

Group Sex, Sex Change, and Parasitic Males: Sexual Strategies Among the Fishes and Their Neurobiological Correlates

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Sexual selection, in the form of intrasexual competition and mate choice, has driven the evolution of a variety of sexual phenotypes amongst the vertebrates (Andersson, 1994). As a result, vertebrate species utilize many different approaches to acquire fertilizations. Humans and other primates show a wide range of sexual behaviors, but this range is dwarfed by the remarkable variation seen in advanced fishes. The goals of this review are (a) to acquaint the reader with the tremendous sexual diversity exhibited by fishes, (b) to demonstrate how this diversity provides unique opportunities to examine the neurobiological correlates of vertebrate sexual strategies, and (c) to highlight the parallels between the neuroendocrine correlates of the sexual strategies of fish with the mechanisms underlying sexual phenotypes in other vertebrates, showing the utility of fish studies for understanding sexual variation in general.

Key Words: alternative morph, GnRH, sneaker, social behavior, teleost, vasopressin, vasotocin.

Fishes Are Sexually Diverse Vertebrates

One way to appreciate the diversity of fishes is to compare fishes to vertebrate taxa with which we are more familiar: birds and mammals. There are approximately 4,400 different species of mammals and over double the number of species of birds, around 9,700. Although this may seem like a large number of species, fishes make up the bulk of the vertebrates, with over 24,500 species, or over five times the diversity seen in mammals (see Figure 1; Nelson, 1994; Pough, Heiser, & McFarland, 1996). In this review, we will focus on euteleost fishes, such as bass, halibut, perch, and salmon, which account for over 90% of all fish species

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(Nelson, 1994). The high species diversity and rapid evolution of the euteleosts resulted in a high degree of phenotypic plasticity, a feature most evident in the realm of phenotypic sex.

Euteleost fishes range in sexual phenotypes from species with permanent sexes, such as cichlids, to species such as the bluehead wrasse and bluebanded gobies that change sex once in their lives. Other fish even change sex multiple times within a lifetime, as in the Japanese goby, *Trimma okinawae* (see Figure 2). Great variation can also exist within a species, with numerous examples showing multiple male morphs and the potential for multiple female morphs (Henson & Warner, 1997; Taborsky, 1994, Taborsky, 2001). In the ocellated wrasse, *Symphodus ocellatus*, four different male strategies have been observed in the same population (Taborsky, Hudde, & Wirtz, 1987). Large males maintain nests and care for eggs while small males are parasitic spawners (sneak-

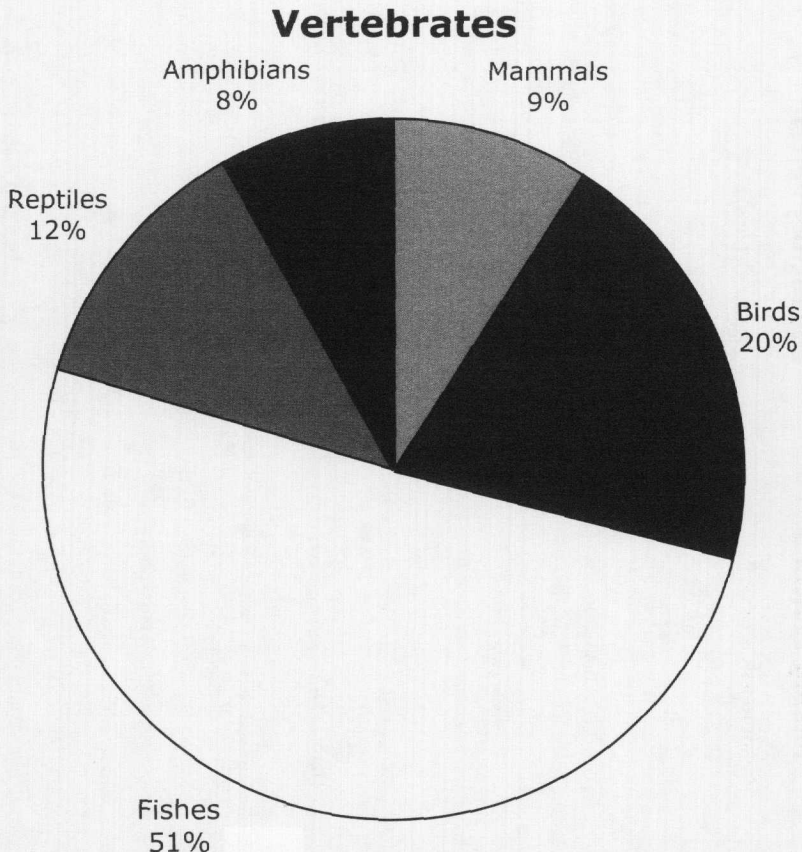


Figure 1. Number of different species in each of the major vertebrate groups as approximate percentages of the total known vertebrates (Data from Nelson, 1994; Pough et al., 1996).

