous ventral flexure was noted in the trunk region. A male approximately two-thirds the length of the female was observed to follow her to the surface.

This pattern was repeated for a period of four to five minutes. Then the male attempted to intertwine with the female. The anal regions were in contact and the snout of the male was in contact with the operculum of the female. This contact lasted a few seconds… The male assumed an arched position on the bottom of the aquarium. The region of the brood pouch was in contact with the bottom but the head and tail regions were raised. The male scraped along the bottom in this position for approximately 10 minutes. No further activity was noted. (Joseph, 1957).

Two of the most conspicuous aspects of courtship were the trunk flexure of the female and her deepened color during spawning. Joseph noticed it was the female that initiated the courtship, and that had nuptial coloration, which, he noted, agreed with the common notion that "spawning coloration is restricted to the sex which is the aggressor in courtship behavior" (Joseph, 1957).

Whately (1969) had greater success in keeping gulf pipefish in aquaria. He reported keeping a male pipefish alive in his aquarium for 27 months, and keeping five others alive for 18 months. Having been the first person to document a breeding population of *Syngnathus scovelli* in a freshwater lake, Whatley decided to keep the fish in freshwater tanks and observe their breeding and growth rate. He attempted to feed them recently-hatched brine shrimp but found that they were difficult to hatch in sufficient quantities. Instead, he fed them plankton that he collected from the location where the fish were taken. Whatley noticed that the fish had an extremely high metabolic rate and did not stop feeding as long as plankton remained in the tank. He noticed that pipefish are tireless hunters whose independent action of their eyes helps them feed. He also had trouble with parasites, losing a few pipefish to flatworms. Whately concluded that "it is possible, though difficult, to maintain *S. scovelli* in fresh water aquaria, chiefly because a readily available supply of live plankton is essential to their maintenance."

Whatley made several observations on the pipefishes that he kept in tanks. On three occasions, he noticed cannibalism after a male that had given birth began eating the

fry almost as rapidly as they were born. He also observed and described a mating that

occurred the day after the male had given birth:

> The female was observed coming to the surface of the bucket and
> violently shaking her head from side to side. After about 20 minutes the male
> swam to the surface and joined the female. Their bodies were erect in the water,
> forming S-shaped curves. There was a much closer resemblance to the sea horse
> body form than I would have believed possible. Dorsal fins of both fish were
> erect and flaring. After swimming around each other and briefly twining bodies
> for some 10 to 15 seconds, the male approached the female from the rear, (her
> ovipositor was apparent all of the time), placed his tail around hers and the pair
> spun around together in the center of the bucket for a very brief interval,
> approximately 3 to 5 seconds. During this period the female's ovipositor was
> placed in the brood pouch of the male. (Whately, 1969)

Other studies of *S. scovelli* have used the collection and preservation of dead

samples to learn more about the pipefish. Some have focused their work on their spatial

distribution (Díaz-Ruiz *et al.,* 2000), or have only looked at the physical aspects of

reproduction (Gasparini & Teixeira, 1999). Jones and Avise (1997a) performed a

detailed study of the mating system of the gulf pipefish by collecting samples, preserving

them, and analyzing the maternity of the embryos through genetic techniques. They

collected male pipefishes from a single locale in the northern Gulf Coast of Florida

during three separate days in July 1994, after which the samples were frozen on dry ice.

Embryos from pregnant males were then removed and analyzed for maternity by using

microsatellite markers. Of the 40 pregnant males they analyzed, only one contained

embryos from more than one female, indicating that the male had mated twice within a

short period of time. However, two pairs of males had received eggs originating from the

same female, indicating that females often distribute their eggs among several males.

Given the high power to detect multiple matings in males, and the low power to detect

multiple matings in females, Jones and Avise concluded that the genetic mating system of

the gulf pipefish was polyandrous, where males have one mate during the course of a

single pregnancy, while females commonly distribute their eggs among more than one male.

However, the study conducted by Jones and Avise (1997a) was comprised of fish collected at a single time point during the summer, when the ratio of females to males was 0.68. We can imagine that when there are excess males, as there were in July 1994, it would be very difficult for a male to obtain additional mates, while females could easily obtain additional mates, as they were outnumbered by males almost 2 to 1. Therefore, this could explain their conclusion that the mating system of the gulf pipefish is polyandrous. However, the sex ratio of the gulf pipefish is known to fluctuate drastically during the year, varying from 0.51 to more than 1.5 females per males (Brown, 1972). Since the OSR is known to affect the mating system of some animals (Emlen & Oring 1977; Vincent, Ahnesjö, & Berglund, 1994), it is probable that the mating system of the gulf pipefish will also vary with changing sex ratios. Consequently, I believe their genetic study provides only a small piece of a complicated mating system puzzle. Because ecological and environmental constraints are essential in determining a species' mating system (Emlen & Oring, 1977), and since these conditions are known to fluctuate in the gulf pipefish (Joseph, 1957; Brown, 1972), I believe that a much more complete mating study of the pipefish is warranted.

This study is the first to use the gulf pipefish, *Syngnathus scovelli*, as an experimental animal in a laboratory setting. As such, it establishes guidelines for the husbandry of the species. It was designed to test whether the gulf pipefish is indeed a polyandrous species, or if its mating system changes according to variations in the OSR. The study sets out to measure the rates of reproduction in males and females, to observe the roles of armaments in intra-sexual competition, and of ornaments in courtship

behavior. This study presents a quantitative analysis of these inquiries. It is based on

daily observations, photographs, and measurements of the gulf pipefish maintained under

standardized conditions.

Methods

Study species

     Syngnathus scovelli is a common pipefish found in shallow grassbeds of the

western Atlantic, from as far north as Georgia (Targett, 1984) to southeastern Brazil

(Gasparini & Teixeira, 1999), and the Gulf of Mexico (Díaz-Ruiz *et al*., 2000) (For more

detailed information, see Introduction). For this study, *Syngnathus scovelli* pipefish were

special-ordered from Aquatic Control (Tampa, Florida). They were collected by running

a small seine across grass flats near Fort DeSoto in Tampa Bay, Florida.

Conditions

     Upon arrival, pipefish were dipped in a 5 ppt solution of Formalin•3 (Kordon

Division of Novalek, Inc.) for one minute to kill parasites and other unwanted organisms.

Only sexually mature fish were used in the experiment. Sexual maturity was determined

in males by the presence of a completely developed brood pouch, and in females by the

presence of silver lateral marks along their trunk rings, which become apparent during the

maturation of the ova (Joseph, 1957; Brown, 1972). Fish were placed into individual 10-

gallon tanks under conditions similar to those in their natural environment (McEachran &

Fechhelm, 1998). The water was maintained at 29 ppt salinity and at an average

temperature of 76°F. Saltwater was made by combining Coralife salt (Energy Savers

Unlimited, Inc) with distilled water. StressZyme (Aquarium Pharmaceuticals) was added

to each tank to promote and help maintain the biological filter in amounts of 10 mL per

tank. Saltwater CopperSafe (Mardel Laboratories, Inc.) was also to added each tank to

prevent growth of unwanted parasites, in amounts of 12.5 mL per tank. The tanks

contained a thin layer of light-colored gravel, a sponge filter with aeration, and two green

plastic plants. Fluorescent bulbs were used to light the tanks, avoiding strong light, as this may disturb pipefish and cause them to bury themselves on the gravel (pers. obs).

Pipefish had a 14-hour light period per day, including 30 minutes of dim light to simulate dawn at the beginning of the day, and 30 minutes of dim light to simulate dusk at the end of the day. Partial water changes of 30% were conducted weekly, as well as siphoning of the gravel and removal of algae from the sides of the tanks. Checks for parasites and proper levels of ammonia, pH, nitrates, and nitrites occurred biweekly. Because tanks were placed next to each other, cardboard was placed between the tanks to prevent any visual contact between the fish. Fish were kept in the tanks for at least one week prior to beginning the experiment to allow them to acclimate to their new conditions and to standardize their reproductive status. Pregnant males were allowed to give birth before beginning the experiment. Females were given also given time to mature a new set of eggs before beginning the experiments. Two separate trials with two different sets of pipefish were conducted. The first trial took place from October 31 until December 21, 2000. The second trial took place from January 17 until March 14, 2001. The details of the feeding regimes differed between the two trials.

Feeding

In the first trial, daily feeding consisted of recently-hatched *Artemia* brine shrimp (Summit Aquaculture Technologies LC) and frozen mysid shrimp (Kyonis), which were thawed before feeding. Mysids were hand-fed because the pipefish would not eat non-moving food. I used small droppers to pick up individual mysids, and moved them in front of each pipefish until the pipefish ate the mysid or swam away. If the pipefish ate the first mysid, two more additional mysids were fed. Fish were also supplemented with

adult gammarids once weekly. This feeding regimen did not meet the nutritional needs of the pipefish and was changed in the second trial (refer to Disease section below).

