

Endocrine control of sexual behavior in teleost fish

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ABSTRACT

Sexual behavior is one of the most profound events during the life cycle of animals that reproduce sexually. After completion of gonadal development that is mediated by various hormones, oviparous teleosts perform a suite of behaviors, often termed as spawning behavior. This is particularly important for teleosts that have their gametes fertilized externally as the behavior patterns ensures the close proximity of both sexes for gamete release, fusion and ultimately the production of offspring. As in other vertebrates, sexual behavior of fish is also under the control of hormones. Testicular androgen is a requirement for male sexual behavior to occur in most fish species that have been studied. Unlike tetrapods, however, ovarian estrogen does not appear to be essential for the occurrence of female sexual behavior for fish that have their gametes fertilized externally. Prostaglandins produced in the ovary after ovulation act as a trigger in some teleosts to induce female sexual behavior. Potentiating effects of gonadotropin-releasing hormone in the brain on sexual behavior are reported in some species.

Under endocrine regulation, male and female fish exhibit gender-typical behavior during spawning, but in some fish species there is also some plasticity in their sexual behavior. Sex changing fish can perform both male-typical and female-typical sexual behaviors during their lifetime and this sexual plasticity can also be observed in non-sex changing fish when undergoing hormonal treatment. Although the neuro-anatomical basis is not clear in fish, results of field and laboratory observations suggest that some teleosts possess a sexually bipotential brain which can regulate two types of behaviors unlike most other vertebrates which have a discrete sex differentiation of their brain and can only perform gender-typical sexual behavior.

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1. Introduction

Sexual behavior is one of the most important events during the completion of the life cycle of animals which undergo sexual reproduction. After completion of gametogenesis, both sexes perform special behaviors for the release and fusion of their gametes, ultimately to create offspring. As in other vertebrates, a series of behaviors consisting of several specific acts are performed in teleost fishes during the process of gamete release. These acts and behaviors are called by various terms depending on the pattern of movement, such as courtship, chasing, clasping, mating, spawning, egg release, sperm release, oviposition, ejaculation, etc. These acts are collectively called “sex behavior”, “sexual behavior” or “reproductive behavior” and the term “spawning behavior” is commonly used for teleosts whose gametes are fertilized in the external environment. It is quite challenging to clearly define “sexual behavior” in fishes largely due to the diversity of behavior patterns observed in fishes (see review by Balon, 1975; Moyle and Cech, 2000). However, it is generally accepted by many researchers that

“sexual behavior” means a series of behavioral acts that are mostly *sex specific*, performed by sexually mature *males and females*, that include actions for *gamete release and fusion*, and are also ultimately for the *production of offspring*.

In vertebrates, there are some behaviors that are closely related to reproductive activity and the occurrence of the behavior is sex specific but are not always categorized as a “sexual behavior”. For example, parturition and lactation in mammals and egg laying in birds and reptiles are performed only by females but these female-specific activities are not usually categorized as “sexual behavior”. Parental care, which is sex specific in some vertebrate species, is not categorized as “sexual behavior” in many cases. These behaviors, as in fishes, performed *after* gamete fusion can be classified as reproductive behaviors in a broader sense but are usually not referred to as a “sexual behavior”. In contrast, nest building and territorial behavior for the maintenance of the nest performed by some fishes *before* gamete fusion are considered parts of sexual behavior that is important for successful spawning to take place. For example, nest building of female salmonids (i.e., digging of gravel beds for oviposition) is considered a part of female-typical sexual behavior since oviposition occurs immediately after the digging event. Some species of fishes move or migrate

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from their living area to a spawning area (Moyle and Cech, 2000). The distance of the movement and conditions of the environment for spawning vary depending on the species. This movement is performed by both the male and female and is not sex specific, but it may be categorized as a sexual behavior in a broader sense as the initial behavior that will ultimately lead towards spawning. Therefore, upstream spawning migration in salmonids fishes can be considered as the first step of a suite of sexual behaviors. In this review, we adopt the definition of “sexual behavior” of fish as defined above, and use the term “spawning behavior” for externally fertilizing teleosts.

Since sexual behavior of fish is innate rather than learned, it can be performed without experience after attaining puberty (i.e., sexual maturity). The behavior, however, is controlled by various physiological and environmental factors as has been demonstrated for other vertebrates (Lamming, 1984). However, one of the things that sets fishes apart from other vertebrates is the existence of species that change their functional sex sometime during their lifetime (Moyle and Cech, 2000; Helfman et al., 1997). In these species, the same individual fish performs both male-typical and female-typical sexual behaviors during their life span. Such sexual plasticity of behavior is not observed in other vertebrate species with the exception of one species of sex-changing amphibian (Francis, 1992) and parthenogenetic lizards (Crews and Fitzgerald, 1980; Crews, 1993). For this reason, elucidation of the underlying mechanism(s) that mediate sexual behavior of fishes, the final phase of the reproductive process, is of great interest among biologists. Investigations focused on fish sexual behavior have resulted in a significant and growing body of information contributing to the advancement of basic vertebrate and comparative biology. There is also significant interest in fields that are more applied in nature, such as for aquaculture and conservation biology (Araki et al., 2007). The present review is intended to describe sexual behavior of teleosts that have their gametes fertilized externally. Information on the physiological and environmental regulation of fish sexual behavior is to be presented that covers the following aspects: (1) physiological prerequisites which enable fish to perform sexual behavior, (2) function of hormones and neuropeptides, (3) endocrine regulation of sexual behaviors of two of the most intensively studied species, the goldfish, *Carassius auratus*, and masu salmon, *Oncorhynchus masou*, (4) regulation of sexual behavior in other species, (5) sexual plasticity of behavior in fishes, and (6) future direction of sexual behavior research in fishes.

2. Prerequisites for sexual behavior

There are four events (Fig. 1) that are physiological prerequisites for sexual behavior to occur and result in the fertilization of spawned eggs and they are: (1) vitellogenesis of oocytes must be

completed in the ovary of females; (2) oocytes must be fully mature and have ovulated as a result of the effects of luteinizing hormone (LH) secreted from the pituitary; (3) spermatogenesis must be completed in the testis of the male individual; (4) sufficient amount of milt (i.e., seminal plasma and mature sperm) must be produced and stored in the sperm duct (Kobayashi et al., 2002). If any of these four events are blocked, normal sexual behavior cannot be completed and successful fertilization does not occur in most fish species. The development of these four events is influenced by both the physiological condition of the fish and environmental conditions in which the fish lives. Among these four prerequisites, the most critical process is ovulation in the female which is stimulated by the ovulatory LH surge from the pituitary. In most cases, the occurrence and timing of the appropriate sexual behavior is highly dependent on the ovulation process in the female(s). Likewise, the occurrence of the LH surge, ovulation and spawning are highly dependent on the environmental conditions experienced by the fishes and these processes are often inhibited by inappropriate conditions (i.e., temperature, depth, water quality) such as found in laboratory tanks, aquaculture ponds, rivers and lakes which have been manually reconstructed (Brett, 1971; Stacey et al., 1979a,b; Ikuta et al., 2001; Kitamura and Kobayashi, 2003; Kobayashi et al., 2008).

The four prerequisites described are largely regulated by the endocrine system which has been previously reviewed (Stacey and Sorensen, 2002; Kobayashi et al., 2002). After the above mentioned four prerequisites have been attained, and when the fish encounters the appropriate environmental conditions, normal sexual behavior will be completed resulting in spawning and production of fertilized eggs. Some species will spawn in their living area where they grow and others are known to migrate to a spawning area(s) which have the environmental conditions suitable for performing the appropriate sexual behaviors and also suitable for larval development (Munakata et al., 2001a,b). The type of sexual behavior(s) and conditions that are necessary for them to occur vary depending on the fish species (Balon, 1975; Moyle and Cech, 2000).