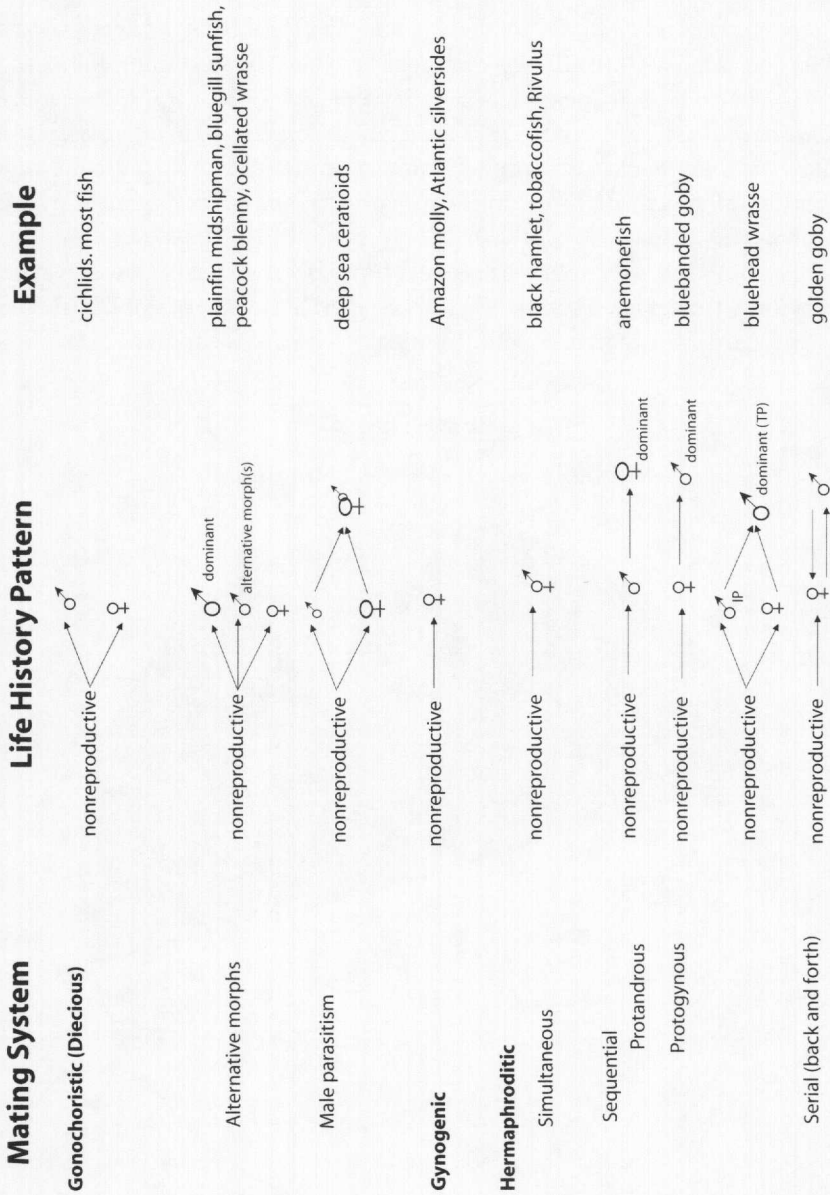


Figure 2. Life history patterns for different species of fish (see text for descriptions, modified from Foran and Bass, 1999).

ers). Mid-sized males, called satellites, help the large males by defending nests against sneakers in exchange for the occasional chance to court and spawn with females. The largest males, dubbed "pirates" dominate over nesting males and take over their nest tubes. In some cases, particularly in other *Symphodus* species, they fertilize eggs in the nesting males' nests and leave them "under the care" of the nesting males (Taborsky, 1994; van den Berghe, 1988). Females can also show variation in sexual phenotype. In the bluehead wrasse, *Thalassoma bifasciatum*, females express two mating tactics: group spawning and pair spawning. Both of these tactics are seen within a single population. As with male strategies, female strategies seem to be size dependent: The largest females only spawn with one male at a time, whereas smaller females primarily spawn in groups (Warner, 1984a, 1985; Warner & Hofman, 1980). The variation in sexual phenotypes for males and females of *S. ocellatus* and *T. bifasciatum* exemplify the diverse alternative reproductive tactics used by fish, and there are many more examples among other fish species (for comprehensive reviews of reproduction and sexuality in fishes, see Breder & Rosen, 1966; Taborsky, 1994).

The environmental potential for polygamy (EPP) provides one useful organizing principle for understanding the evolution of diverse sexual phenotypes and mating systems in fishes. The EPP describes the degree to which resources are distributed or aggregated, such that a limited number of individuals can control access to a large amount of required resources and, as a result, monopolize mates who seek those resources (Emlen & Oring, 1977). Male monopolization of mating resources results in polygyny or the acquisition of multiple females by a limited number of top-ranked males. Because of this, there is tremendous pressure to acquire resources and a resultant skew in male reproductive success greatly benefiting the monopolizers. For males that cannot dominate resources (discussed in detail subsequently), several alternative strategies are available, including (a) maintain nonreproductive status and wait to become large enough to dominate a resource (see the example that follows), (b) help the reproductive dominant and by association get some access to the opposite sex (e.g., satellite tactic in *S. ocellatus*), or (c) cheating the system to obtain resources (e.g., sneak spawning in *S. ocellatus*).

Categories of Sexual Systems

In order to categorize the large amount of sexual variation within fishes, a set of discrete categories, or sexual systems, has been proposed (see Figure 2). In this schema, a sexual system refers to an approach that individuals or species take in attempting to pass their genes on to the next generation. The two major categories are gonochores and her-

maphrodites. Gonochoristic species develop into two separate sexes (are dioecious) and remain the same sex throughout their life. Gonochoristic species can be polygynous and have multiple male reproductive morphs, such as the plainfin midshipman or the bluegill sunfish. Alternatively, gonochores can have dominant males that are reproductive and socially inhibited males that are nonreproductive, such as the African cichlid, *Haplochromis (Astatotilapia) burtoni*. There are also monogamous gonochoristic fish, such as many members of the butterfly fishes (family Chaetodontidae), which are well-known coral reef inhabitants.

Hermaphroditic fishes are less common than gonochoristic species. Some species in this group are simultaneous hermaphrodites, having both male and female gonads at the same time. Simultaneous hermaphrodites include some serranids, like the tobaccofish, *Serranus tabacarius*. Despite having both male and female reproductive organs, *S. tabacarius* mates sexually (i.e., does not self fertilize), and switches on a moment-to-moment basis between mating as a male and then as a female (Petersen, 1995). A single fish species among simultaneous hermaphrodites, *Rivulus marmoratus*, can even fertilize its own eggs (Harrington, 1975).

