Life History, Neuroendocrinology, and Behavior in Fish

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I. INTRODUCTION

Fish make up over half the total number of living vertebrate species and exhibit a level of variation in sexual behavior that is unrivaled among other vertebrates. The early organization (Bass, 1996) and adult reorganization (Grober and Sunobe, 1996; Reavis and Grober, 1999) of neurobiology and behavior generate sexual plasticity in fishes, and these processes can be socially controlled. The major brain areas and neuroendocrine factors that control the development and adult maintenance of reproductive behavior and physiology in fish do not differ substantially from those in mammals (Crews, 1992), suggesting that the examination of fish provides useful insights into the generation of sexual variation in all vertebrates.

II. LIFE HISTORY

Fish make up over half the total number of living vertebrate species and exhibit a level of variation in sexual behavior that is unrivaled among other vertebrates. The early organization (Bass, 1996) and adult reorganization (Grober and Sunobe, 1996; Reavis and Grober, 1999) of neurobiology and behavior generate sexual plasticity in fishes, and these processes can be socially controlled. The major brain areas and neuroendocrine factors that control the development and adult maintenance of reproductive behavior and physiology in fish do not differ substantially from those in mammals (Crews, 1992), suggesting that the examination of fish provides useful insights into the generation of sexual variation in all vertebrates.

The explosive radiation of the percomorph, or advanced, fishes resulted in a tremendous diversity of life histories that has been generated by the invasion of new habitats and characterized by both genotypic adaptation and phenotypic polymorphism. Adaptation to new habitats generates a host of changes in the behavior, anatomy, and physiology of these animals. As a result, percomorph fishes provide a host of natural experiments to investigate the responses of hormones, brain, and behavior to novel environmental challenges. Among fishes, the teleosts include the most commonly recognized fish species (e.g., bass, perch, and flatfish) and represent the most diverse of all vertebrate taxa. In the teleosts, there are at least six classes of reproductive phenotypes (Grober, 1997): (1) reproductive dysfunction, including permanent infertility and environmentally induced (e.g., social or xenobiotic inhibition) reproductive compromises that result in deficits that vary in both duration and intensity; (2) a single phenotype in a sex, the basic pattern common to most fishes and vertebrates (gonochorism); (3) a single bisexual form that functions as a simultaneous hermaphrodite; (4) multiple phenotypes in a sex that often take the form of two distinct male phenotypes that use different behavioral approaches to acquire mates (known as alternative reproductive tactics); (5) singular reproductive transformations resulting from unidirectional adult sex change; and (6) serial reproductive transformations characterized by back-and-forth (or serial) sex change. This review concentrates on the last three categories.
III. CORRELATED CHANGES IN A SUITE OF CHARACTERS

Sexual polymorphism in any given population represents a dynamic interaction between the genetic potential of a species and the environmental-social modulation of this genetic background. For this reason, a holistic approach that examines the system at multiple levels of analysis may be most effective. For example, many fish lay benthic eggs, and this requires one parent to protect the eggs at least until they hatch. Most species exhibit male parental care—the female leaves once the eggs are attached to the bottom and the male is left to care for them. Males attain reproductive success by holding high-quality territories, constituting a mating system known as male resource defense polygyny. A large male that can defend a high-quality territory attracts many females, whereas most males attract very few. This skew in reproductive success can drive the development or evolution of reproductive plasticity (e.g., small sneaker males or sex-role reversal). The generation of this degree of sexual plasticity in turn requires the modification of a host of behavioral, physiological, endocrinological, and anatomical characteristics.

Hence, an important tool in understanding sexual plasticity is delineating the characteristics that are useful for defining sexual phenotype. Even among gonochoristic species, a large number of behavioral, somatic, and gametic characteristics vary in and between the sexes. This is most relevant for studies that use preserved specimens to examine aspects of the mating system or sexual development. Most often these studies rely on the status of one character, the gonad, to determine sexual phenotype or reproductive status. However, the allocation of gonads is not always a good predictor of reproductive behavior or success. For example, the temperate marine goby Lythrypnus dalli was initially considered a simultaneous hermaphrodite based on gonadal histology (St. Mary, 1993, 1997). However, behavioral observations indicate that these gobies are sequential hermaphrodites that exhibit only male or female behavior at any one time (Reavis and Grober, 1999; St. Mary, 1993), regardless of the presence of male and female gonads. It has thus become clear that to identify alternative sexual states, we need to examine a group or suite of characters that, together, give us an unambiguous determination of sex and mode of reproduction (Bass, 1992). These characteristics include body size and coloration, gonad anatomy and physiology, genitalia (usually the genital papilla in fish), sex-specific glands (e.g., accessory gonadal structures or testicular glands), signaling structures (e.g., sound-producing mechanisms), behavior, brain chemistry, and endocrinology.

IV. ALTERNATIVE MALE REPRODUCTIVE MORPHS IN MIDSHPMAN FISH

Perhaps the best-studied system with regard to multiple male reproductive morphs is the plainfin midshipman fish, Porichthys notatus (Fig. 1A). Each of the alternative reproductive male morphs in midshipman fish has been defined at multiple levels, from behavioral to subcellular (Bass, 1992, 1996, 1998) (Table 1). A behavior central to the reproductive tactics of midshipman fish is the production of species- and sex-typical vocalizations. Hence, a central theme in all these studies has been to identify how sexual polymorphisms in the peripheral and central nervous system establish male-morph- and female-specific vocal phenotypes. Both the neural basis of sound production and detection (i.e., hearing) have been subjects of extensive investigation. Although sexual polymorphisms for multiple traits have been identified in the vocal motor system, studies of the peripheral and central auditory systems have focused on species-typical traits (Bass et al., 1999; also see Bodnar and Bass, 1997, 1999; McKibben and Bass, 1998, 1999).