In many cyprinid species, including goldfish, ovulated females induce courtship behavior (i.e., chasing) in the males (Balon, 1975; Moyle and Cech, 2000; Kobayashi et al., 2002). During the chasing process females release oocytes and males release sperm into the water column where the eggs are naturally fertilized. After fertilization eggs soon become adhesive and eventually stick on substrates such as aquatic plants or rocks (i.e., benthic spawners). Alternatively, some species of fishes build nests (i.e., nest spawners) or specifically a place for fertilization of spawned eggs. In salmonid species, such as masu salmon, ovulated females dig and excavate the gravel bottoms of their habitat and make nests called a spawning redd by using their tail (Balon, 1975; Munakata et al., 2001b). Females and males release oocytes and sperm into the nest, respectively, and the fertilized eggs develop among the gravel in the nest. Some cichlids including tilapia make nests that are essentially a circular depression in the gravel substrate. Nile tilapia, *Oreochromis niloticus* make their nests by picking up and transporting the loose gravel with their mouths (Uchida et al., 2005). Some male anabantids, including the dwarf gourami, *Colisa lalia* build a surface bubble nest (i.e., froth nest) using bubbles released from their mouth (Balon, 1975; Yamamoto et al., 1997). Fertilized eggs of this species are placed in the bubble nest where they remain until hatching occurs. Male stickleback, *Gasterosteus aculeatus* build a nest with fragments of aquatic plants and mucous secreted from the kidney that acts as glue (Balon, 1975; Borg, 1994; Moyle and Cech, 2000). Egg and sperm release are performed inside the nest where fertilization takes place. In contrast to nest and benthic spawners, many marine fishes do not exhibit similar nesting behaviors as their oocytes and sperm are scattered into open

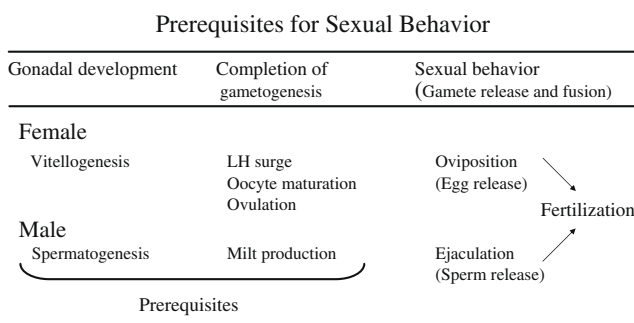


Fig. 1. Physiological prerequisites for the completion of sexual behavior in fish. See text for details.

waters (i.e., pelagic spawners) (Balon, 1975; Moyle and Cech, 2000).

Regardless of the pattern of sexual behavior, most fishes employ external cues from their environment and from biological signals released by conspecific fish. This is done to synchronize spawning interactions among spawning partners that increase the chances for successful production of fertilized eggs. It is well known that fish use environmental cues such as photoperiod, change in temperature, tides, water flow, spawning substrate, and biological signals such as olfactory, visual, acoustical, and behavioral signals from other fish (Helfman et al., 1997; Moyle and Cech, 2000).

3. Functional classification of hormones and neuropeptides in relation to sexual behavior

Sexual behavior of vertebrates is largely regulated by endocrine and neuroendocrine systems and many hormones and neuropeptides are involved in the occurrence of sexual behavior. However, involvement of these chemical mediators in sexual behavior is different among species (Evans, 1998). Also, the molecular forms of hormones vary depending on the species, e.g., different forms of major androgens, such as testosterone, 11-ketotestosterone, 11-ketoandrostendione, 11 β -hydroxyandrostendione, etc., different forms of gonadotropin-releasing hormones with amino acid variation, such as salmon type, chicken-II type, etc. (Borg, 1994; Evans, 1998; Kime, 1998; de Waal et al., 2008).

The actions of hormones for the occurrence of a particular behavior are classified into three categories. The first category is a “physiological trigger” which elicits sexual behavior in relatively short latency when other physiological and environmental conditions are appropriate. One of the typical physiological triggers is prostaglandin (PG) F 2α in female goldfish. Administration of PGF 2α induces the female to exhibit the oviposition act within several minutes after administration. Physiologically, this hormone is produced in the ovary after ovulation by the stimulation of the presence of ovulated oocytes in the oviduct of goldfish (Stacey and Liley, 1974; Stacey, 1976; Sorensen et al., 1995b; Kobayashi et al., 2002). This ovarian PGF 2α acts on the brain of females and induces this specific sexual behavior during natural spawning to occur.

The second category is characterized as a “requirement” or “primer”. This category of hormone does not trigger sexual behavior but the presence of this type of hormone is essential for the occurrence of the behavior. Testicular androgens basically function in this “priming” manner. In many male teleosts, sexual behavior occurs when blood androgen levels are elevated, and it has been demonstrated that castrated males without circulating testicular androgens do not show any signs of an appropriate sexual behavior (Borg, 1994; Munakata et al., 2001b). Administration of androgens can restore male sexual behavior in castrated males, but merely administering the hormones itself does not trigger the behavior to occur. Likewise, after a priming injection of juvenile males with androgen, the male behavior can be triggered by being exposed to external cues, such as pheromones or other behavioral signals from females (Satou, 1987; Satou et al., 1994; Yambe et al., 2003).

The third category is characterized as being a “potentiator”. A potentiating hormone does not trigger the behavior and sometimes it is not even essential for the actual occurrence of the behavior. However, the presence of this particular hormone enhances or increases the effect on the sexual behavior of the fish. Gonadotropin-releasing hormone (GnRH) is well known as one of the potentiator hormones in female goldfish. Without administration of GnRH, sexual behavior of female goldfish can still be triggered by PGF 2α administration. However, PGF 2α -induced spawning activity is enhanced by administration of GnRH agonist and suppressed by GnRH antagonist (Volkoff and Peter, 1999).

Some of these regulators mentioned above are produced by endocrine organs and are transported via the circulatory system where they act directly or indirectly on the brain (Evans, 1998). Others that are produced in the brain will act within the brain.

4. Regulation of sexual behavior in goldfish

4.1. Spawning behavior of goldfish

Physiological and environmental regulation of sexual behavior has been intensively studied in goldfish. Here we describe the regulatory mechanism of sexual behavior of goldfish which is probably the best understood among fish species (Stacey and Sorensen, 2002; Kobayashi et al., 2002). In temperate regions, goldfish spawns several times during their spawning season which occurs during the spring months and when kept under natural environmental conditions. Sexually mature males are sometimes attracted to vitellogenic (maturing) and post-vitellogenic (mature) females by the release of a “recrudescence pheromone” from females and follow or chase the females (Yamazaki, 1990). Ejaculation (sperm release), however, is not performed until females actually ovulate (Kobayashi et al., 2002).

Spawning behavior of goldfish starts several hours prior to ovulation in the females (Kobayashi et al., 2002) (Fig. 2). Females that are in the process of ovulation release a “preovulatory steroid pheromone” that has been described by Dulka et al. (1987). Stimulated by this pheromone, males start to chase and sometimes nudges the females (i.e., courtship behavior) normally during the early period of scotophase. Synchronized to photoperiod, ovulation in females occurs in the middle or latter period of scotophase (Kozuka et al., 1989). After ovulation, females produce PGF 2α in the ovary which triggers the spawning act in the females (Stacey and Liley, 1974; Stacey, 1976). PGF 2α and its metabolites are released into the water as a “postovulatory prostaglandin pheromone” (Sorensen et al., 1988, 1995b). In turn, the PG pheromone stimulates the male(s) where chasing becomes persistent, increases in intensity and is interspersed with the spawning acts of the female. Spawning acts are initiated by an ovulated female when she approaches floating aquatic vegetation near the surface of the water. An ovulated female enters the aquatic vegetation followed by a male. They turn on their sides, releasing eggs (oviposition) and sperm (ejaculation), and then flip their tails to mix spawned eggs and sperm. Released eggs adhere to the vegetation. Female spawning behavior will continue until most ovulated oocytes are released, and this may involve a hundred or more spawning acts over several hours. In this manuscript, the female spawning act is referred to as female sexual behavior, and chasing and the male spawning act is referred to as male sexual behavior in goldfish (Fig. 2).

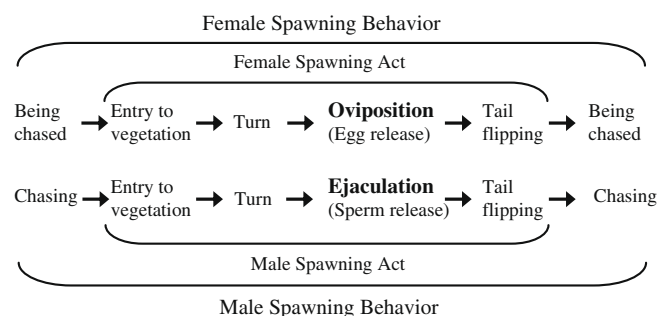


Fig. 2. Diagrammatic representation of the spawning behavior of male and female goldfish (Kobayashi et al., 2002). See text for details.