Rather than being one sex (gonochoristic) or both sexes at the same time (simultaneous hermaphrodites), sequential hermaphrodites are one sex at one stage in their life and then switch to the opposite sex later in life (see Figure 2). These are more common than simultaneous hermaphrodites (Devlin & Nagahama, 2002; Warner, 1984b). At least 19 families of fishes have members that naturally change sex from female to male (protogyny) and even though there are fewer species that change from male to female (protandry), protandry is still seen in at least 10 different families (Devlin & Nagahama, 2002; Policansky, 1982). Sex change is seen in polyandrous, polygynous, and even monogamous species. Sex change often occurs when there is a very limited reproductive resource, such as very few members of the opposite sex or scarce high quality mating territories. In many cases, dominance, usually determined by the relative size of individuals, will determine who changes sex. The individual that changes will experience a dramatic increase in reproductive success (Ghiselin, 1969; Warner, 1984b). As if changing sex were not strange enough, there are those fish that can change sex serially, going back and forth from female to male and back to female, such as the golden goby and several monogamous *Gobiodon* species (Nakashima, Kuwamura, & Yogo, 1995, 1996; Sunobe & Nakazono, 1993).

Why don't all fish change sex? There appear to be constraints on many species of fish that preclude adult sex change. Sex change appears to be a derived trait (e.g., it is represented more in derived lineages, like gobies and wrasses, relative to more ancestral or primitive

fishes, like salmon or even sharks). Most fish do not normally change sex, but even some of these can be chemically induced to change sex, although in most of these cases it is only during development (Piferrer, 2001). This potential for sex change may exist in many gonochoristic fishes because they have a bipotential gonad, or a single primordial tissue from which both testicular and ovarian tissue derives. However, in many of these species, it appears that there may not have been enough selective pressure for the evolution of sex change (see Policansky, 1982, for a more thorough review of this question).

Menage A Trois and Parasitic Males

Sex change is not the only "unusual" reproductive pattern that has emerged amongst the fishes. There are some species of fish in which mating often involves three fish. In many species of Catostomidae (sucker fish), two males join a female in coordinated spawning movements with one male on each side of the female (Jenkins & Jenkins, 1980; Page & Johnston 1990; Reighard, 1920). There are many hypotheses for the function of these coordinated spawns, including the possibility that two males are required to adequately stimulate egg release (for a discussion of possibilities, see Taborsky, 1994). Another extreme that shows the sexual diversity of fishes is the deep-sea anglerfishes (Ceratoidae), which have enormous sexual dimorphism in body size. The largest female known in each family of Ceratoidae is 3 to 13 times longer than the longest known male, and the difference can be even greater within species (Nelson, 1994). The small males have specializations to find females, such as larger eyes, a more developed tail and, in some species, a well developed nasal organ. When a male locates a female, he uses his jaws to permanently attach to the female, drawing sustenance from her for the rest of his life. In some species, such as *Haplophryne mollis* (= *Edriolychnus schmidti*), the vascular systems of the male and female become integrated, most of the male's organs degenerate, and, as a result, he becomes nothing more than a male reproductive organ attached to the female (Bertelsen, 1951). At this point, it is questionable whether what remains is a female with a parasitic male or a single hermaphroditic fish. Other parasitic males retain their identity more than *H. mollis*, and most parasitic males fall into the category of small male morphs.

The Strategies of Small Male Morphs

For the purpose of this review, "small" male morphs are considered those that are 30+% smaller than "large" males in their social groups, yet are adult and reproductively mature (Bass, 1993). In all of these

cases, the larger males dominate a reproductive resource, usually a spawning site. There are many fish in which the small males exhibit alternative male strategies including bluehead wrasse (Warner & Schultz, 1992), plainfin midshipman (Bass, 1992), salmon (Hoar, 1988), poecilids (Ryan, Hews, & Wagner, 1990), blennies (Oliveira, Canario, & Grober, 2001), gobies (Magnhagen, 1992; Jones, Walker, Kvarnemo, Lindstrom, & Avise, 2001), and sunfish (Gross, 1991). In poecilids and salmonids, a heritable basis has been shown for these small males (Ryan, Pease, & Morris, 1992; Thorpe, Morgan, Talbot, & Miles, 1983; Zimmerer & Kallman, 1989). However, as Caro and Bateson (1986) have observed, there are limited cases of alternative tactics where one phenomenon or process (e.g., genes or environment) is solely responsible for the evolution and/or development of small male morphs. In many fish, even if there is a heritable component, the environment still plays a role in whether a fish becomes a small male or not, and this is best demonstrated in salmon. Male Atlantic salmon, *Salmo salar*, make two decisions in the first year of life: (a) whether to migrate to sea (become a smolt) and (b) whether to mature sexually (stay in fresh water and become a sneaker) (Metcalf, Huntingford, Graham, & Thorpe, 1989; Thorpe, 1991; Thorpe, Metcalfe, & Huntingford, 1992). Whether a fish matures or not depends on foraging success, predator avoidance, and success competing with other individuals; the heritable component seems to be the threshold level of these factors used to make the decision (Thorpe, 1986). Thus, in salmon and many poecilids, although genetics plays a role, it is not the sole determining factor. In the bluehead wrasse, it does not even matter if genes drive the initial development of a fish as a male or a female, because either sex can transform into a dominant terminal-phase male later in life.

Alternative male morphs often display a suite of traits that differ from those males that dominate resources (Bass, 1993). For instance, alternative males in midshipman have differences in the neural pathways and sonic muscles associated with the production of sounds used to attract females (Bass, 1992). The competition for mating resources can also result in different types or amounts of sperm. In salmonids, the sperm are more mobile and live longer in small males (Gage, Stockley, & Parker, 1995). Small males of plainfin midshipman have a 900% greater sperm-producing testes to body size ratio than nesting males (Bass, 1993). Small males in the grass goby, *Zosterisessor ophiocephalus*, also have a larger testes/body size ratio, but nesting males have an accessory gonadal structure that produces mucus as a way of generating time-released sperm that helps it compete with sneaker males (Scaggiante, Mazzoldi, Petersen, & Rasotto, 1999). This accessory gonadal structure

found in gobies is analogous to the human prostate in that it provides supporting fluid/mucus that is required for successful reproduction, and it is androgen sensitive (to the unconvertible dihydrotestosterone in humans and unconvertible 11-ketotestosterone in gobies). This serves to highlight the diversity of body systems that respond to sexual selection and the parallels among various vertebrate groups in the way in which these manifold systems respond. Differences in testes size, sperm morphology, and ejaculates in humans have been noted, but their functional significance in terms of different reproductive tactics is still under much debate (reviewed in Baker & Bellis, 1995). Euteleosts have provided useful models to study the evolution of testis size and ejaculate characteristics, and also the changes in these traits within the same individual resulting from social interactions (Pilastro, Scaggiante, & Rasotto, 2002; Stockley, Gage, Parker, & Moller, 1997).