A. Spawning and Vocal Behaviors

The plainfin midshipman has a fairly wide geographic distribution along the western coastline of northern California on into Canada (Walker and Rosenblatt, 1988). Bass and Marchaterre (1989a,b) first reported two male reproductive morphs, type I and type II, on the basis of morphological traits including body size, gonad-body size ratios, and sonic muscle and motoneuron size. This was followed by a series of other studies identifying sexual polymorphisms in a large suite of vocal motor traits (Bass, 1992; also discussed later). Bramley and Bass (1994) later showed that each male morph and females have distinct spawning and vocal behaviors. Type I males build nests under rocks in the intertidal and subtidal zones, where they
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A. Nonreproductive Juvenile

B. Nonreproductive Juvenile

C. Nonreproductive Juvenile

D. Nonreproductive Juvenile

E. Nonreproductive Juvenile

FIGURE 1 A schematic diagram shown the pattern of individual life histories. A. Fixed alternative mating tactics, such as the midshipman, exhibit two permanent distinct male phenotypes (I, II). Midshipman are gonochoristic with separate nonoverlapping developmental trajectories for each class of males (Bass, 1996). B. Conditional male mating strategies, such as in cichlids, include reversible changes in status and breeding condition between territorial (T) or nonterritorial (NT). Developmental observations suggest that cichlids are gonochoristic and that the rate of development is socially mediated in males (Fraley and Fernald, 1982). C. A sex or role changing system (protogyny), found in reef fish such as bluehead wrasse. Initial-phase males (IP) and female can change into dominant territorial terminal-phase males (TP). Studies suggest that all juveniles go through a female stage before differentiating either as a mature female or male (Shapiro and Rasotto, 1993). D. Protandrous sex change, as in the anemone fish Amphiprion. The monogamous male may become the larger dominant female in the group. Work also suggests that gonadal tissue from juveniles passes through an ovarian stage prior to final sexual maturation as either a male or female (Shapiro and Rasotto, 1993). E. Serially sex-changing, such as in the marine goby. Individuals can repeatedly switch between the sexes. Although no evidence is apparently available for nonreproductive juveniles, adult gobies are known to have an ovotestis (Cole, 1990).

fertilize and then guard eggs deposited on the roof of their nest by females. In contrast, type II males neither build nests nor guard eggs; instead, type II males gain access to gravid females and their eggs by essentially parasitizing the type I male's reproductive tactic—they lie perched outside of or sneak into a type I male's nest and shed sperm in an attempt to compete with the type I male for eggs (Fig. 2).

Type I and II males are clearly polygynous; a single nest may contain several thousand eggs even though a gravid female produces only up to 150–200 eggs each breeding season. Females apparently leave their entire clutch of eggs in a single nest and depart soon after spawning is completed. Only sexually mature adults, embryos, and newly hatched fry are found in nests. Nonreproductive juveniles ranging in age from 5 to 12 months are only found in eel grass beds, where adult morphs are also infrequently found.

Nesting type I males generate three major classes of vocalizations (Bramley and Bass, 1994, Bass et al., 1999). Trains of short-duration (ms range) grunts are produced at intervals of approximately 400 ms during defense of a nest against potential intruder males. Type I males also produce a long-duration in the range of minutes to >1 h) multiharmonic humming sound (Hubbs, 1920) or hum (Ibara et al., 1983). Observations of captive populations of nesting type I males, together with playbacks of natural or computer-synthesized acoustic signals through underwater loudspeakers, show that hums and not grunts can attract females to an artificial nest site (Bramley and Bass, 1994; McKibben and Bass, 1998). A number of parameters influence female recognition and preference for a hum-like signal, including fundamental frequency, intensity, and duration (McKibben and Bass, 1998, 2002). Growls are intermediate in duration (in the range of seconds to minutes) between grunts and hums and have not been studied in captive individuals. Field observations, however, show that growls are only associated with nests containing more than one type I male and are likely to function in some agonistic context (A. Bass, unpublished observations). The fundamental frequency of midshipman hums and growls is temperature-dependent, but hovers close to 90–100 Hz in their natural habitat (Bramley and Bass, 1994). A type I male's hum may function in one or more contexts, including female choice of individual males, male–male competition, or as a beacon to assist either females, type II males, or other type I males in locating suitable nest sites. Type II males, like females, do not produce grunt trains or hums, but infrequently generate low-amplitude isolated grunts in nonspawning contexts.
II. Nonmammalian Hormone-Behavior Systems

