# Hormones and Sexual Behavior of Teleost Fishes

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**SUMMARY**

Fishes are an excellent group for studying the mechanisms through which hormones modulate the expression of sexual behaviors in vertebrates. First, they have radiated virtually throughout all aquatic environments and this is reflected in an extraordinary diversity of mating systems and reproductive behaviors. Second, many species present a remarkable plasticity in their sexual displays, as exemplified by fishes that change sex or that adopt more than one reproductive tactic during their lifetime, and this plasticity seems to be mediated by hormones. Third, the fish neuroendocrine system is well conserved among vertebrates and the mechanisms of hormonal action in behavior are likely to share similarities with those of other vertebrates. We review the role of hormones and neuropeptides in the modulation of fish sexual displays. We also try to identify research areas in fish behavioral endocrinology that have the potential to be further developed (e.g., the role of hormones in the regulation of female sexual behavior) and some of the technological developments that promise to increase our knowledge in this field in the near future.

## 1. THEORETICAL CONSTRUCTS: APPETITIVE AND CONSUMMATORY PHASES

In species with sexual reproduction, ‘sexual behavior’ may be generally defined as the set of behavioral acts directed towards the goal of producing offspring (see Munakata & Kobayashi, 2009 for an extended discussion). Classifying these behavioral acts into categories can be useful for descriptive purposes but in fishes the diversity of sexual displays challenges this classification. A widespread dichotomy applied in the context of sexual behavior is the appetitive/consummatory division and we begin by discussing its usefulness in fish behavioral endocrinology.

The appetitive/consummatory division stemmed from the observations of early ethologists, who realized that, in a goal-directed behavioral sequence, behaviors are usually more variable during the initial stages of the sequence and more stereotyped towards its end. To account for this variation, these researchers suggested that an initial appetitive phase, defined as the phase of searching towards the goal, can be distinguished from a final consummatory phase, defined as the stage when the goal is reached (Sherrington, 1906; Craig, 1917). Although this distinction is still widely applied in studies investigating the mechanisms of behavior, there is an ongoing debate on the usefulness of these terms. In a recent review, Sachs (2007) identified some problems in the current use of the appetitive/consummatory dichotomy. These include the difficulties in defining the boundary between the two phases and assigning a particular behavioral element to the appetitive or consummatory stage, and the historical link of these terms to early ethological models that have since been abandoned (e.g., the Lorenz hydraulic model (Lorenz, 1950)). In a reply, Ball and Balthazart (2008) argued in favor of the ongoing use of this terminology using two assumptions: (1) the appetitive/consummatory division is useful in mechanistic studies of animal behavior as it captures an important and real variation in behavioral displays during goal-seeking sequences. The authors agree that defining strict boundaries between the appetitive/consummatory phases may sometimes be difficult and that some overlap should be taken into account when these terms are to be applied, but also suggest that this difficulty is common to other definitions in biology and not serious enough to overcome their usefulness. (2) The link between the appetitive/consummatory terminology and ancient ethological models is probably more historical than conceptual. The authors argue that theories underlying many scientific terms change over time without diminishing the present value of the terms. Likewise, the modern use of the appetitive/consummatory distinction is no longer conceptually linked to the early ethological models of motivation and reflects actual theories of behavior.

**Hormones and Reproduction of Vertebrates, Volume 1—Fishes**

When studying the role played by hormones in the regulation of fish sexual behavior, the use of the appetitive/consummatory dichotomy raises two questions: (1) this distinction has been mainly applied to studies in birds and mammals – can fish sexual behavior also be partitioned into appetitive/consummatory phases? (2) If so, is this distinction useful when trying to understand the hormonal regulation of fish sexual behavior? The answer to the first question seems to be affirmative. Many fishes display a set of behaviors anticipatory to copulation/spawning that fits the definition of appetitive behaviors (i.e., behaviors displayed during the searching–attracting phase for a sexual partner, typically more variable than consummatory behaviors). These include the establishment and defense of a breeding territory, the preparation of a spawning site (e.g., nest), and the expression of courtship displays. Consummatory (goal-reaching) behaviors include copulation in internal fertilizers or the spawning reflex in external fertilizers and are usually highly stereotyped. In some species the distinction and quantification of these behaviors is clear-cut, although the appetitive/consummatory division in the fish literature on sexual behavior is rarely employed. The answer to the second question, on whether this distinction is useful, is more debatable. The division of a continuous behavioral sequence directed towards the goal of mating into appetitive/consummatory phases is artificial and represents only one of many possibilities of partitioning sexual behavior sequences. One important point to consider in this regard is that the original division of sexual behavior into appetitive/consummatory phases made no mechanistic assumptions and merely tried to capture the natural variability of sexual behavior sequences. Some of the later criticism directed at the use of this division stems from the work of Frank Beach, who postulated the existence of two separate mechanisms for sexual processes: a sexual arousal (appetitive) mechanism and a copulatory and ejaculatory (consummatory) mechanism (Beach, 1942). However, the empirical evidence for these separate mechanisms is still not clear, and giving a mechanistic rather than simply descriptive value to the *a priori* appetitive/consummatory distinction seems to be the origin of some of the later criticisms of the use of these terms (e.g., Sachs, 2007).

As an example, an overview of the published literature on the neuroendocrine regulation of sexual behavior in rats, one of the most thoroughly investigated vertebrate models in this field, reveals some of these difficulties. In a review, Everitt (1990) suggests that the medial preoptic area (mPOA) of the hypothalamus is involved in the regulation of consummatory but not appetitive aspects of sexual behavior, whereas the ventral striatum regulates appetitive but not consummatory elements of sexual behavior. However, subsequent experiments have shown that the mPOA is also involved in appetitive aspects of sexual behavior in this species (e.g., Paredes, Highland, & Karam, 1993) and the proposed difference in brain areas regulating appetitive and consummatory behaviors has been questioned (Hull et al., 1999; Paredes, 2003). Further, some studies have revealed that brain aromatization of testosterone (T) has activational effects in both appetitive and consummatory aspects of male rat sexual behavior (e.g., Roselli, Cross, Poonyagariyagorn, & Stadelman, 2003), while others have shown that aromatization of T is necessary to activate consummatory behaviors (copulation) but not appetitive behaviors (Vagell & McGinnis, 1997).

Thus, the available data on the neuroendocrine regulation of sexual behavior in vertebrates is still scarce and partially contradictory, and only a very limited number of species have been investigated in detail. It is thus not possible to generalize on Frank Beach's original proposal or to predict that the appetitive/consummatory division will be more useful than others when trying to understand general mechanisms of hormonal regulation of behavior. For now, it seems more reasonable to apply *a priori* divisions of sexual behavioral sequences that have a descriptive rather than predictive value. In some cases, the appetitive/consummatory division may be considered to be the most helpful for capturing the natural variation in behavioral sequences but other classifications will be more useful in other cases. This seems to be particularly true for fishes, which present wide variation in the patterns of sexual behavior.

## 2. PATTERNS OF SEXUAL BEHAVIOR

Fishes have radiated virtually throughout all aquatic habitats and more than 31 500 species have so far been described (Froese & Pauly, 2009). This large number of species and the wide variation in the ecological conditions of their habitats have resulted in numerous morphological, physiological, and behavioral adaptations. This fact is reflected in their patterns of sexual behavior, which are extraordinarily diverse, ranging from species with nearly absent precopulation/prespawning behaviors to species with elaborate territorial, nest-building, and courtship displays. Although the patterns of sexual behavior in fishes have been reviewed elsewhere (e.g., Breder & Rosen, 1966; Thresher, 1984), a short overview of this diversity is presented here.

### 2.1. Defending and Preparing a Spawning Site

The defense of reproductive resources seems to be the main cause of territoriality in fishes. In a comparative analysis using datasets for freshwater fishes of Canada (Scott & Crossman, 1979), marine fishes of eastern Canada (Scott & Scott, 1988), and tropical fishes (Thresher, 1984), Grant (1997) showed that territories for reproduction are more common than feeding territories. Further, in a comparative analysis between tropical and Atlantic temperate fish fauna, Almada, Henriques, and Gonçalves (1998) demonstrated that the occurrence of permanent territories decreases with latitude and this is likely to relate to the more seasonal reproductive patterns of temperate fish fauna.

Territoriality associated with reproduction is uncommon in pelagic spawning, the main mode of reproduction in teleosts (Breder & Rosen, 1966; Balon, 1984; Thresher, 1984; Grant, 1997). However, in species with demersal eggs, reproduction often depends on the successful acquisition of a breeding territory. This is usually the first stage of the reproductive cycle but there are examples where territories are only established after mating and used not as spawning grounds but for the defense of eggs or juveniles. Breeding territoriality is associated with the defense of resources important for reproduction. These can be nesting structures or substrate, food, or shelters. Male territoriality is prevalent in fishes (e.g., Almada et al., 1998) but many examples of shared territoriality by a male and a female (e.g., Loiselle, 1977), cooperative territoriality (e.g., Taborsky, 1984), and even female territoriality (e.g., Tautz & Groot, 1975) have been described. Males can also defend females rather than territories and this has been documented both in pelagic and demersal spawners. For example, in the triggerfish *Sufflamen chrysopterum*, females compete over and defend territories (presumably for food or shelter) and males guard between one and three females. Males abandon the territories if females are experimentally removed, suggesting that males are guarding females rather than defending a territory (Seki et al., 2009).

When territories are acquired prior to mating, two classes of reproductive behavior can be identified: agonistic displays used to acquire and defend reproductive territories and nest preparation/building behaviors. Within the same species, agonistic behaviors are displayed in many contexts and aggressive patterns exclusively associated with breeding territoriality seem to be rare or nonexistent. The enormous diversity of agonistic displays in fishes makes it difficult to search for 'universal' patterns. Examples of behavioral motor patterns included in the aggressive repertoire of teleosts include charging, butting, chasing, lateral and frontal displays, opercular flare, fin spreading, circling, pendeling (back and forth swimming between two males in frontal position), and mouth-to-mouth fighting (Figure 7.1(a)). Submissive behaviors include fleeing, head-up positions, and body tilting (Figure 7.1(b)). Signals in other sensorial modalities (electrical, acoustic, chemical) may also be used during aggressive interactions. As an example, in the Mozambique tilapia (*Oreochromis mossambicus*) territorial males store urine and release it in pulses during agonistic interactions with other males. As the olfactory potency of the urine correlates with the male's social status, males may be signaling their status to other males using chemical cues in the urine (Barata, Hubbard, Almeida, Miranda, & Canário, 2007; see also Chapter 9, this volume).

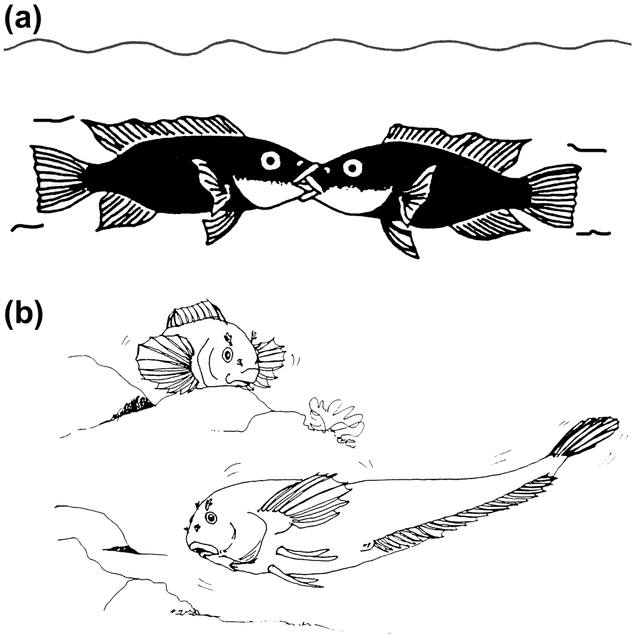

**FIGURE 7.1** Examples of agonistic displays in fish. (a) Mouth-to-mouth fighting in the Mozambique tilapia (*Oreochromis mossambicus*), a high-intensity aggressive display. (b) Dorsal presentation in the blenny *Parablennius sanguinolentus parvicornis*, a submissive posture. Reproduced from Santos and Barreiros (1993).

Fishes that secure breeding territories also may perform behaviors associated with the preparation of spawning or nesting structures. This is more frequent in species with parental care, and the complexity of the nesting structures seems to correlate with the extent of parental care (Potts, 1984). Again, these behaviors are highly diverse among species. In its simplest form, a depression in the substrate where eggs are deposited is dug usually by the male using vigorous tail undulations (lateral or vertical) and/or pushing and mouth digging (Figure 7.2(a)). In some species males additionally build sandcastles or sand-scrape structures as display sites to attract females, and nest features can be assessed by females to select a mating partner (e.g., Fryer & Iles, 1972; Tweddle et al., 1998; Barber, Nairn, & Huntingford, 2001). Parental care of the substrate-deposited eggs may be performed by the male (e.g., *Spondyliosoma cantharus* (Wilson, 1958)), the female (e.g., *Radulinopsis taranetzi* (Abe & Munehara, 2005)), or both (e.g., *Tilapia zilli* (Loiselle, 1977)). In mouth-brooders one or both parents take the eggs (or fry in the case of late mouth-brooders) into the mouth and incubate them for a variable period of time (e.g., Balshine-Earn & Earn, 1998).

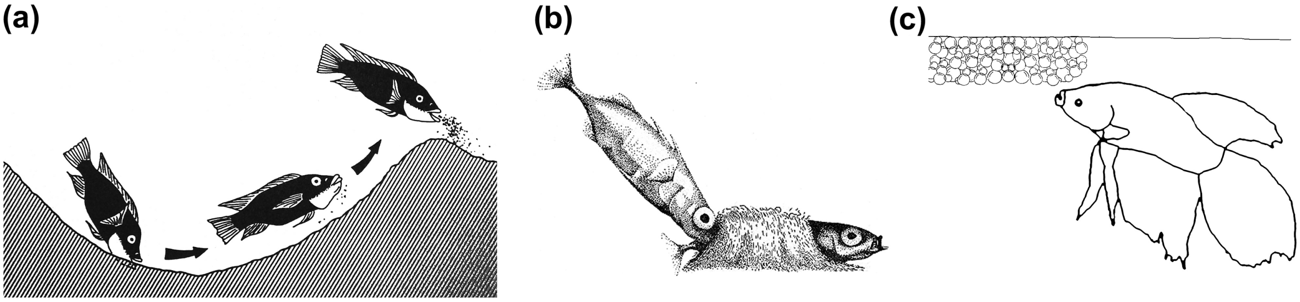

**FIGURE 7.2** Examples of nest-building in fishes. (a) Mouth-digging in *O. mossambicus*. (b) A female three-spined stickleback (*Gasterosteus aculeatus*) inside an algal nest. Reproduced from Pelkwijk and Tinbergen (1937). (c) A male fighting fish (*Betta splendens*) close to its bubble nest.

Natural crevices or holes can also be used as nesting sites, as e.g. in some gobies and blennies (Breder & Rosen, 1966). These natural cavities may receive no or minimal modifications, which include digging underneath a stone or a shell to increase the available space, cleaning the area in front of the nest entrance, and building structures in front or on top of the nest. Typically, the eggs are deposited inside the nest and the male provides parental care of the eggs or juveniles (e.g., Breder & Rosen, 1966; Thresher, 1984).

More complex nesting behaviors also have been described in fishes. The three-spined stickleback (*Gasterosteus aculeatus*) digs a pit in the substrate and then collects with the mouth algal pieces that are bound in a tunnel shape with spiggin, a glycoprotein produced by the kidney (Wootton, 1976) (Figure 7.2(b)). The male leads the female into this tunnel and eggs are laid and fertilized inside the algal nest. After receiving eggs from several females, the male switches into the parental phase and protects the eggs until hatching (Kraak, Bakker, & Mundwiler, 1999). Another species that builds algal nests is the corkwing wrasse (*Symphodus melops*). Males gather algal pieces of several species and deposit them along a crevice. Softer algae are deposited in the deepest part of the nest, where eggs will be laid, presumably because they offer more suitable conditions for egg development. Towards the outer face of the nest harder algae are deposited, forming a network that holds the nest together. After each egg laying, the male expels the female from the nest and covers the egg mass with soft algae, resuming courtship and receiving more eggs afterwards (for details see Potts, 1974; 1984). In a final example, freshwater species such as the fighting fish *Betta splendens* build a bubble nest with a mixture of mucus and water and defend a territory around it (Figure 7.2(c)). Males attract females into the nest and after egg laying the male assumes parental care of the eggs and juveniles (Forselius, 1957).

The examples described here illustrate the diversity of territorial and nest-building behaviors in fishes. After establishing a territory and preparing the breeding ground, fishes have to attract and select a mating partner.

## 2.2. Finding and Choosing a Mate: Species Identification, Sexual Discrimination, and Mate Choice

Reproduction depends on the correct identification of the species and gender of potential partners. As we shall see, this is not always an easy task. Let us illustrate the mechanisms of species and gender recognition in fishes with two examples: electric fish and cichlids.

The Gymnotiformes from South America and the Mormyriformes from Africa produce weak electric signals that are used for electrolocation and in intraspecific communication (Zakon & Smith, 2002). These signals are produced in electric organs located in the tails and received by specialized electroreceptors mainly located in the midline of the fishes (Zakon & Smith, 2002). More than 20 species producing similar signals may occur in sympatry in turbid rivers (Hopkins, 1980) and this presents a challenge for species and gender recognition. The first question is whether the electric organ discharge (EOD) signal has the potential of being used for these purposes. The EOD signal is highly stereotyped and species-specific, and its properties depend on the anatomy and physiology of the electric organ (e.g., Kramer, Kirschbaum, & Markl, 1981; Hopkins, 1988; 1999). In most species there are also sex differences in the EOD signal properties and even individual 'EOD signatures' (Hopkins, 1981; 1988; Crawford, 1992; McGregor & Westby, 1992; Friedman & Hopkins, 1996). Thus, the intrinsic differences in the EOD signal properties between and within species have the potential to be used in species, gender, and even individual recognition. This has been confirmed to some extent by a number of studies. For example, females of the mormyrid *Campylomormyrus compressirostris* have been found to prefer the EOD signal of a conspecific male over an EOD signal produced by males of the closely related sympatric *Campylomormyrus rhynchophorus*. However, females failed to discriminate the EOD signal of conspecific males from that of another sympatric species, *Campylomormyrus tamandua*, the EOD signal of which is more similar to conspecific males (Feulner, Plath, Engelmann, Kirschbaum, & Tiedemann, 2008). Using synthetic playbacks, Hopkins and Bass (1981) showed in a mormyrid species that males can recognize and respond to the signal from conspecific females based only on the temporal properties of the waveform. Further, males of another mormyrid (*Polimyrus isidori*) have been found to discriminate between the electrical signals of conspecific males and females, but in this species the sequence of pulse intervals and not the EOD waveform was used for discrimination (Crawford, 1991). Taken together, the results from these and many other studies (see Carlson, 2002 for a review) show that the EOD signal is used by electric fish to recognize the species and the gender of the sender, a fundamental first step in mating.

Cichlids have rapidly radiated into a large number of diverse species (e.g., Johnson et al., 1996). The coexistence of a large number of species in the same area, often very similar — e.g., more than 500 species of cichlid have been described for Lake Malawi (Martens, 1997) — suggests fine mechanisms must be present for species and gender recognition. Males are usually more colorful than females and color differences are more marked between sexually active males of different species than between females or nonreproductive males (Fryer & Iles, 1972). In a study on the *Pseudotropheus zebra* species complex, females preferred conspecific males over heterospecific males from three closely related species, and, when presented only with the three heterospecific males, females preferred to associate with the male with the most similar color pattern to the conspecific (Couldridge & Alexander, 2002). In another study, differences in color patterns between males of two closely related haplochromine cichlids were manipulated using light conditions, and females were shown to prefer conspecific males when color differences were visible. However, when color differences were masked by light conditions, females of both species did not discriminate between conspecific or heterospecific males (Seehausen & Alphen, 1998). This suggests that color patterns are used by female cichlids for recognizing conspecific mating partners (see also Kidd, Danley, & Kocher, 2006; Stelkens et al., 2008). The males' ability to correctly identify conspecific females based on visual cues alone seems to be poorer than the females' ability to visually discriminate conspecific males (Knight & Turner, 1999), corroborating the predicted stronger selective pressure for correct species and gender identification in females. It should be noted that other studies suggest that both chemical (Plenderleith, Van Oosterhout, Robinson, & Turner, 2005) and acoustic (Amorim, Knight, Stratoudakis, & Turner, 2004; Amorim, Simões, Fonseca, & Turner, 2008) cues also may be important in cichlids for species and gender discrimination, and most likely interspecific mate recognition is mediated by multimodal signals. The above examples show how long-range cues or signals detected in several sensorial modalities are used by animals to find and approach appropriate mates.