Where to Eat, Where to Mate and With Whom

Outside of sperm competition, the social environment can greatly affect fishes; this is seen in the social transmission of information. For example, guppies, *Poecilia reticulata*, learn from conspecifics where to eat and how to get to these feeding sites (Laland & Williams, 1997). Observing and interacting with others can similarly affect sexual behavior. There is mate choice copying in some fish species, where younger females copy the mate choices of mature females (Dugatkin, 1992; Dugatkin & Godin, 1992, 1993). Bluehead wrasse mating sites are culturally transmitted, and traditional sites are used again and again (Warner, 1988), suggesting that cultural transmission can affect reproductive choices for long periods of time. These are just a few examples of the mechanisms by which social interactions cause changes in sexual behavior.

Genes Versus Environment

The relative contribution of the environment and genetics to the sexual phenotype of an individual has been an ongoing controversy in many vertebrate species. As discussed earlier, some alternative male morphs can be more influenced by the environment, whereas others may be more influenced by genetics. Even in gonochoristic fish, temperature, or temperature interacting with genetics, will influence whether the fish develops as male or female in species of at least eight families of fish (Conover, 1984; Conover & Kynard, 1981; Devlin & Nagahama, 2002). High temperatures tend to skew offspring toward male biased numbers, but this depends largely on the species investigated and may bias the sexual development in other ways (Devlin & Nagahama, 2002).

Although environmental flexibility seems to be the common theme

with regard to sexuality in fishes, there are different examples in which sexual phenotypes can be genetically fixed. For instance, in the pygmy swordtail, *Xiphophorus nigrensis*, there is a sex-linked P gene that controls the onset of sexual maturity (Kallman & Schreibman, 1973; Ryan & Causey, 1989; Zimmerer & Kallman, 1989). This Y-linked trait determines the onset of the activation of the hypothalamic-pituitary-gonadal (HPG) axis (see Figure 3). The earlier the onset of activation, the earlier there is circulation of androgens that induce sexual maturity and dramatically reduce growth. With very early activation, the fish remains small and its main strategy is sneaking and chasing the female to copulate, while larger males that mature later in life rely on courtship (Ryan & Causey, 1989; Zimmerer & Kallman, 1989).

Alternative male morphs, such as the pygmy swordtail, have also received a great deal of attention as they provide the opportunity to look

Hypothalamic-pituitary-gonadal (HPG) Axis

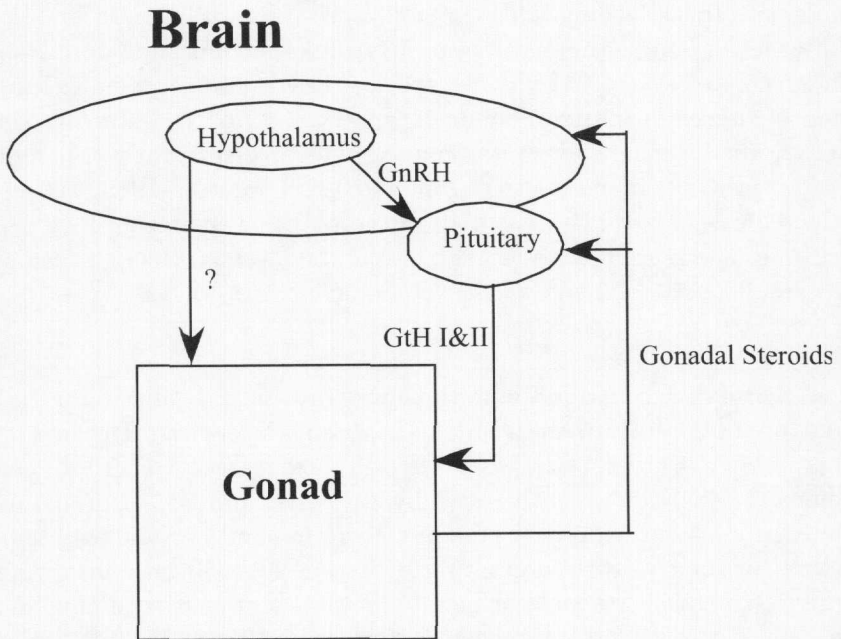


Figure 3. Flow chart of the hypothalamic-pituitary-gonadal (HPG) axis, which regulates reproductive physiology and behavior (modified from Grober, 1998). The question mark represents an unconfirmed and potential direct nervous system signaling pathway between the brain and the gonad (see Nakamura, Specker & Nagahama, 1996; Perez, Fyenzalida, Mendez, & Cerisola, 1983; for examples).

for brain differences between individuals of the same species and sex that display very different sexual behaviors. Fish with less of a genetic basis for their sexual phenotype and more social regulation are also of great interest because of the ways the social environment can shape the brain and the consequent sexual behavior (Grober, 1997, 1998). In the remainder of this paper, we will focus on the role of two neuropeptides, gonadotropin-releasing hormone (GnRH) and arginine-vasotocin (AVT), in the generation of alternative male morphs that involve social regulation of sexual behavior, and the parallels with regard to brain and sexual behavior seen between fish and other vertebrates.