TABLE 1
Sexually Polymorphic Traits in Midshipman Fish

<table>
<thead>
<tr>
<th>Trait</th>
<th>Type I male</th>
<th>Type II male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Behavior</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nest building</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Egg-guarding</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Vocalizations</td>
<td>Hums, grunts, growls</td>
<td>Grunts</td>
<td>Grunts</td>
</tr>
<tr>
<td>Fundamental frequency</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Somatic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body size</td>
<td>Large</td>
<td>Small</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Gonad size and body-size ratio</td>
<td>Small</td>
<td>Large</td>
<td>Large (gravid); small (spent)</td>
</tr>
<tr>
<td>Ventral coloration</td>
<td>Olive-gray</td>
<td>Mottled yellow</td>
<td>Bronze (gravid); mottled (spent)</td>
</tr>
<tr>
<td><strong>Vocal motor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vocal muscle traits</td>
<td>Large</td>
<td>Small</td>
<td>Small</td>
</tr>
<tr>
<td>Vocal neuron traits</td>
<td>Large</td>
<td>Small</td>
<td>Small</td>
</tr>
<tr>
<td>Central vocal discharge frequency</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Neuroendocrine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Circulating steroids</td>
<td>Testosterone; 11-ketotestosterone</td>
<td>Testosterone</td>
<td>Testosterone; estradiol</td>
</tr>
<tr>
<td>Aromatase activity</td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>GNRH-POA neuron size</td>
<td>Large</td>
<td>Small</td>
<td>Small</td>
</tr>
<tr>
<td>GNRH-POA number and body size</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>AVT-POA neuron size</td>
<td>Large</td>
<td>Small</td>
<td>Large</td>
</tr>
<tr>
<td>AVT-POA number and body size</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>AVT/isotocin modulation of vocal circuitry</td>
<td>AVT</td>
<td>Isotocin</td>
<td>Isotocin</td>
</tr>
</tbody>
</table>

B. Somatic and Endocrinological Traits

On average, type I males are two- to threefold larger in body size (length and weight) than type II males at the time of sexual maturity. The gonad-to-body-weight ratio (gonosomatic index) is ninefold greater in type II males, who may invest close to 20% of their weight in testes, compared to only 1% in type I males. Gravid females resemble type II males in having a large gonosomatic index, although theirs is even greater. Both gravid females and type II males have a distended and firm belly, reflecting the large size of their gonads. The dorsal body coloration of midshipman, an olive-gray hue, is fairly similar for all three morphs (Fig. 3). During the breeding season, however, the belly of type I males is typically light to dark gray, whereas that of type II males is mottled yellow. Gravid females have a bronze or golden ventral coloration, whereas spent females are more like type II males (Bass and Marchaterre, 1989a; Brantley and Bass, 1994).

Type I males, type II males, and females have contrasting levels of the two principal classes of vertebrate steroid hormones—androgens such as testosterone and estrogens such as 17β-estradiol (Brantley et al., 1993c; Knapp et al., 1999a). Testosterone is detectable in all three morphs, although at progressively lower levels along the continuum: type II males to females to type I males. 17β-Estradiol is detectable among females and a small percentage of type I males, but at much lower levels than testosterone. Teleosts also have a unique form of testosterone known as 11-ketotestosterone. On average, circulating levels of 11-ketotestosterone are about fivefold greater than testosterone in type I males; type II males and females do not have detectable levels. 11-Ketotestosterone levels vary among type I males in accordance with stages of parental care; they are highest for males in nests without any eggs or with eggs
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When compared to males in nests containing mostly hatched embryos (Knapp et al., 1999a), type I males guard the eggs in their nest while continuing to acoustically court and then spawn with females on successive nights (Brantley and Bass, 1994). Hence, shifting levels of androgens throughout the breeding season may reflect a compromise between investment in paternal care vs courtship and nest defense (Knapp et al., 1999a).

The profile of circulating levels of gonadal steroids resembles that shown for other teleosts with alternative male reproductive tactics (Brantley et al., 1993c). In particular, elevated levels of 11-ketotestosterone characterize the displaying type I male morph vs the nondisplaying type II male morph.

1. Vocal Motor Traits

Sex- and morph-specific vocal behaviors are paralleled by a divergence in neurobiological traits ranging from the size of the sound-producing muscles to the rhythmic firing properties of vocal neurons (Bass, 1992, 1998). The vocal organ of midshipman fish consists of a pair of sonic muscles attached to the lateral walls of their swimbladder. These muscles are the functional analog of the laryngeal and syringeal muscles of terrestrial vertebrates and apparently share a common embryonic origin from occipital head somites (Bass and Baker, 1997). A sixfold greater vocal muscle–body weight ratio in type I males, compared to type II males and females, is paralleled by a fourfold greater muscle–fiber number and fivefold greater muscle–fiber diameter (Brantley et al., 1993b). Dimorphisms in muscle fibers extend to the subcellular level, including the width of Z-lines, which represent the points of overlap of the thin actin filaments of myofilibrils; the branching of the sarcoplasmic reticulum, which is the principal site for calcium exchange; the density of mitochondria; and the concentrations of metabolic enzymes (Brantley et al., 1993c). In particular, elevated levels of 11-ketotestosterone characterize the displaying type I male morph vs the nondisplaying type II male morph.
II. Nonmammalian Hormone-Behavior Systems

The firing frequency of pacemaker neurons is matched 1:1 with that of sonic motoneurons and, in turn, to the fundamental discharge frequency of the vocal motor volley as recorded intracranially from the occipital nerve roots. A single pacemaker neuron innervates the neurons in both motor nuclei, consistent with the hypothesis that their role is to synchronize the firing of motoneurons positioned on both sides of the brain. This in turn, leads to the simultaneous contraction of both sonic muscles at a fundamental frequency that establishes the fundamental frequency of vocalizations. Hence, there is a direct relationship between the rhythmic patterned output of a hindbrain pacemaker-motoneuron circuit and the physical attributes of vocalizations.