Additionally, animals usually display some form of mate choice and the most extravagant morphologic structures and elaborate behaviors in nature seem to be the consequence of sexual selection (Darwin, 1871). Sexual selection is predicted to be stronger in males and mating partner selectivity is expected to be stronger in females (Trivers, 1972; Andersson, 1984). Fishes follow this pattern and males generally exhibit the most elaborate courtship signals and are more ornamented than females. However, many exceptions exist. For example, sexual dimorphism and dichromatism is absent in many species, notoriously in most pelagic spawners (Breder & Rosen, 1966; Thresher, 1984), and even when it occurs both females and males may apparently accept any mate, as long as it is recognized as a conspecific of the correct sex (e.g., Goulet & Green, 1988). Other exceptions discussed below are sex-role-reversed species in which females compete for mates more intensely than males do for females and may be the more ornamented sex (e.g., Berglund, Widemo, & Rosenqvist, 2005). It should be noted that, in species with traditional sex-roles, female traits are also under sexual selection by male choice, although the intensity of sexual selection is expected to be stronger on male traits as male potential reproductive rates are higher than those of females (Amundsen & Forsgren, 2001). The rule in species with sexual dimorphism is nevertheless for males to be more ornamented than females and to take the initiative in courtship (for a review on the function of male ornaments in animals see Berglund, Bisazza, & Pilastro, 1996).

In many fishes, males in reproductive condition react to the presence of females with conspicuous courtship displays that may involve stereotyped motor patterns, color changes, pheromone release, and vocalizations or the production of electrical signals. The properties of these signals may correlate with male traits, and females can use this information to select mates and gain direct or indirect benefits. For instance, in the bicolor damselfish (*Stegastes partitus*), females select males that court at higher rates. This trait, courtship rate, correlates with egg survival, probably because males that court more are in better condition and provide more suitable parental care to the eggs (Knapp & Kovach, 1991). Thus, courtship rate can be considered an honest indicator of male condition and females gain direct (egg survival) or indirect (if variation in male condition has a genetic influence) benefits by selecting high-frequency-courting males.

The same traits used for species/gender discrimination also may be under sexual selection by female choice. In a mormyrid, the sexually dimorphic EOD signal is longer in high-status males than in low-status males (Carlson, Hopkins, & Thomas, 2000). In the pintail knifefish *Brachyhypopomus* *pinnicaudatus*, females prefer larger males over smaller males, and the EOD amplitude and duration are correlated with male body size (Curtis & Stoddard, 2003). Thus, there is the potential for females to use the properties of the EOD signals for both species and gender identification as well as for mate choice. Feulner, Kirschbaum, Mamonekene, Ketmaier, and Tiedemann (2007) suggest for mormyrids of the genus *Campylomormyrus* inhabiting the Congo River that divergent selection acting on the feeding apparatus leads to changes in the EOD signal and to speciation by sexual selection and assortative mating based on the characteristics of this signal. In cichlids, variation in female preference for male colors seems to drive male color polymorphism and sympatric speciation by divergent sensory drive (Dominey, 1984; Seehausen et al., 2008; Seehausen, Van Alphen, & Witte, 1997). The species-specific traits used by females to identify conspecific males have therefore evolved by disruptive sexual selection driven by female mate choice. Similarly, individual variation in male preference for female color morphs has been also shown in the cichlid *Neochromis omnicaeruleus*, suggesting a potential for speciation driven by male mating preferences on female coloration (Pierotti, Martin-Fernandez, & Seehausen, 2009).

Again, searching for general patterns in courtship displays in fishes is difficult due to their enormous interspecific variability. Some examples of male motor patterns displayed during courtship and that illustrate this variability are the sigmoid posture of poeciliids, the zigzag dance of sticklebacks, the lateral body quivering of zebrafish, and the circling and figure-eight swimming of blennioids (Figure 7.3).

## 2.3. Internal and External Fertilization

In teleost fishes, external fertilization is predominant over internal fertilization and is considered to be the ancestral condition. In species with external fertilization, sperm and egg release may be synchronous or asynchronous. Synchronous spawning seems to be predominant in pelagic spawners. When external fertilization takes place in the water-column between paired fish, the male and the female may assume positions that bring their genital pores close and synchronize the release of eggs and sperm (Breder & Rosen, 1966). A common strategy in group spawning species is for several males to follow a gravid female and compete for a privileged position close to her. When the female assumes a spawning posture, males closer to the female try to release sperm synchronously with egg release (Domeier & Colin, 1997; Kiflawi, Mazeroll, & Goulet, 1998). In asynchronous spawning species, males may release sperm prior to or after egg laying. For instance, males of some gobies perform upside-down movements inside the nest, during which they attach sperm trails to the nest walls prior to oviposition by the female (e.g., Marconato, Rasotto, & Mazzoldi, 1996; Ota, Marchesan, & Ferrero, 1996). Sperm release after oviposition occurs, for instance, in haplochromine cichlids. Females deposit the eggs in the male's nesting pit and immediately afterwards collect them with the mouth. Attracted by the anal fin egg-spots characteristic of haplochromine male cichlids, females approach the male's genital region and try to pick up these egg mimics. Males then release sperm and fertilization takes place inside the female's mouth (Fryer & Iles, 1972; see also Salzburger, Braasch, & Meyer, 2007).

In species with internal fertilization, females may cooperate with males or not. In the first case, females expose the genital pore to males and facilitate intromission of the male's copulatory organ. In the second case, a male chases a female while trying to insert the copulatory organ into the female's genital pore. Detailed descriptions of these behaviors for the guppy *Poecilia reticulata* can be found in Liley (1966) and Houde (1997).

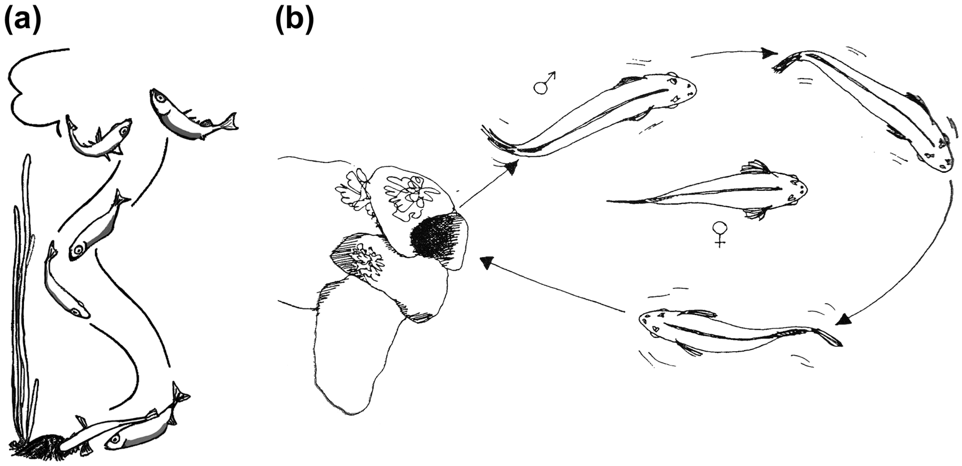

**FIGURE 7.3** Examples of sexual displays in fishes. (a) The zig-zag dance of *G. aculeatus*. Redrawn from Tinbergen (1951). (b) A male *P. sanguinolentus parvicornis* 'circling' a female from his nest. Reproduced from Santos and Barreiros (1993).

Extraordinary exceptions to the general pattern of egg fertilization in animals are seahorses and pipefishes (Syngnathidae). In some genera, males have brood pouches and females transfer the eggs by inserting their ovipositor into the male's brood pouch. Egg fertilization takes place inside the male's pouch, and the male incubates the eggs until hatching (Fiedler, 1954).

Motor patterns associated with gamete release are less variable than behaviors preceding this step. Sperm ejaculation is usually accompanied by stereotyped motor patterns consisting of high-frequency body quivering and are often similar between species with internal and external fertilization (Breder & Rosen, 1966). Egg laying may also be accompanied by quivering, whereas in live bearers parturition consists of lower-frequency contractions of the abdominal cavity, or of the pouch walls in the case of male syngnathids (Breder & Rosen, 1966).

## 2.4. Sex-role Reversal

It is clear from the above examples that in most species females assume a passive role during the prespawning/precopulation phase and have poorly elaborated courtship displays. For this reason, studies on female reproductive behavior in fishes have been mainly focused on consummatory aspects, although a few species, notoriously the goldfish, have been investigated in more detail. This lack of knowledge is somewhat surprising as the wide variation in fish behavior offers an opportunity to overcome this gap. Species with sex-role reversal, where females take the initiative in courtship and display courtship behaviors more frequently than males, are particularly well-suited to studying the proximate mechanisms regulating female reproductive displays.

Sex-role reversal occurs when the males' potential reproductive rates are lower than those of females. In these cases females will compete more intensely over mates, and males are expected to be more selective than females (Clutton-Brock & Vincent, 1991). In teleost fishes, the paradigmatic example of sex-role reversal are syngnathids (for a review see Berglund & Rosenqvist, 2003). Male seahorses and pipefishes carry the eggs until hatching and thus invest more heavily than females in parental care. However, a strong paternal investment seems to be a necessary but not sufficient prerequisite for sex-role reversal, and in syngnathids sex-roles depend on the mating system. In monogamous species, such as seahorses, the female opportunities for additional mating are reduced and the sex-roles are conventional, while in polygamous species, such as pipefish, the sex-roles are reversed (Vincent, 1992; 1994). Two well-studied syngnathids with sex-role reversal are *Nerophis ophidion* and *Syngnathus typhle*. The adult female-to-male ratio for these species in nature is approximately 1 : 1, and females produce more eggs than one male brood pouch can accommodate (Berglund, Rosenqvist, & Svensson, 1989; Berglund & Rosenqvist, 1990). This results in a female-biased operational sex-ratio with more females in reproductive condition than males, as suggested by the observation that most males have a full brood pouch at the peak of the breeding season (Berglund & Rosenqvist, 1993). As a consequence, females compete for mates more strongly than males, and males are more selective than females, preferring to mate with larger females (Berglund, Rosenqvist, & Svensson, 1986; Rosenqvist, 1990). Females are larger and, particularly in *N. ophidion*, more ornamented than males. Female ornamentation consists of a permanent blue coloring along the flanks and the development of a ventral skin fold during the breeding season. Females display towards males, often in lek-like aggregations, by swimming up and down above the eelgrass. Females compete amongst themselves for access to males and may force other females off a male. Female *S. typhle* use these ornaments both for male attraction and to intimidate other females (Berglund & Rosenqvist, 2009). After selecting a female, a male will dance with her above the eelgrass with wriggling and shaking movements, and this dance may precede copulation (Fiedler, 1954; Vincent, Berglund, & Ahnesjö, 1995; Berglund & Rosenqvist, 2003).

Within the same species, the potential reproductive rates of males and females may also vary between populations and throughout the breeding season in the same population. In the two-spotted goby (*Gobiusculus flavescens*), at the beginning of the breeding season the adult female to male ratio is approximately 1 : 1 and sex-roles are conventional. However, as the season progresses the proportion of males sharply decreases, probably due to a higher male than female mortality rate. This is accompanied by a shift in sex-roles with females competing aggressively for the few available males and taking the initiative in courtship (Forsgren, Amundsen, Borg, & Bjelvenmark, 2004). In the peacock blenny *Salaria pavo*, two populations occurring in different ecological scenarios also differ in sex-roles. A population at the Adriatic Sea occurs in a rocky shore area where nest availability is high. Males reproduce in rock crevices and actively defend an area around the nest. Sex-roles are conventional, with males taking the initiative in courtship and performing more courtship displays than females (Saraiva, 2009). In contrast, in southern Portugal, the species inhabits a coastal lagoon where appropriate nesting substrate is scarce. Most males use artificial substrates such as brick holes for nests and do not defend any area around the nest. The scarcity of nests biases the operational sex ratio towards females and in this population females take the initiative and display more courtship behavior than males (Almada, Gonçalves, Oliveira, & Santos, 1995; Saraiva, 2009).

## 2.5. Alternative Reproductive Tactics

In some species, individuals of the same sex, usually males, may adopt alternative tactics to reproduce. Large 'bourgeois' males generally defend reproductive resources such as females or nests whereas smaller males parasitize the investment of bourgeois males (Taborsky, 1997). Alternative reproductive tactics (ARTs) can be categorized as fixed, when individuals adopt one of the tactics for their entire lifetime, or plastic, when individuals change tactics during their lifetime (Moore, 1991; Brockmann, 2001). Plastic tactics can further be classified as irreversible, when individuals switch from one tactic to another at a particular moment in their lifetime, and reversible, when individuals change back and forth between tactics (Moore, 1991; Moore, Hews, & Knapp, 1998; Brockmann, 2001). Teleost fishes are the vertebrate group with the highest incidence of male alternative reproductive tactics (Taborsky, 1994; 2008) and the most common pattern is for males to start reproducing parasitically and then irreversibly switch into the bourgeois tactic (reviewed in Oliveira, 2006).

Traits related to female attraction and monopolization of reproductive resources are selected in bourgeois males, whereas traits prevail in parasitic males that increase the probability of stealing fertilizations. This may result in polyphenisms within the same sex and often parasitic and bourgeois males differ markedly in behavior, morphology, and physiology. The reproductive behavior of bourgeois males follows the general pattern above described for male teleosts, with the access to reproductive resources depending on territorial, nesting, and/or courtship displays. Parasitic males access reproductive resources and fertilize eggs in different ways. These include trying to approach nests or females inconspicuously, darting by fast swims into nests, forcing copulations with females in species with internal fertilization, or relying on a female-like appearance and behavior to approach nesting males (Taborsky, 1999; 2008).

From a behavioral neuroendocrine perspective, species in which parasitic males reproduce by female-mimicry are particularly interesting, as male- and female-like traits coexist in the same individual. These fishes have a female-like appearance and may display female-like behaviors, and simultaneously need to develop functional testes and other male traits. Thus, the neuroendocrine characterization of these species may provide insights into the role played by the endocrine system in the regulation of sexually dimorphic morphologic and behavioral traits. A species for which female-mimicry has been studied in some detail is the peacock blenny. In this species sexual dimorphism is intense, with bourgeois males being larger than females and having a set of well-developed secondary sexual characteristics such as an adipose head crest and an anal gland in the first two rays of the anal fin (Fishelson, 1963; Patzner, Seiwald, Adlgasser, & Kaurin, 1986). For the above-described population in southern Portugal where nest sites are scarce, females court bourgeois males intensively and males assume a passive posture during courtship and often reject females (Almada et al., 1995). The female courtship display is elaborate and markedly different from male displays, and consists of beating the pectoral fins and opening and closing the mouth in synchrony while simultaneously displaying a conspicuous nuptial coloration (Fishelson, 1963; Patzner et al., 1986). Small males, morphologically similar to females, reproduce by mimicking the complex female reproductive displays and nuptial coloration in order to approach large nesting males and achieve parasitic fertilizations of eggs (Gonçalves et al., 1996) (Figure 7.4).

Bourgeois males attack and court matched-for-size parasitic males and females with the same probability, suggesting that they are deceived by these parasitic males (Gonçalves, Matos, Fagundes, & Oliveira, 2005). A mark-recapture study revealed that the parasitic reproductive tactic is only adopted during the males' first breeding season, with parasitic males switching into bourgeois males in their second breeding season onwards (T. Fagundes, J. Saraiva, D. Gonçalves, & R.F. Oliveira, unpublished data). Thus, the same animal experiences a dramatic change in its sexual behavior, displaying female-like sexual behaviors early in its development and typical male ~~displays~~ later on.

The above overview of the patterns of sexual behavior in fishes highlights how sexual displays may differ markedly across species, populations, different individuals of the same population, or even within the same animal

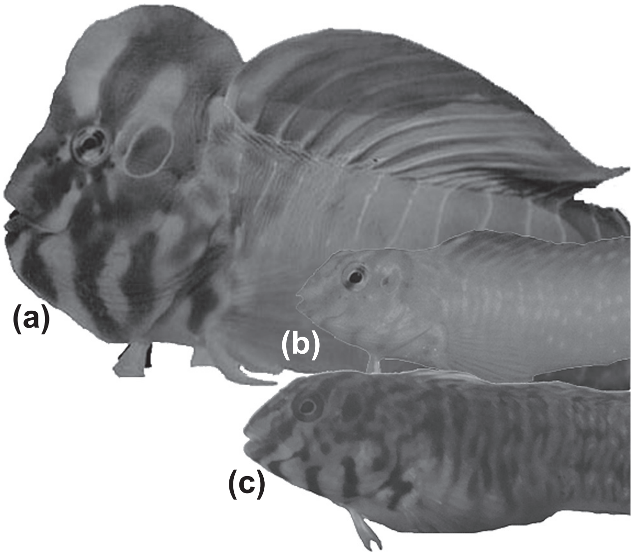

**FIGURE 7.4** Male alternative reproductive tactics in the peacock blenny *Salaria pavo*. Parasitic males (b) reproduce by mimicking the female (c) morphology and behavior in order to approach the nests of larger nesting males (a) and fertilize eggs. See color plate section at the back of the book.

throughout its lifetime. This diversity offers an obvious difficulty when trying to derive general physiological mechanisms for the regulation of sexual behaviors but also provides the opportunity to understand the evolution and organization of neuroendocrine systems underlying the expression of sexual displays. Comparative approaches across different systems may prove a useful basis for understanding both the commonalities and variability of the mechanisms underlying male and female sexual displays. However, although a considerable amount of descriptive data on the ethology of sexual behaviors exists for many fish species, neuroendocrine studies focusing on sexual displays are comparatively scarce and research effort has been put into a reduced number of species. This limits for now the usefulness of comparative approaches, and data for more species are required. With this limitation in mind, an overview of what is known about the neuroendocrine regulation of male and female sexual displays in teleost fishes is presented.

## 3. ENDOCRINE MECHANISMS REGULATING SEXUAL BEHAVIOR

Hormones were classically seen as causal agents of behavior where the presence of a particular hormone was necessary for the expression of a given behavior. However, a deeper understanding of the molecular mechanisms through which hormones influence behavior has changed this view into considering hormones as modulators rather than causal agents of behavior (see Oliveira, 2004 for an historical background). By acting on sensory and motor systems and on the central neural circuitry underlying the expression of specific behavioral patterns, hormones, like other modulators, increase or decrease the probability of pattern occurrence. This view helps us to understand apparently paradoxical observations, such as that the expression of specific displays promoted by specific hormones may not be completely abolished after suppressing hormonal action.

In teleosts, and similarly to other vertebrates, the neuroendocrine system is hierarchically organized, with the hypothalamus controlling the activity of the adenohypophysis, which in turn regulates peripheral endocrine glands. The principal difference between the teleost endocrine system and that of other vertebrates is the lack of a hypothalamic-hypophysial portal vascular system to transport the hypothalamic releasing factors to the adenohypophysis. Instead, the adenohypophysis is controlled by direct innervation by hypothalamic neurosecretory neurons (Peter, 1990). For reasons outlined below, studies on the endocrine regulation of fish sexual behavior have focused on hormones of the hypothalamic-pituitary-gonadal (HPG) axis, in particular of gonadal steroids, and not much is known about the role played by hormones of other axes (e.g., growth hormone, corticosteroids). In some studies sexual behavior has been shown to be independent of gonadal steroids and these observations have promoted the search for other agents potentially involved in the regulation of fish sexual displays. As an example, in the sex-changing bluehead wrasse (*Thalassoma bifasciatum*) both intact and ovariectomized females responded to the removal of territorial males by occupying their territory and rapidly switching into displaying the full suite of male courtship and spawning behaviors, demonstrating that the action of gonadal steroids was not necessary for the expression of typical male displays (Godwin, Crews, & Warner, 1996; see also Chapter 8, this volume). To account for these observations, the role played by some neuropeptides in the control of fish sexual behavior also has been investigated. An emphasis has been placed on the effects of the gonadotropin-releasing hormone (GnRH) and of the two peptides released by the neurohypophysis, arginine vasotocin (AVT) and isotocin (IST), the fish homologs of mammalian arginine vasopressin (AVP) and oxytocin (OXY), respectively. Our overview on the endocrine mechanisms of fish sexual behavior will thus be focused on this set of neuroendocrine agents.