In the second trial, in addition to feeding them recently-hatched *Artemia* brine shrimp and frozen mysids, pipefish were fed adult *Artemia*. The adult Artemia were ordered from Exotic Fish & Pet World (Hadley, MA) and arrived weekly. Adult *Artemia* and frozen mysids were placed for at least 30 minutes in a solution of Vita-Chem (Boyd Enterprises), which is a mixture of natural lipids and amino acids, and Roti-Rich (Pentair Aquaculture, S.A.), which is a mixture of microalgae, food-grade yeast, vitamins, minerals, and nutrients. This was done to enrich the nutritional contents of the adult Artemia and mysids. In addition, fish were supplemented with adult gammarids once weekly.

Disease

Due to the lack of preexisting information about the maintenance of the gulf pipefish in a laboratory setting, I first subjected the fish to conditions that did not satisfy their needs. During the first trial, many pipefish were infected by parasites and later perished as a result of the infection, especially from nematode worms that were visible under a microscope on dead specimens. Nematode infestation has been a problem for captive cultures of other Syngnathid species (Masonjones, 1997) and treatment techniques were modified for use with pipefish. Parasites were also present at very high concentrations in tank water. Several antiparasitic products were used in an attempt to cure and prevent future infections. I first used Formalin•3 by adding 2 mL/gallon to tanks where deaths had occurred due to parasitic infections. I also added 1 mL/gallon of Formalin•3 to all other tanks as a preventive measure. Fish were still becoming infected and dying after the addition of Formalin•3. Because Formalin•3 did not seem to be

working, I changed products and tried using Clout (Aquarium Products). I dissolved one Clout tablet per tank in a cup of water, and added this to all tanks every day for three consecutive days. Microscopic checks of the water at the end of treatment still showed a large presence of parasites, and pipefish were still dying. I increased the concentration of Clout in each tank to 1.5 tablets per tank, but this did not make any noticeable difference. I then conducted small tests in individual tanks with other antiparasitic products, but none seemed to work. In the end, 18 out of a total of 34 pipefish perished, with the great majority having visible nematodes and other parasites upon microscopic inspection of dead samples. Some of the other microorganisms could have been decomposers, but visual inspection of sick individuals indicated a high density of nematodes. Due to this high morbidity and mortality, the experiment was aborted early in the first trial, and final measurements and pictures were not obtained.

Having subjected the pipefish and parasites to all the antiparasitic solutions available and having failed at preventing or curing the infections, I hypothesized that something else must be involved in the spread of infections. There must have been a factor in the pipefish's living conditions that was making them more prone to infections. Having noticed that the pipefish were always aggressive eaters and always seemed hungry, I thought that perhaps a lack of food was responsible for the infections. Perhaps their poor diet weakened their immune systems and made them more susceptible to infections. To test this I decided to increase the variety and quantity of food that was given to the pipefish for the second trial of the experiments. To their diet I added adult brine shrimp, and enriched their nutritional content by soaking them in a solution of nutrients and vitamins (as described in the Feeding section above). None of the experimental fish in the second trial were ever infected by nematodes. Microorganisms

(with the exception of nematodes) were present in very low concentrations of water samples from the tanks, but these were most likely part of the natural tank fauna. It seems that the pipefish are able to coexist with parasites as long as their immune systems are working properly. Any imbalance in this equilibrium, due to poor nutrition and a weakened immune system, may cause the pipefish to become prone to infection. Fish from the second trial were maintained alive and healthy for more than four months (at the time of this writing) under the new feeding regimen, and none have died.

The only parasitic problem encountered in the second trial was due to the water tick *Arugulus*. *Arugulus* were spotted attached to the surface of three pipefish shortly after they arrived. The ticks ranged in size from 3-6 mm. *Arugulus* were removed with tweezers, first immersing these in alcohol, and using them to pull the tick off the fish, careful not to rip the tick in half. All fish that had ticks removed recovered completely. It was essential to check each new fish for visible parasites and remove these if found. If the tick is not removed, fish will gradually lose all color and contrast, silver markings on side will disappear, and it will die within a few days (pers. obs).

Experimental design

The purpose of this study was to observe and describe the interactions in the gulf pipefish between members of the same sex and between members of opposite sex. I was also interested in the role of female ornaments in intrasexual and intersexual competition. In addition, I wanted to quantify the relationship between the operational sex ratio with the mating system of the gulf pipefish, and to compare the rates of reproduction of males and females. To do this, I designed and subjected the fish to four different conditions as described below (Figure 4).

Isolation

Sexually mature pipefish were isolated in individual tanks for one week to get a measure of their baseline contrast and brightness levels without having them be influenced by the presence of other pipefish (Figure 4). In the first trial there were 17 females and 9 males, eight of which had arrived pregnant and given birth during the week of acclimation (showing that they were sexually mature). In the second trial there were 13 females and 11 males. Only two of these males arrived pregnant (although they all seemed to contain fully developed pouches). Mean weights and sizes of fish, along with their ranges, are shown in Table 2. At the end of one week, fish were photographed, sized, and weighed. Pipefish were placed in a small plastic container with a small amount of water, and were then photographed from above to capture their natural contrast and brightness (refer to Image Analysis below for descriptions of how colors and measurements were obtained from the pictures). To size the fish, they were placed on a narrow, shallow plastic container filled with water, which contained a small ruler glued to the bottom as a reference for size. They were then also photographed from above. To weigh the pipefish, they were quickly placed onto a KimWipe and blotted dry to remove any excess water, and were then transferred to a container filled with water, which was placed on a scale. Weights were measured to the nearest 0.1 g.
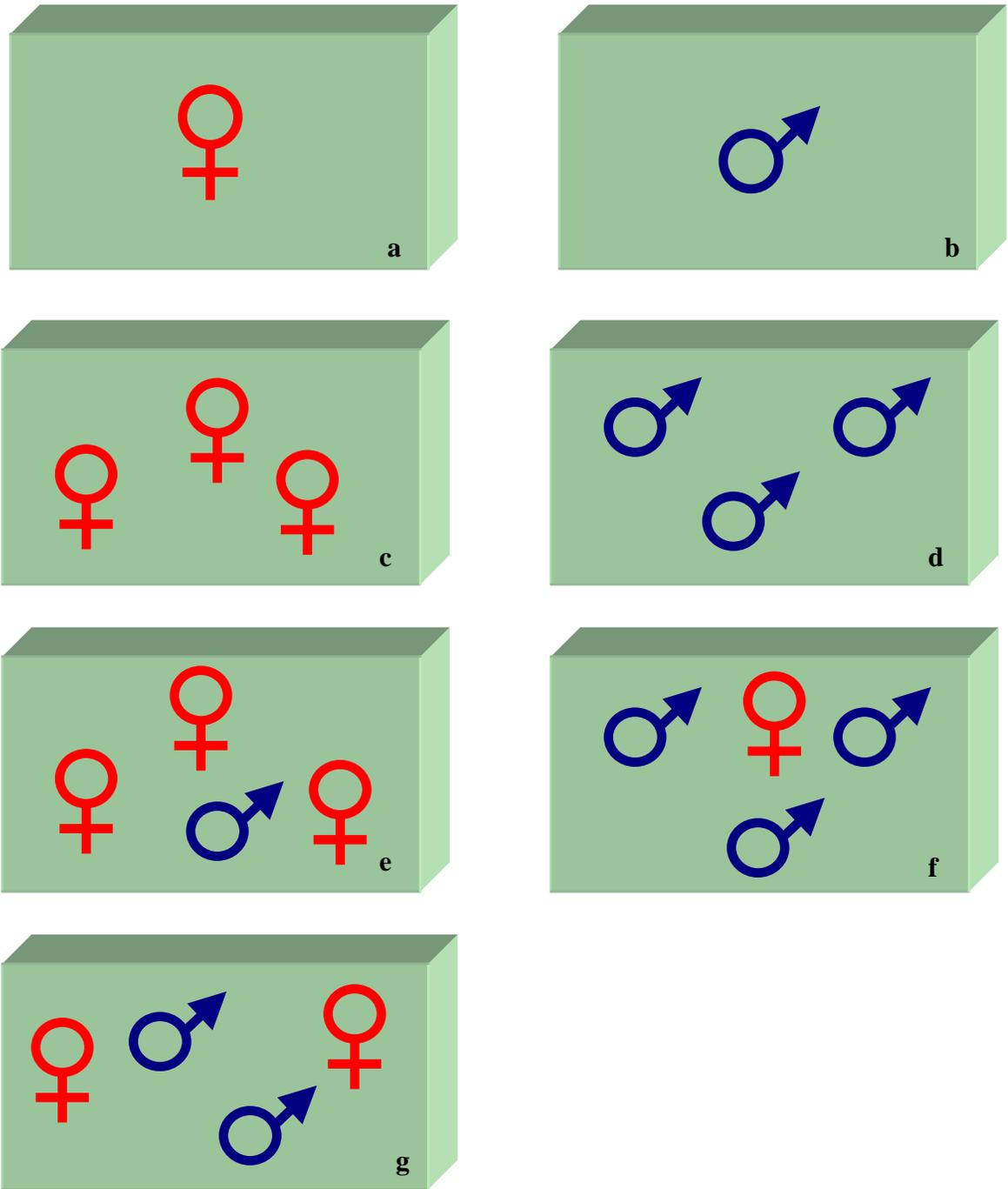
Figure 4. Overview of the different conditions in the present experiment. Female (a) and male (b) in isolation. Same-sex female (c) and male (d) tanks. Female-biased (e) and male-biased (f) tanks. Even-ratio control tank (g).

