

# A Qualitative Model of the Interaction of Sexual Behavior and Hormone Gene Transcription in Male Blue Gourami during Reproduction

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## Abstract

In the present study, a model is suggested to describe hormone control in male blue gourami (*Trichogaster trichopterus*) along the gonadotropic brain-pituitary-gonad axis (BPG axis) and the hypothalamic-pituitary-somatotropic axis (HPS axis). This model is based on the cloning and transcription of genes encoding hormones of the two axes involved in spermatogenesis during blue gourami reproduction. Gene transcription is affected by environmental, biological, and behavioral factors. Mature males were examined in two different stages—nonreproductive in high-density habitats and reproductive in low-density habitats. Based on gene transcription, gonadotropin-releasing hormone 1 (GnRH1) was involved in controlling spermatogenesis (spermatogonia to spermatids) via the BPG axis in nonreproductive and reproductive stages by controlling follicle-stimulating hormone (FSH), 11-ketotestosterone (11KT) and 17 $\beta$ -estradiol (E<sub>2</sub>). However, GnRH3 had a larger effect during the reproductive stage via the BPG axis (spermatids to sperm) on luteinizing hormone (LH), 11KT, and 17 $\alpha$ -hydroxyprogesterone (17P). At the same time, the HPS axis was involved in spermatogenesis via pituitary adenylate cyclase-activating polypeptide (PACAP) and its related peptide PRP (formerly known as GHRH-like peptide) in the brain, and growth hormone (GH) in the pituitary affected synthesis of insulin-like growth factor 1 (IGF1) in the liver.

## Keywords

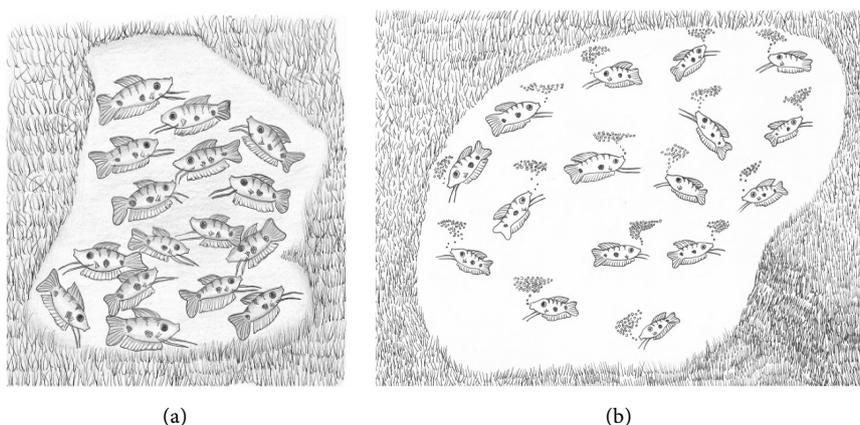
Qualitative Model, Anabantidae, Hormone, Gene, Trichogaster, Spermatogenesis, Sperm, GnRH, 11-Ketotestosterone, Gonadotropic Brain Pituitary Gonad Axis

## 1. Introduction

Blue gourami (*Trichopodus trichopterus*) belongs to the Labyrinthici fish, order Anabantiformes. It is characterized by a specialized organ termed labyrinth, located above its gills, for the respiration of atmospheric oxygen [1]. The geographical distribution of suborder Anabantoidei fish is Central Africa, India, and southern Asia [2]. In its natural habitat, the Anabantoidei fish adapts to an unpredictable environment in which water oxygen concentration varies throughout the year, and can reach a very low percentage, prompting development of the labyrinth organ [1].

Labyrinthici fish undergo two different periods during their life cycle: 1) before labyrinth organ development, from eggs to juveniles, when oxygen is retained over the entire surface by diffusion; 2) after labyrinth development, when the organ becomes important for breathing [1] [3] [4]. The adaptation for the development of eggs and fry in water with low oxygen concentrations involves laying eggs in a bubble nest. The complex sexual and nest-building behaviors of fish belonging to the Anabantoidei species have been described in detail [2] [5] [6] [7]. In natural habitats, when there is a low density of mature males, they become territorial, build a bubble nest and protect it from other males (Figure 1). The function of the bubble nest is to supply oxygen to the eggs and larvae in water with very low oxygen concentrations [1].