Neuroendocrine Correlates of Sexual Strategies and Phenotypes

GnRH: Axis Conservation, Male Morphs and Modulation by Social Influences

Gonadotropin-releasing hormone is at the apex of the HPG axis, a signaling pathway that has a central role in controlling reproductive behavior and sexual development, and has been conserved among vertebrates (see Figure 3; Demski, 1984; Muske, 1993). One place GnRH is found is in the preoptic area of the hypothalamus, which is located in the medial-ventral forebrain and is a key regulator of reproductive behavior and physiology. Upregulated GnRH can increase endogenous levels of circulating gonadal steroids, positively influencing the development of secondary sex characteristics (see Figure 3, reviewed in Sherwood, Parker, McRory, & Lescheid, 1994; Silverman, Livne, & Witkin, 1994). In addition, GnRH acts directly in the brain to modulate reproductive behavior (Pfaff et al., 1994; Silverman & Witkin, 1994). GnRH is critical for sexual development and adult reproductive behavior, as demonstrated by the reproductive dysfunction of animals with dysfunctional GnRH preoptic systems, such as the hypogonadal mouse mutant and hypogonadal humans with Kallman's syndrome (Cattanach, Iddon, Charlton, Chiappa, & Fink, 1977; Schwanzel-Fukuda, Brick, & Pfaff, 1989). In both of these cases, the improper development of the forebrain GnRH axis results in the absence of the GnRH peptide and a complete inability to produce mature gametes. Interestingly, in laboratory mice this loss of function is due to an error in gene transcription, whereas in Kallman's syndrome the problem is the inability for the GnRH cells to migrate to their appropriate target during early development. A loss of function tells us something, but the recovery of function magnifies our understanding of this relationship: The hypogonadal mouse mutant can undergo sexual maturation if brain grafts or recombinant DNA technol-

ogy is used to introduce GnRH preoptic cells into their brain. These "rescued" hypogonadal mice begin to express secondary sex characteristics (Livne, Gibson, & Silverman, 1992; Livne, Silverman, & Gibson, 1992; Mason et al., 1986) and can perform all normal reproductive functions. Remarkably, a very small percentage of the normal number of GnRH cells needs to be transplanted in order to rescue the hypogonadal mouse mutant (Livne, Gibson, & Silverman, 1992).

In euteleost fish, variation in the GnRH forebrain axis is associated with the expression of alternative phenotypes that can be induced either developmentally or through social interactions. Platyfish, a poeciliid, shows differences in GnRH-ir (GuRH-immunoreactive) between male morphs. The platyfish has two male morphs, a large morph and a small sneaker morph. The small sneaker males mature faster and have 50% more GnRH-ir cells in the preoptic area relative to large males (Halpern-Sebold, Schreiber, & Margolis-Nunno, 1986). For both of the male morphs, changes in GnRH cells in the preoptic area are necessary for development of the pituitary-gonadal axis, so the platyfish show age-related increases in GnRH, and sneakers and large morphs level off at similar values when they've both sexually matured (Halpern-Sebold et al., 1986).

Another gonochoristic fish, the plainfin midshipman, shows a similar pattern. The plainfin midshipman has two types of males, Type I males, which build nests, guard eggs, and court females by acoustic communication in the form of humming, and Type II males, which are smaller and spawn by sneaking instead of courting. In both of these types of males, and in females, the maturation from juvenile to adult involves a 50% increase in the preoptic GnRH-ir cell number and 100% or more increase in cell size (Grober, Fox, Laughlin, & Bass, 1994). In gonochoristic fishes, it seems that increases in GnRH neuron size and number begin a series of events that end in sexual maturation of the individual, whether it is early, as in sneakers, or later, as in the nesting Type I males in midshipman (Bass, 1993). In addition, there are differences in the GnRH neurons between morphs. Type I males have larger GnRH neurons than Type II males or females, even after controlling for body size, and when body size is taken into account, Type II males have a higher number of GnRH cells per gram body mass than Type I males or females (Foran & Bass, 1999; Grober et al., 1994).

The bluehead wrasse, *Thalassoma bifasciatum*, has three sexual morphs as well. There are initial-phase males and initial-phase females that share the same yellow body coloration (Warner, 1984a). Under appropriate social conditions, either of these initial-phase phenotypes transform into a territorial terminal-phase male, which has a blue

head, a green body color, and a black-white-black banding pattern dividing the head and body. As seen in the midshipman fish, the smaller initial-phase males try to sneak in sperm to fertilize eggs of the female who pair spawns with the terminal-phase male. These sneaker males have larger gonads for their body size and the terminal-phase males have a higher number of GnRH neurons than initial-phase males and females (Grober & Bass, 1991).

The ballan wrasse, *Labrus berggylta*, a protogynous sex changer, differs from the bluehead wrasse in that there are no initial-phase males. All fish begin life as females. Additionally, the ballan wrasse spawns seasonally in temperate waters, unlike the bluehead, which spawns all year long and lives in the tropics. In the case of the ballan wrasse, males that had been through the reproductive season (postspawning) had larger GnRH-ir cells than females or those males just prior to the reproductive season (prespawning). The temporary size changes in GnRH-ir cells were coupled to the male spawning cycle, yet the total number of GnRH cells appeared to be fixed (Elofsson, Winberg, & Nilsson, 1999).

Amphiprion melanopus, an anemone fish, lives in groups with a spawning pair and several nonreproductive individuals. The largest fish is female, the others in the group are male, but there is only one reproductive male. The female is the dominant member of the group and helps to repress sex change in the other group members. When the female dies, a male changes to female and one of the nonreproductive males becomes a reproductive male. In these groups, the dominant female has the largest GnRH-ir cell size, but the reproductive male has a greater number of cells than the female or the nonreproductives (Elofsson, Winberg, & Francis, 1997).