The pacemaker-motoneuron circuit fires at a fundamental frequency that is approximately 15–20% higher in type I males than in type II males and females, which parallels sex differences in the fundamental frequency of natural vocalizations (Bass and Baker, 1990, 1991; Brantley and Bass, 1994). The swimbladder itself may contribute to the spectral properties of hums and grunts by influencing the higher-frequency components (harmonics) of the signals.

Intracellular injections of horseradish peroxidase (HRP) show that the somata, dendrites, and axons of vocal neurons are up to threefold larger in magnitude in type I males than in females and type II males (Bass and Baker, 1990); electron microscopic studies show comparable polymorphisms at the level of sonic neuromuscular junctions (Fluet and Bass, 1990). The use of low-molecular-weight tracers (e.g., biocytin and neurobiotin) that are transported transneuronally in the vocal motor system, have permitted a delineation of the entire complement of neurons in the hindbrain vocal circuit of midshipman fish (Bass et al., 1994). Thus, the application of biocytin or neurobiotin crystals to the cut end of a single sonic nerve results in a mapping of the entire bilateral extent of the pacemaker-motoneuron circuitry. The biocytin studies extend and confirm, for a large sample size, our initial demonstration of sexual polymorphisms using intracellular HRP injections.
(Bass et al., 1996). These analyses also demonstrate sexual polymorphisms in a previously unidentified group of ventral medullary neurons just rostral to the sonic motor nucleus that extensively link the pacemaker-motoneuron circuitry across the midline (Bass et al., 1996). There are no significant adult sex- or morph-specific differences in motoneuron number after controlling for the effects of body size (Bass and Andersen, 1991; also see Grober et al., 1994). Hence, inter- and intrasexual variation in the organization of the vocal motor system is dependent on a divergence in the morphophysiological properties of individual neurons. Most important, these studies together show that the morphological and physiological traits of a rhythmically active vocal-pattern generator in the hindbrain of type II males and females are similar to one another but strikingly divergent from that of type I males. This pattern of structural traits reflects the divergence in vocal behaviors exhibited by type I males vs type II males and females.

The transneuronal biocytin method for mapping was exploited to trace the ontogeny of the vocal circuit. These studies revealed that sexual maturation of the type I male’s mate-calling circuit parallels the ontogeny of its sonic muscle (Brantley et al., 1993b; Bass et al., 1996). Hence, among juvenile type I males, the motoneuron size and volume of the sonic motor nucleus increase most during a stage prior to sexual maturation that parallels a fourfold increase in the number of sonic muscle fibers. A more modest growth phase for motoneurons is coupled to the greatest increase in pacemaker neuron size at a stage coincident with the onset of sexual maturity that parallels a fivefold increase in the size of sonic muscle fibers. Ventral medullary neurons show similar growth increments during both stages. It is important to note that juvenile to type II male and juvenile to adult female transformations are accompanied by no or little change in vocal neuron or muscle size. Transneuronal transport of biocytin and neurobiotin has shown that male dimorphisms in motoneurons may arise very early in development, soon after fry become free swimming (Knapp et al., 1999b). Together, the ontogenetic studies demonstrate alternative growth trajectories for the neurons and muscles that determine morph-specific vocal behaviors.

An individual fish’s age can be determined by counting the number of growth increments that appear on its otoliths, much like the rings in the trunk of a tree. The aging of saccular otoliths shows that type I males, type II males, and females overlap in age, although type II males and females can become sexually mature at an earlier age and smaller size (Bass et al., 1996). Together with the delineation of vocal neuron ontogeny, the results support the hypothesis that alternative male morphs in midshipman fish adopt nonsequential, mutually exclusive growth patterns during their first year of life.

C. Neuroendocrine Traits

The two neuropeptides that have been most commonly studied in the context of sexual plasticity among teleost fish, including midshipman fish are gonadotropin-releasing hormone (GnRH) and arginine vasotocin (AVT) (Foran and Bass, 1999; Bass and Grober, 2002).

1. Gonadotrophin-releasing Hormone

It appears, at least for mammals, that the proper development, adult location, and physiological function of GnRH preoptic cells are necessary for adult reproductive function. Disproportionate variation in any of these parameters often leads to reproductive dysfunction. In contrast to the restrictive organization of GnRH brain nuclei required for normal sexual development in mammals, variability in the adult status of the GnRH centers in the forebrain’s preoptic (POA) in fish and may play a role in the production of the wide range of sexual systems (Foran and Bass, 1999; also see Francis et al., 1993, Halpern-Sebold et al., 1986) and, as discussed later, sex reversal (Grober and Bass, 1991).