### 3.1. Gonadal Steroids

Sex steroids have long been recognized as key hormones regulating sexual differentiation, physiological aspects of reproduction, and the development of primary and secondary sexual characteristics (Nelson, 2005). This recognition has established gonadal steroids as prime candidates for the regulation of sexual behavior. The traditional view of the action of sex steroids on behavior was that steroids produced in the gonads exert their effects by acting via nuclear receptors on sensorial, central, or motor output neural systems (Nelson, 2005). However, data suggesting some important exceptions to this general pattern of steroidal action have been accumulating. First, steroids may act via nonclassic membrane receptors (Moore & Evans, 1999). The existence of these receptors was suspected for a long time as steroids were shown to induce physiological and behavioral changes too rapidly to be compatible with their classical genomic action via nuclear receptors (Klein & Henk, 1964; Spach & Streeten, 1964). However, it was not until 2003 that the first membrane steroid receptor, a progestin receptor, was conclusively isolated and cloned in a teleost, the spotted sea trout (*Cynoscion nebulosus*) (Zhu, Rice, Pang, Pace, & Thomas, 2003). Data on the rapid effects of steroid hormones have been rapidly accumulating over the past years, and a few studies report fast effects of steroid hormones on fish sexual displays (see Section ~~X~~). Second, central mechanisms may regulate the action of steroids produced in the periphery. For example, many behavioral actions of T depend on its conversion to estradiol ($E_2$) by the P450 enzyme aromatase ($P450_{aro} = CYP19$) (e.g., Naftolin et al., 1975). In birds, $P450_{aro}$ has been suggested to be stored in an inactive form in presynaptic boutons and its activity to be quickly regulated by changes in intracellular $Ca^{2+}$ levels triggered by neurotransmission (Balthazart & Ball, 2006). In a recent study, the rapid modulation of T and $E_2$ concentrations in the brain by social interactions was demonstrated in zebra finches using a microdialysis technique (Remage-Healey, Maidment, & Schlinger, 2008), corroborating this hypothesis. Neural regulation of $P450_{aro}$ activity is thus a possible mechanism to rapidly control local estrogen synthesis from androgens and estrogenic effects in the central nervous system (CNS) (for details see Balthazart & Ball, 2006). Finally, *de novo* synthesis of steroids from cholesterol in the brain has been suggested for a few species. For instance, all enzymes necessary for the synthesis of adrenal steroids from cholesterol have been identified in the CNS of mammals (Compagnone & Mellon, 2000). It is thus possible that the regulation of brain circuits underlying the expression of sexual behaviors by steroids is partially independent from their peripheral secretion. Nevertheless, steroids produced in the gonads are still logical candidates for synchronizing gonadal development and gamete maturation with the expression of sexual behavior, and there is ample evidence for their effect on sexual displays.

The role of gonadal steroids in fish behavior has been investigated using several complementary approaches. One is to correlate plasma steroid levels with the frequency of behavioral displays (which assumes that the gonads are the main source of the steroids measured in the plasma). Correlational data provide only limited evidence of the role played by specific hormones in specific behavioral displays. For example, in most species dominant males have higher plasma androgen levels and perform aggressive displays more frequently than subordinate males. However, dominant and subordinate males also differ in other aspects (relative testicular development, secondary sexual characteristics, feedback from the social environment) and it is difficult to pinpoint what the functional role of the androgen difference is, based only on correlational data. Since the pioneering experiments of Arnold Berthold (1849), who, by castrating, reimplanting, and transplanting testes in cockerels, demonstrated an effect of a 'secretory blood-borne product' in the development of male sexual behavior and morphological traits, castration–hormone replacement experiments have become a standard procedure for testing the effects of gonadal hormones. However, even with this experimental approach it may be difficult to disentangle the effects of hormonal manipulations in the several components of sexual behavior. For instance, a reduction in copulation frequency due to castration and its restoration by hormone replacement may result from a direct effect of that hormone on copulation behavior or from an effect of the hormone on other aspects of sexual behavior (e.g., nuptial coloration, courtship displays) that indirectly influence copulation success. Direct evidence for the role of a specific hormone in a specific behavior implies demonstrating the site of hormonal action and the mechanism through which the hormone modifies the display. This is only possible using a combination of techniques such as electrophysiological and molecular methods, some of which are technically demanding. For this reason, a detailed knowledge of the way in which specific hormones influence specific sexual behaviors in fishes has been gathered for only a very few well-studied models. With these limitations in mind, a brief overview of the role played by gonadal steroids in male and female sexual behavior is presented below.

### 3.1.1. Gonadal steroids and male sexual behavior

The principal sex steroids detected in the plasma of male teleosts are T, 11β-hydroxytestosterone (OHT), and the nonaromatizable 11-ketotestosterone (11-KT) (Borg, 1994). 11-ketotestosterone is usually the most abundant sex steroid in male plasma and is thought to be the most relevant androgen in male teleosts, whereas T is the major circulating androgen in females (Borg, 1994; Lokman et al., 2002). Similarly, in species with polymorphic male ARTs, bourgeois males have higher plasma levels of 11-KT, but not of T, than parasitic males (Oliveira, 2006). Besides playing a role in the regulation of spermatogenesis (e.g., Miura, Yamauchi, Takahashi, & Nagahama, 1991), gonadal differentiation (e.g., Strussmann & Nakamura, 2002), and the development of male secondary sex characteristics (e.g., Mayer, Borg, & Schulz, 1990), gonadal androgens also have been implicated in the activation of male sexual behavior in fishes (for reviews see Liley & Stacey, 1983; Borg, 1994; Oliveira & Gonçalves, 2008).

Aggressive behavior associated with reproductive territory acquisition may be facilitated by androgens (e.g., Cardwell & Liley, 1991). However, the available data suggest that the effect of androgen manipulation in male aggression in fishes is largely variable. For example, in sixteen studies where males were castrated, the frequency of aggressive displays decreased in six, remained unchanged in nine, and increased in one study (Table 7.1). Nevertheless, a meta-analysis has confirmed a positive effect of exogenous androgen administration on male aggressiveness in teleosts, and this effect was larger for territorial male–male aggression than for nest defense aggression (Hirschenhauser & Oliveira, 2006). The central effects of androgens in aggressive behavior are unlikely to depend on T aromatization. Peripheral administration of $E_2$

**TABLE 7.1** Effects of castration on the frequency of aggressive, nest-building, and sexual behaviors in fishes

| Species | Reference |
|---|---|
| **Aggressive behavior** | |
| *No effect* | |
| *Betta splendens* (breeding males)[a] | Weiss and Coughlin (1979) |
| *Macropodus opercularis* (breeding males)[a] | Villars and Davis (1977) |
| *Trichogaster trichopterus* (breeding males) | Johns and Liley (1970) |
| *Gasterosteus aculeatus* (pre-nesting males; short-days) | Hoar (1962) |
| *Gasterosteus aculeatus* (pre-nesting males) | Baggerman (1966); Wootton (1970) |
| *Lepomis megalotis* (territorial males) | Smith (1969) |
| *Lepomis gibbosus* (territorial males) | Smith (1969) |
| *Salaria pavo* (parasitic males) | Gonçalves et al. (2007) |
| *Increased* | |
| *Gasterosteus aculeatus* (pre-nesting males; long-days) | Hoar (1962) |
| *Decreased* | |
| Gasterosteus aculeatus (nesting males) | Baggerman (1966); Wootton (1970) |
| Astatotilapia burtoni (males) | Francis et al. (1992) |
| Pseudocrenilabrus multicolor (males) | Reinboth and Roxner (1970) |
| Xiphophorus maculatus (males) | Chizinsky (1968) |
| Bathygobius soporator (breeding males) | Tavolga (1955) |
| **Nest building** | |
| *No effect* | |
| Sarotherodon melanotheron melanotheron (breeding males) | Aronson (1951) |
| Sarotherodon melanotheron heudelotii (breeding males) | Heinrich (1967) |
| Oreochromis upembae (breeding males) | Heinrich (1967) |
| Gasterosteus aculeatus (breeding males) | Ikeda (1933) |
| Macropodus opercularis (breeding males) | Villars and Davis (1977) |
| *Decreased* | |
| Lepomis gibbosus and L. megalotis (breeding males) | Smith (1969) |
| Pseudocrenilabrus multicolor multicolor (breeding males) | Reinboth and Rixner (1970) |
| Gasterosteus aculeatus (breeding males) | Baggerman (1957); Hoar (1962); Wai and Hoar (1963); Baggerman (1966); Borg (1987) |
| Trichogaster trichopterus (breeding males) | Johns and Liley (1970) |
| **Sexual behavior** | |
| *No effect* | |
| Hemichromis bimaculatus (breeding males) | Noble and Kumpf (1936) |
| Gasterosteus aculeatus (breeding males) | Baggerman (1968); Páll et al. (2002) |
| Bathygobius soporator (breeding males) | Tavolga (1955) |
| Thalassoma bifasciatum (breeding males) | Semsar and Godwin (2003) |
| *Decreased* | |
| Pseudocrenilabrus multicolor multicolor (breeding males) | Reinboth and Rixner (1970) |
| Gasterosteus aculeatus (breeding males) | Baggerman (1957); Hoar (1962) |

(*Continued*)

**TABLE 7.1** Effects of castration on the frequency of aggressive, nest-building, and sexual behaviors in fishes—cont'd

| Species | Reference |
|---|---|
| Morone Americana (breeding males) | Salek (2001) |
| Trichogaster trichopterus (breeding males) | Johns and Liley (1970) |
| Oncorhynchus mykiss (breeding males) | Mayer et al. (1994) |

[a] *Incomplete gonadectomy or evidence for rapid testicular regeneration.*

has an inhibitory effect on male aggression in fishes (Bell, 2001; Clotfelter & Rodriguez, 2006; Gonçalves, Alpedrinha, Teles, & Oliveira, 2007). In a sex-changing goby, brain P450$_{aro}$ activity decreases with aggression, suggesting that a decrease in $E_2$ synthesis (or a higher availability of T) facilitates aggressive behavior (Black, Balthazart, Baillien, & Grober, 2005). Both T and 11-KT promote aggression in teleosts (Oliveira & Gonçalves, 2008), although in a recent study pre-fight levels of T but not of 11-KT correlated positively with aggressive displays in the internally self-fertilizing hermaphroditic killifish, *Kryptolebias marmoratus* (Earley & Hsu, 2008).

The role of gonadal steroids in nest-building behavior has been investigated in only a few species but in greatest detail in *G. aculeatus*. The results from twelve castration studies in several species range from no effect (five studies) to a suppression of nest-building behavior (seven studies) (Table 7.1). Both T and 11-KT administration to castrates restores the behavior (Oliveira & Gonçalves, 2008), and in sticklebacks 11-ketoandrostenedione (11-KA) seems to be more effective than T in restoring nest-building behavior (Borg, 1987). In *G. aculeatus*, males exposed to $E_2$ start building nests later, although $E_2$ does not affect the percentage of males that build nests (Wibe, Rosenqvist, & Jenssen, 2002). Also in this species, the males' exposure to the antiandrogen flutamide decreases nest-building behavior at low concentrations of the substance and completely abolishes the behavior at higher levels, although this may be a consequence of lower spiggin production at these concentrations (Sebire, Allen, Bersuder, & Katsiadaki, 2008). Similarly, exposure to fenitrothion, an organophosphorus pesticide structurally similar to flutamide, reduces spiggin production and nest-building behavior in this species (Sebire et al., 2009). Taken together, the available data suggest that the central action of androgens secreted by the testis promotes nest-building behavior in males.

Hormonal manipulations also induce largely variable effects in male courtship displays. In eleven studies in which males were castrated, the frequency of male sexual displays was maintained in five and reduced in six cases (Table 7.1). In five out of these six cases, some castrated males were also administered with androgens. Testosterone (two studies), methyltestosterone (MT) (one study), and 11-KT (one study) at least partially recovered male sexual displays, and in one case 11-KA failed to do so. In intact males, T administration failed to promote male sexual behavior in four different species whereas 11-KT or 11-KA promoted male sexual displays in two out of four species. Blocking androgen action with cyproterone acetate, flutamide, or vinclozolin reduced male sexual displays in four out of five cases (Oliveira & Gonçalves, 2008). Hormonal manipulations in females and immature males have also produced variable results. As an example, female goldfish treated with 11-KT (but not with T) displayed male-like sexual behaviors in response to a stimulus female (Stacey & Kobayashi, 1996), while in *G. aculeatus* 11-KA (Borg & Mayer, 1995) or MT (Wai & Hoar, 1963) administration to females failed to induce male-like behaviors. Further, the role of central aromatization of T into $E_2$ in the regulation of male sexual displays, well established in mammals and birds (reviewed in Baum, 2003; Ball & Balthazart, 2004), is not clear in fishes. In a study in guppies (*Poecilia reticulata*) two of three male sexual displays were reduced by P450$_{aro}$ inhibition (Hallgren, Linderoth, & Olsen, 2006), suggesting that, similarly to other vertebrates, T aromatization to $E_2$ facilitates some aspects of male sexual behavior. On the other hand, for five species in which exogenous $E_2$ was administered to males, sexual displays were reduced in four cases and remained unchanged in one case (Oliveira & Gonçalves, 2008). Moreover, in weak electric fish, androgens masculinize the EOD signal in females, castrated males, juvenile males, or males in nonreproductive conditions (e.g., Bass & Hopkins, 1983; Meyer, 1983; Bass & Hopkins, 1985; Bass & Volman, 1987; Dunlap & Zakon, 1998; Herfeld & Moller, 1998; Few & Zakon, 2001) whereas estrogens feminize the signal (Dunlap, McAnelly, & Zakon, 1997), probably via transcriptional regulation of ionic membrane channels in electrocytes, the cells of the electric organ (e.g., Liu, Wu, & Zakon, 2008). Thus, the role of central T aromatization in the regulation of male sexual behavior in fishes is for now unclear and more experiments are necessary to assess its relevance.

A role for progestins in male courtship displays also has been suggested. Plasma levels of the progestin 17,20β-dihydroxy-4-pregen-3-one (17,20βP) correlate with male courtship displays in several species (e.g., *Oncorhynchus mykiss* (Liley, Breton, Fostier, & Tan, 1986), *Salmo salar* (Mayer et al., 1990b), *Salmo trutta* (Olsén, Järvi, Mayer, Petersson, & Kroon, 1998), and *Chromis dispilus* (Barnett & Pankhurst, 1994)). Further, in species with male ART, differences in circulating levels of the progestins 17,20βP, 17,20β,21-trihydroxy-4-pregen-3-one (17,20β,21P), and 17,20α-dihydroxy-4-pregen-3-one (17,20αP) occur (for a review see Oliveira, 2006). However, because progestins play a role in reproductive functions, namely in spermatogenesis and spermiation (e.g., Miura, Higuchi, Ozaki, Ohta, & Miura, 2006), it is difficult to assess whether these differences are a consequence of the progestins' actions in the testis and/or whether they reflect the central action of progestins in the modulation of male sexual displays. Evidence for a central regulation of behavior by progestins comes from a study in the rainbow trout (*O.mykiss*), where the administration of 17,20βP, but not of 11-KA, to castrated males restored male sexual behavior (Mayer, Liley, & Borg, 1994).

Additionally, a pheromonal role for progestins has been suggested. In females, 17,20βP plasma levels peak prior to ovulation and are released into the water. Recently, putative progestin receptor genes with potential to respond to steroid pheromones have been identified in the goldfish olfactory epithelium transcriptome (Kolmakov, Kube, Reinhardt, & Canário, 2008), and 17,20βP has been shown to act as a potent stimulator of the males' olfactory system (e.g., Sorensen, Scott, Stacey, & Bowdin, 1995). In contrast, exposure of male goldfish to 17,20βP induced an increase in gonadotropin (GTH) levels and milt production but had only minor effects on behavior (Sorensen, Stacey, & Chamberlain, 1989), and thus the putative pheromonal role of 17,20βP needs to be further investigated (see Chapter 6, this volume).

Rapid effects of steroid manipulation on behavior, consistent with a nongenomic action, have also been documented in fishes. In both the Gulf toadfish (*Opsanus beta*) and the closely related plainfin midshipman (*Porichthys notatus*), nesting males (type I) produce vocalizations to attract females into their nests, and in *P. notatus* parasitic female-like males (type II) that do not emit mating calls also have been described. In these species, it is possible to study the pattern of 'fictive vocalizations' in electrophysiological preparations. Electrical microstimulation in forebrain/midbrain vocal nuclei induces a rhythmic motor volley that can be intracranially recorded from occipital nerve roots descending into the sonic muscles. As the temporal pattern of the vocal motor volley determines the properties of the natural calls, the vocal motor volley is designated as a 'fictive vocalization' (Bass, 2008; see below for details). Intramuscular injections of 11-KT increased, within minutes, the duration of fictive vocalizations in both species and this effect was restricted to type I males, the morphotype with higher plasma 11-KT levels (Brantley, Wingfield, & Bass, 1993; Remage-Healey & Bass, 2004; 2006a; 2007). Fictive vocalizations in females of both species and in type II males of *P. notatus* were rapidly responsive to T instead of 11-KT, and T is the main circulating androgen in these morphs (Remage-Healey & Bass, 2007). This is consistent with a field experiment in toadfish where 11-KT administered to males (using food containing 11-KT crystals) increased their calling behavior within minutes (Remage-Healey & Bass, 2006a; for reviews see Remage-Healey & Bass, 2006b; Bass & Remage-Healey, 2008).

### 3.1.2. Gonadal steroids and female sexual behavior

In females, both $E_2$ and T are major circulating steroids, while 11-KT, the main androgenic steroid in males, is usually undetectable or present only in low concentrations (Borg, 1994 but see Lokman et al., 2002; Desjardins, Hazelden, Van der Kraak, & Balshine, 2006). Stacey (1981) has proposed that prostaglandins (PGs) produced by mature oocytes in external fertilizing species may signal a ready-to-spawn state and thus promote female sexual behaviors. In internal fertilizers, sexual behavior and fertilization are temporally dissociated and estrogens produced during follicular development may induce sexual behaviors in anticipation of ovulation. Although studies on the endocrine regulation of female sexual behavior in fishes are scarce, effects of gonadal steroids on various aspects of female sexual displays in both internal and external fertilizers have been reported.

In the cichlid *Neolamprologus pulcher*, the male and the female jointly defend their breeding territory. Females are more aggressive than males and also have higher plasma T levels than their mates, suggesting a role for T in female aggression (Desjardins et al., 2008). Accordingly, in the blue acara (*Aequidens pulcher*), T increases and $E_2$ decreases the frequency of female aggressive behaviors (Munro & Pitcher, 1985). In the fighting fish (*Betta splendens*), daily injections of T to females for nine weeks progressively increased aggressiveness towards males but decreased aggressiveness towards females (Badura & Friedman, 1988). Androgens also induce nest-building behavior in females in species where only males typically build nests. For instance, in two cichlids (*Astotilapia burtoni* and *Pseudocrenilabrus multicolor multicolor*), T induces nest-building behavior in females (Reinboth & Rixner, 1970; Wapler-Leong & Reinboth, 1974). However, the role of gonadal steroids in this aspect of female sexual behavior in species where females naturally exhibit nest building has not been investigated. These results suggest that in females, androgens, particularly T, promote aggressive and nest-building behaviors.