4.2. Hormonal and pheromonal control of female sexual behavior in goldfish

During the process of ovarian development, vitellogenic and post-vitellogenic females release a “recrudescent pheromone” which attract males and induces “nudging”, which is one of the typical courtship behaviors in goldfish (Yamazaki, 1990; Kobayashi et al., 2002). Males will exhibit a behavioral response to the water where vitellogenic females or estradiol-17 β (E₂)-treated ovariectomized females are kept but not to the water from either males or ovariectomized females. The production of this recrudescent pheromone is induced by E₂ but the chemical identity of the recrudescent pheromone remains unknown.

When post-vitellogenic females are exposed to certain external cues, such as a change in water temperature and encountering spawning substrate, they will exhibit an ovulatory LH surge that lasts about 15 h, terminating with ovulation and spawning (Fig. 3) (Stacey et al., 1979a,b; Kobayashi et al., 1989a,b; Stacey and Sorensen, 2002; Kobayashi et al., 2002). The LH surge in turn stimulates the production of the maturation-inducing steroid, 17 α ,20 β -dihydroxy-4-pregnen-3-one (17,20-P) in the ovarian follicles, and this ovarian progestin induces oocyte maturation (Kobayashi et al., 1987a). 17,20-P and its conjugates, 17,20-P sulfate (17,20-PS) are released into the water as “preovulatory steroid pheromones” (Dulka et al., 1987; Sorensen et al., 1990, 1995a). These steroid pheromones induce LH release in male goldfish (i.e., male LH surge) (Kobayashi et al., 1986a,b) and weak chasing by males (Sorensen et al., 1989; DeFraipont and Sorensen, 1993; Poling et al., 2001). Increased release of LH further induces milt production in sexually mature males that are ready for spawning. This female to male pheromone interaction is thought to function as a signal to males announcing that ovulation is imminent and stimulates the preparatory steps required for ejaculation.

17,20-P and its conjugates appear to act not only on males but also on females as well. Waterborn 17,20-P is known to stimulate occurrence of an LH surge in other females, which may supplement and/or synchronize the effect of external cues on the occurrence of the ovulatory LH surge among females (Sorensen and Stacey, 1987; Stacey and Sorensen, 2002; Kobayashi et al., 2002). It is possible that an increased number of ovulated females, which in turn release pheromones, attract additional sexually mature males to the spawning area, resulting in higher spawning success. While ovulatory females are known to release androstenedione (AD), which has been shown to induce aggressiveness among males, its exact role remains unknown because greater amounts of AD have

been shown to be released by males during spawning (Poling et al., 2001; Sorensen et al., 2005).

It has been demonstrated that ovulation in goldfish occurs at the peak of the LH surge (Stacey et al., 1979a,b; Kobayashi et al., 1987a), and the presence of ovulated oocytes in the oviduct induces the synthesis of PGF₂ α (Stacey and Liley, 1974; Stacey, 1976; Sorensen et al., 1995b). PGF₂ α is transported to the brain via blood circulation and triggers the female spawning act (Stacey and Peter, 1979). Spawning behavior is terminated when the ovulated oocytes are mostly shed by the spawning act or when manually stripped (Stacey and Liley, 1974). It is thought that PGF₂ α conveys the information of the presence of ovulated oocytes in the ovarian cavity and that they are ready to be oviposited to the brain of female goldfish.

PGF₂ α and its metabolite, 15-keto-prostaglandin F₂ α (15K-PGF₂ α) are released into the water as “postovulatory prostaglandin pheromones” (Sorensen et al., 1988, 1995b; Sorensen and Goetz, 1993; Stacey and Sorensen, 2002). These pheromones have a strong effect in inducing male sexual behavior in goldfish. When exposed to these PG pheromones, male goldfish exhibit persistent chasing of the ovulated female and perform their spawning act with an ovulated female. The effect of these PG pheromones on LH release in males is very weak compared to that of the preovulatory steroid pheromones (Sorensen et al., 1989). This would indicate that the postovulatory PG pheromone’s main function is a signal from female to male announcing that the female has ovulated oocytes and is ready to spawn. Interestingly, when PGF₂ α is intramuscularly injected to a non-ovulated female, this female starts to perform the female sexual behavior (i.e., oviposition act) within several minutes although no egg release is accompanied under these conditions (Stacey, 1976). The injected PGF₂ α is released into the water as a pheromone just as PGF₂ α is released in ovulated females and stimulates males to perform their sexual behavior (i.e., chasing and ejaculation act with sperm release). The males do not discriminate between ovulated females and PG-injected females in performing male specific sexual behavior, and thus, sexual behavior of goldfish can be artificially induced all through the year regardless of maturity of females by using the PG-injection method and if the sexual maturity of males can be maintained in the laboratory. It is by the use of this method that study of sexual behavior in goldfish has been greatly advanced.

Estrogens are required for sexual behavior in many female vertebrates (Sakuma, 1997), but ovarian sex steroids are not essential for the occurrence of sexual behavior in female goldfish. Ovariectomized female goldfish exhibited female sexual behavior (i.e., spawning act) after injection of PGF₂ α , while treatment with sex steroids (i.e., E₂, testosterone (T), or 17,20-P) did not trigger any signs of sexual behavior or potentiate the effect of PGF₂ α (Kobayashi and Stacey, 1993).

Sexually mature female goldfish exhibit high blood levels of T produced in the ovary. This T is important for the occurrence of the ovulatory LH surge (Kobayashi et al., 1989a,b, 2002) but is not involved in female sexual behavior as shown by the experiments mentioned above. Interestingly, administration of androgen induces male-typical sexual behavior (chasing and ejaculation act) in female goldfish, but androgens do not inhibit the occurrence of female-typical sexual behavior (Stacey and Kobayashi, 1996).

While progesterone is known to complement the expression of proceptive behavior in female rats (Sakuma, 1997), administration of 17,20-P did not enhance the activity of PG-induced spawning behavior in female goldfish (Kobayashi and Stacey, 1993).

GnRH shows potentiating effects on female sexual behavior in the goldfish. Intracerebroventricular injection of salmon-type GnRH and chicken-II-type GnRH enhanced PG-induced spawning activity, and GnRH antagonist suppressed the activity (Volkoff and Peter, 1999). Although goldfish have been reported to have

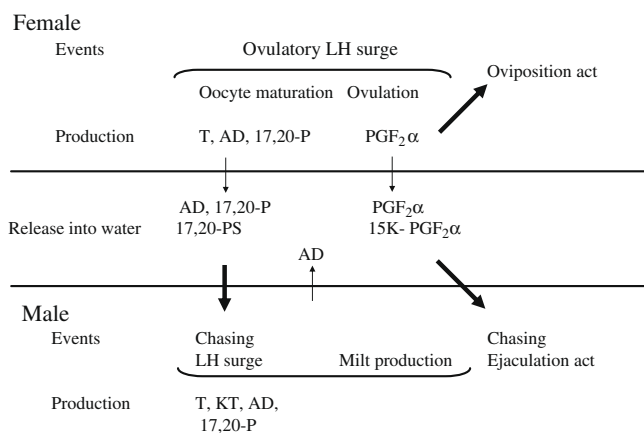


Fig. 3. Diagrammatic representation of the hormonal and pheromonal actions during spawning in goldfish. Thick arrows indicate the action of hormones and pheromones inducing sexual behavior. See text for details.

three GnRH neuronal populations in the brain, terminal nerve (TN) GnRH, preoptic GnRH, and midbrain GnRH (Kim et al., 1995a), it is unclear which of the three neuronal GnRH populations is physiologically involved in behavioral facilitation. Since the female goldfish ovulate and spawn even after the axonal transport of GnRH from TN to the other brain areas is blocked by using an olfactory tract section, GnRH of TN origin apparently is not essential for the occurrence of the female behavior (Stacey and Kyle, 1983; Kobayashi et al., 1992, 1994; Kim et al., 1995b, 2001). However, the latency to the onset of the behavior after PGF2 α injection became longer compared to that of sham-operated females (Stacey and Kyle, 1983; Kobayashi, unpublished data). These results suggest that GnRH of TN origin plays a role in maintaining the threshold for the behavior to occur in response to the sexual stimuli as shown in the male dwarf gourami (Yamamoto et al., 1997; Abe and Oka, 2007).