POA GnRH neurons directly innervate the pituitary in teleosts, unlike most other vertebrates in which a blood portal system mediates the access of GnRH to the anterior pituitary. GnRH can then modulate the release of gonadotropins from the anterior lobe of the pituitary gland, which, in turn, controls the secretion of gonadal steroid hormones. The POA-pituitary-gonadal axis is thus considered to regulate the expression of secondary sex characteristics via the influence of gonadal steroids on sexual differentiation of steroid-sensitive structures such as those of the vocal motor system. Halpern-Sebold et al. (1986) first showed in platyfish, which also have two male morphs that differ in age at sexual
maturity, a cascade of GnRH-gonadotropin events along the brain-pituitary axis that are temporally linked to the onset of sexual maturation in each morph. Studies in midshipman fish are consistent with this developmental scenario. Both immunocytochemical and in situ hybridization studies have identified the position and extent of GnRH in the forebrain of midshipman fish (Grober et al., 1994, 1995; Foran et al., 1997). There are increases in the number and size of POA-GnRH neurons at the time of juvenile to adult transformations (Fig. 4). However, unlike vocal motor neurons, there is a convergence in GnRH traits among all sexually mature individuals; type I males, type II males, and adult females have a similar GnRH phenotype (number and cell size), consistent with all morphs being sexually mature. Together, the data support the hypothesis that transformations in the organization of the juvenile POA-pituitary-GnRH-gonadotropin axis could initiate a cascade of events leading to sexual maturation.

2. Arginine Vasotocin

Neuropeptides of the vasopressin-oxytocin family (AVT-isotocin in fish) have been implicated in the modulation of a variety of reproductive-related behaviors, including vocalization (Moore, 1992; Boyd, 1997; Goodson and Bass, 2001). As in other vertebrate classes (Moore and Lowry, 1998), AVT-containing neurons are localized to the POA in midshipman fish (Foran and Bass, 1998; Goodson and Bass, 2000a). AVT-immunoreactive (-ir) neurons are larger in type I males and females than in type II males. Unlike the case for GnRH neurons (Grober et al., 1994), AVT cell-size differences among morphs can be explained by differences in body size. However, as is the case for GnRH phenotypes (reanalysis of Grober et al., 1994; data by Foran and Bass, 1999), type II males have more AVT-ir neurons per gram body weight than do type I males (on average almost a sixfold difference).

Ultimately, functional interpretations of the significance of morph- and sex-specific differences in neuropeptide phenotypes rely on neurophysiological studies. A series of studies have now begun to assess AVT influences on the midshipman fish’s vocalization circuitry. An immunocytochemical study in midshipman fish has provided a complete mapping of AVT-ir fiber pathways and terminal fields (Goodson and Bass, 2000a). Sites of dense AVT-ir include the anterior hypothalamus (ventral tuberal nucleus) and the midbrain (paralemniscal tegmentum adjacent to the lateral lemniscus). A rhythmic vocal motor output from the hindbrain pacemaker circuitry can also be evoked by electrical stimulation in these same brain regions (which are also linked to the central acoustic circuitry; Bass et al., 2000; Goodson and Bass, 2000a, unpublished observations). Whereas picospritzing AVT into the ventral tuberal hypothalamus of type I males inhibits electrically evoked vocal motor output from this region, a V1 receptor antagonist facilitates such output.
In contrast, isotocin, another nine-amino-acid peptide that differs from AVT in only one amino acid, is the active substance in type II males and females. Other studies in type I males alone show that both AVT and vasoactive intestinal polypeptide (VIP) influence electrically evoked vocal output from the midbrain tegmentum by reducing the number of vocal motor bursts obtained and increasing response latency; VIP also leads to a reduction in the duration of vocal bursts (Goodson and Bass, 2000a,c). Antagonists have no effect on vocal output evoked from the midbrain, suggesting that AVT and VIP action are not necessary for normal call initiation or vocal motor patterning but rather operate in a condition-dependent manner.

3. Gonadal Steroids and Aromatase

Anatomical and physiological studies of vocal motor traits show that androgens contribute to the masculinization of the type I male vocal circuit among juvenile males; androgens can induce an elevation of the fundamental discharge frequency of the pacemaker-motoneuron circuit and an increase in the size of vocal neurons and muscles in juveniles (Brantley et al., 1993a; Bass, 1995).

One enzyme important to androgen metabolism in the brain is aromatase, which converts testosterone to estrogen. Teleost fishes have the highest known levels of aromatase (Callard et al., 1990). Studies in midshipman fish have identified male morph- and sex-specific patterns of aromatase activity (Schlinger et al., 1999). Aromatase levels were determined for three grossly dissected brain regions: (1) the olfactory bulb and telencephalon, inclusive of the preoptic area; (2) an expansive region including the diencephalon, midbrain, and cerebellum; and (3) a vocal hindbrain region defined approximately by the extent of the ventral medullary-pacemaker-motoneuron circuit identified by Bass et al. (1994). The levels are highest in the first two regions, with a modest difference in region 2 between females and type I males. The most striking findings are for the vocal hindbrain where levels are three- to fivefold higher in type II males and females than in type I males. The results suggest that the aromatase-driven conversion of testosterone to estrogen may effectively protect the vocal hindbrain of type II males and females from the masculinizing effects of testosterone on vocal motor traits that characterize type I males. Studies based on the cloning of a partial cDNA sequence for midshipman fish aromatase show the anatomical distribution of brain aromatase and support the biochemical studies of dimorphisms in the vocal hindbrain region (Forlano et al., 2000).

D. Summary Comments

In summary, our studies of adult reproductive morphs in midshipman fish show that (modified from Bass, 1992):

1. Alternative mating tactics among sexually mature males are paralleled by alternative phenotypes for the neurons that determine tactic-specific types of vocal behavior—type I singing males have a suite of vocal motor traits distinct from those of sneak-spawning type II males and of females.

2. Reproductive maturation is not obligatorily linked to the expression of neuronal secondary sex characteristics—sexual maturation of type II male or female morphs does not involve the expression of type-I-male-specific traits.