The fish in which the most is known about GnRH is the African cichlid, *Haplochromis burtoni*. In this gonochoristic fish, GnRH-immunoreactive (-ir) cell size increases when a fish changes from a nonterritorial nonreproductive subordinate to a territorial reproductive dominant. The GnRH-ir cell size and reproductive status of subordinate males are normally inhibited by the dominant male. The GnRH-ir cells can also decrease in size if a dominant again becomes subordinate and nonreproductive (Francis, Soma, & Fernald, 1993). This is one case in which the size of the gonad divided by the size of the body (gonosomatic index, or GSI) is greater in dominants than in the small males, which is not surprising, because the subordinates are nonreproductive (Fox, White, Kao, & Fernald, 1997). Investigations into the expression of the three different GnRH forms in the brain show that only GnRH1 mRNA showed significant increased expression as a male changed from nonterritorial to territorial (White & Fer-

Table 1
Preoptic Gonadotropin Releasing Hormone (GnRH) in Fish With Different Life History Patterns

Species	Group Members	Gonad Weight/Body Weight (Gonadosomatic Index, or GSI)	GnRH Cells in the Preoptic Area
African cichlid ^a	Territorial male (T) Nonterritorial males (NT) Females	T > NT	T > NT (cell size) (females vary with reproductive status)
Plainfin midshipman ^b	Territorial (Type I) male Nonterritorial (Type II) males Females	Type II > Type I	Type I > Type II and females (cell size) Type II > Type I and females (cell number/body mass)
Bluehead wrasse ^c (protogynous hermaphrodite)	Terminal phase (TP) male Initial phase (IP) males Initial phase females	IP > TP ^d	TP > IP and females (cell number)
Anemone fish ^e (protandrous hermaphrodite)	Female Reproductive male (R) Nonreproductive males (NR)	R > NR ^f	R > NR and females (cell number) Females > R and NR (cell size)
Ballan wrasse ^g (protogynous hermaphrodite)	Male Females	- ^h	postspawning males > prespawning males and females (cell size)
Platyfish ⁱ	Males (L) Small males (S)	S > L	S > L (cell number); S > L (cell size) at same age but when both are mature, S = L for cell size

Note. Modified from Foran and Bass (1999). Female GSI varies with reproductive condition and is larger prespawning than post

^aAfrican cichlid (*Haplochromis burtoni*): Davis & Fernald, 1990; Francis et al., 1993; Fox et al., 1997; White & Fernald, 1993

^bPlainfin midshipman (*Porichthys notatus*): Bass, 1992; Bass, 1996; Bass & Andersen, 1991; Grober et al., 1994

^cBluehead wrasse (*Thalassoma bifasciatum*): Grober & Bass, 1991; Henson & Warner, 1997

^dActually for a closely related species, *Thalassoma lucasanum*: Warner, 1982

^eAnemone fish (*Amphiprion melanopus*): Elofsson et al., 1997; Shapiro, 1992

^fActually from a closely related species, *Amphiprion akallopisos*: Fricke, 1979

^gBallan wrasse (*Labrus bergyllta*): Elofsson et al., 1999

^hMale prespaw GSI > male postspaw GSI

ⁱPlatyfish (*Xiphophorus maculatus*): Halpern-Sebold et al., 1986; Schreibman & Kallman, 1977

nald, 1998; White, Nguyen & Fernald, 2002). GnRH1 codes for [Ser⁸]GnRH and is expressed in the preoptic area neurons projecting to the pituitary (White & Fernald, 1998). White et al. (2002) also found that both behavior and GnRH cell anatomy changed rapidly in one direction, but lagged in returning to its previous condition, a trend that is also seen in sex changing fish (see the subsequent discussion that follows). Female cichlids showed changes in GnRH cell size, depending on their reproductive state. Postspawning females have the largest GnRH cell size, followed by spawning females, who have cells two times as large as females caring for offspring (White & Fernald, 1993). Thus, a general rule amongst the fishes is that forebrain GnRH structure and function appears to be closely tied to reproductive state and alternative reproductive morphs show predictable differences in the GnRH system (see Table 1).

Vasotocin (AVT) and Its Homologue, Vasopressin (AVP), Exhibit an Evolutionary Conservation in Their Anatomical Characteristics and Sexual Differentiation

Alternative reproductive morphs also show consistent differences in the vasotocin system. Vasotocin (AVT) and its mammalian homologue, vasopressin (AVP), show similar peptide structure across vertebrate taxa and the homologous peptides can give insight into their common functions (Acher & Chauvet, 1988; Moore, 1992). AVT/AVP can act as a hormone in the blood and/or as a classical neurotransmitter, a signaling molecule between neurons. We will focus on AVT/AVP's role within the brain. AVT/AVP are found in the preoptic area and the anterior hypothalamus throughout vertebrate taxa and males of many vertebrate species have higher levels of AVT or AVP than females (DeVries & Al-Shamma, 1990; Goodson & Bass, 2001). For example, males have larger AVT-producing cell size than females in the preoptic area of the blue-banded goby, a marine fish (Reavis & Grober, 1999; also see Table 2). In parallel to this, the supraoptic nucleus of the preoptic area has higher vasopressin activity in human males than females (Swaab, Chung, Kruijver, Hofman, & Ishunina, 2001).

Vasotocin is Associated With Social and Reproductive Behavior

The association of vasotocin and vasopressin with social and reproductive behavior is well documented among vertebrates (see the review by Goodson & Bass, 2001). Fish, amphibians, birds, and mammals all have species that exhibit social and sexual behavior changes after exogenous administration of vasopressin/vasotocin peptide (Castagna, Absil, Foidart, & Balthazart, 1998; Goodson & Bass, 2001; Moore &

Table 2
Preoptic Arginine-Vasotocin (AVT) in Fish With Different Life History Patterns

Species	Group Members	Behaviors	Preoptic Area AVT	Injection Effects
Pecock blenny ^a	Territorial nesting male (T) Sneaker males (S) Females	egg care courtship courtship	T and S > females (number and size) S and females > T (mRNA expression)	S and females: increased female courtship and nuptial coloration duration TI: AVT decreased number of fictive bursts (neural pattern for accoustic sounds) and summed duration of all bursts; AVP-antagonist ^c did the reverse TII: no effect of AVT Female: decreased number of fictive bursts; AVP antagonist had no effect TP:AVT increased male courtship behavior and AVP-antagonist decreased courtship; AVT increased territoriality in nonterritorial TP males; AVP-antagonist decreased territoriality in territorial TP males
Plainfin midshipman ^b	Territorial (Type I) male	egg care, courtship hums	Type II > Type I and females (cell number/body mass)	
Bluehead wrasse ^d (protogynous hermaphrodite)	Terminal phase (TP) male Initial phase (IP) males Initial phase females	often territorial aggression, pair spawns group spawns pair spawn		
Bluebanded goby ^e (protogynous hermaphrodite)	Male Females	courtship jerks, nest care visit nests	Male > female (cell size)	
Golden goby ^f (serial hermaphrodite)	Male Female	courtship skipping, nest care visit nests	Male > female (cell size)	

Note. Modified from Foran and Bass (1999).