Table 2. Mean lengths and weights (and ranges) of pipefish in first and second trials.

Condition	Mean length (range)	Mean weight (range)
First Trial		
Females (n=17)	115.6 mm (96-135)	1.17 g (0.7-2.3)
Males (n=9)	106.7 mm (94-124)	0.89 g (0.5-1.5)
Second Trial		
Females (n=13)	118.1 mm (100-138)	1.20 g (0.6-2.5)
Males (n=11)	110.4 mm (99-124)	1.07 g (0.7-1.7)

Same-sex tanks

Following the week of isolation, fish were placed into same-sex tanks to observe behavioral interactions and to get a measure of the change in baseline contrast and brightness at the end of one week (Figure 4). Three pipefish of similar weights were placed into each tank, with average differences in weights and lengths as seen in Table 3. I attempted to match the weights as closely as possible, given the small sample size of my population, to promote competition between individuals. It has been shown in other species that no competitive behaviors are displayed when one of the individuals has a large size advantage (Riechert, 1977), and that the most competitive behaviors occur when the individuals are very closely matched (Austad, 1983). In the first trial five female tanks and three male tanks were constructed. In the second trial, four female tanks and three male tanks were constructed. The tanks were videotaped for the first three mornings for a period of 1.5 hours. At the end of one week, all fish were photographed again as described above to get a measure of their baseline contrast and brightness.

Table 3. Differences in weights and lengths (and SE) of same-sex tanks and control tanks in first and second trials.

Condition	Mean difference in weights (SE)	Mean difference in lengths (SE)
First Trial		
Female tanks (n=5)	18.18% (4.45)	16.08% (9.54)
Male tanks (n=3)	17.55% (3.54)	21.05% (10.53)
Control tank (n=1)	48.78%	30.24%
Second Trial		
Female tanks (n=4)	9.92% (1.85)	21.67% (11.78)
Male tanks (n=3)	11.91% (0.70)	22.71% (3.11)
Control tank (n=1)	21.62%	19.01%

Biased sex ratio tanks

Following the week of the same-sex tank treatment, a member of the opposite sex was introduced into each same-sex tank, of a size and weight within the range of the other pipefish in the tank (Figure 4). For females placed in the all-male tanks, I wanted to observe how many males she could impregnate during the course of the experiment. For males placed in all-female tanks, I wanted to observe if the male would mate with more than one female at a time. In the first trial this individual had been isolated before its introduction. In the second trial, this individual had previously been in a same-sex tank. The first trial had four female-biased tanks and two male-biased tanks. The second trial had three female-biased tanks and two male-biased tanks. The tanks were videotaped for the first three mornings for a period of 1.5 hours. Male pipefish were checked daily for the presence of embryos in the pouch. The dates of mating and birth, and the number of offspring were recorded. This treatment lasted for three weeks, after which all fish were weighed, sized, and photographed as described above. Pregnant

males at the end of the three weeks were allowed to give birth before being measured and photographed.

Control tanks

Tanks containing two males and two females were made to observe mating behaviors with even sex ratios (Figure 4). The pipefish used for this condition had been previously isolated. Only one control tank was set up in both first and second trials due to a short supply of fish. Average weights and lengths of all four fish can be seen in Table 2. They were weighed, sized, and photographed before the treatment in both trials, and were again measured at the end of the treatment in only the second trial. Tanks were videotaped for the first three mornings for a period of 1.5 hours. Male pipefish were checked daily for the presence of eggs in the pouch. This treatment lasted two weeks.

Image analyses

To obtain color photographs of the pipefish, they were first videotaped, and the video was then captured from a VCR to a PC by using an Iomega Buz device (Iomega Corporation). Individual images were then extracted from the video using VideoWave software (MGI). The fish were sized by using Scion Image software (Scion Corporation) and setting the scale in the program to match the ruler in the photograph. Pipefish were measured in millimeters from the tip of the snout to the end of the caudal fin using short lines that ran through the middle of their body, to the nearest 1mm (Figure 5).

Adobe Photoshop 5.5 (Adobe Systems Inc.) was used to obtain information about the brightness and contrast of each fish. I selected a square segment on the upper surface of the pipefish that began behind the gills and ended with the dorsal fin,

Figure 5. Pipefish in measuring container. Red line shows how I measured the length of the fish.

and which had the width of the pipefish (Figure 6). This short segment was representative of the overall brightness and contrast values of the pipefish. Using the Histogram feature of the software, I was able to obtain a value of average luminosity of the selected segment, which is essentially a measure of the brightness of the fish. However, because each picture varied slightly in overall brightness, I had to standardize the values and correct for the differing brightnesses of the pictures. I accomplished this by dividing the value of the brightness of a segment by the brightness of the entire picture, divided by the average brightness of all the pictures [Brightness of fish / (brightness of picture / average brightness of all pictures)]. This corrected value will simply be called "brightness" hereon.

I was also able to obtain a value for the contrast levels of each fish. With the same square segment as described above (Figure 6), a value for the standard deviation of luminosity was calculated using the Histogram feature of Photoshop. This value

represents a measure of the difference in brightness of the pixels in the segment, which can be equated with contrast. Because contrast does not vary with the brightness of the picture, these values did not need to be corrected. The standard deviation of luminosity will be simply referred to as "contrast."

Figure 6. Pipefish segment selected for analysis of contrast and brightness.

Behavioral analyses

All behaviors were described and quantified from 49 videotapes (over 100 hours of footage) that I recorded during the first and second trials of the experiment. Five distinct female competitive behaviors were observed and counted, all of which were displayed to other females as part of intrasexual competition, or to males as part of courtship. These behaviors were never displayed while in isolation. The first characteristic is a change in standard color pattern to a high-contrast, zebra-like pattern, here called the *ornament*. This pattern consists of alternating light and dark colored bands, which run vertically across the entire length of the body. The second behavior is

extreme trunk flexure, which I have called *posing*. This behavior consists of bending the upper body outwards while at the same time expanding the dorsal fin. The third competitive behavior is *rising*, where two or more females rise upwards in the water column. The fourth behavior is *approaching* another female aggressively. The final competitive behavior is *quivering*, where females violently shake their bodies from side to side for less than half a second. Males presented three of the five behaviors that females displayed, performing *quivering*, *approaching*, and *rising*. These behaviors were counted and tallied. Behaviors from the first and second trials were pooled together, as there were no noticeable differences between them.

Statistical analyses

Statistical analyses were performed using two different statistical packages: Minitab 13 (Minitab Inc.) and SigmaStat 2.03 (SPSS, Inc.). I performed independent t-tests to compare the difference of the means between two independent groups or populations. This included comparing measurements between the sexes, or between populations. I ran paired t-tests to compare the means between related samples, where assumption of normality and equal variances were met. This was done especially for before-and-after measurements of size, weight, brightness, and contrast.

To analyze competitive behaviors, I first conducted a Pearson correlation analysis to determine whether behaviors are independent from one another. I found that there were significant correlations in 8 out of a possible of 10 correlations, which suggested that they are not independent. Therefore, I pooled together female behaviors and from those I ran a two-way ANOVA using tanks and females as fixed effects to see whether individuals differed significantly in their competitive behavior involvement.

All statistical analyses were two-tailed, except for analyses of female competitive behaviors. These were one-tailed because I expected the presence of a dominance hierarchy, which would make less dominant females perform fewer behaviors than more dominant females.

Results

Measurements

There were no significant differences between the populations of pipefish used in the first and second trials in regards to weight, length, brightness, or contrast. There were no significant differences within the different conditions in each trial.

Isolation

First trial

After one week of isolation, I weighed, sized, and photographed all fish (Table 4). Mean female size tended to be larger than male size, although this difference was not significant (Two-sample t-test: t=1.73, df=16, p=0.103). Females showed a trend of being heavier than males, which was almost significant (Two-sample t-test: t=1.93, df=20, p=0.068). There were no differences in brightness or contrast between the sexes.

Second trial

Female length was larger than male length (Two-sample t-test: t=2.14, df=21, p=0.044). Otherwise, there were no differences between the sexes in weight, contrast, or brightness (Table 4).