3. Although type II males and females have morph-specific spawning tactics, they are convergent in a number of traits, including vocal motor phenotype, neurochemical phenotypes (isotocin modulation of vocal motor circuitry), brain aromatase levels, circulating plasma steroids (testosterone), large investment in gonad, absence of parental care, and dependence on type I males for nest construction.

4. Alternative male morphs adopt nonsequential, mutually exclusive growth patterns during their first year of life.

One of the key issues in the midshipman system, and in others including bluegill sunfish and salmon (see Gross, 1996), is whether the male morphs are genetically determined to develop at different rates and thus into different types, or whether there is some social or environmental modulation of the development of variable sexual phenotypes. There is a suggestion that the density of animals during the juvenile phase may determine the type of male that develops in the midshipman fish (Foran, 1998). Similar data have been obtained for a European blenny that exhibits a
similar system of two alternative male morphs (Almada et al., 1994, 1995). In the Peacock blenny, two research groups independently began studies on populations of these fish that recruited to sandflats that were modified to support aquaculture. Both populations of this blenny exhibited the same alternative male morph at the same time in response to similar environmental modifications, namely changes in population density. These data are more consistent with phenotypic, environmentally driven plasticity generating the variation in male function rather than a genetic mechanism. One difference between the blenny and the midshipman is that there appears to be a difference between the blenny and the midshipman at which males become fixed in their specific morphology (see Bass et al., 1999). The blenny use one tactic when small (sneaking and female mimicry) and then switch to the alternative tactic when large (aggressive nest holding) (Oliveira et al., 2002). Another example of the likely effects of population density on reproductive tactics among males is a Peacock blenny population studied in southern Portugal. Here, large males form nest aggregations that are so dense that the territories are virtually absent, resulting in sex-role reversal with respect to courtship (Almada et al., 1994, 1995). Although males retain the capacity to court when they have no eggs in the nest, they usually assume a passive role in courtship, the females compete aggressively among themselves and actively court males, displaying a female nuptial coloration (Almada et al., 1995). Smaller and younger males adopt female nuptial coloration and female courting behavior toward nest-holder males as a means of entering the nest during or shortly after the spawning of a female ( Goncalves et al., 1996). We know that there are major differences in the physiology of these male morphs and that differences between morphs are mediated in part by androgenic steroids (Oliveira et al., 2002) and forebrain neuropeptides (George et al., 1999).

The midshipman fish story highlights the potential level of sexual plasticity exhibited among fish with multiple male morphs and the multiple levels at which the male morphs diverge from one another and from females. The remainder of this review focuses on fish species that exhibit even more extreme degrees of sexual plasticity, including fish that show adult sex change and thus switch from the entire suite of characters of one sex to those of the other.

V. PRIMACY OF THE BEHAVIOR—BRAIN CASCADE: REVERSIBLE SEX CHANGE IN GOBIES

Of the many fish species that exhibit socially mediated sex reversal (Smith, 1975), the marine goby, Trinma okinawae, is one of only a handful of species known to change sex more than once and in either direction (see Fig. 1E) (Kuwamura et al., 1994; Sunobe and Nakazono, 1993). These changes in sexual phenotype are under strict social control and involve rapid changes in the production of sex-specific mating behavior (Sunobe and Nakazono, 1993). Male-typical behavior includes the occupation of a nest, aggression toward other males, and a skipping courtship directed toward females. Female-typical behavior is limited to visiting the nests of males. The presence of these sex-typical behaviors and the anatomy of the gonads, accessory gonadal structure, and genital papillae are used as indicators of complete sex change. The accessory gonadal structure is unique to this family of fish (Gobiidae) and is a reliable characteristic of male sexual identity (Sunobe and Nakazono, 1993). The genital papilla is a sexually dimorphic structure that aids in the delivery of sperm or eggs and also provides a clear indication of sexual state in this species (Grober and Sunobe, 1996).

By using established methods for manipulating small social groups under laboratory conditions (removing or replacing the large male, or both; Sunobe and Nakazono, 1993), we evaluated changes in the gonads, accessory gonadal structure and genital papilla in individuals that experienced male to female (M→F), female to male (F→M), and female to male to female (F→M→F) sexual transformations. During these transitions, behavioral changes are rapid (within minutes to hours of the social trigger), and the time for a complete sex change (behavioral and morphological) depends on the direction and sequence of the change (Grober and Sunobe, 1996). F→M transitions are associated with increased production of male-typical behaviors, whereas M→F transitions involve the loss of these same behavior patterns and the onset of a female-specific behavior (nest visitation). During sex change, the genital papilla and gonads show correlated changes that are consistent with the reproductive behavior of each individual (Grober and Sunobe, 1996). The degree to which the opposite-sex gonad regresses depends on the
time since the onset of sex change (Grober and Sunobe, 1996). As predicted by the male-specific activity of the accessory gonadal structure, this organ regresses in females and reexpands in males (Grober and Sunobe, 1996; Sunobe and Nakazono, 1993).