4.3. Hormonal and pheromonal control of male sexual behavior in goldfish

Sexually mature male goldfish follow or chase vitellogenic and post-vitellogenic females stimulated by the recrudescence pheromone or preovulatory steroid pheromone (Yamazaki, 1990; Kobayashi et al., 2002). Preovulatory steroid pheromones (17,20-P and 17,20-PS) stimulate LH release in males and induce weak chasing (Sorensen et al., 1989; DeFraipont and Sorensen, 1993). The spawning act, however, is performed only after ovulation of females which is the source of the postovulatory PG pheromone that gets released into the water column. Androgen(s) is considered to be a requirement for the occurrence of male behavioral responses. Although complete castration of male goldfish is impractical, because of testicular regeneration, involvement of androgen(s) is supported by the fact that male-typical sexual behavior is performed by sexually mature males with secondary sex characteristics and also androgen-treated females (Stacey and Kobayashi, 1996). 11-Ketotestosterone (KT) and T are found in the plasma of male goldfish, and KT is the more potent androgen than T (Stacey and Kobayashi, 1996). Based on the studies with KT-implanted female goldfish, KT is considered to be essential for LH release in response to the preovulatory steroid pheromone and male sexual behavior in response to the postovulatory PG pheromones. In addition to preovulatory pheromones, LH release in males is stimulated by behavioral interactions with ovulated females (Sorensen et al., 1989; Zheng and Stacey, 1997). Increased blood levels of LH stim-

ulate synthesis of 17,20-P, T, and AD, and among these steroids, a large amount of AD is released into the water (Sorensen et al., 2005). Sorensen et al. (2005) provided the following interpretation of the function of AD: This male pheromone acts on other males by the induction of agonistic behavior. This male to male pheromone seems to be an indicator of male sexuality providing a warning to other males and preventing being chased by other males during the pursuit of suitable females. In turn, females release preovulatory and postovulatory pheromones to stimulate physiological readiness for spawning in males. Since goldfish spawn during late scotophase and sexual dimorphism is not distinct, these pheromones also seem to function as a means to discriminate both the sex and maturational status of individuals among the population of fishes maximizing the encounters of appropriate mates.

Inhibitory effects of estrogen on male sexual behavior have been reported to occur in relation to the effects of estrogenic endocrine disrupting chemicals (Bjerselius et al., 2001). It should be mentioned that the physiological involvement of estrogen in male sexual behavior is still not known. Although GnRH exhibited a potentiatory effect on sexual behavior in female goldfish, this peptide does not show any clear effect on the sexual behavior in male goldfish (Volkoff and Peter, 1999).

5. Regulation of sexual behavior in salmonid fishes

5.1. Life cycle and sexual behavior of masu salmon

Most salmonid fishes spawn in the upper part of their home rivers (Machidori and Katou, 1985; Helfman et al., 1997; Munakata et al., 2001a). Some salmonid species (mainly anadromous salmonids) spawn only once in their lifetime while other species spawn several times annually during their lifetime. In some anadromous salmonids, such as masu salmon, a Pacific salmonid, juveniles that hatched in fresh water migrate to the ocean or lakes (downstream migration) (Machidori and Katou, 1985). After staying several months or more in the ocean or a lake, these salmon return to their home rivers for spawning (upstream migration).

Masu salmon juveniles (i.e., parr) grow in the river for about 1.5 years, and after parr-smolt transformation (i.e., smoltification) most of the juveniles (i.e., smolts, migratory form) migrate down to the Pacific Ocean. Here some juveniles start to mature sexually at an early age (i.e., precocious males, non-migratory form) and remain in the river (Fig. 4). It is well known that hormones (i.e., thyroid, cortisol, growth hormone, etc.) are involved in the regulation

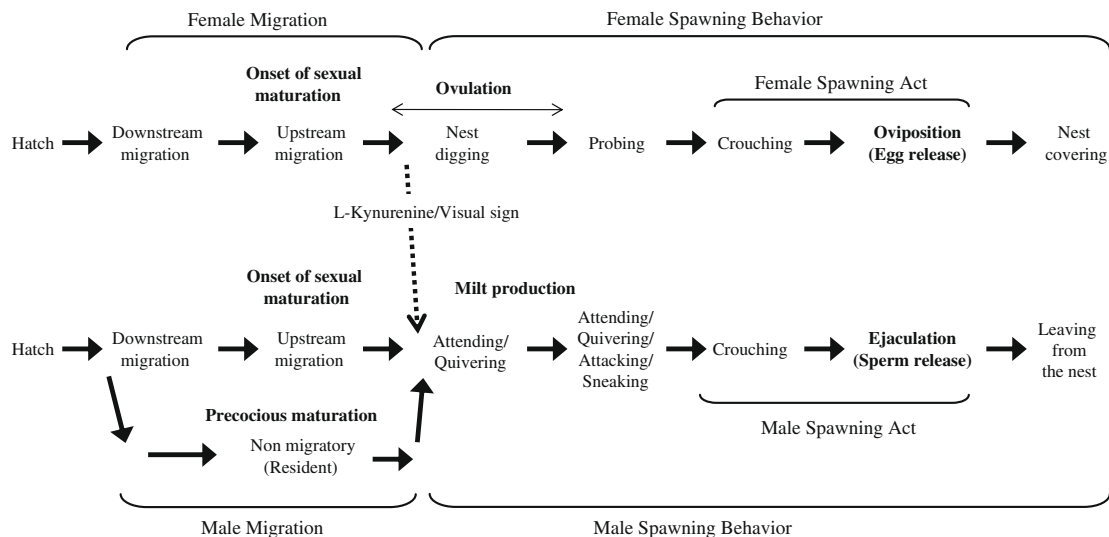


Fig. 4. Diagrammatic representation of the sexual behaviors of male and female masu salmon. See text for details.

of smoltification following the downstream migration of salmonid fishes (Ikuta et al., 1985; Dickhoff et al., 1997; McCormick, 2001; Munakata et al., 2007). It is also known that administration of sex steroids inhibits the smoltification process and interferes in the downstream migration of salmon smolts (Ikuta et al., 1985; Ikuta, 1994; Munakata et al., 2000, 2001a). After a one-year stay in the ocean, masu salmon begin their upstream migratory behavior (i.e., homing behavior) together with the onset of gonadal maturation. As mentioned previously, this upstream migratory behavior is considered to be the initial step for spawning to take place.

When reaching their spawning grounds, two forms of sexually mature males are observed and they are the (1) migratory form and (2) non-migratory (resident) form. The migratory males were smolts that had completed their migration to the ocean and returned. In contrast, the non-migratory males were those that remained and began to mature precociously. Migratory males attain a body length of 40–60 cm during their life in the ocean and become behaviorally dominant in the spawning area. Non-migratory males are smaller in body size (15–25 cm) and become subordinate fish (Gross, 1982, 1985). Most females are observed to become the migratory form (Munakata et al., 2000).

When sexually mature masu salmon arrive at a suitable spawning area in the upper parts of their native river, females that are ready to spawn start their “nest digging behavior”. The females dig a nest (redd) or what is called a spawning bed, by using their tails as well as hime salmon (landlocked sockeye salmon), *Oncorhynchus nerka* (Satou et al., 1984). Females frequently check the shapes (i.e., depth and width) and substrates of the spawning bed by using their abdominal fins. This type of sexual behavior is called “probing behavior”. During and after nest digging, it has been reported that sexually mature females release the pheromone, L-kynurenine in their urine (Yambe et al., 2006). In contrast, “attending” and “quivering” are typical male-typical sexual behaviors that males display parallel to the female. These courtship behaviors that are characteristic of males have been shown to be triggered not only by the visual signals exhibited by female specific sexual behaviors (Satou et al., 1984, 1994; Satou, 1987) but also by some of the reported pheromonal substances (Stacey and Sorensen, 2002; Yambe et al., 2006).

During the spawning period, dominant migratory males frequently exhibit attending and quivering behaviors alongside nest digging females. In contrast, most non-migratory resident males participate in spawning in a behavior that can be characterized as “sneaking” and do not exhibit any of the characteristic courtship behaviors that migratory males display towards nest digging females. Dominant migratory males also exhibit “aggressive” behaviors (i.e., attacking, chasing, and nipping) against other males. After the masu salmon arrive at their spawning ground, females will ovulate and males will complete milt production in preparation for fertilizing the eggs. When nest digging is completed, both females and dominant males crouch on the spawning bed and the females release their eggs (oviposition) and males release their sperm (ejaculation). During these periods, “sneaker” males swim rapidly to the spawning bed and release their sperm on the released eggs. After oviposition and ejaculation, females will cover the fertilized eggs with small stones and pebbles by use of their tail and males leave from the spawning bed. Masu salmon females will repeat this spawning act several times over a period of a few weeks until most ovulated oocyte are released.