Females take a passive role during courtship and lack stereotyped courtship displays in many teleost fishes. As a consequence, the neuroendocrine control of female courtship displays has been poorly investigated but sex-role-reversed species offer the possibility to overcome this difficulty. In the above-described sex-role-reversed population of the peacock blenny in southern Portugal, females take the initiative in courtship and exhibit a stereotyped courtship display and a typical nuptial coloration when approaching a male's nest (Almada et al., 1995). The principal circulating sex steroid in females is $E_2$ and both T and 11-KT levels are significantly lower than in nesting males (Gonçalves, Teles, Alpedrinha, & Oliveira, 2008). To test the roles of gonadal hormones in female sexual behavior, females were either sham-operated, ovariectomized and implanted with vehicle, or ovariectomized and implanted with $E_2$, and their courtship behavior towards a nesting male was tested for one hour every two days for fourteen days. Ovariectomy significantly reduced the frequency of the female courtship displays although 75% of ovariectomized vehicle-implanted females still courted a nesting male at least once after 14 days in comparison with approximately 90% of sham-operated females. Estrogen partially restored female sexual behavior as the frequency of courtship displays and the time spent in nuptial coloration was intermediate in $E_2$-treated females when compared with sham-operated and vehicle-implanted females (D. Gonçalves, M. Teles, S. Costa, and R.F. Oliveira, unpublished data). These results suggest that $E_2$ produced in the ovaries promotes female sexual behavior in *S. pavo* but also show that the behavior may persist after 14 days without gonadal steroid production. In this species, female-like sneaker males mimic the females' displays, and the effects of steroids in the sneakers' behavior also have been tested. Estradiol implants to castrated sneakers failed to promote female-like displays (Gonçalves et al., 2007). Interestingly, castration increased female-like displays while T or 11-KT implants decreased the frequency of these displays, suggesting an inhibitory role for androgens in the sneakers' female-mimicking behavior (Oliveira, Carneiro, Gonçalves, Canário, & Grober, 2001; Gonçalves et al., 2007) (Figure 7.5). These results show that similar courtship displays in females and sneakers may be under the regulation of different endocrine mechanisms.

The results presented above for males and females stress the enormous variability in the effects of endocrine manipulation on appetitive aspects of sexual behavior in teleost fishes. It seems likely that part of this variability reflects interspecific variation in the mechanisms of hormonal regulation of sexual behavior, but some is certainly derived from methodological differences among studies. For example, an inverted U-shaped function has been reported for the effects of cortisol and AVT on fish behavior, with maximal stimulatory effects occurring at intermediate dosages of AVT (Santangelo & Bass, 2006) or cortisol (Bass, 2008; see also Volkoff & Peter, 1999). In most studies investigating the behavioral effects of hormonal manipulations, dose-response curves are not considered and only a single dosage is used. Thus, hormonal concentrations, time between hormonal manipulation and behavioral observations, or methods of hormone delivery are just a few of the many technical aspects that vary between studies and that make their comparison difficult. The field would certainly benefit from standardizing some of these procedures.

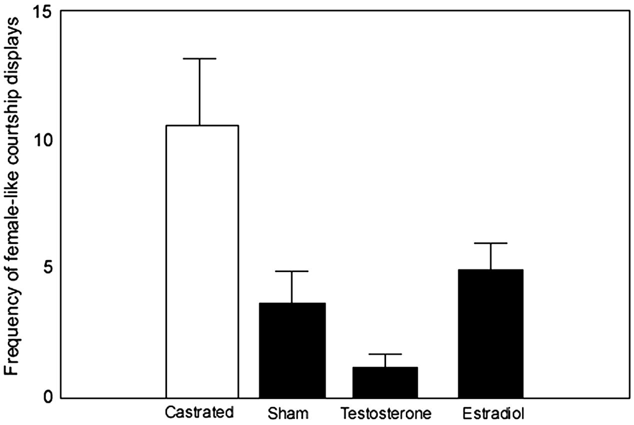

**FIGURE 7.5** Frequency of female-like displays directed by parasitic males towards a nesting male. Parasitic males were either castrated, sham operated, castrated and implanted with testosterone, or castrated and implanted with estradiol. Adapted from Gonçalves, Alpedrinha, Teles, and Oliveira (2007).

## 3.2. Neuropeptides

### 3.2.1. Gonadotropin-releasing hormone (GnRH)

Gonadotropin-releasing hormone is a highly conserved decapeptide that appears in three forms in vertebrates: GnRH1, GnRH2, and GnRH3. A gene duplication event early in the teleost lineage gave rise to the third isoform, GnRH3 (White, Kasten, Bond, Adelman, & Fernald, 1995; Parhar, 1997). The three isoforms have different distributions in the teleost brain and perform different functions (for reviews see Hofmann, 2006; Chen & Fernald, 2008; see also Chapter 2, this volume). GnRH1 neurons occur mainly in the preoptic area (POA)/anterior hypothalamus and project to the adenohypophysis, regulating the activity of the gonadotrope cells that synthesize follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Release of FSH and LH into the bloodstream stimulates normal gonadal development and gonadal steroid production in both males and females, and as we have seen gonadal steroids are relevant modulators of sexual behavior. Alternatively, GnRH2 and GnRH3 may directly modulate sexual behavior by acting on central mechanisms. GnRH2 is found throughout the brain, especially in the midbrain system, and is thought to play a neuromodulatory role. This isoform stimulates female sexual behavior in mammals (Barnett, Bunnell, Millar, & Abbott, 2006) and fishes (Volkoff & Peter, 1999). In the goldfish brain, mRNA expression levels of GnRH2 correlated with female spawning behavior (Canosa, Stacey, & Peter, 2008) and intracerebroventricular injections of low doses of GnRH2 and GnRH3 stimulated female spawning behavior, while higher dosages inhibited the behavior (Volkoff & Peter, 1999). GnRH3 neurons are found in the terminal nerve system with projections throughout the brain and this peptide is also thought to act as a neuromodulator (Oka & Matsushima, 1993). Lesioning of the terminal nerve impairs nest-building behavior in male dwarf gouramis (*Colisa lalia*) (Yamamoto, Oka, & Kawashima, 1997). In male tilapia (*Oreochromis niloticus*) immunoneutralization of GnRH3 (but not of GnRH1 or GnRH2) has the same suppressive effect on male nest-building behavior and also reduces aggressive displays (Ogawa et al., 2006). It seems possible that the effects of gonadal steroids on fish sexual behavior mentioned above are partly the result of an interaction with GnRH neurons, and several studies report a modulation of GnRH by sex steroids (e.g., Amano et al., 1994; Parhar, Tosaki, Sakuma, & Kobayashi, 2001; Levavi-Sivan, Biran, & Fireman, 2006; Vetillard, Ferriere, Jego, & Bailhache, 2006).

### 3.2.2. Arginine-vasotocin (AVT)

Besides its well-established role in osmoregulation, the nonapeptide AVT has been implicated in the regulation of social and sexual behavior in vertebrates (for review see Foran & Bass, 1999; Goodson & Bass, 2001; Balment, Lu, Weybourne, & Warne, 2006). In teleost fishes, AVT neurons occur in the POA and project to the neurohypophysis and to many other brain regions. Within the POA, three subpopulations of AVT neurons can be identified: parvocellular, magnocellular, and gigantocellular, and different roles for these populations have been suggested (e.g., Greenwood, Wark, Fernald, & Hofmann, 2008).

Arginine vasotocin has been implicated in male aggression in fishes, though with variable effects. For example, in the bluehead wrasse, AVT administration to territorial terminal phase (TP) males decreased aggression towards initial phase (IP) males while AVT administration to nonterritorial terminal phase males had the opposite effect (Semsar, Kandel, & Godwin, 2001) (see Chapter 8, this volume, for discussion of TP and IP in sex-changing fishes). In another field study, AVT injections to territorial male beaugregory damselfish (*Stegastes leucostictus*) promoted aggressive behavior towards intruders at medium dosages but had no effect at low and high dosages (Santangelo & Bass, 2006). In several other species, exogenous AVT administration decreased aggressive behavior. Arginine vasotocin reduced the production of an EOD signal used in agonistic contexts in the weakly electric fish *Apteronotus leptorhynchus* (Bastian, Schniederjan, & Nguyenkim, 2001), and decreased aggressive behavior in the Amargosa pupfish (*Cyprinodon nevadensis amargosae*) (Lema & Nevitt, 2004) and in juvenile rainbow trout (*O. mykiss*) (Backström & Winberg, 2009). In the plainfin midshipman, type I territorial males use grunt vocalizations as aggressive acoustic displays (Brantley & Bass, 1994) and AVT inhibited induced fictive grunt vocalizations in electrophysiological preparations (Goodson & Bass, 2000a; 2000b) (Figure 7.6). In *A. burtoni,* AVT mRNA expression in the gigantocellular layer was higher in territorial than in nonterritorial males, while in the parvocellular layer an opposite pattern was found (Greenwood et al., 2008). The authors of the study suggest that this difference may relate to a role of the gigantocellular AVT neurons in the promotion of dominance traits (aggression, courtship, and/or upregulation of reproductive physiology) in contrast to the activation of subordinate behavior or of the stress axis by parvocellular AVT neurons. If this is the case, exogenous administration of AVT by implants or injections may have unexpected effects as the targets of both gigantocellular and parvocellular neurons are activated. This is corroborated by the between- and within-species variable effects of AVT manipulation on the regulation of aggressive behavior in teleosts and in other vertebrates (Goodson & Bass, 2001).

Arginine vasotocin also has been implicated in the regulation of courtship behavior. Females of the bluehead wrasse may undergo sex change when a territorial TP male is removed (Warner & Swearer, 1991) and, as mentioned above, the female-to-male behavioral switch is fast and independent of the gonads (Godwin et al., 1996). Arginine vasotocin mRNA expression increases in the brain of sex-changing females (Godwin, Sawby, Warner, Crews, & Grober, 2000) and is higher even in ovariectomized dominant sex-changing females when compared with subordinate females (Semsar & Godwin, 2003). Arginine vasotocin administration to both territorial and nonterritorial TP males promoted male courtship behavior, further supporting an activational role of AVT in male sexual displays in this species (Semsar et al., 2001). However, this activational role seems to be context-dependent, as AVT administration to IP males or females fails to induce male-like behaviors under social conditions that inhibit sex change (i.e., in the presence of a territorial TP male) (Semsar & Godwin, 2004; see also Chapter 8, this volume).

Arginine vasotocin facilitates male sexual displays in other fish species. In the weakly electric fish *A. leptorhynchus*, AVT increased the production of electrical

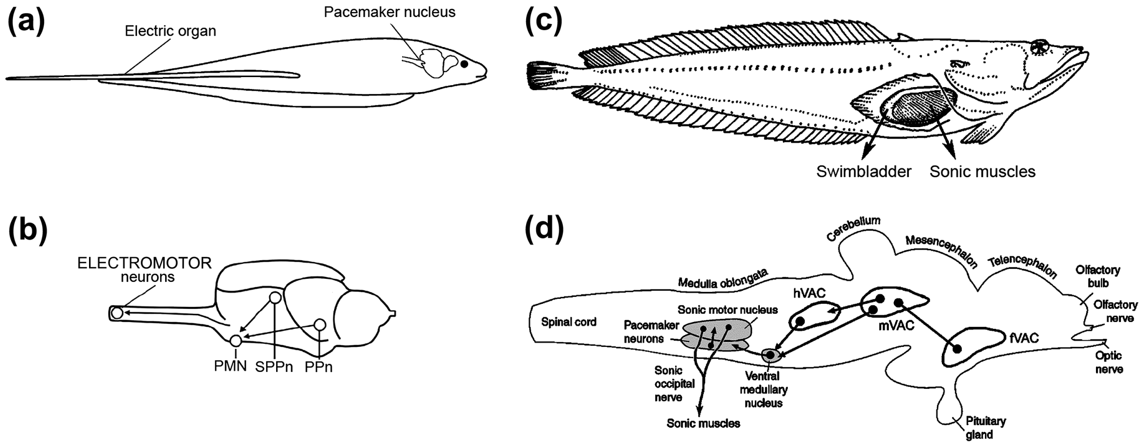

**FIGURE 7.6** (a) Schematic representation of the prepacemaker nucleus and electric organ in a gymnotiform. Redrawn from McAnelly and Zakon (2000). (b) The neural circuitry regulating electric organ discharge (EOD) in gymnotiforms. The pacemaker nucleus (PMN) sets the EOD frequency. The firing rate of the PMN is in turn modulated by the thalamic prepacemaker nucleus (PPn) and by the sublemniscal prepacemaker nucleus (SPPn). Redrawn from Zakon, Oestreich, Tallarovic, and Triefenbach (2002). (c) The type I male midshipman produces vocalizations by vibrating the swim bladder lateral walls through a pair of sonic muscles. Reproduced from Bass and Zakon (2005). (d) The vocal motor network includes vocal−acoustic integration centers (VACs) at the forebrain (f), midbrain (m), and hindbrain (h) level that input, via a ventral medullary nucleus, into the hindbrain-spinal sonic motor nucleus, which innervates the sonic muscles (for details and other references see Bass and Zakon, 2005).

signals used in female attraction (Bastian et al., 2001), and in male white perch (*Morone americana*) intracerebroventricular AVT administration promoted male sexual displays (Salek, Sullivan, & Godwin, 2002). In the grass puffer (*Takifugu niphobles*), brain AVT mRNA expression increases in prespawning females, suggesting a relationship with female sexual behavior (Motohashi, Hamabata, & Ando, 2006). In the above-mentioned sex-role-reversed population of the peacock blenny (*S. pavo*), AVT mRNA was higher in the courting morphs (females and female-like sneaker males) than in nest-holders, suggesting that AVT is correlated with the expression of female and female-like courtship displays (Grober, George, Watkins, Carneiro, & Oliveira, 2002). Accordingly, exogenous AVT administration to females and sneakers promoted female courtship displays, whereas AVT administration had no effects on the sexual displays by nest-holders (Carneiro, Oliveira, Canário, & Grober, 2003).

Taken together, these results suggest that AVT generally promotes courtship behavior in teleost fishes, but future studies are necessary to clarify how the different subpopulations of AVT neurons regulate male and female sexual behavior. It is also possible that the modulation of courtship behavior by the AVT system is under the regulation of GnRH as, in *O. mykiss*, *in-vitro* GnRH administration to POA-AVT neurons stimulated their electrical activity (Saito, Hasegawa, & Urano, 2003).

### 3.2.3. Isotocin (IST)

Oxytocin, the mammalian homolog of IST, has been implicated in the activation of female sexual behavior and in the inhibition of female aggression in rats (Pedersen & Boccia, 2002). The effects of IST in fish sexual behavior, however, have been investigated in only a few studies. In electrophysiological studies of the plainfin midshipman CNS, IST inhibited fictive grunt vocalizations (used in an agonistic context) in type II parasitic males and females but not in type I territorial males (Goodson & Bass, 2000a). In the beaugregory damselfish (*S. leucosticus*), intramuscular injections of IST had no effect on territorial male aggressive behavior (Santangelo & Bass, 2006). In the goldfish, intraperitoneal injections of IST increases plasma $E_2$ levels (Mennigen et al., 2008), and $E_2$ inhibits aggressive behavior in fishes (e.g., Gonçalves et al., 2007). The results thus suggest that IST reduces aggressive behavior in fishes, and there is the possibility that these effects are partially mediated by steroids.

In females of the grass puffer, brain IST mRNA expression did not change during the breeding period (Motohashi et al., 2006), although brain IST levels measured with high performance liquid chromatography (HPLC) in female (but not male) three-spined stickleback were maximal during the reproductive season (Gozdowska, Kleszczynska, Sokolowska, & Kulczykowska, 2006). In spite of these correlational data, the role of IST in fish courtship displays is for now unclear as experimental studies are missing.

Neurohypophysial hormones are also thought to play a role in consummatory aspects of fish sexual behavior. The spawning reflex has been induced by the administration of pituitary extracts to hypophysectomized male killifish (*Fundulus heteroclitus*) (Pickford, 1952) and this result was later confirmed for both males and females of the same species (Wilhelmi, Pickford, & Sawyer, 1955). Similar results were afterward obtained for female medaka

(*Oryzias latipes*) (Egami, 1959), female bitterling (*Rhodeus sericeus*) (Egami & Ishii, 1962), and male and female flagfish (*Jordanella floridae*) (Crawford, 1975). In the killifish, AVT was more effective than IST in eliciting the spawning reflex (Pickford & Strecker, 1977). In several other species, however, neurohypophysial hormones failed to induce the spawning reflex (*G. aculeats* (T. J. Lam and Y. Nagahama, personal communication in Liley & Stacey, 1983), *C. auratus* (G. E. Pickford in Macey, Pickford, & Peter, 1974), *Misgurnus fossilis*, and *S. salar* (Egami & Ishii, 1962)). In male seahorses (*Hippocampus hippocampus*), both OXY and IST failed to induce spawning behavior, although these substances did induce parturition reflexes (Fiedler, 1970).

Interestingly, in *Clarias batrachus* intraovarian pressure induces the hypertrophy of POA neurosecretory neurons (Subhedar, Krishna, & Deshmukh, 1987) and pharmacologically blocking putative mechanosensory channels in the ovary inhibits the effect in POA cells (Subhedar, Deshmukh, Jain, Khan, & Krishna, 1996; see also Deshmukh & Subhedar, 1993). This could be a potential mechanism for synchronizing ovarian development and oocyte maturation with the putative endocrine-induced spawning reflex.

### 3.3. Prostaglandins (PGs)

In mammals, the fatty-acid-derived PGs promote luteolysis, facilitate female sexual behavior (e.g., Buntin & Lisk, 1979), and mediate the perinatal brain masculinization induced by steroids (Amateau & McCarthy, 2004). In fishes, plasma PGs, in particular $PGF_{2\alpha}$, peak during ovulation (e.g., Goetz & Cetta, 1983). Prostaglandins produced in the mature ovary may serve three main functions in teleosts: to act in a paracrine fashion to stimulate follicular rupture; to enter the bloodstream and act on the nervous system, activating female sexual receptivity and spawning behavior; and to be released into the water as a pheromone in order to stimulate male sexual behavior (for a review see Sorensen & Goetz, 1993; see also Chapter 6, this volume). The role of $PGF_{2\alpha}$ in sexual behavior has been investigated in more detail in the goldfish. Injecting non-ripe female goldfish with mature oocytes (Stacey & Liley, 1974) or with $PGF_{2\alpha}$ (e.g., Stacey & Liley, 1974; Stacey, 1976; 1981; Sorensen & Goetz, 1993; Kobayashi, Sorensen, & Stacey, 2002) stimulates female behavior associated with oviposition. Response to $PGF_{2\alpha}$ administration is similar in ovariectomized, intact, or sham females, suggesting that oviposition behavior is probably induced by $PGF_{2\alpha}$ produced in the reproductive tract during ovulation (Kobayashi & Stacey, 1993). Additionally, sexually mature male goldfish treated with $PGF_{2\alpha}$ display female-like spawning behavior similar to $PGF_{2\alpha}$-injected females (Stacey, 1976; 1977; Stacey & Kyle, 1983). The facilitation of female reproductive behavior by $PGF_{2\alpha}$ has been extended to other species, namely the cichlid *Cichlasoma bimaculatum* (Cole & Stacey, 1984), the paradise fish (*Macropodus opercularis*) (Villars, Hale, & Chapnick, 1985), and the Java barb (*Barbonymus gonionotus*) (Liley & Tan, 1985). In spite of this well-established role of $PGF_{2\alpha}$ in facilitating female sexual behavior, the neural targets of $PGF_{2\alpha}$ responsible for this effect are for now unknown.

Additionally, $PGF_{2\alpha}$ produced by ovulated females is released into the water and acts as a potent olfactory stimulant for males (Sorensen, Hara, Stacey, & Goetz, 1988; Sorensen et al., 1989). Exposure to $PGF_{2\alpha}$ upregulates GnRH3 mRNA expression in the male brain (Chung-Davidson, Rees, Bryan, & Li, 2008) and thus the pheromonal action of $PGF_{2\alpha}$ on male sexual displays may be mediated by a GnRH system. By promoting receptivity and spawning behavior in females and activating sexual responses in males, $PGF_{2\alpha}$ produced in the oviduct of mature females is thought to synchronize male and female reproductive behavior (for reviews see Kobayashi et al., 2002; Stacey, 2003).