As a first approach to understanding the neuroendocrine mechanisms that control the sex-change process in this species, immunocytochemical methods were used to examine possible changes in the size of AVT-producing cells in the POA following single and serial sex changes. Females, regardless of their prior sexual status, have significantly larger AVT forebrain cells than males and the area of these cells is greatest following M→F transitions, whereas F→M transitions result in a significantly smaller mean cell size. The reversal of a subset of the F→M males back to females results in a return to the larger female-specific mean cell size, and these differences are independent of body size (Grober and Sunobe, 1996). These results are noteworthy for the speed of the changes and their reversibility in fully functional adults. The primary goal for this study was to correlate changes in forebrain AVT cells with changes in a variety of sexual characteristics that were indicative of sex change. As a result, fish were not sampled until they were completely sex reversed (e.g., had spawned successfully as the opposite sex). The advantage of this approach was that it provided an assurance that sex change had occurred. This led to two conclusions: (1) AVT forebrain centers appear to be organized in a sex-specific manner and, 2) sequential transitions between the sexes involve reversible neuroendocrine changes, as well as modifications of a wide range of sex-specific characteristics. The disadvantage of this approach was that it did not provide any indication of how quickly the neurochemistry could change, or an indication of the temporal relationship between behavioral, neurobiological, and gonadal changes. These issues can be addressed in a Caribbean labrid fish, the bluehead wrasse, that also exhibits socially controlled sex change.

VI. TEMPORAL AXIS FOR SOCIAL MODULATION OF AVT PHENOTYPE IN THE BLUEHEAD Wrasse

The bluehead wrasse, Thalassoma bifasciatum, has been the subject of intensive study regarding the evolution and ecology of sex change (Warner, 1975; Warner and Hofman, 1980; Warner and Swearer, 1991), and has been the focus of studies addressing how behavior and neuroendocrinology regulate the sex-change process (Godwin et al., 1996; Grober et al., 1991; Grober and Bass, 1991). Populations are composed of two distinct color phases: terminal-phase (TP) males have blue heads, a black-white-black banding behind the head, and green bodies, whereas initial-phase (IP) males and females show a similar yellow coloration. All individuals first reproduce as either IP males or females. TP individuals (supermales) arise from one of two irreversible processes: (1) sex change in an IP female, or 2) role change in an IP male (see Fig. 1C). In addition to the striking differences in color between IP and TP individuals, there are also dramatic differences in body size, reproductive behavior, and fecundity (Petersen et al., 1992; Warner and Schulz, 1992). TP males aggressively defend breeding sites, gain exclusive access to females, and as a result may mate with up to 100 females per day. IP males show no breeding-site defense and thus are less aggressive than TP males; they mate in large aggregations (~50 IP males per female) or they sneak-mate with a TP male–IP female pair. This difference in access to females may explain the much higher gonosomatic index (gonad weight/body weight) in IP males, which is probably a response to the intense sperm competition associated with mass spawnings. Finally, IP females show no breeding-site defense and little aggression toward IP fish; and they visit TP-male or IP-mass-spawning sites Fig. 5 (Fig. 5).

The removal of the TP male from a social group acts as the behavioral trigger for both sex and role change (Warner and Swearer, 1991). Within minutes after the removal of the TP male, the largest IP fish (either male or female) initiates behavioral sex-role change, as evidenced by increased aggression toward other large IP fish and courtship directed toward smaller females (Godwin et al., 1996). The increased aggression probably leads to social dominance, which may be a key factor in the inhibition of sex-role change in subordinates. This functions to limit the number of fish that undergo sex-role change after the loss of a TP male. Gonadal sex reversal in this species follows this rapid behavioral change and can be completed in 7–10 days. This sequence of events highlights the role of social cues
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Initial phase (IP) Transition Terminal phase

Sex change Role change

FIGURE 5 Overview of the life history of the bluehead wrasse, Thalassoma bifasciatum, a sex-role-changing protogynous fish found in the Caribbean Sea. Both initial-phase (IP) males (via role change) and females (via sex change) can transform into dominant territorial terminal-phase (TP) males. IP males obtain fertilizations by either mating in large aggregations of IP males or sneak-mating during pair spawns of IP females–IP males. This sexual transformation is mediated by social interactions and involves changes in coloration, as well as a host of other sexually polymorphic characteristics.

in the regulation of sexuality in individual members of the social group.

Both sex and role changes in the bluehead wrasse are associated with significant increases in the number of POA GnRH cells (Grober and Bass, 1991), and exogenous androgen implants in IP males and females can trigger increases in GnRH cell number to levels seen in TP males, but have no effect on GnRH cell number in TP males fish (Grober et al., 1991). These early studies have been replicated in other species of sex-changing fish with similar results (Elofsson et al., 1997, for a protandrous fish; Elofsson et al., 1999, for a protogynous fish) and set the stage for work that has examined the temporal relationship between changes in the POA-AVT phenotype and transformations in behavioral phenotypes.