Same sex tanks

First trial

Fish were photographed after one week of being with two other fish of the same sex and of similar size. There were no significant differences between brightness and

Table 4. Measurements (and SE) of fish after isolation, same-sex tanks, and biased sex ratio tanks in both trials. Pregnant males were allowed to give birth before being photographed and measured.
* Indicates statistically significant changes from previous measurements

Condition	After Isolation (SE)	**After Same Sex (SE)**	**After Opposite Sex (SE)**
First Trial			
Females (n=17)			
Mean weight	1.17 g (0.20)		
Mean length	115.6 mm (3.1)		
Mean brightness	75.08 (6.97)	75.16 (5.20)	
Mean contrast	11.40 (1.01)	12.33 (1.01)	
Males (n=9)			
Mean weight	0.89 g (0.09)		
Mean length	106.7 mm (4.2)		
Mean brightness	72.77 (7.22)	62.55 (2.54)	
Mean contrast	13.68 (2.56)	12.65 (1.72)	
Second Trial			
Females (n=13)			
Mean weight	1.25g (0.14)		1.43g (0.11) *
Mean length	118.1 mm (2.3)		125.0 mm (1.6) *
Mean brightness	71.19 (7.51)	84.44 (6.13) *	79.41 (5.24)
Mean contrast	12.87 (1.77)	13.54 (1.34)	12.90 (1.00)
Males (n=11)			
Mean weight	1.07 g (0.10)		1.24 g (0.11) *
Mean length	110.4 mm (2.8)		119.0 mm (3.5) *
Mean brightness	75.80 (8.40)	78.5 (11.0)	63.30 (9.8) *
Mean contrast	9.41 (0.67)	15.72 (1.62) *	10.24 (1.29)*

contrast in either sex (Table 4). Percentage change in brightness and contrast are shown on Table 5.

Second trial

Female average contrast was not significantly different from previous measurements (Table 4). However, females in this group did experience a significant increase in brightness from isolation measurements (Paired t-test: t = 2.37, n=12, p = 0.037). Males significantly increased in contrast from isolation measurements (Paired t-

Table 5. Percentage of males and females that showed increases in contrast and brightness from isolation to same-sex tanks, in first and second trials.

* Indicates statistically significant increases.

Condition	Increase in contrast	Increase in brightness
First Trial		
Females (n=14)	50%	43%
Males (n=8)	38%	25%
Second Trial		
Females (n=12)	75%	75% *
Males (n=7)	86% *	63%

test: t= 5.61, n=7, p= 0.001), but did not change in brightness. Percentage of

change in brightness and contrast are shown on Table 5.

Biased sex ratio tanks

First trial

Due to morbidity and mortality because of infection, the experiment was aborted

early, and no pictures of the fish were taken at the end of this condition.

Second trial

At the end of three weeks in the biased sex ratio tanks, females had significantly

increased in weight from initial measurements (Paired t-test: t= 2.52, n=12, p= 0.028;

Table 4). They also experienced a significant increase in length (Paired t-test: t= 4.33,

n=10, p=0.002). Their brightness or contrast did not change significantly from

measurements in same-sex tanks. Males also experienced significant weight gain from

initial measurements (Paired t-test: t = 4.33, n=10, p=0.002) as well as significant

increases in length (Paired t-test: t = 2.69, n=10, p = 0.025; Table 4). They also had a

significant decrease in their brightness from after the same-sex condition (Paired t-test: t

= -2.82, n=10, p = 0.031) and had a significant decrease in contrast as well (Paired t-test: t= -5.20, n=10, p=0.002).

Control tanks

First Treatment

Due do morbidity and disease, I did not perform final measurements on these fish.

Second Treatment

Male and female fish showed no significant increase in contrast, brightness, weight, or size during the two-week period of the control treatment.

Differences between treatments

There were no significant differences in contrast, brightness, length, or weight across the different treatments for males and females (Table 6). However, females in the excess female treatment seemed to be of higher contrast than those in the excess male treatments (Two-sample t-test: t = 1.91, df=8, p = 0.092).

Behavior

Isolation

I did not notice any noteworthy behavior in isolated females other than feeding and swimming. Males, however, had a peculiar habit of repeatedly poking at the sides of the tank with their snouts, especially after dawn. On several times I observed this poking behavior continued for over an hour. This same behavior was present in same-sex tanks, biased sex ratio tanks, as well as in the controls.

Table 6. Final measurements (and SD) after biased sex ratio tanks, by condition, in the second treatment. Pregnant males were allowed to give birth before being photographed and measured.

Condition	Mean contrast (SD)	Mean brightness (SD)	Mean weight (SD)	Mean length (SD)
Females				
Excess female (n=9)	13.80 (3.38)	76.48 (18.50)	1.48 (0.42)	125.5 mm (6.58)
Excess male (n=2)	11.63 (0.23)	80.44 (14.63)	1.50 (0.00)	123.6 mm (1.11)
Control (n=2)	7.86 (0.67)	106.14 (3.42)	0.95 (0.07)	116.3 mm (10.10)
Males				
Excess female (n=3)	11.67 (4.62)	85.74 (32.21)	1.23 (0.23)	119.2 mm (13.50)
Excess male (n=6)	11.49 (3.90)	60.74 (22.77)	1.31 (0.40)	121.0 mm (9.16)
Control (n=2)	8.70 (4.29)	65.49 (41.46)	0.90 (0.14)	107.8 mm (7.99)

Same sex competitive behavior

Females

Intrasexual competition took place everyday in tanks with more than one mature

female, regardless if there were males present or if these were pregnant. These behaviors

took place shortly after dawn, and usually lasted no more than 30 minutes. Female gulf

pipefish showed five distinct behaviors during intrasexual competition. The first

characteristic, which marked the beginning of competitive or courtship interactions, was

the change in standard color pattern to a high-contrast, zebra-like pattern which I will

refer to as the *ornament* (Figure 7). This pattern consisted of alternating light and dark

colored bands, which ran vertically across the entire length of the body. Generally, only

one female developed this zebra pattern, although I noticed one case when two females
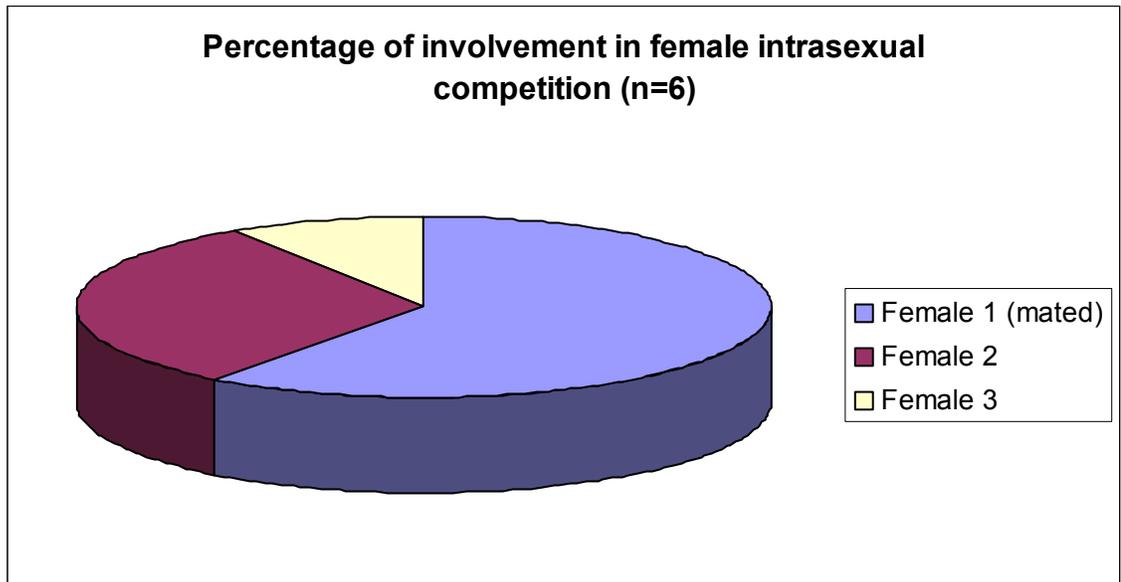
Figure 7. Female posing with ornament. Notice the zebra-pattern, extended dorsal fin, and extreme trunk flexure.

developed the pattern at the same time. The second behavior evident in intrasexual competition was the extreme trunk flexure, which I have called "*posing*" (Figure 7). This posture consisted of an arched back, which accentuated the chest as it was directed forwards. In addition, the dorsal fin was always extended during posing. The third competitive behavior was *rising*, where two or more females rose upwards in the water column. Rising was generally performed while posing. The fourth behavior was *approaching* another female aggressively, generally followed by posing or rising. The final competitive behavior was *quivering*, where females violently shake their bodies from side to side for less than half a second. Quivering was only seen performed by the ornamented female.