## 4. BRAIN CIRCUITS UNDERLYING SEXUAL BEHAVIOR IN FISHES

To understand how hormones modulate sexual behaviors it is fundamental to identify the neural substrates upon which hormones act. The techniques available to study the brain areas implicated in the expression of behaviors in fishes include electrophysiological and immunohistochemical techniques, gene expression, directed lesions, and more recently *in-vivo* imaging and gene manipulation techniques (see Section 5). Since the 1950s, ablation and lesion studies have shown that telencephalic regions are important for the expression of sexual behaviors. Bilateral ablation of the telencephalon does not abolish sexual behaviors but reduces the frequency of their expression (e.g., Kamrin & Aronson, 1954; Aronson & Kaplan, 1968; Davis & Kassel, 1980). Studies in which partial lesions directed to various regions of the telencephalon were conducted suggest that the suppressive effects of bilateral telencephalon ablation on sexual behavior are a consequence of damage to the ventral regions of the telencephalon, including those containing part of the POA (e.g., Davis, Kassel, & Martinez, 1981; Koyama, Satou, Oka, & Ueda, 1984). Lesions to the POA reduce the male spawning reflex in *Fundulus heteroclitus* (Macey et al., 1974) and lesions to ventral telencephalon regions dorsal and anterior to the anterior commisure (the area ventralis telencephali pars supracommisuralis and the posterior part of the area ventralis telencephali pars ventralis) impair male spawning behavior in the goldfish (Kyle & Peter, 1982; Kyle, Stacey, & Peter, 1982). Electrical stimulation of these brain areas, on the other hand, evokes courtship and spawning behaviors in several species (e.g., Demski & Knigge, 1971; Demski, Bauer, & Gerald, 1975; Demski & Hornby, 1982; Satou et al., 1984). In spite of these early studies and of the more recent technical possibilities, detailed knowledge on the neural circuitry underlying the expression of specific sexual behaviors is available for only a few species. The mechanism of EOD signal production of electric fishes and the sound-emitting behavior of sound-producing fishes are probably the best-described models so far (for a comparative review see Bass & Zakon, 2005). The wave-type EOD signal of Gymnotiformes and the vocal signaling behavior of the plainfin midshipman are presented as examples, although other electric (i.e., mormyrids) and acoustic (e.g., toadfish) systems have been well characterized.

The electrical signal of Gymnotiformes is produced by electrocyte cells displayed bilaterally in a serial arrangement in the tail. These cells are activated by electromotor neurons, which, in turn, receive input from relay cells adjacent to a small set of ca. 50−100 neurons located in the pacemaker nucleus (Pn) of the medulla oblongata (Figure 7.6). The pacemaker neurons discharge spontaneously and set the EOD timing in a 1 : 1 fashion. The pulse duration of the EOD, on the other hand, is determined by the ion current kinetics of the electrocytes. Within the same animal, consecutive EODs fire at a highly regular rate, and the wave-type EOD signal of Gymnotiformes is probably the most regular pacemaker system known in vertebrates (Moortgat, Keller, Bullock, & Sejnowski, 1998). This continuous firing serves the role of electrosensing, but the properties of the EOD waveform also communicate species, sex, and social status (Wong & Hopkins, 2007). Besides this function, the EOD signal is used in communication by changing its temporal and spectral properties. For example, as part of their courtship displays, males of the genus *Brachyhypopomus* increase the frequency of the EODs in early phases of the courtship sequence (‘acceleration’) and reduce the amplitude of the EODs in later phases (‘chirp’) (for review see Stoddard, 2002; Stoddard, Zakon, Markham, & McAnelly, 2006). The Pn receives input from two other nuclei: the midbrain sublemniscal prepacemaker nucleus (SPPn) and the thalamic prepacemaker nucleus (PPn) (Figure 7.6). In *Eigenmannia*, cells from the SPPn mediate smooth decreases in the frequency of the EOD. Within the PPn, two subpopulations of neurons have been identified: a medial population that mediates gradual increases in EOD frequency (PPn-G) and lateral magnocellular neurons (PPn-C) that evoke interruptions in the EOD. Prepacemaker nuclei in turn receive input from the ventral telencephalon and POA (reviewed in Zupanc & Maler, 1997; Zupanc, 2002). In *Eigenmannia*, electrical stimulation of rostral POA regions, but not of other forebrain regions, evoke interruptions in the EOD discharges, similar to those naturally observed during courtship, suggesting that the POA may control some aspects of the EOD modulation observed during courtship (Wong, 2000).

The wave-type EOD signal is sexually dimorphic, with the EOD pulse being longer in males than in females (e.g., Hopkins, Comfort, Bastian, & Bass, 1990). Additionally, rapid, intermediate, or long-term changes in the properties of the EOD occur and are regulated by different neuroendocrine mechanisms. For example, the rapid production of chirps in *A. leptorhynchus* is mediated by AVT (Bastian et al., 2001); the intermediate (6−45 minute) changes in EOD properties observed during agonistic interactions in *Brachyhypopomus pinnicaudatus* are mediated by melanocortin peptide hormones such as corticotropin (ACTH) (Markham & Stoddard, 2005); and the long-term changes in the EOD signal (seasonal, ontogenetic) are mediated by sex steroids (e.g., Few & Zakon, 2001; 2007). As androgen administration decreases EOD frequency, set by Pn neurons, and also increases pulse duration, determined by ionic properties of the electrocyte membranes, androgens are predicted to act on both sites. Indeed, electrocytes express androgen receptors (Gustavson, Zakon, & Prins, 1994), small androgen implants in the electric organ increase EOD pulse duration but not frequency (Few & Zakon, 2001), and androgens induce gene expression changes in $K^+$ ionic channels in electrocytes that ~~explain~~ the EOD pulse duration increase (Few & Zakon, 2007). Although androgen receptors are not expressed in the Pn (Gustavson et al., 1994), they are located in the upstream SPPn and PPn nuclei (Zakon, 1996), and thus it is possible that androgens masculinize the EOD frequency acting on these nuclei or even in other brain regions afferent to the prepacemaker nuclei.

In the plainfin midshipman and in other Batrachoididae, sounds are produced by the contraction of a pair of sonic muscles attached to the swim bladder. Unlike the electrical signal of Gymnotiformes, sounds are only produced in social contexts. The sonic muscles are commanded by ipsilateral motor neurons originating from a bilateral hindbrain−spinal cord nucleus. The firing rate of the sonic motor neurons is established by adjacent pacemaker-like neurons that innervate the sonic motor nucleus. Together, the pacemaker and sonic motor neurons form the vocal pattern generator (Figure 7.6). Modulation of the firing rate of pacemaker neurons occurs via more anterior regions. The pacemaker nucleus receives input from a rostro-ventral medullary nucleus and this in turn receives input from vocal−acoustic integration centers (VACs) located in the mid and hindbrain (Figure 7.6). A third forebrain VAC located in the POA/anterior hypothalamus inputs into the midbrain VAC. The synchronous activity of the sonic motor neurons produces compound action potentials that can be easily recorded from the descending occipital nerve roots and that are matched 1 : 1 with the sonic muscle contraction and sound pulses. The compound action potentials can be elicited by electric stimulation of forebrain and midbrain VACs ('fictive calls') (for details see Bass, 2008). During the breeding season, nesting males produce long-lasting (one-hour) advertisement 'hums' used in female attraction and short (50—100 ms defense 'grunts' (Bass & McKibben, 2003). The surge in circulating 11-KT observed in nesting males during the breeding season is thought to trigger the 'hum' calls (Knapp, Marchaterre, & Bass, 2001). In accordance, intramuscular injections of 11-KT (and also $E_2$ and cortisol) to nesting males caused within five minutes a significant increase in the duration of midbrain-elicited fictive calls (Remage-Healey & Bass, 2004). Contrarily, T is the predominant circulating androgen during the reproductive period in females and sneaker males, and T, but not 11-KT, increases ~~VPG~~ output (Remage-Healey & Bass, 2007). Using intact and semi-intact CNS preparations, the hindbrain—spinal cord region has been found to be necessary and sufficient for these fast effects of steroids, while midbrain input seems necessary for the continuation (up to 120 minutes) of these effects (Remage-Healey & Bass, 2004).

These two examples testify how understanding of the neural circuitry underlying behaviors may help us to draw testable hypotheses related to their endocrine regulation, and how this contributes to an integrated view of the neurophysiological mechanisms of behavior. Nevertheless, the extraordinary research being done in electric and acoustic fishes is for the time being not paralleled in other species, and in general our knowledge about the brain areas underlying the expression of sexual behaviors in fishes and their neuroendocrine regulation is still poor.

## 5. PROSPECTS FOR FUTURE RESEARCH

As in other fields, behavioral neuroendocrinology is experiencing exciting technological developments that promise to significantly increase our knowledge in this area within the next few years. These include developments in molecular biology, genetics, and brain imaging.

### 5.1. *In-vivo* Imaging of Brain Activity

As we have seen, identifying the brain regions implicated in the regulation of specific behavioral patterns is a fundamental step for understanding their endocrine regulation. In the last 20 years, noninvasive imaging techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have been applied in humans and primates in order to identify brain areas underlying the expression of particular behaviors, and have revolutionized our understanding of brain function (for a review see Otte & Halsband, 2006). The development of scans that fit small animals has allowed extension of this technology to other mammalian and nonmammalian species (for a review see Van der Linden, Van Camp, Ramos-Cabrer, & Hoehn, 2007). Functional magnetic resonance imaging has already been applied to fishes (Van den Burgh et al., 1999) but behaviorally relevant questions have not been addressed. It should be pointed out that fMRI requires the immobilization of the animal, limiting the questions that can be tested. Nevertheless, as in humans and primates, brain activation by external cues (e.g., pheromones, sight of a mating partner) in immobilized animals may provide interesting data on the brain areas activated by different social contexts. Endocrine manipulations of immobilized animals exposed to social stimuli could also provide insights into the roles played by hormones in the modulation of brain activity. Manganese-enhanced MRI techniques also can be applied to the study of brain activation in nonimmobilized animals. Manganese enters the cells during voltage-gated $Ca^{2+}$ channel opening and has a low clearance time, and can thus be used as a contrast agent in MRI to assess brain electrical activity. By administering $Mn^{2+}$ to animals before the behaviors are elicited and performing MRI scans immediately afterwards it is possible to visualize the brain areas that have just been active. Nevertheless, as in immunohistochemical or *in situ* hybridization studies using immediate early gene expression for the same purposes, manganese-enhanced MRI also lacks specificity (see Van der Linden et al., 2007 for details). Combining fMRI and manganese-enhanced MRI with more classical techniques (electrophysiology, lesions, immunohistochemistry, immunoneutralization) seems a promising strategy for identifying the neural substrates underlying the expression of specific behaviors in fishes.

### 5.2. Molecular Biology

Molecular biological tools are valuable technical resources when trying to understand the mechanisms through which hormones change cells and ultimately behavior. There are aspects of hormonal action in relation to behavior for which molecular biological tools are particularly valuable. These techniques can be applied to understanding how changes in gene expression and ultimately protein production influence behavior, and how hormones might mediate these changes. They may also help us to understand how the same hormone may have very different effects in different individuals of the same species or even in the same animal at different times. Hormonal action is influenced by a multitude of factors, including the action of transport proteins, sensitivity of the target tissues to hormones, and interaction with other modulators (neuropeptides, enzymes, other hormones), and molecular biological tools may be useful in characterizing some of these aspects, providing a more integrative view of hormonal action.

Currently, knowledge of the species genome/transcriptome is growing at an exponential rate, due mostly to new massive sequencing techniques (Morozova & Marra, 2008). This knowledge facilitates the design of methods for gene-expression profiling (e.g., microarrays, RNAseq) and also the interpretation of results from gene-expression studies. These recent advances have allowed documentation of differences in brain gene expression profiles in animals with divergent sexual behaviors (e.g., males and females, dominants and subordinates, bourgeois and sneaker males) (see St-Cyr & Aubin-Horth, 2009 for a list of studies). It seems likely that most of these differences are under hormonal regulation and that some will be behaviorally relevant. Fish behavioral genomics is therefore a growing field and data for non-model species will certainly accumulate over the next few years, allowing comparative functional genomic studies that may provide important insights into the neuroendocrine mechanisms mediating behavior. Nevertheless, knowledge of protein function is clearly falling behind DNA/RNA sequencing, and further effort will be necessary in this respect.

## 5.3. Genetics

New genetic approaches applied to fish behavior are also very promising. There are now a multitude of techniques available for manipulating gene expression in many animals, including fishes, and some recent advances are worth noting. A landmark of gene manipulation techniques in fishes was the development of the first transgenic teleost in 1984 by Zhu, Li, He, and Chen (1985), where a recombinant human growth hormone gene was introduced into a goldfish genome. Since then, many stable transgenic lines have been produced in species such as zebrafish, medaka, carp, and salmon. In 1999, the first zebrafish GAL4-UAS stable transgenic line was produced (Sheer & Campos-Ortega, 1999). In the GAL4-UAS transgenic binary system, the gene encoding a yeast transcription activator protein (Gal4) is placed under the control of a native gene promoter, or driver gene. In another line, a short sequence of the promoter region activated by Gal4 (UAS) is placed upstream of a target gene. By crossing both lines, it is possible to obtain transgenics where the activation of the target gene by Gal4 binding to UAS is restricted to cells expressing the driver gene (i.e., synthesizing Gal4). This system is now highly refined in *Drosophila* and has been used with enormous success in the study of the neural control of this genus' behavior, including the neuroendocrine regulation of sexual behavior (e.g., Certel, Savella, Schlegel, & Kravitz, 2007). In zebrafish, the system is not as refined, but recent advances have been made. As an example, in a 2008 study this system was used in zebrafish to assess the role played by distinct populations of neurons in a specific behavior, the touch-response behavior. Gene and enhancer trap methods were first applied to generate fish expressing Gal4 in specific cells. Gal4 gene expression was visualized by crossing Gal4 transgenics with another transgenic line carrying the green or red fluorescent protein (GFP, RFP) gene downstream of the Gal4 recognition sequence (UAS). Finally, parental lines where Gal4 was expressed in relevant cell types were crossed with another transgenic line carrying a synaptic transmission blocker gene (the tetanus toxin light chain gene) downstream of UAS. Gal4 expressed in different neural populations, and thus blocking synaptic transmission in different brain regions, caused distinct abnormalities in the touch-response behavior (for details see Asakawa et al., 2008). Transgenic Gal4 medaka lines combined with a heat-shock promoter that allows a temporal control by temperature of the expression of the gene downstream to UAS have also been developed (Grabher & Wittbrodt, 2004).

These examples illustrate the potential of genetic manipulation for studying behavior in fishes. The field of behavioral neuroendocrinology will certainly benefit from this technology in the near future.

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## ABBREVIATIONS

| | |
|---|---|
| **11-KA** | 11-ketoandrostenedione |
| **11-KT** | 11-ketotestosterone |
| **17,20β,21P** | 17α,20β,21-trihydroxy-4-pregnen-3-one |
| **17,20βP** | 17,20β-dihydroxy-4-pregen-3-one |
| **17,20αP** | 17,20α-dihydroxy-4-pregen-3-one |
| **ACTH** | Corticotropin |
| **ART** | Alternative reproductive tactic |
| **AVP** | Arginine vasopressin |
| **AVT** | Arginine vasotocin |
| **CNS** | Central nervous system |
| **CYP19** | See $P450_{aro}$ |
| **$E_2$** | Estradiol |
| **EOD** | Electric organ discharge |
| **fMRI** | Functional magnetic resonance imaging |
| **FSH** | Follicle-stimulating hormone |
| **GFP** | Green fluorescent protein |
| **GnRH** | Gonadotropin-releasing hormone |
| **GTH** | Gonadotropin |
| **HPG** | Hypothalamic-pituitary-gonadal |
| **HPLC** | High pressure liquid chromatography |
| **IP** | Initial phase |
| **IST** | Isotocin |

| | |
|---|---|
| **LH** | Luteinizing hormone |
| **mPOA** | Medial preoptic area |
| **MT** | Methyltestosterone |
| **OHT** | 11β-hydroxytestosterone |
| **OXY** | Oxytocin |
| **$P450_{aro}$** | P450 enzyme aromatase |
| **PET** | Positron emission tomography |
| **PG** | Prostaglandin |
| **$PGF_{2\alpha}$** | Prostaglandin $F_{2\alpha}$ |
| **Pn** | Pacemaker nucleus |
| **POA** | Preoptic area |
| **PPn** | Prepacemaker nucleus |
| **RFP** | Red fluorescent protein |
| **SPPn** | Sublemniscal prepacemaker nucleus |
| **T** | Testosterone |
| **TP** | Terminal phase |
| **VAC** | Vocal–acoustic integration center |
| **VPG** | |

## REFERENCES

Abe, T., & Munehara, H. (2005). Spawning and maternal-care behaviours of a copulating sculpin, *Radulinopsis taranetzi*. *Journal of Fish Biology, 67*, 201–212.

Almada, V. C., Gonçalves, E. J., Oliveira, R. F., & Santos, A. J. (1995). Courting females - ecological constraints affect sex-roles in a natural population of the blenniid fish *Salaria pavo*. *Animal Behaviour, 49*, 1125–1127.

Almada, V. C., Henriques, M., & Gonçalves, E. J. (1998). Ecology and behaviour of reef fishes in the temperate north-eastern Atlantic and adjacent waters. In V. C. Almada et al. (Eds.), *Behaviour and conservation of littoral fishes* (pp. 33–69). Lisboa: ISPA.

Amano, M., Hyodo, S., Urano, A., Okumoto, N., Kitamura, S., Ikuta, K., et al. (1994). Activation of salmon gonadotropin-releasing hormone synthesis by 17 alpha-methyltestosterone administration in yearling masu salmon, *Oncorhynchus masou*. *General and Comparative Endocrinology, 95*, 374–380.

Amateau, S. K., & McCarthy, M. M. (2004). Induction of PGE(2) by estradiol mediates developmental masculinization of sex behavior. *Nature Neuroscience, 7*, 643–650.

Amorim, M. C. P., Knight, M. E., Stratoudakis, Y., & Turner, G. F. (2004). Differences in sounds made by courting males of three closely related Lake Malawi cichlid species. *Journal of Fish Biology, 65*, 1358–1371.

Amorim, M. C. P., Simões, J. M., Fonseca, P. J., & Turner, G. F. (2008). Species differences in courtship acoustic signals among five Lake Malawi cichlid species (*Pseudotropheus* spp.). *Journal of Fish Biology, 72*, 1355–1368.

Amundsen, T., & Forsgren, E. (2001). Male mate choice selects for female coloration in a fish. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 13155–13160.

Andersson, M. (1984). *Sexual selection*. Princeton: Princeton University Press.

Aronson, L. R., & Kaplan, N. (1968). Functions of the teleostean forebrain. In D. Ingle (Ed.), *The central nervous system and fish behavior* (pp. 107–125). Chicago: University of Chicago Press.

Aronson, L. R. (1951). Factors influencing the spawning frequency in the female cichlid fish *Tilapia macrocephala*. *American Museum Novitates, 1484*, 1–26.

Asakawa, K., Suster, M. L., Mizusawa, K., Nagayoshi, S., Kotani, T., Urasaki, A., et al. (2008). Genetic dissection of neural circuits by Tol2 transposon-mediated Gal4 gene and enhancer trapping in zebrafish. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 1255–1260.

Backström, T., & Winberg, S. (2009). Arginine-vasotocin influence on aggressive behavior and dominance in rainbow trout. *Physiology and Behavior, 96*, 470–475.

Badura, L. L., & Friedman, H. (1988). Sex reversal in female *Betta splendens* as a function of testosterone manipulation and social influence. *Journal of Comparative Psychology, 102*, 262–268.

Baggerman, B. (1957). An experimental study on the timing of breeding and migration in the three-spined stickleback (*Gasterosteus aculeatus* L.). *Archives Ne´erlandaises de Zoologie, 12*, 105–318.