A. Arginine Vasotocin

Studies in the bluehead wrasse used in situ hybridization to identify cells that were labeled with a degenerate probe that is complementary to AVT mRNA (Godwin et al., 2000). This technique allows an examination of the regulation of AVT production over the course of sex change. These studies, conducted in the field by manipulating naturally occurring social groups, show that both the number of cells expressing AVT mRNA and the amount of expression per cell increase in a similar manner from females to IP males to TP males. These differences are not due to body size allometry. Rather, TP males appear to have more AVT-mRNA-producing cells and these cells have a higher per cell message production. Godwin et al. (2000) sacrificed fish at 2, 3, and 5 days after removal of the TP male from a social group and quantified the level of AVT mRNA expression in each fish. Compared to unmanipulated females, levels of AVT mRNA expression increase progressively 2 and 3 days after the removal of the TP male. After 3 days, there is a significant increase in mRNA production relative to preremoval levels, resulting in an overproduction of mRNA compared to what is found in established TP males. Moreover, the levels begin dropping after 4 days, suggesting that the increase in AVT mRNA expression can be short-lived and may only be required during the period of social instability, when overproduction of behavior may be critical to acquiring TP male status. The correlation between AVT expression and behavior is striking and suggests a possible causal link between increased AVT mRNA and increases in TP-male-specific behavior patterns. Comparative support for these results comes from work on the Hawaiian congener, T. duperrey, in which TP males have significantly more and larger AVT-mRNA-producing cells than either IP sex (Grober, 1998). This difference parallels the results from the bluehead wrasse and suggests that TP males
regulate AVT production in a different way than the IP sexes.

Finally, other studies have investigated the effects of exogenous treatment with gonadal steroids on AVT production in the bluehead wrasse. In control fish that were either housed separately or given sham implants, TP males have more peptide-producing cells than either IP sex (McIntyre, 1998). Steroid implants are effective in significantly elevating circulating levels of 11-ketotestosterone. However, these increased hormone levels do not have a significant effect on the number or size of AVT cells in IP males and females. These results indicate that the changes in the brain are not driven by gonadal hormones and support the idea that brain changes precede gonadal transformation. Conversely, elevated levels of 11-ketotestosterone in TP males have a negative effect on AVT peptide production as evidenced by a significant decrease in the number of labeled cells in steroid-treated TP males. This suggests that the higher number of AVT cells in TP males is not a direct result of the increase in androgens that accompanies sex change.

These findings provide an interesting contrast to our earlier work on GnRH (Grober and Bass, 1991; Grober et al., 1991). For both GnRH and AVT, TP males have more and larger cells than IP males and females, indicating a consistent and robust sexual polymorphism in brain areas that are critical for the control of reproductive behavior and physiology. However, androgen implants have much different effects on GnRH than on AVT cells. 11-Ketotestosterone implants increase the number of GnRH cells in both IP sexes to levels characteristic of field-collected TP males, whereas androgen implants have no effect on GnRH cell number in TP males. Both of these results are the exact opposite effect from the other AVT studies discussed. One possible resolution of this contradiction in regulation concerns the function of each peptide and, hence, the temporal nature of its expression. AVT may be primarily involved in producing early changes in behavior, which precede changes in the gonad and thus the ability to produce significant levels of androgens. Conversely, GnRH may primarily be involved in the regulation of gonadal function, and this process occurs later in the sex-change process and may be more sensitive to steroidal regulation.

An integration of available results suggests the following temporal model of socially controlled sex change. Decreases in the amount of aggressive behavior received by the largest subordinate result in rapid changes in brain peptide expression (e.g., AVT; Godwin et al., 2000; NPY, Kramer and Imbriano, 1997). These early changes in the brain permit the production of higher rates of male-typical behavior in the early stages of sex change. The combination of the decreased aggression received and the increased production of male behavior patterns triggers a cascade of changes in the hypothalamic-pituitary-gonadal axis. These would include independent changes in the brain (e.g., the GnRH axis), gonad anatomy and physiology, gonadal steroid levels, and body coloration. This general model conforms quite well to an empirical model that is being tested in ongoing studies of the bluebanded goby. Thus, removal of a dominant male results in rapid increases in aggressive behavior and a cessation of submissive behavior in the dominant female (Reavis and Grober, 1999). These early changes in behavior lead to further changes in forebrain AVT cells (Reavis and Grober, 1999), gonad and accessory gland anatomy (Marxer-Miller et al., 2002), and the structure of the external genitalia (Carlisle et al., 2000). This work can lead to an integrative model of sex change based on early behavioral changes with other systems mapped onto it (e.g., external genitalia, gonad anatomy and physiology, steroid hormones, and neuropeptides; Reavis and Grober, 1999).

VII. SUMMARY

The rapid diversification of the advanced fishes has been associated with the ability in these fishes to express a wide range of sexual phenotypes. These phenotypes can be generated by genetic change over time or the expression of phenotypic variants in response to environmental or social input. Differences between sexual morphs extend to almost all levels of biological organization and can include behavior, coloration, signalling structures, brain chemistry and anatomy, endocrinology, body size, gonad size and anatomy, accessory gonadal structures, and external genitalia. Many or most of these traits are expressed as a suite of characteristics that are typical of a given sex or sexual morph, and transitions between the morphs involve orderly transitions of the host of associated traits. More generally,
there appears to be a high degree of neurochemical and neuroendocrinological conservation associated with the production of sexual polymorphism across the vertebrate taxa (Foran and Bass, 1999; Bass and Grober, 2002). AVT and AVP treatment induces sex-specific reproductive behaviors in a range of vertebrate species, including the spawning reflex in fishes, amplexus in newts, and pair-bonding and parental care in voles (Carter et al., 1995; Egami, 1959; Macey et al., 1974; Moore, 1992). Perhaps the most striking comparison is between our results in fishes, which show a strict correlation between sexual behavior and AVT neurochemistry, and studies showing differences in forebrain peptidergic cell populations in human males who exhibit heterosexual vs homosexual (Swaab and Hofman, 1990) or transsexual (Zhou et al., 1995) behavior.

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