Female competitive behavior in same-sex tanks was an excellent predictor of mating success. I was able to observe which female mated first in two female-biased tanks. Using the videotapes of those tanks during the same-sex treatment, I tallied the number of each behavior that each female performed. There was a significant difference between the numbers of competitive interactions that each female performed (Two-way ANOVA with females and tanks as fixed effects, df=5, p=0.028). The dominant female performed significantly more behaviors than did the second-dominant (One-tailed Tukey Simultaneous Tests, p=0.035) and the third-dominant female (One-tailed Tukey Simultaneous Tests, p=0.013). However, the second- and third-dominant females did not differ from each other. Most importantly, the most dominant female (the female that was involved in the greatest number of behaviors) was the first to mate (Figure 8).

Males

Percentage of involvement in female intrasexual competition (n=6)

Legend:
- Female 1 (mated)
- Female 2
- Female 3

Figure 8. Female involvement in intrasexual competitive behavior (posing, rising, gaining of the ornament, approaching, and quivering). Notice that the female involved in the greatest number of behaviors was the first to mate. Data was combined from two tanks observed over a period of three days.

Male competitive behavior in same-sex tanks was also an excellent predictor of mating success. However, because I only observed which male mated first in one male-biased tank, I could not conduct statistical analyses of these behaviors. In any case, I observed that the male that quivered the most was also the male that rose the most and that approached other males the most. More importantly, it was this male that mated first.

Courtship Behavior

During courtship, males and females exhibited all of the competitive behaviors as described above, except that they were directed at each other. These courtship behaviors took place shortly after a new fish was introduced into a tank, or during the first hour of

Figure 9. Mating sequence (a-g) of ornamented female (dark with zebra pattern) and male.

dawn on subsequent days. Successful courtship culminated in the sexual embrace, here called *entwining*, where the female wrapped herself around the male in a sexual embrace, inserted her ovipositor into the male's pouch, and delivered the eggs (Figure 9). Following the entwining, the male rested on his pouch on the bottom of the tank, with both his head and tail raised, making a wide u-shape with his body. He was often seen shaking slightly from side to side while the brood pouch scraped on the bottom, presumably as he settled the eggs in his pouch.

The delay between introduction of the fish and first mating varied from 0 to 9 days (n=9, mean=5, SE=1.4). However, some of the matings observed were not successful, as no eggs were evident in the male pouch after the sexual embrace. This is not the first time that dry matings have been observed in this species (Joseph, 1957), although I do not know if this occurs frequently in the pipefish's natural environment or if it is a result of laboratory conditions.

From videotapes that I recorded of biased sex tanks, I counted the number of times that an individual quivered to a member of the opposite sex. I found that females quivered significantly more often than did males (Paired t-test: $t = 3.23$, n=20, p= 0.004). While females quivered more often than did males, I found that the proportion of female-to-male quivers increased in female-biased tanks, and decreased in male-biased tanks (Two-sample t-test: $t = 2.78$, n=20, p = 0.027).

Control tanks

No competitive or courtship behaviors were evident in the control tanks of either trial. Females did not pose, rise, approach, quiver, or gain a zebra pattern, and males did not quiver, rise, or approach females or each other.

Rates of reproduction

Males and females were able to mate within a very short period of time after introduction, as soon as 12 minutes. Gestation length was found to be between 15 to 16 days at 76°F (variation depending on what time of the day the male gave birth and when I noticed the offspring). Females were able to produce more eggs than males could brood during one brood cycle. In the excess male tanks of the second trial, both females were able to fill the three males before the first one gave birth. In these tanks it took the females exactly eight days (n=2) to fill all three males. With a gestation period of 16 days, this means that at this rate a female can completely fill six male pouches in the time it takes one male to brood his embryos.

Number of mates

I found evidence that males frequently (n=1, out of 2) mated with more than one female when the OSR is highly biased towards females, although I did not witness a mating of one male with more than one female. During the second trial, one out of the two males in excess female tanks showed evidence of having mated more than once. A few days after the mating I first noticed that his pouch appeared to be divided into three separate bunches. Several days later I noticed that the eggs in his pouch seemed to be at different stages of development. He then gave birth to a few offspring on one day, and delivered the rest of his pouch on the following day. I did not notice any of these peculiarities in males that only had access to one female (those in the excess male tanks).

I also found evidence on three separate occasions of males having mated with multiple females during my observations prior to the beginning of the experiment. I observed three different males that seemed to have mated with more than one female (out

of a total of 12 pregnant males that I examined). The first male had a horizontal space in his pouch, separating it into two bunches. The second male gave birth to half of his pouch, after which he died. Examining the specimen under the microscope, I opened his brood pouch and found that the rest of the embryos were in a much earlier stage of development. Finally, I observed one male give birth to four young, and deliver the rest of his pouch on the following day.

Additional observations

In the second trial, I saw two males (out of eight males that became pregnant) had drop their embryos during the early stages of their pregnancy. Their pouches were filled on the following day. I also noticed that both males and females cannibalized their offspring as soon as they were born, so it was necessary to remove the young fry from the adult tanks as soon as possible.

Discussion

This study was successful in many aspects. First, I established the requirements for the proper maintenance and breeding of the gulf pipefish, *Syngnathus scovelli*, in a laboratory setting. Second, I created a method of quantifying the degree to which female pipefish exhibit their ornaments, and determined whether these ornaments were honest indicators of quality. Third, I observed and described same-sex competitive behavior, which, although it has been briefly mentioned in articles, has never been described in any species of pipefish. Fourth, I determined the mating system of the gulf pipefish, and discovered several factors that may affect it. Finally, I concluded whether sex role reversal is present in the gulf pipefish by comparing the relative reproductive rates of males and females.

Husbandry

The maintenance of the gulf pipefish in saltwater aquaria proved to be a challenge (see Methods). The main difficulty was providing enough live food for the pipefish, which was also a problem encountered by Whately (1969) in his research. Poor nutrition made pipefish susceptible to infections that were not curable with a myriad of antiparisitic products. The addition to their diet of adult brine shrimp soaked in a solution of vitamins and nutrients made a huge difference in morbidity and mortality, as no pipefish died during the second trial. Furthermore, the careful monitoring of temperature, salinity, and water chemistry, along with weekly water changes and siphoning of the gravel, proved to be essential in providing a healthy environment for the pipefish.

Ornaments and armaments

Visual cues have been found to be crucial in determining dominance and mate choice in pipefishes. For example, in female *N. ophidion* blue coloration is a status badge that works to repel females as well as attract males (Rosenqvist, 1990). In *S. typhle*, males avoid mating with females that have many black spots, which are caused by a parasitic trematode that also reduces egg production in females (Rosenqvist & Johansson, 1995). However, males also avoided females with artificially produced tattoo spots, showing that it is the visual cue more than anything else that attracts or repels mates. These findings stress the importance of studying visual signals in pipefish, as they provide reliable information of quality.

Females

Female gulf pipefish used the contrast on their bodies as armaments in intrasexual competition and as ornaments during courtship. Traits such as these of dual utility have been documented in several species (Berglund *et al.* 1996). Female gulf pipefish possess baseline contrast levels that were greatly amplified during competitive and courtship behaviors. They obtained a zebra-like pattern (the ornament) that consisted of light and dark bands, which ran vertically across their entire bodies. I measured their baseline contrast as a means of determining potential increases in contrast. It has been shown in a different species of pipefish that baseline contrast is a good predictor of the extent to which the ornament will be shown (Berglund *et al.*, 1997).

Females in the first treatment, which received very little food, were unable to brighten or increase in their baseline contrast when placed in same-sex tanks. In addition, females that were sick lost all baseline contrast. On the other hand, females in the second

treatment, which received proper amounts of food, were able to have significant increases in baseline brightness when placed in same sex tanks, and three-fourths of all females increased in contrast as well. Consequently, both of these displays were energetically costly to produce because starved or sick individuals could not display these signals. Furthermore, it has been shown that females with higher contrast contain more mature eggs than those with a dull contrast (Berglund *et al.* 1997), thus supporting my hypothesis that contrast and brightness are reliable signs of fitness.

However, a previous study (Berglund *et al.*, 1997) has suggested that the high contrast ornament is not energetically costly to produce because females in a low food regimen were able to obtain the same degree of ornamentation as those in a high food diet. In that study, however, Berglund and others assessed contrast while the ornament was expressed, while in the present study I measured baseline contrast. As stated above, levels of baseline contrast are good predictors of the degree to which females will develop the ornament, so there is no reason why our results should disagree (assuming that *S. scovelli* and *S. typhle* show similar characteristics). Their observations might be explained by the fact that they measured ornamentation levels when the females where in contact with males, while I measured baseline contrast while they were in same-sex tanks. In the presence of males, although the ornament might have been costly to produce, starved females had no choice but to produce it since females that do not produce the ornament are not able to excite the male to mate (Bernet *et al*., 1998). Instead, I measured contrast levels when females had the option of increasing in contrast without directly affecting their future reproductive success. Furthermore, I believe it would have been very difficult for Berglund and others (1997) to assess small differences in contrast because they were making their measurements by eye instead of relying on

imaging software like I did. For these reasons, I believe that their conclusions may have been imprecise. In my study, the fact that starved females did not increase in contrast as much as those that were fed appropriate amounts suggests that the ornament in the gulf pipefish was energetically expensive to produce.