Baggerman, B. (1966). On the endocrine control of reproductive behaviour in the male three-spined stickleback (*Gasterosteus aculeatus* L.). *Symposia of the Society for Experimental Biology, 20*, 427–456.

Baggerman, B. (1968). Hormonal control of reproduction and parental behaviour in fishes. In E. J. W. Barrington, & C. Barker Jørgensen (Eds.), *Perspectives in endocrinology: hormones in the lives of lower vertebrates* (pp. 351–404). New York: Academic Press.

Ball, G. F., & Balthazart, J. (2004). Hormonal regulation of brain circuits mediating male sexual behavior in birds. *Physiology and Behavior, 83*, 329–346.

Ball, G. F., & Balthazart, J. (2008). How useful is the appetitive and consummatory distinction for our understanding of the neuroendocrine control of sexual behavior? *Hormones and Behavior, 53*, 307–311, author reply 315–308.

Balment, R. J., Lu, W., Weybourne, E., & Warne, J. M. (2006). Arginine vasotocin a key hormone in fish physiology and behaviour: A review with insights from mammalian models. *General and Comparative Endocrinology, 147*, 9–16.

Balon, E. K. (1984). Patterns in the evolution of reproductive styles in fishes. In G. W. Potts, & R. J. Wootton (Eds.), *Fish reproduction: strategies and tactics* (pp. 35–53). New York: Academic Press.

Balshine-Earn, S., & Earn, D. J. D. (1998). On the evolutionary pathway of parental care in mouth-brooding cichlid fish. *Proceedings of the Royal Society of London Series B-Biological Sciences, 265*, 2217–2222.

Balthazart, J., & Ball, G. F. (2006). Is brain estradiol a hormone or a neurotransmitter? *Trends in Neurosciences, 29*, 241–249.

Barata, E. N., Hubbard, P. C., Almeida, O. G., Miranda, A., & Canário, A. V. M. (2007). Male urine signals social rank in the Mozambique tilapia (*Oreochromis mossambicus*). *BMC Biology, 12*, 5–54.

Barber, I., Nairn, D., & Huntingford, F. A. (2001). Nests as ornaments: revealing construction by male sticklebacks. *Behavioral Ecology, 12*, 390–396.

Barnett, C. W., & Pankhurst, N. W. (1994). Changes in plasma levels of gonadal steroids and gonad morphology during the spawning cycle of male and female demoiselles *Chromis dispilus* (Pisces: Pomacentridae). *General and Comparative Endocrinology, 93*, 260–274.

Barnett, D. K., Bunnell, T. M., Millar, R. P., & Abbott, D. H. (2006). Gonadotropin-releasing hormone II stimulates female sexual behavior in marmoset monkeys. *Endocrinology, 147*, 615–623.

Bass, A. H., & Hopkins, C. D. (1983). Hormonal control of sexual differentiation: changes in electric organ discharge waveform. *Science, 220*, 971–974.

Bass, A. H., & Hopkins, C. D. (1985). Hormonal control of sex differences in the electric organ discharge (EOD) of mormyrid fishes. *Journal of Comparative Physiology A, 156*, 587—604.

Bass, A. H., & McKibben, J. R. (2003). Neural mechanisms and behaviors for acoustic communication in teleost fish. *Progress in Neurobiology, 69*, 1—26.

Bass, A. H., & Remage-Healey, L. (2008). Central pattern generators for social vocalization: Androgen-dependent neurophysiological mechanisms. *Hormones and Behavior, 53*, 659—672.

Bass, A. H., & Volman, S. F. (1987). *From behavior to membranes: testosterone-induced changes in action potential duration in electric organs, 84*, 9295—9298.

Bass, A. H., & Zakon, H. H. (2005). *Sonic and electric fish: At the crossroads of neuroethology and behavioral neuroendocrinology Hormones and Behavior, 48*, 360—372.

Bass, A. H. (2008). Steroid-dependent plasticity of vocal motor systems: Novel insights from teleost fish. *Brain Research Reviews, 57*, 299—308.

Bastian, J., Schniederjan, S., & Nguyenkim, J. (2001). Arginine vasotocin modulates a sexually dimorphic communication behavior in the weakly electric fish *Apteronotus leptorhynchus*. *Journal of Experimental Biology, 204*, 1909—1923.

Baum, M. J. (2003). Activational and organizational effects of estradiol on male behavioral neuroendocrine function. *Scandinavian Journal of Psychology, 44*, 213—220.

Beach, F. A. (1942). Analysis of factors involved in the arousal, maintenance and manifestation of sexual excitement in male animals. *Psychosomatic Medicine, 4*, 173—198.

Bell, A. M. (2001). Effects of an endocrine disrupter on courtship and aggressive behaviour of male three-spined stickleback, *Gasterosteus aculeatus*. *Animal Behaviour, 62*, 775—780.

Berglund, A., & Rosenqvist, G. (1990). *Male limitation of female reproductive success in a pipefish: Effects of body-size differences, 27*, 129—133.

Berglund, A., & Rosenqvist, G. (1993). *Selective males and ardent females in pipefishes, 32*, 331—336.

Berglund, A., & Rosenqvist, G. (2003). Sex-role reversal in pipefish. *Advances in the Study of Behavior, 32*, 131—167.

Berglund, A., & Rosenqvist, G. (2009). An intimidating ornament in a female pipefish. *Behavioral Ecology, 20*, 54—59.

Berglund, A., Bisazza, A., & Pilastro, A. (1996). Armaments and ornaments: An evolutionary explanation of traits of dual utility. *Biological Journal of of the Linnean Society, 58*, 385—399.

Berglund, A., Rosenqvist, G., & Svensson, I. (1986). Reversed sex roles and parental energy investment in zygotes of two pipefish (Syngnathidae) species. *Marine Ecology-Progress Series, 29*, 209—215.

Berglund, A., Rosenqvist, G., & Svensson, I. (1989). Reproductive success of female limited by males in two pipefish species. *The American Naturalist, 133*, 506—516.

Berglund, A., Widemo, M. S., & Rosenqvist, G. (2005). *Sex-role reversal revisited: choosy females and ornamented, competitive males in a pipefish Behavioral Ecology, 16*, 649—655.

Berthold, A. A. (1849). Transplantation der Hoden. *Archiv für Anatomie, Physiologie und wissenschaftliche Medicin*, 42—46.

Black, M. P., Balthazart, J., Baillien, M., & Grober, M. (2005). Socially induced and rapid increases in aggression are inversely related to brain aromatase activity in a sex-changing fish, *Lythrypnus dalli*. *Proceedings of the Royal Society of London Series B-Biological Sciences, 272*, 2435—2440.

Borg, B., & Mayer, I. (1995). Androgens and behaviour in the three-spined stickleback. *Behaviour, 132*, 1025—1035.

Borg, B. (1987). Stimulation of reproductive behaviour by aromatizable and non-aromatizable androgens in the male three-spined stickleback, *Gasterosteus aculeatus* L. *Proceedings of the V Congress of European Ichthyologists, Stockholm, 1985*, 269—271.

Borg, B. (1994). Androgens in teleost fishes. *Comparative Biochemistry and Physiology, 109C*, 219—245.

Brantley, R. K., & Bass, A. H. (1994). Alternative male spawning tactics and acoustic signals in the plainfin midshipman fish *Porichthys notatus* Girard (Teleostei, Batrachoididae). *Ethology, 96*, 213—232.

Brantley, R. K., Wingfield, J. C., & Bass, A. H. (1993). Sex steroid levels in *Porichthys notatus*, a fish with alternative reproductive tactics, and a review of the hormonal bases for male dimorphism among teleost fishes. *Hormones and Behavior, 27*, 332—347.

Breder, C. M., Jr., & Rosen, D. E. (1966). *Modes of reproduction in fishes*. New York: The Natural History Press.

Brockmann, H. J. (2001). The evolution of alternative strategies and tactics. *Advances in the Study of Behavior, 30*, 1—51.

Buntin, J. D., & Lisk, R. D. (1979). Prostaglandin-E2-induced lordosis in estrogen-primed female hamsters - relationship to progesterone action. *Physiology & Behavior, 23*, 569—575.

Canosa, L. F., Stacey, N., & Peter, R. E. (2008). Changes in brain mRNA levels of gonadotropin-releasing hormone, pituitary adenylate cyclase activating polypeptide, and somatostatin during ovulatory luteinizing hormone and growth hormone surges in goldfish. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 295*, R1815—R1821.

Cardwell, J. R., & Liley, N. R. (1991). Androgen control of social status in males of a wild population of stoplight parrotfish, *Sparisoma viride* (Scaridae). *Hormones and Behavior, 25*, 1—18.

Carlson, B. A. (2002). Electric signaling behavior and the mechanisms of electric organ discharge production in mormyrid fish. *Journal of Physiology - Paris, 96*, 405—419.

Carlson, B. A., Hopkins, C. D., & Thomas, P. (2000). Androgen correlates of socially induced changes in the electric organ discharge waveform of a mormyrid fish. *Hormones and Behavior, 38*, 177—186.

Carneiro, L., Oliveira, R., Canário, A. V. M., & Grober, M. (2003). The effect of arginine vasotocin on courtship behaviour in a blenniid fish with alternative reproductive tactics. *Fish Physiology and Biochemistry, 28*, 241—243.

Certel, S. J., Savella, M. G., Schlegel, D. C. F., & Kravitz, E. A. (2007). Modulation of *Drosophila* male behavioral choice. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 4706—4711.

Chen, C. C., & Fernald, R. D. (2008). GnRH and GnRH receptors: distribution, function and evolution. *Journal of Fish Biology, 73*, 1099—1120.

Chizinsky, W. (1968). Effects of castration upon sexual behavior in male platyfish, *Xiphophorus maculatus*. *Physiological Zoology, 41*, 466—475.

Chung-Davidson, Y., Rees, C. J., Bryan, M. B., & Li, W. (2008). Neurogenic and neuroendocrine effects of goldfish pheromones. *The Journal of Neuroscience, 28*, 14492—14499.

Clotfelter, E. D., & Rodriguez, A. C. (2006). Behavioral changes in fish exposed to phytoestrogens. *Environmental Pollution, 144*, 833—839.

Clutton-Brock, T. H., & Vincent, A. C. (1991). Sexual selection and the potential reproductive rates of males and females. *Nature, 351*, 58—60.

Cole, K. S., & Stacey, N. E. (1984). Prostaglandin induction of spawning behavior in *Cichlasoma bimaculatum* (Pisces cichlidae). *Hormones and Behavior, 18*, 235–248.

Compagnone, N. A., & Mellon, S. H. (2000). Neurosteroids: Biosynthesis and function of these novel neuromodulators. *Frontiers in Neuroendocrinology, 21*, 1–56.

Couldridge, V. C. K., & Alexander, G. J. (2002). Color patterns and species recognition in four closely related species of Lake Malawi cichlid. *Behav. Ecol., 13*, 59–64.

Craig, W. (1917). Appetites and aversions as constituents of instincts. *Proceedings of the National Academy of Sciences of the United States of America, 3*, 685–688.

Crawford, J. D. (1991). Sex recognition by electric cues in a sound-producing mormyrid fish, *Pollimyrus isidori*. *Brain Behavior and Evolution, 38*, 20–38.

Crawford, J. D. (1992). Individual and sex specificity in the electric organ discharges of breeding mormyrid fish (*Pollimyrus isidori*). *Journal of Experimental Biology, 164*, 79–102.

Crawford, S. (1975). *An analysis of the reproductive behaviour of the female American Flagfish, Jordanella floridae (Pisces: Cyprinodontidae), including preliminary studies of the hystology of the reproductive tract and of the internal factors regulating spawning behaviour. B. Sc. thesis thesis*. Vancouver, Canada: University of British Columbia.

Curtis, C. C., & Stoddard, P. K. (2003). Mate preference in female electric fish, Brachyhypopomus pinnicaudatus. *Animal Behaviour, 66*, 329–336.

Darwin, C., 1871. The descent of man and selection in relation to sex Murray: London.

Davis, R. E., & Kassel, J. (1980). Behavioral functions of the teleostean telencephalon. (Vol. 2). Higher brain functions. In R. E. Davis, & R. G. Northcutt (Eds.), *Fish neurobiology and behavior*. Ann Harbor: University of Michigan Press.

Davis, R. E., Kassel, J., & Martinez, M. (1981). The telencephalon and reproductive behaviour in the teleost *Macropodus opercularis* (L.): effects of lesions on the incidence of spawning and egg cannibalism. In P. R. Laming (Ed.), *Brain mechanism of behaviour in lower vertebrates* (pp. 239–255). Cambridge: Cambridge University Press.

Demski, L. S., & Hornby, P. J. (1982). Hormonal control of fish reproductive behavior: brain-gonadal steroid interactions. *Canadian Journal of Fisheries and Aquatic Science, 38*, 36–47.

Demski, L. S., & Knigge, K. M. (1971). The telencephalon and hypothalamus of the bluegill (*Lepomis macrochirus*): evoked feeding, aggressive and reproductive behavior with representative frontal sections. *Journal of Comparative Neurology, 143*, 1–16.

Demski, L. S., Bauer, D. H., & Gerald, J. W. (1975). Sperm release evoked by electrical stimulation of the fish brain: a functional-anatomical study. *Journal of Experimental Biology, 191*, 215–232.

Deshmukh, M. K., & Subhedar, N. (1993). Stretch sensory ovarian preoptic pathway in the teleost, *Clarias Batrachus* (Linn) - transection studies. *Journal of Experimental Zoology, 267*, 359–364.

Desjardins, J. K., Hazelden, M. R., Van der Kraak, G. J., & Balshine, S. (2006). Male and female cooperatively breeding fish provide support for the "Challenge Hypothesis". *Behavioral Ecology, 17*, 149–154.

Desjardins, J. K., Stiver, K. A., Fitzpatrick, J. L., Milligan, N., Van Der Kraak, G. J., & Balshine, S. (2008). Sex and status in a cooperative breeding fish: behavior and androgens. *Behavioral Ecology and Sociobiology, 62*, 785–794.

Domeier, M. L., & Colin, P. L. (1997). Tropical reef fish spawning aggregations: Defined and reviewed. *Bulletin of Marine Science, 60*, 698–726.

Dominey, W. (1984). Effects of sexual selection and life history on speciation: species flocks in African cichlids and Hawiian Drosophila. In A. A. Echelle, & I. Kornfield (Eds.), *Evolution of fish species flocks* (pp. 231–249). Orono: University of Maine Press.

Dunlap, K. D., & Zakon, H. H. (1998). Behavioral actions of androgens and androgen receptor expression in the electrocommunication system of an electric fish, *Eigenmannia virescens*. *Hormones and Behavior, 34*, 30–38.

Dunlap, K. D., McAnelly, M. L., & Zakon, H. H. (1997). Estrogen modifies an electrocommunication signal by altering the electrocyte sodium current in an electric fish, *Sternopygus*. *The Journal of Neuroscience, 17*, 2869–2875.

Earley, R. L., & Hsu, Y. Y. (2008). Reciprocity between endocrine state and contest behavior in the killifish, Kryptolebias marmoratus. *Hormones and Behavior, 53*, 442–451.

Egami, N., & Ishii, S. (1962). Hypophyseal control of reproductive functions in teleost fishes. *Gen. Comp. Endocrinol.,* (Suppl. 1), 248–253.

Egami, N. (1959). Preliminary note on the induction of the spawning reflex and oviposition in *Oryzias latipes* by the administration of neurohypophyseal substances. *Annot. Zool. Japon, 32*, 13–17.

Everitt, B. J. (1990). Sexual motivation: a neural and behavioural analysis of the mechanisms underlying appetitive and copulatory responses of male rats. *Neurosci. Biobehav. Rev., 14*, 217–232.

Feulner, P. G. D., Kirschbaum, F., Mamonekene, V., Ketmaier, V., & Tiedemann, R. (2007). Adaptive radiation in African weakly electric fish (Teleostei: Mormyridae: Campylomormyrus): a combined molecular and morphological approach. *Journal of Evolutionary Biology, 20*, 403–414.

Feulner, P. G., Plath, M., Engelmann, J., Kirschbaum, F., & Tiedemann, R. (2008). Electrifying love: electric fish use species-specific discharge for mate recognition. *Biol. Lett*.

Few, W. P., & Zakon, H. H. (2001). Androgens alter electric organ discharge pulse duration despite stability in electric organ discharge frequency. *Hormones and Behavior, 40*, 434–442.

Few, W. P., & Zakon, H. H. (2007). Sex differences in and hormonal regulation of Kv1 potassium channel gene expression in the electric organ: Molecular control of a social signal. *Developmental Neurobiology, 67*, 535–549.

Fiedler, K. (1954). Vergleichende verhaltensstudien an seenadeln, schlangennadeln und seepferdchen (Syngnathidae). *Zeitschrift fur Tierpsychologie, 11*, 358–416.

Fiedler, K. (1970). Hormonale Auslosung der Gebursbewegungen beim Seepferdchen (*Hippocampus*, Syngnathidae, Teleostei). *Z Tierpsychol, 27*, 679–686.

Fishelson, L. (1963). Observations on littoral fishes of Israel I. Behaviour of *Blennius pavo* Risso (Teleostei, Blenniidae). *Israel Journal of Zoology, 12*, 67–91.

Foran, C. M., & Bass, A. H. (1999). Preoptic GnRH and AVT: axes for sexual plasticity in teleost fish. *Gen. Comp. Endocrinol., 116*, 141–152.

Forselius, S. (1957). Studies on anabantid fishes. *Zoologiska Bidrag fran Uppsala, 32*, 593–597.

Forsgren, E., Amundsen, T., Borg, A. A., & Bjelvenmark, J. (2004). Unusually dynamic sex roles in a fish. *Nature, 429*, 551–554.

Francis, R. C., Jacobson, B., Wingfield, J. C., & Fernald, R. D. (1992). Castration lowers aggression but not social dominance in male *Haplochromis burtoni* (Cichlidae). *Ethology, 90*, 247–255.

Friedman, M. A., & Hopkins, C. D. (1996). Tracking individual mormyrid electric fish in the field using electric organ discharge waveforms. *Animal Behaviour, 51*, 391−407.

Froese, R., and Pauly, D. (Eds.) FishBase. World Wide Web electronic publication. www.fishbase.org.,04/2010.

Fryer, G., & Iles, T. D. (1972). *The cichlid fishes of the Great Lakes of Africa: their biology and evolution*. Edinburgh: Oliver and Boyd.

Godwin, J., Crews, D., & Warner, R. R. (1996). Behavioural sex change in the absence of gonads in a coral reef fish. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 263*, 1683−1688.

Godwin, J., Sawby, R., Warner, R. R., Crews, D., & Grober, M. S. (2000). Hypothalamic arginine vasotocin mRNA abundance variation across sexes and with sex change in a coral reef fish. *Brain, Behavior and Evolution, 55*, 77−84.

Goetz, F. W., & Cetta, F. (1983). Ovarian and plasma PGE and PGF levels in naturally ovulating brook trout (*Salvelinus fontinalis*) and the effects of indomethacin on prostaglandin levels. *Prostaglandins, 26*, 387−395.

Gonçalves, E. J., Almada, V. C., Oliveira, R. F., & Santos, A. J. (1996). Female mimicry as a mating tactic in males of the blenniid fish *Salaria pavo*. *Journal of the Marine Biological Association of the United Kingdom, 76*, 529−538.

Gonçalves, D., Alpedrinha, J., Teles, M., & Oliveira, R. F. (2007). Endocrine control of sexual behavior in sneaker males of the peacock blenny *Salaria pavo*: effects of castration, aromatase inhibition, testosterone and estradiol. *Hormones and Behavior, 51*, 534−541.

Gonçalves, D., Matos, R., Fagundes, T., & Oliveira, R. (2005). Bourgeois males of the peacock blenny, *Salaria pavo*, discriminate female mimics from females? *Ethology, 111*, 559−572.

Gonçalves, D., Teles, M., Alpedrinha, J., & Oliveira, R. F. (2008). Brain and gonadal aromatase activity and steroid hormone levels in female and polymorphic males of the peacock blenny Salaria pavo. *Hormones and Behavior, 54*, 717−725.

Goodson, J. L., & Bass, A. H. (2000a). Forebrain peptides modulate sexually polymorphic vocal circuitry. *Nature, 403*, 769−772.