5.2. Hormonal control of upstream migration and sexual behavior in female masu salmon

Reproductive activity of female masu salmon begins with the upstream migration in accordance with the onset of gonadal maturation.

During the period of vitellogenesis, plasma levels of T and E₂ gradually increase (Munakata et al., 2001a). After vitellogenesis, plasma E₂ levels rapidly decline and there is an increase in 17,20-P which induces oocyte maturation. Plasma T levels remain high until oviposition. When sexually immature female (post-smolt) masu and hime salmon were treated with T, E₂, and KT in the autumn, these treated fish exhibited the upstream migratory behavior against the flow of water in an experimental raceway (Ikuta et al., 2001; Munakata et al., 2001a). Since salmonid females normally do not have KT in the plasma, T and E₂ are thought to play significant roles in the induction of the upstream migratory behavior in females of both of these salmonids. In addition, T is reported to be a requirement in the occurrence of female sexual behavior in masu salmon as indicated by the induction of the nest digging behavior in sexually immature female masu salmon reared in experimental spawning tanks and when treated with T (Munakata et al., 2001b). Apparently, T treatment itself does not trigger the behavior but T-treated fish will begin the nest digging behavior when placed in tanks with suitable gravel and water flow. The involvement of the other sex steroids, such as estrogen and progesterone, in nest digging behavior has yet to be examined.

Sexually mature female masu salmon are known to release the sex pheromone, L-kynurenine which attracts sexually mature males (Yambe et al., 2006). Experimentally, urine from sexually mature females attracted males but urine from immature females did not. It is not known how the production of this pheromone is regulated but this pheromone is released mainly from females just before and after ovulation. The timing of its production in the reproductive cycle would indicate that this sex pheromone produced in sexually mature females could be a signal to conspecific males indicating gonadal maturity, location and receptiveness of females.

In salmonids, there is no clear evidence whether PG is involved in female sexual behavior. Likewise, the role(s) of GnRH in mediating sexual behaviors are also not fully understood. Administration of GnRH analog enhanced the occurrence of upstream migratory behavior in sexually mature females and T-treated immature hime salmon (Sato et al., 1997; Munakata et al., unpublished data), but it is not clear whether the GnRH acted directly on the brain or stimulated the production of sex steroids via the pituitary gland.

5.3. Hormonal control of upstream migration and sexual behavior in male masu salmon

Male masu salmon that underwent the downstream migration start upstream migratory behavior (i.e., homing behavior) with the onset of gonadal maturation as do the females. During the same period, precocious males that inhabited the middle sections of the river also move upward towards the spawning area. Androgens are considered to be essential for this upstream migratory behavior to occur in precocious males (Munakata et al., 2001a). Castration of such fish impaired the upstream behavior while administration of T and KT treatments restored the behavior. Interestingly, E₂ and 17,20-P had no influence on the occurrence of upstream behavior in these fish. As discussed previously, E₂ was effective in inducing the upstream behavior in females but was ineffective in males.

After sexually mature male masu salmon arrive at the spawning grounds, they exhibit courtship behaviors (i.e., attending and quivering) towards the females that are exhibiting the nest digging behavior. Noticeably, some dominant males show aggressive behaviors towards the subordinate males up until the end of the spawning acts. It has been reported that the series of male sexual behaviors are elicited by visual signals from female specific sexual behaviors (Satou et al., 1984, 1994; Satou, 1987) and a pheromone (Stacey and Sorensen, 2002; Yambe et al., 2006). Involvement of sex steroids in the sexual behaviors exhibited at the spawning grounds has been elucidated by using castrated sexually mature

male masu salmon. Under this treatment, the attending and quivering behaviors were clearly suppressed and treatment of T restored the behaviors in masu salmon (Munakata et al., 2001b). However, in male rainbow trout, *Oncorhynchus mykiss*, attending and quivering behaviors of castrated males were induced by treatment with 17,20-P but not by 11-ketoandrostendione (KA) which is converted to KT in the plasma (Mayer et al., 1994). Clearly, there is a need to examine the effectiveness of other sex steroids other than T in the occurrence of male sexual behavior in masu salmon.

It has been demonstrated that androgens are also a requirement for male salmonids to respond to the sex pheromone, L-kynurenine. Precociously mature male masu salmon exhibited a clear behavioral response when L-kynurenine is released into the water (Yambe et al., 2006). Treatment of sexually immature masu salmon and rainbow trout males with methyltestosterone (MT) has been shown to elicit the expected sexual behavior when the fish are exposed to urine that contains L-kynurenine (Yambe et al., 2003). Based on these studies, gonadal steroids clearly play important roles in the regulation of sexual behaviors in masu salmon. In particular, the gonadal sex steroids are clearly involved in the series of spawning behaviors leading up to spawning such as digging, attending, and quivering. It is interesting that T, a sex steroid common to both the male and female sex, stimulates the expected behaviors from the respective sex. It remains to be determined why the sexual behavior of the opposite sex does not occur in the same individual when both sexually mature male and female salmon experience high plasma levels of T (Munakata et al., 2001a,b). Unlike goldfish, such findings seem to be similar to the case of other vertebrate classes (Pfaff et al., 2002) that once the brain is sexually differentiated, the brain of a male and a female exhibits a differential sensitivity to sex steroids and regulates sex-typical behavior.

It is also interesting to note that the sex steroids apparently also regulate rheotaxis of fish. Fish without sex steroids, sexually immature or gonadectomized, swim with the downstream current or do not exhibit upstream swimming behavior. In contrast, fish that possess sex steroids swim against the current, resulting in reproduction in the river (resident and upstream migration) (Munakata et al., 2000, 2001a,b).

6. Hormonal control of female sexual behavior in other fishes

6.1. Sex steroids

Hormonal control of female sexual behavior is not intensively studied in other species of fishes other than goldfish and salmonids and may be in large part due to the challenges faced in the maintenance of sexually mature females. Although involvement of T in female sexual behavior has been shown in masu salmon (Munakata et al., 2001b), there are no other reports showing a stimulatory effect of T on female sexual behavior in fish. Unlike females in other vertebrates (Sakuma, 1997), estrogens do not seem to be a requirement for the female sexual behavior in many externally fertilizing teleosts. In fact, plasma E_2 show signs of decreased levels during the spawning period and coincides with completion of vitellogenesis (Kobayashi et al., 1987b, 1988; Moyle and Cech, 2000; Munakata et al., 2001a). This is in contrast to internal fertilizing fishes such as the guppy *Poecilia reticulata*, where estradiol maintains receptivity in females just as it does in internally fertilizing tetrapods (Liley, 1972).

Major functions of estrogens in vertebrates are synthesis of vitellogenin in the liver of oviparous animals and expression of proceptive behavior (Moyle and Cech, 2000; Sakuma, 1997). It is conceivable that the effect of estrogen to induce estrous (i.e., receptive) in females may have appeared with the development of the means for internal fertilization during the evolution of vertebrate

reproduction. The effect of estrogen in external fertilizing teleosts is mainly the synthesis of vitellogenin, and oviposition in fishes is not considered a passive behavior (i.e., receiving of gametes) but rather an active behavior (i.e., extruding of gametes) without an influence of estrogen. In this sense, sexual behavior of female fish may be homologous to parturition of mammals and oviposition in reptiles and birds rather than copulation. On the other hand, in most male vertebrates androgen appears to be a requirement of spermatogenesis and the occurrence of male-typical sexual behavior, namely the release of sperm which can take place either into the surrounding environment or into the reproductive tract of females.