At the end of the experiment, females that were in female-biased tanks showed slightly higher baseline contrast that did females in male-biased tanks. These results support the idea that the baseline contrast is a good measure of competition, because females that faced more competition had higher levels of baseline contrast. In a study on *S. typhle*, Berglund *et al.* (1997) showed that grouped females maintained their baseline contrast levels throughout the course of their experiment, whereas females in isolation decreased theirs. In addition, it has been shown that females are more likely to display the ornament under female-female competition than otherwise, probably to intimidate rivals (Bernet *et al.*, 1998). Accordingly, we can see that contrast levels in female pipefish are a phenotypically plastic trait that can be expressed under intrasexual competition. This supports my hypothesis that the contrast is costly, because condition-dependent expression of the ornament is essential for an honest trait, as females will vary the expression according to their fitness (Fitzpatrick, Berglund, & Rosenqvist, 1995). It is interesting that generally only one female in each of the female-biased tanks displayed the ornament. The ornament probably serves as a status badge that reflects the presence of a dominance hierarchy, where one female is able to develop the ornament while repressing its expression by other females. My results are similar to those found in *N. ophidion*, where one female develops a skin fold and blue nuptial coloration and is able to suppress skin fold and color development in other females (Rosenqvist, 1990). However, I noticed on one occasion that two females had developed the ornament,

though one to a lesser extent. The female with the fully developed ornament was seen repeatedly approaching and harassing the second one. Consequently, I believe the development of the zebra pattern has costs of social punishment, and can therefore be considered a reliable signal of dominance. Low-status females who display the ornament would be constantly put to the test during female-female competition. This, in addition to the energetic costs of the trait, allows the ornament to be an honest signal of quality. As we have seen, the ornament is not merely war paint, but instead it is a reliable status badge that is effectively used to repel rival females.

In addition to serving as a status badge, the zebra pattern in female gulf pipefish may serve as an amplifier of body size. As explained above (see Introduction), amplifiers do not magnify or augment a particular attribute, but they help in differentiating between small differences in individuals. The "handicaps" in these amplifiers do not have to be energetic costs, because the costs come from amplifying the defects and flaws as well as the virtues (Zahavi & Zahavi, 1997). A study by Berglund (2000) showed that it was easier to differentiate sizes between females when they adopted the high-contrast ornament than with their ordinary baseline contrast. Therefore, one of the reasons for the development of the zebra pattern might be to amplify their size so that other pipefish can discriminate between small differences in individuals. In addition, the fact that the stripes run vertically rather than lengthwise seems not to be mere coincidence. Zahavi suggested that vertical stripes in fish enhance the height of the body and make it easier to differentiate heights between fish, while lengthwise stripes accentuate the length of the fish. Since pipefish stripes run vertically, female pipefish are accentuating their trunk height. Accordingly, Berglund (2000) found that the stripes in *S. typhle* made it easier for

people to discriminate small differences in trunk heights between females. Furthermore, they showed that body height was more strongly correlated with fecundity than was body length. Therefore, amplifying this feature may be beneficial to large females by intimidating rivals as well as attracting mates.

In addition to the ornament, the posing might be yet another way for a female to emphasize the size of her trunk. Posing (which is commonly performed in conjunction with the ornament) seems to require extreme flexibility as well as strength to keep the rigid posture. By virtue of its difficulty, the signal can be considered honest because only fit females will be able to express it. I also noticed that ornamented females always posed to a greater extent than non-ornamented females, and therefore the degree of posing could be used to distinguish small differences in quality between females (Zahavi & Zahavi, 1997). This use of posture as an amplifier of physical condition has been shown to be common in other animals as well (Taylor *et al.*, 2000).

The ornament and the posing, like all reliable signals, showed differential costs depending on quality. It would be very costly for small females to display the zebra-pattern amplifier because they would only be making their small size more apparent. However, larger females that show the pattern while posing would benefit by amplifying their large size, thereby using the ornament as a status badge to intimidate rivals, while at the same time attracting mates. Males that use these signals in choosing their mates would benefit by being able to make quicker, more accurate decisions on a mate, and by choosing a more dominant, fitter female.

Males

Contrast and brightness in male pipefish also seem to be honest signals of fitness. Males in the low food regimen (first trial) did not increase in baseline contrast or in brightness when placed with other males. However, males in the high food regimen (second trial) increased in contrast significantly when placed with other males, and most of them increased in brightness as well. These results indicate that contrast and brightness may be costly signals and therefore honest indicators of fitness in both sexes.

Male contrast has never been previously studied in pipefishes. Although male pipefish have been shown to be choosier than females (Berglund & Rosenqvist 1993), little work has been done on mate choice by females (the more competitive sex). However, a recent study by Sandvik, Rosenqvist, and Berglund (2000) suggests that both sexes are able to discriminate between potential partners. Male pipefish as well as female pipefish were able to distinguish between better and worse partners. Moreover, the criteria pipefish used to choose their mates was not based on size but on some other attribute that Sandvik *et al.* did not address. Perhaps the pipefish were choosing their mates based on their levels of contrast and brightness. In that case, it would benefit males as well as females to increase in contrast and appear more attractive to the opposite sex. Since I have shown that contrast in pipefish is a costly signal because starved males and females could not produce it, both females and males would benefit by choosing high-contrast, brighter partners.

Males showed significant decreases in brightness and contrast at the end of the experiment. Because most of these males had been pregnant before I photographed them, this decrease in contrast and brightness might have been due to a purposeful reduction in visibility to predators when in the vulnerable pregnant state. Alternatively, the reduction

in brightness and contrast might have been a result of the high costs of brooding, which may not have allowed males to "waste" energy to increase their attractiveness to mates.

Intrasexual competition

Female

Female gulf pipefish showed five distinct behaviors during intrasexual competition: gaining of the *ornament* (high-contrast zebra pattern), *posing, rising, approaching*, and occasionally *quivering*. The female with the ornament was always the most dominant aggressor, as she performed the greatest number of behaviors in each tank. Her posing led other females to pose, and her rising led other females to rise as well.

Rising and posing were generally performed simultaneously, which made it easier to compare sizes between the females. Similar rising behaviors have been documented in field studies of *S. typhle*. Vincent, Berglund, and Ahnesjö (1995) reported that they saw groups of 2-4 females aligning themselves parallel to each other vertically, while moving up and down. The reason for this behavior, they explained, was that it allowed females to assess each other's size, since smaller females were usually the first to leave.

The approaching behavior, such as the one that was performed by dominant pipefish, is a very common way of threatening an opponent (Zahavi & Zahavi, 1997). It is a reliable signal because by approaching, the individual is opening itself to attack. According to Zahavi, approaching is an animal's way of saying: "C'mon big shot, lay one on me!"

Male

Males also showed aggression toward each other while in same-sex tanks by *approaching* other males, *rising* with them, and *quivering* at them. Furthermore, I observed in one tank that the most aggressive male (the one that took part in most of the competitive behaviors) was also the first to mate. I found it surprising that males were competing against each other, considering that in the gulf pipefish males are supposed to limit female reproductive success. However, changes in the OSR have been known to alter the intensity of competition of either sex without having to shift the sex roles (Kvarnemo & Ahnesjö, 1996). My results show that males still compete among themselves when the OSR is highly biased toward males. This supports the idea that males are not merely passive players in the game of life, but that they adjust to their surroundings and act accordingly, especially when they sense competition from other males.

Control tanks

I never noticed any competitive or courtship behavior between any of the pipefish in the control tanks. This might have been caused by the fact that the pipefish in these tanks were immature. These pipefish were slightly (but not significantly) smaller than the pipefish in the other conditions. However, it was difficult to determine whether it was the females, the males, or both, that were not sexually mature in these tanks. The smallest males that I have seen carry broods of embryos weighed between 0.7-0.8 g and measured as little as 90 mm, which lends support to previous findings of minimum male size for sexual maturity (Brown, 1972). Males that I placed in the control tanks were all larger than this. However, it was a little more difficult to determine what the minimum

size and weights are for mature females, since they do not have visible pregnancies. The smallest females that I saw give eggs to males were 1.5 g and over 120 mm in length. Females in the control tanks were smaller than these, with an average weight of 1.0 g and mean lengths of 116 mm. However, Brown (1972) reported that most females over 110 mm were sexually mature, so females in the control tanks should have been mature according to his findings. Nevertheless, since sexual maturity is obtained at different times in different populations of the gulf pipefish (Joseph, 1957), perhaps females in my population were not yet mature at that point, although they appeared to show silver markings have been suggested to correlate with sexual maturity (Joseph, 1957; Brown, 1972).