Goodson, J. L., & Bass, A. H. (2000b). Vasotocin innervation and modulation of vocal-acoustic circuitry in the teleost *Porichthys notatus*. *The Journal of Comparative Neurology, 422*, 363−379.

Goodson, J. L., & Bass, A. H. (2001). Social behavior functions and related anatomical characteristics of vasotocin/vasopressin systems in vertebrates. *Brain Res. Brain Res. Rev., 35*, 246−265.

Goulet, D., & Green, J. M. (1988). Reproductive success of the male lumpfish (*Cyclopterus lumpus* L) (Pisces, Cyclopteridae) - evidence against female mate choice. *Canadian Journal of Zoology-Revue Canadienne De Zoologie, 66*, 2513−2519.

Gozdowska, M., Kleszczynska, A., Sokolowska, E., & Kulczykowska, E. (2006). Arginine vasotocin (AVT) and isotocin (IT) in fish brain: Diurnal and seasonal variations. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology, 143*, 330−334.

Grabher, C., & Wittbrodt, J. (2004). Efficient activation of gene expression using a heat-shock inducible Gal4/Vp16-UAS system in medaka. *Bmc Biotechnology,* 4.

Grant, J. W. A. (1997). Territoriality. In J.-G. J. Godin (Ed.), *Behavioural ecology of teleost fishes* (pp. 81−103). Oxford: Oxford University Press.

Greenwood, A. K., Wark, A. R., Fernald, R. D., & Hofmann, H. A. (2008). Expression of arginine vasotocin in distinct preoptic regions is associated with dominant and subordinate behaviour in an African cichlid fish. *Proceedings of the Royal Society B-Biological Sciences, 275*, 2393−2402.

Grober, M. S., George, A. A., Watkins, K. K., Carneiro, L. A., & Oliveira, R. F. (2002). Forebrain AVT and courtship in a fish with male alternative reproductive tactics. *Brain Research Bulletin, 57*, 423−425.

Gustavson, S., Zakon, H. H., & Prins, G. (1994). Androgen receptors in the brain electroreceptors and electric organ of a wave-type electric fish. *Society of Neuroscience Abstracts, 20*, 371.

Hallgren, S. L., Linderoth, M., & Olsen, K. H. (2006). Inhibition of cytochrome p450 brain aromatase reduces two male specific sexual behaviours in the male Endler guppy (*Poecilia reticulata*). *General and Comparative Endocrinology, 147*, 323−328.

Heinrich, W. (1967). Untersuchungen zum Sexualverhalten in der Gattung Tilapia (Cichidae, Teleostei) und bei Arbastarden. *Zeitschrift für Tierpsychologie, 24*, 684−754.

Herfeld, S., & Moller, P. (1998). Effects of 17α-methyltestosterone on sexually dimorphic characters in the weakly discharging electric fish, *Brienomyrus niger* (Gunther, 1866) (Mormyridae): Electric organ discharge, ventral body wall identation, and anal-fin ray bone expansion. *Hormones and Behavior, 34*, 303−319.

Hirschenhauser, K., & Oliveira, R. F. (2006). Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis. *Animal Behaviour, 71*, 265−277.

Hoar, W. S. (1962). Hormones and the reproductive behaviour of the male three-spined stickleback (*Gasterosteus aculeatus*). *Animal Behaviour, 10*, 247−266.

Hofmann, H. A. (2006). Gonadotropin-releasing hormone signaling in behavioral plasticity. *Current Opinion in Neurobiology, 16*, 343−350.

Hopkins, C. D., & Bass, A. H. (1981). Temporal coding of species recognition signals in an electric fish. *Science, 212*, 85−87.

Hopkins, C. D. (1980). Evolution of electric communication channels of Mormyrids. *Behavioral Ecology and Sociobiology, 7*, 1−13.

Hopkins, C. D. (1981). On the diversity of electric signals in a community of mormyrid electric fish in West-Africa. *American Zoologist, 21*, 211−222.

Hopkins, C. D. (1988). Neuroethology of electric communication. *Annual Review of Neuroscience, 11*, 497−535.

Hopkins, C. D., Comfort, N. C., Bastian, J., & Bass, A. H. (1990). Functional-analysis of sexual dimorphism in an electric fish, *Hypopomus pinnicaudatus*, Order Gymnotiformes. *Brain Behavior and Evolution, 35*, 350−367.

Hopkins, C. D. (1999). Signal evolution in electric communication. In M. D. Hauser, & M. Konishi (Eds.), *Design of Animal Communication* (pp. 461−491). Cambridge, Massachusetts: M.I.T. press.

Houde, A. E. (1997). *Sex, color, and mate choice in guppies*. Princeton: Princeton University Press.

Hull, E. M., Lorrain, D. S., Du, J., Matuszewich, L., Lumley, L. A., Putnam, S. K., et al. (1999). Hormone-neurotransmitter interactions in the control of sexual behavior. *Behav. Brain. Res., 105*, 105−116.

Ikeda, K. (1933). Effect of castration on the secondary sexual characters of anadromous three-spined stickleback, *Gasterosteus aculeatus* (L): *Japanese Journal of Zoology, 5*, 135−157.

Johns, L. S., & Liley, N. R. (1970). The effects of gonadectomy and testosterone treatment on the reproductive behavior of the male blue gourami *Trichogaster trichopterus*. *Canadian Journal of Zoology, 48*, 977−987.

Johnson, T. C., Scholz, C. A., Talbot, M. R., Kelts, K., Ricketts, R. D., Ngobi, G., et al. (1996). Late pleistocene desiccation of Lake Victoria and rapid evolution of Cichlid fishes. *Science, 273*, 1091–1093.

Kamrin, R. P., & Aronson, L. R. (1954). The effects of forebrain lesions on mating behavior in male platyfish, *Xiphophorus maculatus*. *Zoologica, 39*, 133–140.

Kidd, M. R., Danley, P. D., & Kocher, T. D. (2006). A direct assay of female choice in cichlids: all the eggs in one basket. *Journal of Fish Biology, 68*, 373–384.

Kiflawi, M., Mazeroll, A. I., & Goulet, D. (1998). Does mass spawning enhance fertilization in coral reef fish? A case study of the brown surgeonfish. *Marine Ecology-Progress Series, 172*, 107–114.

Klein, K., & Henk, K. (1964). Klinisch-experimentelle untersuchungen uber den einfluss von aldosteron auf haemodynamik and gerinnung. *Zeitschrift für Kreislaufforschung,* 40–53.

Knapp, R. A., & Kovach, J. T. (1991). Courtship as an honest indicator of male parental quality in the bicolor damselfish. *Stegastes partitus, 2*, 295–300.

Knapp, R., Marchaterre, M. A., & Bass, A. H. (2001). Relationship between courtship behavior and steroid hormone levels in a parental male plainfin midshipman fish. *Hormones and Behavior, 39*, 335.

Knight, M. E., & Turner, G. F. (1999). Reproductive isolation among closely related Lake Malawi cichlids: can males recognize conspecific females by visual cues? *Anim. Behav., 58*, 761–768.

Kobayashi, M., & Stacey, N. (1993). Prostaglandin-induced female spawning behavior in goldfish (*Carassius auratus*) appears independent of ovarian influence. *Hormones and Behavior, 27*, 38–55.

Kobayashi, M., Sorensen, M. W., & Stacey, N. E. (2002). Hormonal and pheromonal control of spawning behavior in the goldfish. *Fish Physiology and Biochemistry, 26*, 71–84.

Kolmakov, N. N., Kube, M., Reinhardt, R., & Canário, A. V. M. (2008). Analysis of the goldfish Carassius auratus olfactory epithelium transcriptome reveals the presence of numerous non-olfactory GPCR and putative receptors for progestin pheromones. *BMC Genomics, 9*, 429.

Koyama, Y., Satou, M., Oka, Y., & Ueda, K. (1984). Involvement of the telencephalic hemispheres and the preoptic area in sexua behavior of the male golfish, *Carassius auratus*, a brain-lesion study. *Behavioral and Neural Biology, 40*, 70–86.

Kraak, S. B. M., Bakker, T. C., & Mundwiler, B. (1999). Correlates of the duration of the egg collecting phase in the three-spined stickleback. *Journal of Fish Biology, 54*, 1038–1049.

Kramer, B., Kirschbaum, F., & Markl, H. (1981). Species specificity of electric organ discharges in a sympatric group of gymnotoid fishes from Manaus (Amazonas). In T. Szabo, & G. Czeh (Eds.), *Sensory physiology of aquatic lower vertebrates* (pp. 195–219). Budapest: Pergamon.

Kyle, A. L., & Peter, R. E. (1982). Effects of brain lesions on spawning behaviour in the male goldfish. *Physiology and Behavior, 28*, 1103–1109.

Kyle, A. L., Stacey, N., & Peter, R. E. (1982). Ventral telencephalic lesions: effects on bisexual behavior, activity, and olfaction in the male goldfish. *Behavioral and Neural Biology, 36*, 229–241.

Lema, S. C., & Nevitt, G. A. (2004). Exogenous vasotocin alters aggression during agonistic exchanges in male Amargosa River pupfish (Cyprinodon nevadensis amargosae). *Horm. Behav., 46*, 628–637.

Levavi-Sivan, B., Biran, J., & Fireman, E. (2006). Sex steroids are involved in the regulation of gonadotropin-releasing hormone and dopamine D2 receptors in female tilapia pituitary. *Biology of Reproduction, 75*, 642–650.

Liley, N. R., & Stacey, N. E. (1983). Hormones, pheromones, and reproductive behavior in fish. In W. S. Hoar (Ed.), *Fish Physiology IX Part B* (pp. 1–63). New York: Academic Press, Inc.

Liley, N. R., & Tan, E. S. P. (1985). The induction of spawning behaviour in *Puntius gonionotus* (Bleeker) by treatment with prostaglandin $PGF_{2\alpha}$. *Journal of Fish Biology, 26*, 491–502.

Liley, N. R. (1966). Ethological isolating mechanisms in four sympatric species of poeciliid fishes. *Behaviour, 13*, 1–197.

Liley, N. R., Breton, B., Fostier, A., & Tan, E. S. P. (1986). Endocrine changes associated with spawning behavior and social stimuli in a wild population of rainbow trout (*Salmo gairdneri*). I. Males. *Gen. Comp. Endocrinol., 62*, 145–156.

Liu, H., Wu, M., & Zakon, H. H. (2008). A novel Na+ channel splice form contributes to the regulation of an androgen-dependent social signal. *The Journal of Neuroscience, 28*, 9173–9182.

Loiselle, P. V. (1977). Colonial breeding by an African substratum-spawning cichlid fish, *Tilapia zilli* (Gervais). *Biology of Behaviour, 2*, 129–142.

Lokman, P. M., Harris, B., Kusakabe, M., Kime, D. E., Schulz, R. W., Adachi, S., et al. (2002). 11-Oxygenated androgens in female teleosts: prevalence, abundance, and life history implications. *Gen. Comp. Endocrinol., 129*, 1–12.

Lorenz, K. (1950). The comparative method in studying innate behavior patterns. *Symposia of the Society of Experimental Biology, 4*, 221–268.

Macey, M. J., Pickford, G. E., & Peter, R. E. (1974). Forebrain localization of the spawning reflex response to exogenous neurohypophysial hormones in the killfish, *Fundulus heteroclitus*. *J. Exp. Zool., 190*, 269–280.

Marconato, A., Rasotto, M. B., & Mazzoldi, C. (1996). On the mechanism of sperm release in three gobiid fishes (Teleostei: Gobiidae). *Environmental Biology of Fishes, 46*, 321–327.

Markham, M. R., & Stoddard, P. K. (2005). Adrenocorticotropic hormone enhances the masculinity of an electric communication signal by modulating the waveform and timing of action potentials within individual cells. *Journal of Neuroscience, 25*, 8746–8754.

Martens, K. (1997). Speciation in ancient lakes. *Trends in Ecology and Evolution, 12*.

Mayer, I., Borg, B., & Schulz, R. (1990a). Seasonal changes in and effect of castration/androgen replacement on the plasma levels of five androgens in the male three-spined stickleback, *Gasterosteus aculeatus* L. *General and Comparative Endocrinology, 79*, 23–30.

Mayer, I., Liley, N. R., & Borg, B. (1994). Stimulation of spawning behavior in castrated rainbow trout (*Oncorhynchus mykiss*) by 17a, 20b-dihydroxy-4-pregnen-3-one, but not by 11-ketoandrostenedione. *Hormones and Behavior, 28*, 181–190.

Mayer, I., Lundqvist, H., Berglund, I., Schmitz, M., Schulz, R., & Borg, B. (1990b). Seasonal endocrine changes in Baltic salmon, *Salmo salar*, immature parr and mature male parr. I. Plasma levels of five androgens, 17α-hydroxy-20β-dihydroprogesterone, and 17β-estradiol. *Canadian Journal of Zoology, 68*, 1360–1365.

McAnelly, L., & Zakon, H. H. (2000). Coregulation of voltage-dependent kinetics of Na+ and K+ currents in electric organ. *The Journal of Neuroscience, 20*, 3408–3414.

McGregor, P. K., & Westby, G. W. M. (1992). Discrimination of individually characteristic electric organ discharges by a weakly electric fish. *Animal Behaviour, 43*, 977–986.

Mennigen, J. A., Martyniuk, C. J., Crump, K., Xiong, H., Zhao, E., Popesku, J., et al. (2008). Effects of fluoxetine on the reproductive axis of female goldfish (*Carassius auratus*). *Physiological Genomics, 12*, 273–282.

Meyer, J. H. (1983). Steroid influences upon the discharge frequencies of a weakly electric fish. *Journal of Comparative Physiology, 153*, 29–37.

Miura, T., Higuchi, M., Ozaki, Y., Ohta, T., & Miura, C. (2006). Progestin is an essential factor for the initiation of the meiosis in spermatogenetic cells of the eel. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 7333–7338.

Miura, T., Yamauchi, K., Takahashi, H., & Nagahama, Y. (1991). Hormonal induction of all stages of spermatogenesis in vitro in the male Japanese eel (*Anguilla japonica*). *Proceedings of the National Academy of Sciences of the United States of America, 88*, 5774–5778.

Moore, F. L., & Evans, S. J. (1999). Steroid hormones use non-genomic mechanisms to control brain functions and behaviors: A review of evidence. *Brain Behavior and Evolution, 54*, 41–50.

Moore, M. C. (1991). Application of organization-activation theory to alternative male reproductive strategies: a review. *Hormones and Behavior, 25*, 154–179.

Moore, M. C., Hews, D. K., & Knapp, R. (1998). Hormonal control and evolution of alternative male phenotypes: generalizations of models for sexual differentiation. *Integrative and Comparative Biology, 38*, 133–151.

Moortgat, K. T., Keller, C. H., Bullock, T. H., & Sejnowski, T. J. (1998). Submicrosecond pacemaker precision is behaviorally modulated: The gymnotiform electromotor pathway. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 4684–4689.

Morozova, O., & Marra, M. A. (2008). Applications of next-generation sequencing technologies in functional genomics. *Genomics, 92*, 255–264.

Motohashi, E., Hamabata, T., & Ando, H. (2006). Structures of neurohypophysial hormone genes and there expression patterns during spawning of *Takifugu niphobles*. *Zoological Science, 23*. 1212-1212.

Munakata, A., & Kobayashi, M. (2009). Endocrine control of sexual behavior in teleost fish. *General and Comparative Endocrinology, 165*, 456–468.

Munro, A. D., & Pitcher, T. J. (1985). Steroid hormones and agonistic behavior in a cichlid teleost, *Aequidens pulcher*. *Hormones and Behavior, 19*, 353–371.

Naftolin, F., Ryan, K. J., Davies, I. J., Reddy, V. V., Flores, F., Petro, Z., et al. (1975). The formation of estrogens by central neuroendocrine tissues. *Recent Progress in Hormone Research, 31*, 295–319.

Nelson, R. (2005). *An introduction to behavioural endocrinology* (3rd ed.). Sunderland, MA: Sinauer.

Noble, G. K., & Kumpf, K. F. (1936). The sexual behavior and secondary sex characters of gonadectomized fish. *Anatomical Record, 67*, 113.

Ogawa, S., Akiyama, G., Kato, S., Soga, T., Sakuma, Y., & Parhar, I. S. (2006). Immunoneutralization of gonadotropin-releasing hormone type-III suppresses male reproductive behavior of cichlids. *Neuroscience Letters, 403*, 201–205.

Oka, Y., & Matsushima, T. (1993). Gonadotropin-releasing-hormone (GnRH) immunoreactive terminal nerve-cells have intrinsic rhythmicity and project widely in the brain. *Journal of Neuroscience, 13*, 2161–2176.

Oliveira, R. F., & Gonçalves, D. (2008). Hormones and social behaviour of teleost fish. In C. Magnhagen, et al. (Eds.), *Fish Behaviour* (pp. 61–125). Enfield, N.H: Science Publishers.

Oliveira, R. F., Carneiro, L. A., Gonçalves, D. M., Canário, A. V., & Grober, M. S. (2001). 11-Ketotestosterone inhibits the alternative mating tactic in sneaker males of the peacock blenny, *Salaria pavo*. *Brain, Behavior and Evolution, 58*, 28–37.

Oliveira, R. F. (2006). Neuroendocrine mechanisms of alternative reproductive tactics in fish. In K. Sloman, et al. (Eds.), *Behaviour and Physiology of Fish* (pp. 297–357). New York: Elsevier.

Oliveira, R. F. (2004). Social modulation of androgens in vertebrates: Mechanisms and function. In P. J. B. Slater, et al. (Eds.), *Advances in the Study of Behavior* (pp. 165–239). New York: Academic Press.

Olsén, H. K., Järvi, J. T., Mayer, I., Petersson, E., & Kroon, F. (1998). Spawning behaviour and sex hormone levels in adult and precocious brown trout (*Salmo trutta* L.) males and the effect of anosmia. *Chemoecology, 8*, 9–17.

Ota, D., Marchesan, M., & Ferrero, E. A. (1996). Sperm release behaviour and fertilization in the grass goby. *Journal of Fish Biology, 49*, 246–256.

Otte, A., & Halsband, U. (2006). Brain imaging tools in neurosciences. *Journal of Physiology - Paris, 99*, 281–292.

Páll, M. K., Mayer, I., & Borg, B. (2002). Androgen and behavior in the male three-spined stickleback, *Gasterosteus aculeatus*. II. Castration and 11-ketoandrostenedione effects on courtship and parental care during the nesting cycle. *Hormones and Behavior, 42*, 337–344.

Paredes, R. G. (2003). Medial preoptic area/anterior hypothalamus and sexual motivation. *Scandinavian Journal of Psychology, 44*, 203–212.

Paredes, R. G., Highland, L., & Karam, P. (1993). Socio-sexual behavior in male rats after lesions of the medial preoptic area: evidence for reduced sexual motivation. *Brain Research, 618*, 271–276.

Parhar, I. S. (1997). GnRH in tilapia: three genes, three origins and their roles. In I. S. Parhar, & Y. Sakuma (Eds.), *GnRH neurons: gene to behavior* (pp. 99–122). Tokyo: Brain Shuppan.

Parhar, I. S., Tosaki, H., Sakuma, Y., & Kobayashi, M. (2001). Sex differences in the brain of goldfish: gonadotropin-releasing hormone and vasotocinergic neurons. *Neuroscience, 104*, 1099–1110.

Patzner, R. A., Seiwald, M., Adlgasser, M., & Kaurin, G. (1986). The reproduction of *Blennius pavo* (Teleostei, Blenniidae) V. Reproductive behavior in natural environment. *Zoologischer Anzeiger, 216*, 338–350.

Pedersen, C. A., & Boccia, M. L. (2002). Oxytocin maintains as well as initiates female sexual behavior: Effects of a highly selective oxytocin antagonist. *Hormones and Behavior, 41*, 170–177.

Pelkwijk, J. J.t., & Tinbergen, N. (1937). Eine reizbiologische analyse einiger verhaltensweisen von *Gasterosteus aculeatus* L. *Zeitschrift für Tierpsychologie, 1*, 193–200.