^aPecock blenny (*Slaria pavos*): Carneiro et al., in press; Grober, George, Watkins, Carneiro & Oliveira, 2002

^bPlainfin midshipman (*Porichthys notatus*): Bass, 1996; Goodson & Bass, 2000

^cAVP-antagonist=Arginine-vasopressin antagonist

^dBluehead wrasse (*Thalassoma bifasciatum*): Godwin, Sawby, Warner, Crews, & Grober, 2000; McIntyre, 1998; Semsar, Kandel, & Godwin, 2001

^eBluebanded goby (*Lythrypnus dalli*): Reavis & Grober, 1999

^fGolden goby (*Trimma okinawae*): Grober & Sunobe, 1996; Sunobe & Nakazono, 1990, 1993

Miller, 1982; Semsar, Kandel, & Godwin, 2001; Winslow, Hastings, Carter, Harbaugh, & Insel, 1993).

As will be described subsequently, the different sexual phenotypes of fishes show different vasotocin forebrain anatomy. Interestingly, different human sexual phenotypes also show different vasopressin forebrain anatomy in forebrain areas similar to fish (Grober, 1997). There are differences in vasopressin production in human heterosexual versus homosexual males in the forebrain, but the reason for these differences remains unknown (Swaab & Hofman, 1990). Likewise, human male to female transsexuals have female-typical-size forebrain peptidergic cell populations (Zhou, Hofman, Gooren, & Swaab, 1995). Because of the sexual variation among fishes, mechanistic investigations can be used to elucidate both how pathways of sexual differentiation diverge to generate variation within a sex and how they may be modified based on social input.

Sexually Varied Vasotocin

A gonochoristic fish in which AVT has been well studied is the peacock blenny, *Salario pavo*. In the population studied in a coastal lagoon in southern Portugal, nest resources are so scarce that nesting males are in such demand that they no longer need to court females (Almada, Goncalves, Oliveira, & Santos, 1995). The result is that larger males in this species defend nests and females now compete for access to these males via female-typical courtship behavior, whereas smaller males mimic female nuptial coloration and courtship behavior to sneak spawn (Almada et al., 1995; Goncalves, Almada, Oliveira, & Santos, 1996). AVT mRNA expression is higher in females and sneaker males than in nesting males, but there are more AVT-ir cells/body size in males (both sneakers and nesting) than in females (Grober, George, Watkins, Carneiro, & Oliveira, 2002). This suggests that high levels of AVT expression are associated with the production of courtship behavior (regardless of sex), whereas the number of cells in the forebrain is associated with the initial development of each sex in that species (regardless of behavior). In support of this idea, AVT injected into females and sneaker males increases female courtship and duration of female nuptial coloration (Carneiro, Oliverira, Canário, & Grober, in press).

As a comparison, plainfin midshipman, as described earlier, have males that court females by humming (Type I males), and an alternative male that sneak spawns instead of courting (Type II male). Type II males have a greater number of AVT-ir cells per body size than females or nesting males (Foran & Bass, 1998). Based on the results of the *S. pavo* study, it would be interesting to see if the AVT mRNA was higher in nesting males. Goodson and Bass (2000) suggest that this might not

be the case, as injected AVT decreased the neural firing pattern for total courtship hum (combined busts) and an AVP-antagonist increased the total courtship hum (combined bursts) in a dose dependent manner. However, AVT may be modulated in response to the other behaviors the nesting male expresses, such as parental care or nest defense.

Lythrypnus dalli, the bluebanded goby, is functionally a protogynous sex changer (but see St. Mary, 1993). Males care for eggs and court females with a male-typical jerk swim. Intrasexual aggression occurs among females, but they are subordinate to the male (Behrents, 1983). When females are ready to lay eggs, they also may solicit males for courtship and spawning. Removal of a male from the group results in a dominant female changing sex to become the new male. Male AVT-ir cells are larger than those of females and if a female changes to male, the size of AVT-ir cells in the sex-changed fish is not significantly different from other males. As a result of a social manipulation, the brain has changed from smaller female-size AVT cells to the larger male-typical cell size. The behavior during sex change in *Lythrypnus dalli* has been modeled such that the process can be broken into different behavioral timepoints and neuroendocrinological changes can be mapped onto or correlated with the established changes in behavior (see Figure 4; Grober, 1997; Reavis & Grober, 1999).

Like the goby, the dominant, courting male bluehead wrasse shows higher AVT expression relative to the other sexual phenotypes. AVT mRNA expression in bluehead wrasse is greater in territorial courting males than females or initial-phase males and rises as a fish transforms into the territorial courting male (Godwin, Sawby, Warner, Crews, & Grober, 2000). In addition, the courting terminal-phase males had more AVT-ir cells in the preoptic area (McIntyre, 1998). These results match the pattern seen in *S. pavos* between AVT mRNA expression and courtship. Further supporting this link is research showing that injections of AVT enhance and AVP-antagonist injections reduce courtship in territorial males (Semsar, Kandel, & Godwin, 2001). Semsar et al. also found a "ceiling" effect, where territorial males that already had high rates of courtship did not increase levels of courtship with AVT administration.

The golden goby, *Trimma okinawae*, is able to change sex more than once and in either direction. The changes are socially controlled and result in rapid changes in both behavior and forebrain vasotocin. Males display aggression toward other males, maintain nests, and court females with a "skipping" behavior while females visit nests (Sunobe & Nakazono, 1993). Females have larger forebrain AVT cells than males, regardless of body size, prior sexual status, or if they sex changed multiple times (Grober & Sunobe, 1996). These experiments suggest that sex-

ual phenotype in this fish is associated with a specific size of AVT fore-brain cells and when a new sexual phenotype is adopted, AVT cells are modified accordingly.