Courtship

It was interesting that both female and male courtship behaviors were identical to those behaviors exhibited during intrasexual competition, except that they were directed at each other. Behaviors of dual purpose such as these are known to occur as well in other animals, for example sexual calls in mammals and birds repel competitors as well as attract mates (reviewed in Berglund *et al*., 1996). Berglund and others argued that armaments and competitive behaviors probably first evolved to repel competitors, and that over time, potential mates benefited from these cues by being able to accurately assess quality in their potential mates. In female pipefish, the ornament was displayed during intrasexual competition as a sign of dominance. In addition, the ornament also seemed to attract mates, as males were observed to court and mate only with ornamented females. By using ornaments as a way of choosing potential partners, males would benefit by mating with more dominant, higher-quality females, because female

intrasexual competition ensures that the ornament is only displayed in the most dominant female.

During courtship, it was evident that females were the main aggressors. Quivering, which was by far the most violent and aggressive behavior exhibited by either sex, was performed significantly more often by females than by males. However, it is interesting to note that the ratio of female to male quivers was significantly higher in female-biased than in male-biased tanks. From my data, it was not possible to distinguish whether females increased quivers in female-biased tanks, or whether males increased quivers in male-biased tanks, but it is possible that they both responded accordingly. This finding supports previous observations in *S. typhle* that the sexes are able to vary their aggressiveness according to the sex ratios. Vincent *et al*. (1994) noticed in field studies that female-female competition for mates increased when the OSR was more female-biased. Females had increased their exposure under these conditions, as they performed meeting, rising, and displaying more often. Similarly, male pipefish have also been observed to alter their behavior in accordance with the OSR (Berglund, 1994, 1995). Under a female-bias, males preferred larger females, and copulated with them sooner and more often. However, under a male-bias, these preferences disappeared and males mated at random with respect to female size. These findings indicate that aggressiveness and choosiness in pipefishes, including in *S. scovelli*, are not a genetically fixed trait, but they are able to be varied in accordance with changing sex ratios.

Mating system of the gulf pipefish

This study provides supporting evidence that the mating system of the gulf pipefish, *Syngnathus scovelli*, is highly dependant on the operational sex ratio, the ratio of

sexually mature females to sexually mature males. In tanks where females had access to more than one male, they were able to deliver eggs to all of them within one male brooding cycle. Of the two tanks where males had access to more than one female, there was evidence that one of them had received eggs from all three females. Although I did not witness this male multiply mate, I noticed that his full pouch was divided into three sections, possibly due to three separate matings within a short period. I later noticed that these sections were at different stages of development, and when he finally gave birth he delivered half of his pouch on one day, and half on the following day (See Results for further evidence of multiple matings).

Interestingly, the male who mated with only one female in the female-biased tank had access to larger females than the male who possibly mated with three females. These results agree with Jones, Rosenqvist, Berglund, and Avise's (2000) findings in *S. typhle* that males with access to smaller females mate with more females (2.1) than males with access to larger females (1.3). To explain their findings, Jones and others suggested that males might intentionally accept fewer eggs from smaller females, hoping that his next mate would be larger. Since larger females produce larger eggs, which give rise to larger offspring (Berglund *et al.* 1986a), this would explain a male's preference for a larger female. Alternatively, they also suggested that it might be more difficult for a male to assess quality in smaller females. I believe that it is the absence of well-developed secondary sexual characteristics in smaller females that make it more difficult for males to assess female quality before mating.   This result confirms the importance of dimorphisms in assessing quality in mates. In addition, Jones *et al.*'s and my findings suggest that variation in the size of mates is an important factor that influences the mating system of a species. The abundance of large females in a population may decrease the

number of partners that males mate with, while an abundance of smaller females may increase polygyny.

This evidence lends support to the idea that mating systems are not stable, but that they fluctuate according to environmental conditions, as suggested by Darwin (1871) and Emlen and Oring (1977). I classify the gulf pipefish as polygynandrous, where both males and females can mate with several mates at once, if circumstances allow it. These findings contradict Jones and Avise's (1997) genetic study in which they argued that the gulf pipefish is polyandrous, and that only very rarely do males mate with more than one female. I believe their study was incomplete because they collected samples at a point when the sex ratio was biased toward males. Since the sex ratio is known to fluctuate dramatically during the year for the gulf pipefish (Joseph, 1957; Brown, 1972), it is imperative to study their mating system when the ratios are biased. Here I show that polygyny occurs in the gulf pipefish when the sex ratios are female-biased or in the presence of several smaller mates.

Mating systems and sexual dimorphisms

The gulf pipefish was able to mate within minutes of the introduction of an opposite-sexed member into the tank. In contrast, it takes three days for the dwarf seahorse, *H. zosterae*, to go through courtship and mating (Masonjones & Lewis, 1996). The difference in the time it takes for mating in each species may be accounted for by the time it takes each individual to assess the quality of its potential mate. Ornaments and other secondary sexual characteristics may aid in this process by providing an easy, quick, and reliable way to distinguish between individuals and pick the fittest mate among many in a population. Female gulf pipefish possess extensive secondary sexual

traits such as larger size, silver markings along their sides, the zebra-pattern ornament (which serves as a status badge), and the depth of the trunk. Therefore, it is probably easy for a male pipefish to accurately assess female quality by using these traits in choosing a mate. On the other hand, the dwarf seahorse is a monomorphic species in which neither sex has extensive secondary sexual traits. The courtship process may take longer in the dwarf seahorse because they do not have ornaments by which to accurately assess fitness. Instead, they assess quality through a series of complex courtship behaviors that include pumping, quivering, and pointing (Masonjones & Lewis, 1996). These behaviors allow females to compare small differences in quality between individuals. The lack of ornaments in seahorses makes it essential to have an extensive period courtship whereby assess a potential partner's quality, and it might result in the three-day courtship that we observe.

Female seahorses have been observed to follow courtship behaviors with a male, yet after three days fail to mate with him (Reece, N., pers. comm.). This supports the hypothesis that it is more difficult and takes longer to choose a high-quality mate in a species with no sexual dimorphisms. Furthermore, I believe their monogamous mating system may be a result of their sexual monomorphism. Monogamous pairs of seahorses skip the three-day courtship that is required by new pairs, and hence have higher rates of reproduction than if they were to change mates after every mating. If seahorses had secondary sexual traits by which to quickly assess mate quality, they would have the opportunity of being polygamous.

Similarly, other syngnathid species that have a monogamous mating systems lack strong sexual dimorphisms (reviewed in Vincent *et al.,* 1992). Pipefishes, such as the six *Corythoichthys* species and *F. tigris*, all exhibit monogamous mating systems and are

basically monomorphic. Vincent and others argue that monogamy in pipefishes and seahorses may have evolved from a combination of low rates of mate-encounter (due to low population densities and low mobility) and increased reproductive efficiencies (because monogamous pairs do not need to court after they have mated a first time). I believe that the lack of sexual dimorphisms in these species is an overlooked factor that may have forced the monogamous mating system. Because these species lack obvious indicators of quality (ornaments), a long courtship is required before mating to assess mate quality. In turn, these individuals have foregone the long courtship required to assess quality in new partners, by forming pair bonds that increase their rate of reproduction by being able to remate shortly after the male has given birth (Reece, N., pers. comm.).

Of course, it is difficult to speculate whether the dimorphisms gave rise to the short courtship in gulf pipefish, or whether the short courtship caused the dimorphisms to evolve. Similarly, it is hard to say whether the mating system in seahorses is such because of a lack of dimorphisms, or if it is the lack of dimorphisms that forced the mating system on the species. Either way, ornaments (or their absence) appropriately reflect the mating system and length of courtship in both species. These observations support Lein's (1973, cited in Zahavi, 1975) finding that it takes monomorphic species of warblers longer to mate than dimorphic species with colorful plumage. It could also explain why northern duck species, which have a short breeding season and therefore a short period of pair formation, have evolved such striking male plumage (Zahavi, 1975).

Rates of reproduction and sex role reversal

Are gulf pipefish a sex role reversed species? Sex role reversal occurs when females compete more intensely than males for access to mates, as males limit female reproductive success. Accordingly, females should be more modified by sexual selection because intrasexual competition will be stronger between females than between males (Vincent *et al.,* 1992). However, it is premature to judge the extent of sex role reversal solely from the presence of sexual dimorphisms in a species. Sexual dimorphisms (such as one sex being larger) may arise from increased fecundity or reduced food competition, not necessarily from sexual selection pressures (Darwin, 1871; Hedrick & Temeles, 1989).   If male gulf pipefish limit female reproductive success by having a slower potential reproductive rate than females, then females should compete for access to males, and the species should then be classified as sex-role reversed.