6.2. Prostaglandins

Female sexual behavior can be induced by PG injection in other species of female fishes as described previously for the female goldfish and they are: Japanese loach, *Misgurnus anguillicaudatus* (Kitamura et al., 1994), paradise fish, *Macropodus opercularis* (Villars et al., 1985), *Cichlasoma bimaculatum* (Cole and Stacey, 1984), dwarf gourami (Yamamoto et al., 1997), *Puntius gonionotus* (Liley and Tan, 1985), and medaka, *Oryzias latipes* (Oshima et al., 2003). Injection of PG into the females of these species induces the sexual behavior between females and males. From the fact that the action of PG is rapid and is more effective when administered to the ventricle of the brain (Stacey and Peter, 1979), it is felt that PG functions in the stimulation of specific parts of the brain to trigger female sexual behavior in these species. Among these species, PG endogenously produced in the females is also known to be released externally as a pheromone and induces sexual behavior in males of the Japanese loach just as in the goldfish (Kitamura et al., 1994). However, such a pheromone-like response of males does not seem to occur in the dwarf gourami. In the dwarf gourami, an anosmic male can perform male sexual behavior with PG-injected females, suggesting that PG induces female sexual behavior by acting centrally but does not function as a sex pheromone in this species (Yamamoto et al., 1997). It seems that the approach of ovulated females or PG-injected females to a bubble nest visually stimulates males and induces the appropriate sexual behavior in this species. It is not known how males of other species will react and/or are stimulated by PG-injected females.

6.3. Neuropeptides

Arginine vasotocin (AVT) was once believed to act as a trigger of sexual behavior (i.e., spawning reflex) in female killifish, *Fundulus heteroclitus* (Pickford, 1952; Pickford and Strecker, 1977). However, it was found that AVT does not act at the level of the brain but peripherally in the induction of the spawning reflex (Knight and Knight, 1996).

Effects of GnRH on female sexual behavior has not been studied in teleosts other than in goldfish and salmon as mentioned previously. It has been shown that in the female white-crowned sparrow, *Zonotrichia leucophrys* that gonadotropin-inhibiting hormone (GnIH) suppresses female sexual behavior (Tsutsui and Osugi, 2009; Bentley et al., 2006). Although the presence of GnIH homologs were identified in the brain of goldfish (Sawada et al., 2002), the involvement of these peptides in controlling sexual behavior has yet to be examined in fish species.

7. Hormonal control of male sexual behavior in other fishes

7.1. Sex steroids

Testicular androgen is one of the important hormones for mediating the reproductive activity in male fish and apparently is a

requirement in the regulation of male sexual behavior in most fish species (Borg, 1994). Castration suppresses the occurrence of male sexual behavior and administration of androgen enables castrated males, sexually immature males and females to perform male-typical sexual behavior (Kindler et al., 1991; Borg, 1994; Borg and Mayer, 1995; Stacey and Kobayashi, 1996; Kobayashi and Nakaniishi, 1999; Yambe et al., 2003; Munakata et al., 2001b). As mentioned previously, androgen itself does not induce the male-typical sexual behavior. When fish have sufficient levels of circulating androgen(s) or after androgen(s) have acted on the brain at sufficient levels and/or over a sufficient time period, sexual behavior in these fish is triggered by external cues, such as pheromones or other behavioral signals provided by ovulatory females and by other environmental factors.

In addition to functioning as a “requirement”, androgens reportedly function as a “potentiator” and “trigger” in some species. It is known that androgen treatment increases olfactory sensitivity to sex pheromones in some cyprinids (Cardwell et al., 1995; Irvine and Sorensen, 1993). In these reports, the impact of the androgen was to facilitate male fishes to initiate sexual behavior.

Recent studies in plainfin midshipman, *Porichthys notatus* demonstrated the rapid action of sex steroids (Remage-Healey and Bass, 2004, 2007). Sexually mature males (type-I males) of this species generate calls that advertise their presence, and is a male-typical sexual behavior to attract females. This vocal activity was induced within 5 min by administration of KT, E₂, and cortisol but not by T. It is thought that a rapid increase in plasma sex steroids elicits the vocal activity of the males in the natural environment. This rapid action of steroids, most likely through a non-genomic mechanism, may be called one of the trigger hormones.

The functional molecular/chemical form of an androgen differs among fish species. KT and KA are known as major androgens in fishes (Borg, 1994). However, these androgens are not detected in gobies, *Glossogobius olivaceus* and *Gobius paganellus*, and sardine *Sardinops melanostictus* (Asahina et al., 1985; Colombo et al., 1970; Matsuyama et al., 1991). 5 α -Dihydrotestosterone and T are considered to be the major androgens in their respective species. de Waal et al. (2008) reported that 11 β -hydroxyandrostenedione, KT, and KA are potential ligands for the zebrafish androgen receptor. MT, a synthetic androgen, is often used for investigations in fish and highly potent in the induction of sexual behaviors (Borg, 1994). In contrast, T is shown to have weak androgenic effects in some studies (Borg, 1994). Although both sexually mature males and females have high circulating blood levels of T, females do not normally perform male-typical sexual behavior and the reason why high blood T levels do not mediate the occurrence of male-typical sexual behavior has yet to be determined.

There are some recent studies on the involvement of brain aromatase in influencing sexual behavior in fish. Although KT and KA are non-aromatizable androgens, there is a possibility that in some fish species E₂, which is aromatized from T, mediates the expression of male sexual behavior as shown in mammals and birds (Vagell and McGinnis, 1997; Ball and Balthazart, 2004). In the guppy, *P. reticulata*, administration of an aromatase inhibitor, fadrozole, was shown to reduce male sexual behavior (Hallgren et al., 2006). However, effects of fadrozole on the behavior in the male peacock blenny, *Salarias pavo* was not conclusive (Goncalves et al., 2007). Further investigations are necessary to elucidate whether aromatization in the brain plays a key role in the expression of male sexual behavior or aromatization in the brain mediates the hypothalamus–pituitary–gonad function for the production of androgens.

It is reported that some species of sex changing fish can perform sexual behavior without testicular androgens and other sex steroids. The cleaner wrasse, *Labroides dimidiatus*, is a protogynous sex changing fish that exhibits female-to-male sex change that is under social control (Nakashima et al., 2000). In this species, it is

known that one dominant territorial male spawns with many females. When the dominant male disappears or is removed, the largest female in the social group changes its sex to become a functional male. Interestingly, when the dominant male was removed experimentally, the largest female possessing ovulated oocytes started to perform male-typical sexual behavior within 1–2 h, although no sperm release occurred at this time (Nakashima et al., 2000). Although plasma steroid levels were not measured in this study, it is unlikely that the pattern of steroid production in the ovary could have changed in such a short time frame. It is known that gonadal sex change takes a few weeks in this hermaphroditic species (Nakashima et al., 2000; Robertson, 1972).

In the study with bluehead wrasse, *Thalassoma bifasciatum*, a protogynous sex changing fish similar to the cleaner wrasse, ovariectomized females performed male-typical sexual behavior within a few days after they acquired social dominancy (Godwin et al., 1996). These results collectively suggest that gonadal sex steroids are not a requirement for the occurrence of male-typical sexual behavior in these species and social cues apparently triggers the sexual behavior.

Estrogen stimulates the early phases of spermatogenesis in the testis of fish (Schulz and Miura, 2002). However, the involvement of testicular estrogens in male sexual behavior is not fully understood. Inhibitory effects of exogenous estrogens and estrogenic endocrine disrupting chemicals on male sexual behavior of fish have been extensively studied (Bayley et al., 1999; Gray et al., 1999; Loomis and Thomas, 2000; Bjerselius et al., 2001; Oshima et al., 2003; Kristensen et al., 2005; Tilton et al., 2005; Martinovic et al., 2007). Most of these studies show suppression of androgen production by the testis and the resulting reduction in androgen levels are considered to be the primary cause of the failure in sexual behavior.

7.2. Neuropeptides

Unlike the male goldfish in which the sexual behavior is not influenced by GnRH, the involvement of GnRH in male sexual behavior acting as a potentiator has been suggested in investigations involving the dwarf gourami (Yamamoto et al., 1997; Abe and Oka, 2007). The male dwarf gourami builds a nest with bubbles released from the mouth and small pieces of water plants, and when ovulated or when PG-injected females approach the nest, they spawn under the bubble nest in response to the male clasping of the female. The fertilized eggs float and are protected by bubbles in the nest. The GnRH producing neurons of the terminal nerve (TN-GnRH neurons) project their axons to various areas of the brain, and lesion of the TN-GnRH neurons depletes GnRH of TN origin in the brain (Yamamoto et al., 1995). The TN-lesioned fish can still perform all the repertoires characteristic of male sexual behavior, but there is a significant difference between the intact and the TN-lesioned male. The TN-lesion did not affect the occurrence of mating trials but decreased the frequency of nest-building behavior (bubbling behavior). These results lead the authors to suggest that GnRH of TN origin is not a requirement for the general performance of the male sexual behavior, but that it is involved in controlling the threshold for the initiation of the nest-building behavior. The male dwarf gourami actively maintains the nest by frequent bubbling when paired with a sexually mature female. This can be regarded as a highly motivated state of the male for sexual behavior. After removal of the female, the frequency of bubbling decreases with time as the motivated state spontaneously declines. Thus, the frequency of the solitary nest-building may be regarded as an index of the motivation for nest building. This spontaneous decline in the frequency of bubbling behavior is delayed by GnRH agonist and shortened by GnRH antagonist administrations (Oka et al., personal communication).