Peter, R. E. (1990). *Direct neural regulation of the teleost adenohypophysis. The Journal of Experimental Zoology, 4*(suppl.), 84–89.

Pickford, G. E., & Strecker, E. L. (1977). The spawning reflex response of the killifish, *Fundulus heteroclitus*: isotocin is relatively inactive in comparison with arginine vasotocin. *General and Comparative Endocrinology, 32*, 132–137.

Pickford, G. E. (1952). Induction of a spawning reflex in hypophysectomized killifish. *Nature, 170*, 807–808.

Pierotti, M. E., Martin-Fernandez, J. A., & Seehausen, O. (2009). Mapping individual variation in male mating preference space: multiple choice in a color polymorphic cichlid fish. *Evolution, 63*, 2372–2388.

Plenderleith, M., Van Oosterhout, C., Robinson, R. L., & Turner, G. F. (2005). Female preference for conspecific males based on olfactory cues in a Lake Malawi cichlid fish. *Biology Letters, 1*, 411−414.

Potts, G. W. (1974). The colouration and its behavioural significance in the corkwing wrasse, *Crenilabrus melops*. *Journal of the Marine Biological Association of the United Kingdom, 54*, 925−938.

Potts, G. W. (1984). Parental behaviour in temperate marine teleosts with special reference to the development of nest structures. In G. W. Potts, & R. J. Wootton (Eds.), *Fish reproduction: strategies and tactics* (pp. 223−244). New York: Academic Press.

Reinboth, R., & Rixner, W. (1970). *Verhalten des kleinen Maulbruters Hemihaplochromis multicolor nach Kastration und Behandlung mit Testosteron.Unpublished manuscript, Göttingen*.

Remage-Healey, L., & Bass, A. H. (2004). Rapid, hierarchical modulation of vocal patterning by steroid hormones. *Journal of Neuroscience, 24*, 5892−5900.

Remage-Healey, L., & Bass, A. H. (2006a). From social behavior to neural circuitry: steroid hormones rapidly modulate advertisement calling via a vocal pattern generator. *Hormones and Behavior, 50*, 432−441.

Remage-Healey, L., & Bass, A. H. (2006b). A rapid neuromodulatory role for steroid hormones in the control of reproductive behavior. *Brain Research, 1126*, 27−35.

Remage-Healey, L., & Bass, A. H. (2007). Plasticity in brain sexuality is revealed by the rapid actions of steroid hormones. *Journal of Neuroscience, 27*, 1114−1122.

Remage-Healey, L., Maidment, N. T., & Schlinger, B. A. (2008). Forebrain steroid levels fluctuate rapidly during social interactions. *Nature Neuroscience, 11*, 1327−1334.

Roselli, C. E., Cross, E., Poonyagariyagorn, H. K., & Stadelman, H. L. (2003). Role of aromatization in anticipatory and consummatory aspects of sexual behavior in male rats. *Hormones and Behavior, 44*, 146−151.

Rosenqvist, G. (1990). Male mate choice and female−female competition for mates in the pipefish *Nerophis ophidion*. *Animal Behaviour, 39*, 1110−1115.

Sachs, B. D. (2007). A contextual definition of male sexual arousal. *Hormones and Behavior, 51*, 569−578.

Saito, D., Hasegawa, Y., & Urano, A. (2003). Gonadotropin-releasing hormones modulate electrical activity of vasotocin and isotocin neurons in the brain of rainbow trout. *Neuroscience Letters, 351*, 107−110.

Salek, S. J., Sullivan, C. V., & Godwin, J. (2001). Courtship behavior of male white perch, *Morone americana*: evidence for control by androgens. *Comparative Biochemistry and Physiology, Part A: Molecular & Integrative Physiology, 130*, 731−740.

Salek, S. J., Sullivan, C. V., & Godwin, J. (2002). Arginine vasotocin effects on courtship behavior in male white perch (*Morone americana*). *Behavioral Brain Research, 133*, 177−183.

Salzburger, W., Braasch, I., & Meyer, A. (2007). Adaptive sequence evolution in a color gene involved in the formation of the characteristic egg-dummies of male haplochromine cichlid fishes. *BMC Biology*, 5.

Santangelo, N., & Bass, A. H. (2006). New insights into neuropeptide modulation of aggression: field studies of arginine vasotocin in a territorial tropical damselfish. *Proceedings of The Royal Society of London Series B-Biological Sciences, 273*, 3085−3092.

Santos, R. S., & Barreiros, J. P. (1993). The ethogram of *Parablennius sanguinolentus parvicornis*(Valenciennes in Cuvier & Valenciennes, 1836) (Pisces: Blenniidae) from the Azores. *Arquipélago - Série Ciências da Natureza, 11A*, 73−90.

Saraiva, J., 2009. Variação inter-populacional no comportamento reprodutor do blenídeo *Salaria pavo*. PhD thesis. Universidade do Porto, Porto.

Satou, M., Oka, Y., Kusunoki, M., Matsushima, T., Kato, M., Fujita, I., et al. (1984). Telencephalic and preoptic areas integrate sexual behavior in the hime salmon (landlocked red salmon, *Oncorhynchus nerka*): results of electrical brain stimulation experiments. *Physiology and Behavior, 33*, 441−447.

Scott, W. B., & Crossman, E. J. (1979). Freshwater fishes of Canada. *Bulletin of the Fisheries Board of Canada, 184*, 966.

Scott, W. B., & Scott, M. G. (1988). Atlantic fishes of Canada. *Canadian Bulletin of Fisheries and Aquatic Sciences, 219*, 731.

Sebire, M., Allen, Y., Bersuder, P., & Katsiadaki, I. (2008). The model anti-androgen flutamide suppresses the expression of typical male stickleback reproductive behaviour. *Aquatic Toxicology, 90*, 37−47.

Sebire, M., Scott, A. P., Tyler, C. R., Cresswell, J., Hodgson, D. J., Morris, S., et al. (2009). The organophosphorous pesticide, fenitrothion, acts as an anti-androgen and alters reproductive behavior of the male three-spined stickleback, *Gasterosteus aculeatus*. *Ecotoxicology, 18*, 122−133.

Seehausen, O., & Alphen, J. J. M. (1998). The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex). *Behavioral Ecology and Sociobiology, 42*, 1−8.

Seehausen, O., Terai, Y., Magalhães, I. S., Carleton, K. L., Mrosso, H. D. J., Miyagi, R., et al. (2008). Speciation through sensory drive in cichlid fish. *Nature, 455*, 620−626.

Seehausen, O., Van Alphen, J. J. M., & Witte, F. (1997). Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science, 277*, 1808−1811.

Seki, S., Kohda, M., Takamoto, G., Karino, K., Nakashima, Y., & Kuwamura, T. (2009). Female defense polygyny in the territorial triggerfish *Sufflamen chrysopterum*. *Journal of Ethology, 27*, 215−220.

Semsar, K., & Godwin, J. (2003). Social influences on the arginine vasotocin system are independent of gonads in a sex-changing fish. *Journal of Neuroscience, 23*, 4386−4393.

Semsar, K., & Godwin, J. (2004). Multiple mechanisms of phenotype development in the bluehead wrasse. *Hormones and Behavior, 45*, 345−353.

Semsar, K., Kandel, F. L., & Godwin, J. (2001). Manipulations of the AVT system shift social status and related courtship and aggressive behavior in the bluehead wrasse. *Hormones and Behavior, 40*, 21−31.

Sheer, N., & Campos-Ortega, J. A. (1999). Use of the Gal4-UAS technique for targeted gene expression in the zebrafish. *Mechanisms of Development, 80*, 153−158.

Sherrington, C. (1906). *The integrative action of the nervous system*. New York: Scribner.

Smith, R. J. F. (1969). Control of prespawning behaviour of sunfish (*Lepomis gibbosus* and *L. megalotis*). I. Gonadal androgen. *Animal Behaviour, 17*, 279−285.

Sorensen, P. W., & Goetz, F. W. (1993). Pheromonal and reproductive function of F prostaglandins and their metabolites in teleost fish. *Journal of Lipid Mediators, 6*, 385−393.

Sorensen, P. W., Hara, T. J., Stacey, N. E., & Goetz, F. W. (1988). F prostaglandins function as potent olfactory stimulants that comprise the postovulatory female sex pheromone in goldfish. *Biology of Reproduction, 39*, 1039−1050.

Sorensen, P. W., Scott, A. P., Stacey, N. E., & Bowdin, L. (1995). Sulfated 17,20β-dihydroxy-4-pregnen-3-one functions as a potent and specific olfactory stimulant with pheromonal actions in the goldfish. *General and Comparative Endocrinology, 100*, 128−142.

Sorensen, P. W., Stacey, N. E., & Chamberlain, K. J. (1989). Differing behavioral and endocrinological effects of two female sex pheromones on male goldfish. *Hormones and Behavior, 23*, 317−332.

Spach, C., & Streeten, D. H. (1964). Retardation of sodium exchange in dog erythrocytes by physiological concentrations of Aldosterone in vitro. *Journal of Clinical Investigation, 43*, 217−227.

Stacey, N., & Kobayashi, M. (1996). Androgen induction of male sexual behaviors in female goldfish. *Hormones and Behavior, 30*, 434−445.

Stacey, N. E., & Kyle, A. L. (1983). Effects of olfactory tract lesions on sexual and feeding behavior in the goldfish. *Physiology and Behavior, 30*, 621−628.

Stacey, N. E., & Liley, N. R. (1974). Regulation of spawning behaviour in the female goldfish. *Nature, 247*, 71−72.

Stacey, N. E. (1976). Effects of Indomethacin and prostaglandins on spawning behavior of female goldfish. *Prostaglandins, 12*, 113−126.

Stacey, N. E. (1977). *The regulation of spawning behavior in the female goldfish, Carassius auratus. Ph.D. thesis*. Vancouver, Canada: University of British Columbia.

Stacey, N. E. (1981). Hormonal regulation of female reproductive behavior in fish. *American Zoologist, 21*, 305−316.

Stacey, N. (2003). *Hormones, pheromones and reproductive behavior Fish Physiology and Biochemistry, 28*, 229−235.

St-Cyr, S., & Aubin-Horth, N. (2009). Integrative and genomics approaches to uncover the mechanistic bases of fish behavior and its diversity. *Comparative Biochemistry and Physiology A, 152*, 9−21.

Stelkens, R. B., Pierotti, M. E. R., Joyce, D. A., Smith, A. M., Van der Sluijs, I., & Seehausen, O. (2008). Disruptive sexual selection on male nuptial coloration in an experimental hybrid population of cichlid fish. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 12*, 2861−2870.

Stoddard, P. K. (2002). Electric signals: predation, sex, and environmental constraints. In P. J. B. Slater, et al. (Eds.), *Advances in the Study of Behavior* (pp. 201−242). San Diego: Academic Press.

Stoddard, P. K., Zakon, H. H., Markham, M. R., & McAnelly, L. (2006). Regulation and modulation of electric waveforms in gymnotiform electric fish. *Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology, 192*, 613−624.

Strussmann, C. A., & Nakamura, M. (2002). Morphology, endocrinology, and environmental modulation of gonadal sex differentiation in teleost fishes. *Fish Physiology and Biochemistry, 26*, 13−29.

Subhedar, N., Deshmukh, M. K., Jain, M. R., Khan, F. A., & Krishna, N. S. R. (1996). Activation of hypothalamic neurons by intraovarian pressure signals in a teleost fish, Clarias batrachus: Role of mechanosensitive channels. *Brain Behavior and Evolution, 47*, 179−184.

Subhedar, N., Krishna, N. S. R., & Deshmukh, M. K. (1987). The response of nucleus preopticus neurosecretory cells to ovarian pressure in the teleost, *Clarias batrachus* (Linn). *General and Comparative Endocrinology, 68*, 357−368.

Taborsky, M. (1984). Broodcare helpers in the cichlid fish *Lamprologus brichardi*: Their costs and benefits. *Animal Behaviour, 32*, 1236−1252.

Taborsky, M. (1997). Bourgeois and parasitic tactics: Do we need collective, functional terms for alternative reproductive behaviours? *Behavioral Ecology and Sociobiology, 41*, 361−362.

Taborsky, M. (2008). Alternative reproductive tactics in fish. In R. F. Oliveira, et al. (Eds.), *Alternative reproductive tactics: an integrative approach* (pp. 251−299). Cambridge: Cambridge University Press.

Taborsky, M. (1999). Conflict or cooperation: what determines optimal solutions to competition in fish reproduction? In V. C. Almada, et al. (Eds.), *Behaviour and conservation of littoral fishes* (pp. 301−349) Lisboa: ISPA.

Taborsky, M. (1994). Sneakers, satellites and helpers: parasitic and cooperative behavior in fish reproduction. In P. J. B. Slater, et al. (Eds.), *Advances in the Study of Behavior* (pp. 1−100). San Diego: Academic Press.

Tautz, A. F., & Groot, C. (1975). Spawning behavior of chum salmon (*Oncorhynchus keta*) and rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada, 32*, 633−642.

Tavolga, W. N. (1955). Effects of gonadectomy and hypophysectomy on prespawning behavior in males of the Gobiid fish, *Bathygobius soporator. Physiological Zoology, 28*, 218−233.

Thresher, R. E. (1984). Combtooth Blennies (Blenniidae). In R. E. Thresher (Ed.), *Reproduction in reef fishes* (pp. 314−333). London: T.H.F. Pub.

Tinbergen, N. (1951). *The study of instinct*. Oxford: Clarendon Press.

Trivers, R. L. (1972). Parental investment and sexual selection. In B. Campbell (Ed.), *Sexual selection and the descent of man* (pp. 52−93). Chicago: Aldine Publishing Company.

Tweddle, D., Eccles, D. H., Frith, C. B., Fryer, G., Jackson, P. B. N., Lewis, D. S. C., et al. (1998). Cichlid spawning structures - bowers or nests? *Environmental Biology of Fishes, 51*, 107−109.

Vagell, M. E., & McGinnis, M. Y. (1997). The role of aromatization in the restoration of male rat reproductive behavior. *Journal of Neuroendocrinology, 9*, 415−421.

Van den Burgh, E. H., Peeters, R. R., Verhoye, M., Meek, J., Flik, G., & Van der Linden, A. (1999). Brain responses to ambient temperature fluctuations in fish: reduction of blood volume and initiation of a whole-body stress response. *Journal of Neurophysiology, 93*, 2849−2855.

Van der Linden, A., Van Camp, N., Ramos-Cabrer, P., & Hoehn, M. (2007). Current status of functional MRI on small animals: application to physiology, pathophysiology, and cognition. *NMR in Biomedicine, 20*, 522−545.

Vetillard, A., Ferriere, F., Jego, P., & Bailhache, T. (2006). Regulation of salmon gonadotrophin-releasing hormone gene expression by sex steroids in rainbow trout brain. *Journal of Neuroendocrinology, 18*, 445−453.

Villars, T. A., & Davis, R. E. (1977). Castration and reproductive behavior in the paradise fish, *Macropodus opercularis* (L.) (Osteichthyes: Belontiidae). *Physiology & Behavior, 19*, 371−375.

Villars, T. A., Hale, N., & Chapnick, D. (1985). Prostaglandin-$F_{2\alpha}$ stimulates reproductive behavior of female paradise fish (*Macropodus opercularis*). *Hormones and Behavior, 19*, 21−35.

Vincent, A. C. J. (1992). Prospects for sex role reversal in teleost fishes. *Netherlands Journal of Zoology, 42*, 392−399.

Vincent, A. C. J. (1994). Seahorses exhibit conventional sex roles in mating competition, despite male pregnancy. *Behaviour, 128*, 135−151.

Vincent, A. C., Berglund, A., & Ahnesjö, I. (1995). Reproductive ecology of five pipefish species in one eelgrass meadow. *Environmental Biology of Fishes, 44*, 347−361.

Volkoff, H., & Peter, R. E. (1999). Actions of two forms of gonadotropin releasing hormone and a GnRH antagonist on spawning behavior of the goldfish *Carassius auratus*. *General and Comparative Endocrinology, 116*, 347−355.

Wai, E. H., & Hoar, W. S. (1963). The secondary sex characters and reproductive behavior of gonadectomized sticklebacks treated with methyltestosterone. *Canadian Journal of Zoology, 41*, 611−628.

Wapler-Leong, D. C. Y., & Reinboth, R. (1974). The influence of androgenic hormone on the behavior of *Haplochromis burtoni* (Cichlidae). *Fortschritte der Zoologie, 22*, 334−339.

Warner, R. R., & Swearer, S. E. (1991). Social control of sex change in the bluehead wrasse, *Thalassoma bifasciatum* (Pisces: Labridae). *The Biological Bulletin, 181*, 199−204.

Weiss, C. S., & Coughlin, J. P. (1979). Maintained aggressive behavior in gonadectomized male siamese fighting fish (*Betta splendens*). *Physiology & Behavior, 23*, 173−177.

White, S. A., Kasten, T. L., Bond, C. T., Adelman, J. P., & Fernald, R. D. (1995). Three gonadotropin-releasing-hormone genes in one organism suggest novel roles for an ancient peptide. *Proceedings of the National Academy of Sciences of the United States of America, 92*, 8363−8367.

Wibe, A. E., Rosenqvist, G., & Jenssen, B. M. (2002). Disruption of male reproductive behavior in threespine stickleback *Gasterosteus aculeatus* exposed to 17 beta-estradiol. *Environmental Research, 90*, 136−141.

Wilhelmi, A. E., Pickford, G. E., & Sawyer, W. H. (1955). Initiation of the spawning reflex response in *Fundulus* by the administration of fish and mammalian neurohypophysial preparations and synthetic oxytocin. *Endocrinology, 57*, 243−252.

Wilson, D. P. (1958). *Notes from the Plymouth Aquarium. III, 37*, 299−307.

Wong, C. J. H. (2000). Electrical stimulation of the preoptic area in Eigenmannia: Evoked interruptions in the electric organ discharge. *Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology, 186*, 81−93.

Wong, R. Y., & Hopkins, C. D. (2007). Electrical and behavioral courtship displays in the mormyrid fish Brienomyrus brachyistius. *Journal of Experimental Biology, 210*, 2244−2252.

Wootton, R. J. (1970). Aggression in the early phases of the reproductive cycle of the male three-spined stickleback (*Gasterosteus aculeatus*). *Animal Behaviour, 18*, 740−746.

Wootton, R. J. (1976). *The biology of the sticklebacks*. London: Academic Press.

Yamamoto, N., Oka, Y., & Kawashima, S. (1997). Lesions of gonadotropin-releasing hormone-immunoreactive terminal nerve cells: effects on the reproductive behavior of male dwarf gouramis. *Neuroendocrinology, 65*, 403−412.

Zakon, H. H., & Smith, G. T. (2002). Weak electric fish: behavior, neurobiology and neuroendocrinology. In D. W. Pfaff, et al. (Eds.), *Hormones, Brain and Behavior* (pp. 349−374). New York: Academic Press.

Zakon, H. H. (1996). Hormonal modulation of communication signals in electric fish. *Developmental Neuroscience, 18*, 115−123.

Zakon, H. H., Oestreich, J., Tallarovic, S., & Triefenbach, F. (2002). EOD modulations of brown ghost electric fish: JARs, chirps, rises, and dips. *Journal of Physiology - Paris, 96*, 451−458.

Zhu, Y., Rice, C. D., Pang, Y. F., Pace, M., & Thomas, P. (2003). Cloning, expression, and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 2231−2236.

Zhu, Z., Li, G., He, L., & Chen, S. (1985). Novel gene transfer into the fertilized eggs of goldfish (*Carassius auratus* L. 1758). *Journal of Applied Ichtyology, 1*, 31−34.

Zupanc, G. K. H., & Maler, L. (1997). Neuronal control of behavioral plasticity: The prepacemaker nucleus of weakly electric gymnotiform fish. *Journal of Comparative Physiology A-Sensory Neural and Behavioral Physiology, 180*, 99−111.

Zupanc, G. K. H. (2002). From oscillators to modulators: behavioral and neural control of modulations of the electric organ discharge in the gymnotiform fish, *Apteronotus leptorhynchus*. *Journal of Physiology-Paris, 96*, 459−472.