The potential reproductive rates of males and females are influenced primarily by the relative amounts of time or energy that the two sexes spend on their progeny (Bateman, 1948; Williams, 1966; Trivers, 1972). When there is no parental care, the main source of competition results from the different rates of gamete production in the two sexes. As shown by Bateman (1948), the smaller size of sperm allows males to produce gametes more rapidly than females can produce eggs, which leads females to limit male reproductive success. Accordingly, males will compete among themselves for the possession of females. However, when one sex spends a lot of time or energy in rearing offspring, its potential rate of reproduction may decrease, and the OSR will be biased towards the other sex (Clutton-Brock & Parker, 1992). Nevertheless, although male seahorses spend time and energy in brooding their offspring, they follow traditional courtship roles because their rate of reproduction is higher than that of females

(Masonjones & Lewis, 2000). Therefore, determining the potential reproductive rate of males and females is imperative in determining which sex is the predominant competitor for mates (Clutton-Brock and Parker, 1992; Parker & Simmons, 1996; Masonjones & Lewis, 2000).

In the gulf pipefish, the reproductive rates of both sexes differed widely. It took males 15-16 days to brood one set of eggs, while females were able to fill three male pouches in 8 days. At this rate, female rate of reproduction would be six times that of males. However, I am not sure if egg production rate is continuous throughout, so it may not be correct to assume that they would be able to fill six males during one male brooding cycle. In any case, I can say with certainty that females could at least completely fill three males within one brooding cycle. These results show that males were the limiting factor in female reproductive success. As a result, theory predicts that females will have developed secondary sexual traits, show aggression, and would compete against themselves for the possession of a mate (Darwin, 1871; Bateman 1948; Williams, 1966). This is in fact what I observed: females were much more aggressive than males in intrasexual competition, and even during courtship they were more aggressive and willing to mate than were males. In addition, females had extensive ornaments and other secondary sexual characteristics such as deep trunks and large size, which males did not possess.

These rates of reproduction are much more skewed in the gulf pipefish than what Berglund *et al.* (1989b) showed for *N. ophidion* and *S. typhle*. Although males limit female reproductive success in all three species, female *N. ophidion* and *S. typhle* can fill about two males during one male brooding cycle, while the present study suggests that *S. scovelli* females can fill at least three males and possibly more. The main difference

between the gulf pipefish and both *N. ophidion* and *S. typhle* that may affect the reproductive rate is that the latter two species live off the Swedish coast in temperatures of 49-62°F, as opposed to 76°F for the gulf pipefish. Temperature is known to affect potential reproductive rates in pipefish (Ahnesjö, 1995). It takes males longer (58 days) to brood embryos in colder temperatures than in warmer temperatures (35 days), and accordingly their potential rate of reproduction decreases in colder temperatures. However, females were able to produce the same number of eggs in both temperatures, apparently because egg production is more affected by food availability than by temperatures (Wootton, 1979, cited in Ahnesjö, 1995). Therefore, at low temperatures, the rates of reproduction would be more skewed because the OSR would become more female biased, and as a result female-female competition would become more intense.

It takes much less time to brood embryos in the gulf pipefish than it does in either *N. ophidion* or in *S. typhle*, probably because of the increase in temperatures. However, with an increase in temperatures for the gulf pipefish as compared to those in Swedish waters, we might have expected that males would have been able to brood eggs faster than females can make them, which is clearly not the case. The increase in temperature did bring about a much faster rate of brooding, but female reproductive rate also appeared to increase dramatically at a much faster rate than male brooding time. This resulted in the highly skewed rates of reproduction that I observed, which are likely to be responsible for the development of extensive sexual dimorphisms in the gulf pipefish, as compared to other species.

Comparisons between species

The gulf pipefish is the most dimorphic species in its genus (Brown, 1972). I have shown that females can fill at least three (and possibly six) males during one brooding cycle. This makes the OSR highly biased towards females, which results in greater female intrasexual competition (Emlen & Oring, 1977; Vincent *et al.*, 1994). Their highly skewed OSR may be the reason why the species is so sexually dimorphic. The reproductive rates of female gulf pipefish are much greater than the reproductive rates of both *N. ophidion* and *S. typhle* (Berglund *et al.*, 1989b). Accordingly, gulf pipefish females have been under greater pressure from sexual selection to develop armaments to compete against other females, and ornaments to attract mates. Hence, females in this species have evolved silver markings, high-contrast ornaments, large size, and trunk depth as a result (although large size and trunk depth may be a result of fecundity selection as well).

These findings still do not explain why *N. ophidion* females have the skin fold and sexual coloration whereas *S. typhle* females do not. Females in both of these species have similar rates of reproduction, so this does not seem to be the cause of the dimorphisms in these two species. Recent work has proposed that increased levels of polyandry will result in greater inequalities in sexual selection pressures between males and females, and thus will result in sexual dimorphisms (Jones & Avise, 1997a, 1997b; Jones, Rosenqvist, Berglund, & Avise, 1999). Therefore, polyandrous species will exhibit greater levels of sexual dimorphism than species that are less polyandrous (monogamous or polygynandrous). This theory helps explain the presence of the dimorphisms in *N. ophidion*, which is polyandrous, and it explains the lack of dimorphisms in *S. typhle*, which is polygynandrous. However, they also argued that *S.*

scovelli fits the theory as well because they assumed it is a polyandrous species (and is therefore highly dimorphic). As I have shown, however, the gulf pipefish is really polygynandrous, which does not explain the degree of dimorphisms found in this species. I believe that the highly skewed reproductive rates (which have not been measured before this study) is the reason for the extensive secondary sexual characters that female gulf pipefish possess.

Conclusions

As I have shown, the gulf pipefish is a sex-role reversed species because males limit female reproductive success. As a result, females are more modified than males through sexual selection: females exhibit silver markings along their sides, and have zebra-pattern ornaments that they display during intrasexual competition and during courtship. In turn, males who have used these signals in choosing their mates will have been more successful, as by doing so they were ensured to mate with high-quality partners. Females have also adopted traditional male characteristics such as larger size, aggressiveness, and willingness to mate. Female intrasexual competition is more prevalent and more frequent than male intrasexual competition, and females were more aggressive during courtship as well. Accordingly, the gulf pipefish can be classified as a sex role reversed species.

As we have seen, however, males also competed against themselves for access to females when the sex ratio was biased toward males. In addition, although females were more aggressive than males during courtship, males increased courtship aggression when they were in tanks with other males. This shows that although males limit female

reproductive success, the extent to which a species shows sex role reversal depends on environmental cues such as the OSR, as they are not as fixed as we make them seem.

Future directions

With the husbandry guidelines that I have provided, it is now possible to keep a healthy, breeding population of the gulf pipefish in the laboratory. The next step to this work should be to perform additional behavioral observations to increase sample size. Although I found evidence that males often mate with more than one female when the sex ratio is female-biased, I did not observe this behavior. Further laboratory experiments should subject the pipefish to tanks with different sex ratios (including even ratios), and genetic studies should be performed to accurately assess maternity from the embryos.

Additional mating studies should be performed on the gulf pipefish, especially looking to see whether both males and females prefer partners with higher baseline contrast and brightness levels. Using the methods of measuring contrast and brightness that I have developed, a study such as this would be very simple to conduct, yet its results would be very important in furthering our understanding of the role of visual cues in choosing mates. In addition, further research should measure the energetic costs of these signals in the gulf pipefish.

Although I have shown that male *S. scovelli* limit female reproductive success, it would be very interesting to measure whether males spend more energy in brooding the embryos than females do in making the eggs. Previous studies on other sex-role-reversed species of pipefish have shown that males do not always spend more energy than females

on brooding (Berglund *et al*. 1989b), which shows that extensive parental investment cannot be used to predict sex-role-reversal, as argued by Trivers (1972).

Furthermore, studies should be performed to investigate whether female pipefish are able to repress growth in less-dominant females. I saw some evidence that females in same-sex tanks grew more slowly than those in isolation or with males, although I did not get conclusive results because of my small population size. It would be very interesting to study these observations, as they would have fascinating implications on the growth of pipefish in the wild. Furthermore, if such a trend were observed, it would be essential to find out by what mechanisms are dominant females repressing growth in other females.

Most importantly, it is essential to verify if the behaviors that I observed were a result of laboratory conditions, or whether these behaviors are common in the wild. Evidence from other studies suggests that pipefish behavior in their natural environment is similar to that encountered in the lab (Vincent *et al.,* 1995). However, field observations of *S. scovelli* do not exist, and they would be incredibly helpful in gaining more information on how their mating system varies with the OSR in the wild.

In addition, further studies should investigate the roles of secondary sexual characteristics in the mating system of other animals. Are monogamous species monogamous because they lack secondary sexual characteristics with which to easily choose high-quality mates, or do they lack secondary sex characteristics because of their monogamous mating system? Is courtship quicker in a species with sexual dimorphisms? Do highly skewed potential rates of reproduction contribute to the development of sexual dimorphisms?

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