These results suggest that TN-GnRH is not a requirement for the sexual behavior in males, but controls the motivational state or the threshold for the initiation of a certain repertoire of sexual behavior, e.g., the nest-building behavior. An inhibitory effect of GnRH on sexual behavior has been shown in the male rat (Johnson et al., 2007) but the effect of the same peptide in male fish has not been examined yet.

8. Sexual plasticity of behavior in fishes

8.1. Sex change in fishes

Among vertebrates, one of the unique characters in fish is that some species experience being both a functional male and a functional female during their lifetime (i.e., hermaphroditism) (Chan and Yeung, 1983; Helfman et al., 1997; Moyle and Cech, 2000). Therefore, in these species, one individual fish regularly performs both male-typical and female-typical sexual behavior which is rarely observed in other non-sex changing vertebrates (i.e., gonochorism). It is reported that hermaphroditism is observed within at least 23 families belonging to seven teleostean orders (Helfman et al., 1997). Hermaphroditism in fish is classified into two categories: sequential hermaphroditism and simultaneous (synchronous) hermaphroditism. In sequential hermaphroditism, the ovary and the testis develop at different times resulting in a male phase and female phase in each individual. Sequential hermaphroditism are further classified into three types according to the direction of sex change: (1) protandrous hermaphroditism (i.e., male to female sex change), (2) protogynous hermaphroditism (i.e., female-to-male sex change), and (3) serial sex change (both-way sex change, i.e., sex change in either direction) (Sunobe and Nakazono, 1993; Kuwamura et al., 1994; Nakashima et al., 1995; Grober and Sunobe, 1996). In sequential hermaphrodites, sex change occurs at a certain age or in response to social stimuli. In simultaneous hermaphroditism, the ovary and the testis develop at the same time in one individual fish making fish bisexual when they are mature. Two ways of fertilization are observed in simultaneous hermaphroditism: (1) mating and (2) self-fertilization. In fish which exhibit mating for fertilization, two partners perform the appropriate sexual behavior switching between male and female roles and taking turns fertilizing each other's egg (Cheek et al., 2000). In the self-fertilizing type, the fish does not need partners and releases fertilized eggs without performing any sexual behavior (Minamimoto et al., 2006). In these hermaphroditic fishes, except for the self-fertilizing one, individual fish performs male-typical and female-typical sexual behavior during their lifetime. It is of great interest to understand how the neural and endocrine systems of these fish regulate both types of sexual behaviors in comparison to the regulatory systems of sexual behavior in non-sex changing vertebrates.

8.2. Plasticity of behavior and LH secretion in non-sex changing fish

Although gonochoristic (i.e., non-sex changing) teleosts normally do not exhibit heterotypical reproductive functions, heterotypical patterns of behavior and the LH secretion can be induced in adult goldfish by hormonal treatments (Kobayashi et al., 2002). For example, PGF₂α injection induces female-typical sexual behavior in male goldfish at a rate similar to that seen in females (Stacey and Kyle, 1983; Fig. 5) but does not inhibit performance of male-typical sexual behaviors. Similarly, androgen implants induce male-typical sexual behaviors in mature females without reducing their capacity to exhibit PG-induced female-typical sexual behavior (Stacey and Kobayashi, 1996; Fig. 5). Interestingly, such androgen treatments do not prevent ovarian development nor the occurrence of the ovulatory LH surges (Stacey and Kobayashi,

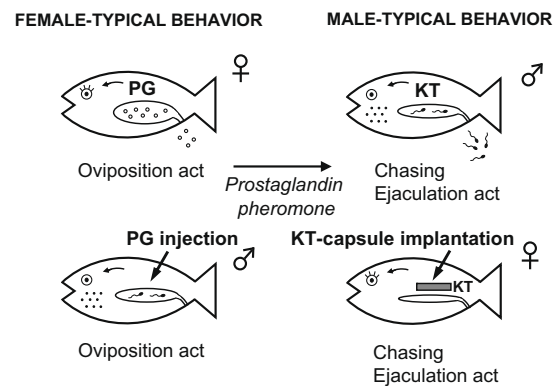


Fig. 5. Diagrammatic representation of the hormonal control and plasticity of sexual behavior in goldfish. Upper: Homotypical behaviors. Ovarian prostaglandin triggers female sexual behavior, oviposition act, in ovulated females. Sexually mature males with high blood levels of 11-ketotestosterone start to perform male sexual behavior, chasing and ejaculation act, stimulated by prostaglandin pheromone. Lower: Heterotypical behavior. When males are injected with prostaglandin, these males perform female sexual behavior although no egg release accompanied. When females are implanted with 11-ketotestosterone capsule, these females start to perform male sexual behavior stimulated by prostaglandin pheromone although no sperm release accompanied (Kobayashi et al., 2002). See text for details. PG, prostaglandin; KT, 11-ketotestosterone.

1996; Kobayashi et al., 1997). Finally, although female goldfish do not usually exhibit a male-typical LH release in response to pheromonal 17,20β-P, they do if implanted with KT (Kobayashi et al., 1997). It is not yet known if androgen-treated females also exhibit the male-typical LH release induced by behavioral interaction with ovulated females, or if hormone treatment can induce a female-typical ovulatory LH surge in males. The potential for heterotypical reproductive function also occurs in adult gynogenetic crucian carp, *C. auratus langsdorffii*, where KT treatment induces male-typical behaviors and 17,20-P-induced LH release in females without inhibiting PG-induced female-typical sexual behavior (Kobayashi and Nakanishi, 1999). This would mean that a species that has evolved or has been created as an all genetically female population can exhibit male-typical functions. Adult goldfish and crucian carp exhibit heterotypical behavior and LH release, and this sexual plasticity extends to the level of the gonad. Androgen treatment has been shown to be able to induce testicular development in the ovary of adult female goldfish (Kobayashi et al., 1991) and such bipotentiality of the gonad is comparable to that of hermaphroditic fishes (Chan and Yeung, 1983; Strussmann and Nakamura, 2002).

Gonochoristic teleosts other than goldfish and crucian carp also exhibit sexual bipotentiality of behavior as adults. For instance, androgen treatment induces male-typical sexual behavior in adult female stickleback (Wai and Hoar, 1963), guppy (Landsman et al., 1987) and medaka, *O. latipes* (Kang, 2003), and exposure to paper mill effluents containing androgenic chemicals causes female mosquitofish, *Gambusia affinis holbrooki*, to display male-typical sexual behavior (Howell et al., 1980). In gonochoristic fish, induction of female-typical sexual behavior in males has only been shown in goldfish. It would appear that some adult teleosts retain relatively more bisexual potential than in mammals, where the normal processes of male development typically involve inhibition of female-typical potential (Yamanouchi, 1997).

Studies of hermaphroditic fish suggest that brain GnRH and arginine vasotocin (AVT) are involved in the sex change process (Foran and Bass, 1999). After sex change, the number and size of GnRH-immunoreactive neurons change in the bluehead wrasse (Grober et al., 1991), ballan wrasse, *Labrus berggylta* (Elofsson et al., 1999), and dusky anemone fish, *Amphiprion melanopus* (Elo-

fsson et al., 1997). Similar changes are seen in the number and size of AVT neurons in bluehead wrasse (Godwin et al., 2000) and marine goby, *Trimma okinawae* (Grober and Sunobe, 1996) at the time of sex change. It is unknown, however, whether these peptidergic cell changes are the cause or the result of behavioral sex changes to maintain a particular sexual status. When female goldfish were implanted with KT and induced to perform male sexual behavior, no change was observed in GnRH- and AVT-immunoreactive cells (Parhar et al., 2001).