Going Back: The Timing of Changes

In this same experiment, Grober and Sunobe (1996) used spawning as a different sex as an indicator of sex change in *T. okinawae*. *T. okinawae*'s male to female (M->F) transformations took longer than female to male (F->M) transformations (not statistically different, but an average of 5 versus 9 days, respectively). However, F->M->F transformations took longer than the two added together. The initial F->M transition was the same length, but the following M->F took 18 days instead of 5 (Grober & Sunobe, 1996). This suggests there is a mechanistic hurdle to going back after a trajectory in one direction has started. A similar increase in the time to change back from male to female after becoming male is seen in *Lythrypnus dalli*, so much so that the change back is unlikely to happen in the wild (Reavis & Grober, 1999). The behavioral model of Reavis and Grober (1999) could be used to replace the male at different stages of sex change to determine exactly when this mechanistic hurdle occurs relative to behavioral changes (see Figure 4). A similar mechanistic hurdle is present in other fish with socially regulated reproduction. A *Haplochromis burtoni* that has changed from a nonreproductive male to a reproductive

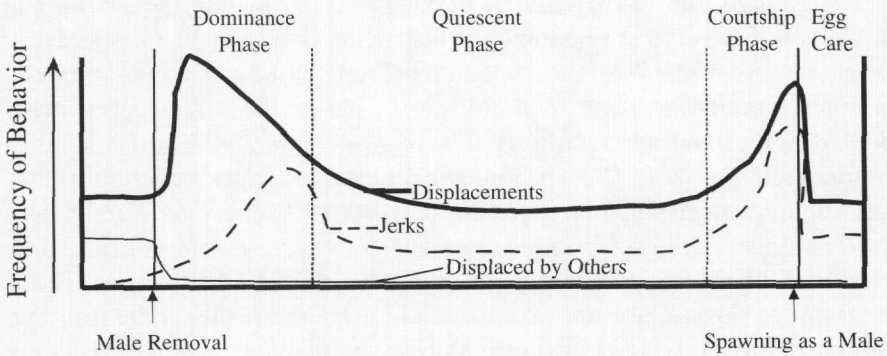


Figure 4. A behavioral model of female to male sex change in *Lythrypnus dalli* (modified from Reavis & Grober, 1999), with phases based on the jerk and displacement behavior of a focal female fish. Jerks are male-typical courtship behaviors and displacements are aggressive behaviors where a fish comes within 5cm of another fish such that the other fish moves away. The model represents a dominant female fish's behavior and the changes that occur over time following removal of a male. The end line is spawning as a male (determined by fertilized eggs), after which the fish takes care of eggs as a male. Fish with the highest initial displacement behavior were selected as dominant fish. Time from male removal to spawning as a male was 7-10 days. Neurochemical and physiological changes can be compared in time to different behavioral phases during the sex change process.

male can change back to the nonreproductive form and its behavior changes rapidly. The corresponding GnRH expression changed quickly in the initial direction, but lags in returning to its previous condition (White et al., 2002).

Like *H. burtoni*, the aggressive behavior of the bluebanded goby and the sex-specific mating behavior of the golden goby change within minutes to hours of a social trigger. This suggests that there are rapid brain changes that affect behavior, followed by downstream or independent slower changes involved in sex change of the gonads and secondary sex characters. Godwin, Crews and Warner (1996) supported this idea: Bluehead wrasse with their gonads removed still underwent behavioral sex change from female-typical behavior to male-typical behavior, without the influence of gonadal steroids (or any products of the gonad). In addition, androgen implants in female bluehead wrasse changed secondary sex characteristics but did not influence AVT peptide or mRNA production (McIntyre, 1998). Semsar and Godwin (2003) proposed that in the bluehead wrasse AVT and gonadal steroids are acting independently of each other because gonadal steroids change slowly, the social hierarchies and mating opportunities change quickly, and a female must assume dominant behavior rapidly to keep others in the group from trying to change sex.

Why Study Fishes?

Sex change and the tremendous variations in phenotypic sex among fishes are interesting phenomena, but what is the utility of studying these aspects of fish? At the applied level, one can imagine the very different dynamics involved in regulating a fishery consisting of a large-bodied, long-lived sex changing fish (e.g., grouper) versus a fish that cannot change sex. In the sex-changer, the largest individual (and therefore the most desirable to fishermen) represents the rare male amongst a much larger number of smaller females. However, the gonochoristic fish (e.g., cod) that cannot change sex has both males and females in the largest size classes, and there is normally a 1:1 sex ratio. Understanding the reproductive biology of these diverse species is critical to managing food resources from the ocean (Rowe & Hutchings, 2003).

At the comparative level, the diversity of fishes is ripe for the study of sexual variation in vertebrates. Many of these fish are also small and can therefore be observed and manipulated under controlled conditions in aquaria. There are alternative reproductive strategies of males and variation among females of the same species. There are groups with external fertilization and those with internal fertilization; fish that spawn once in their lives and fish that spawn multiple times; isolated

individuals or social groups in which a male is dominant, or a female is dominant, or a fish that is simultaneously male and female is dominant. Social learning and cultural transmission can affect choice of mate and/or mating site. There is great variation in parental care: Species in which only the female cares for offspring, both the male and female care for offspring, or only the male cares for offspring (Breder & Rosen, 1966; Taborsky, 1994). Euteleost fishes also share many parallel structures in the brain, both with each other and with the other major vertebrate groups. There are remarkable parallels between fishes and mammals in their responsiveness to complex social issues and the brain areas and chemicals that both transduce these social signals and then regulate reproductive physiology and behavior accordingly. These parallels suggest that sexual expression amongst all vertebrates is based upon a set of highly conserved principles and structures and that the study of sexually liberal species (e.g., euteleost fishes) can provide useful insights into the lives of more conservative species (e.g., mammals).

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