8.3. Sexual bipotentiality in the fish brain

In mammals and birds, the potential for gender-typical behavior and LH secretion is determined by *organizational* actions of perinatally secreted steroids that induce sexual differentiation of the brain, and by *activational* effects of hormones on the adult (Yamanouchi, 1997; Sakuma, 1997; Pfaff et al., 2002). Adults of these vertebrate types exhibit brain sex differentiation that normally exhibit only gender-typical behavior and LH secretion and do not show heterotypical functions. Recent studies in plainfin midshipman, *P. notatus*, strongly suggest that the brain of this species sexually differentiate as in mammals. Sexually mature males generate calls that advertise their presence to attract females, and this vocal activity is induced by KT but not T, whereas agonistic calls of females are induced not by KT but T, consistent with the predominant plasma androgen in males (KT) versus females (T) (Remage-Healey and Bass, 2004, 2007). Salmonid species may also have the brain sex differentiation as mentioned previously. In masu salmon, T is a requirement of both males and females for the occurrence of sexual behaviors but T elicits homotypical behavior but not heterotypical behavior (Munakata et al., 2001a,b). These studies suggest that the brain sex differentiation occurs in an organization/activation principle in some species of fishes just as in mammals and birds.

However, laboratory and field observations, suggest that some teleosts possess a sexually bipotential brain and do not conform to the general tetrapod paradigm. Francis (1992) proposed an idea of brain ability of fish and suggested that neural changes occur in the brain prior to gonadal sex change in sex changing fish. Although the neuroanatomical basis for the sexual bipotentiality of the brain of teleosts remains to be determined, we think that short response latencies of performing heterotypical behavior reflect activation of pre-existing mechanisms rather than development of new ones (Kobayashi et al., 2002) (Fig. 6). In protogynous hermaphrodites, for example, display of heterotypical behavior occurs rapidly in response to social cues (1–2 h in the cleaner wrasse, Nakashima et al., 2000; 1–2 days in the bluehead wrasse, Godwin et al., 1996), and in the gonochoristic goldfish, KT can induce male-typical sexual behavior in females within 3 days (Kobayashi, unpublished data) whereas PGF2 α induces female-typical sexual behavior in males within several minutes (Stacey and Kyle, 1983).

Based upon the knowledge available to date, the following explanation is being provided as to the brain function of teleosts in comparison to that of mammals (Kobayashi et al., 2002). In the rat, the undifferentiated brain sex is believed to normally be female. In the presence of androgen or estrogen during the neonatal period, the brain develops neural systems which regulate male functions and inhibit female functions (Fig. 6). Some teleost fishes, on the other hand, appear to possess a sexually bipotential brain. When a protogynous hermaphroditic fish is in a female phase, the female portion of the brain is active and the male portion is quiescent. At the time the individual starts to behave as a male, the male portion of the brain is activated and the female portion becomes quiescent. External factors (age, social status, etc.) that regulate sex change vary among species. Although gonochoristic

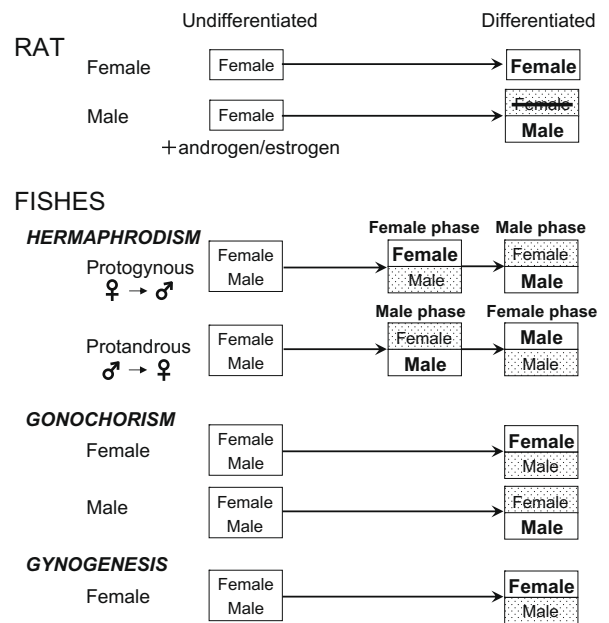


Fig. 6. A hypothesis of sexual bipotentiality of the brain in teleosts (adapted from Kobayashi et al., 2002). In the rat, the undifferentiated brain sex is believed to normally be female but in the presence of androgen and estrogen during the perinatal period, the brain develops neural systems which regulate male functions and inhibit female functions (crossed out area). Teleost fish on the other hand, appear to possess a sexually bipotential brain. When a protogynous hermaphroditic fish is in the female phase, the female portion of the brain is active and the male portion is quiescent (shaded area). At the time the individual starts to behave like a male, the male portion of the brain is activated and the female portion becomes quiescent (shaded area): External factors (age, social status, etc.) that regulate the sex change vary among species. Although gonochoristic and gynogenetic teleosts normally use only brain areas controlling homotypical behaviors during their lifetime, brain areas controlling heterotypical behaviors can be activated by hormonal treatments. See text for details.

and gynogenetic teleosts normally use only brain areas controlling homotypical behaviors during their lifetime, brain areas controlling heterotypical behaviors can be activated by hormonal treatments.

Although examples of adult sexual bipotentiality in teleost behavior admittedly are limited, we are suggesting that brain bisexuality is typical of teleosts regardless of their sexual system (i.e., hermaphroditic, gonochoristic, gynogenetic) (Kobayashi et al., 2002). From this perspective, fish hermaphroditism could be viewed not as the result of specializations that depart dramatically from normal reproductive function, but rather as the opportunistic expression of latent behavioral, endocrine and gonadal bisexuality in ecological situations where hermaphroditism is more adaptive than gonochorism. One of the evolutionary questions that arises during the examination of sexual behavior in fishes is whether only fish obtained the brain bipotentiality in vertebrates during their evolution or have other vertebrates simply lost this bipotentiality during their evolution. There may be several explanations on these two possibilities, but we propose an explanation for the latter possibility. Considering the fact that the extant animals, including invertebrates, which conduct both asexual and sexual reproduction are mostly hermaphrodites, it is possible that the first animal which reproduced sexually appeared during evolution as hermaphroditic (Brusca and Brusca, 2002). If the primitive hermaphroditic animal has the ovary and the testis and neural systems each for regulation of ovarian development and testicular development, this neuronal system is considered as the prototype of a bisexual neural system. It is not surprising that many invertebrates and some fishes retain this neural bisexuality, and other ver-

tebrates including fishes, such as plainfin midshipman and masu salmon, have lost this system with a loss of the hermaphroditic reproductive strategy.

9. Future directions for the study of fish sexual behavior

The underlying regulatory mechanism(s) of sexual behavior of fishes clearly needs to be elucidated for the various categories covered in this review. The justification for such future work is not only for the advancement in basic biology but also for its potential application the practical fields of aquaculture and conservation. One of the major concerns which still require clarification is how hormones and neuropeptides act on the brain during the induction of sexual behaviors. Early studies by electrophysiology revealed the brain areas that control sexual behavior (Koyama et al., 1985; Satou et al., 1984; Satou, 1987) but these studies fall far short of showing the localization of hormone receptors or neurons and genes that are involved in mediating sexual behavior. Use of molecular techniques currently available to biologists is one of the effective approaches to identify these cellular and subcellular matrices that are taking part in this complex process. A major constraint for the study of behavior are that current methods rely on sacrificing specimens and are limiting in the measurement of changes of a target molecule during behavior changes. Use of a transgenic model might be a powerful tool for revealing the interplay of neurons and genes that regulate sexual behavior especially if a method of induction of sexual behavior is established in a species such as has been done in goldfish.

It is also important to study the involvement of environmental factors and their impacts on sexual behavior for the development and use of the information in such applied fields as aquaculture and environmental conservation. Understanding the environmental parameters that control maturation and spawning of a fish species targeted for culture can be exploited and used to result in the production of fertilized eggs and larvae on demand. Likewise, the same understanding would result in principles and practices that would identify important parameters that would need to be preserved in conservation efforts where habitats are being impacted. Although effects of manmade chemicals on sexual behavior of fish have been intensively studied (Kime, 1998; Bjerselius et al., 2001; Oshima et al., 2003; Larsen et al., 2008), only a handful of investigations have been undertaken that examine the effects of modifications (e.g., physical) to the aquatic environment and their impacts on fish sexual behavior (Stacey et al., 1979b; Kitamura and Kobayashi, 2003; Araki et al., 2007; Kobayashi et al., 2008). Clearly, the basic information to date is only in its infancy and clearly an area of future research.

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