In blue gourami, as a model Labyrinthici fish for hormonal control of reproduction, the male's sexual behavior [10] and pheromones [11] [12] affect the gonadotropic axis—the brain-pituitary-gonadal (BPG) axis [9] [13], and the somatotropic axis—the brain-pituitary-liver and body (BPLB) axis. These axes are very complex and they control gametogenesis [1] [10] [14]. In the present study, a model is suggested to describe hormone control in male blue gourami.



**Figure 1.** The two habitats, with high and low densities of males. (a) At high density, the male does not build a nest; (b) At low density, the male builds a nest and sexual behavior [1] [5] [8] [9]. After courting and fertilization, the female swims under the bubble nest and spawns eggs into it. The male guards the nest with the eggs. If an egg falls out, the male returns it to the nest. It also protects the fish in the first period, immediately after hatch (Figure 2).

## 2. Material and Methods

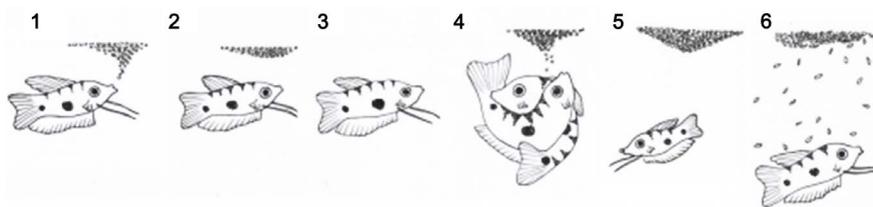
The sexual and nest-building behaviors of blue gourami males have been described in detail in their natural habitat [1] and under experimental conditions [5] [10]. The cloning of genes encoding various hormones and their mRNA levels have been presented in various papers for different organs of the male blue gourami: in the brain—kisspeptin (KISS1 and 2) [9] [15], gonadotropin-releasing hormone (GnRH1 and 3) [9] [16] [17] and pituitary adenylate cyclase-activating polypeptide (PACAP) [18] and its related peptide (PRP) [18]; in the pituitary gland—follicle-stimulating hormone (FSH) [19] [20], luteinizing hormone (LH) [19] [21], growth hormone (GH) [22] and prolactin (PRL) [23]. Levels of the following steroids have been measured by radioimmunoassay in the plasma and testes: 17 $\beta$ -estradiol (E<sub>2</sub>), testosterone (T) and 17 $\alpha$ , 20 $\beta$ -dihydroxy-4-pregnen-3-one (17, 20P) [9] [24] [25] [26], and in the liver: synthesis vitellogenin (VTG) and insulin-like growth factor 1 (IGF1) [22] (Table 1).

## 3. Hormones Involved in the Control of Male Reproduction

Many factors have differential effects on the brains of mature males vs. females [5], including environmental factors such as temperature and water quality, and biological factors such as pheromones and the behavior of other males. Those factors affect the male's sexual behavior, territoriality and nest-building [1] [5] [6] [7] [8] [27] (Figure 1 and Figure 2). Some of the factors are physical, e.g., temperature, water volume, and objects, such as plants, that can uphold the hindernest building. Other parameters involve interactions with other fish, such

**Table 1.** Describe hormones described involve in male blue gourami (*Trichogaster trichopterus*) along the gonadotropic brain-pituitary-gonad axis (BPG axis) and the hypothalamic-pituitary-somatotropic axis (HPS axis).

Name of hormones	The organ or tissue gene transcription	reference
Kisspeptin (KISS1 and 2)	Brain	[24]
Gonadotropin-releasing hormone (GnRH1 and 3)	Brain	[16] [17]
Pituitary adenylate cyclase-activating polypeptide (PACAP) and its related peptide (PRP)	Brain	[18]
Follicle-stimulating hormone (FSH)	Pituitary	[19] [22]
luteinizing hormone (LH)	Pituitary	[19] [21]
Growth hormone (GH)	Pituitary	[22]
Prolactin (PRL)	Pituitary	[23]
17 $\beta$ -estradiol (E2)	Ovary	[24] [25] [26]
Testosterone (T)	Ovary	[24] [25] [26]
17 $\alpha$ , 20 $\beta$ -dihydroxy-4-pregnen-3-one (17, 20P)	Ovary	[24] [25] [26]
Vitellogenin (VTG)	Liver	[22]
Insulin-like growth factor 1 (IGF1)	Liver	[22]



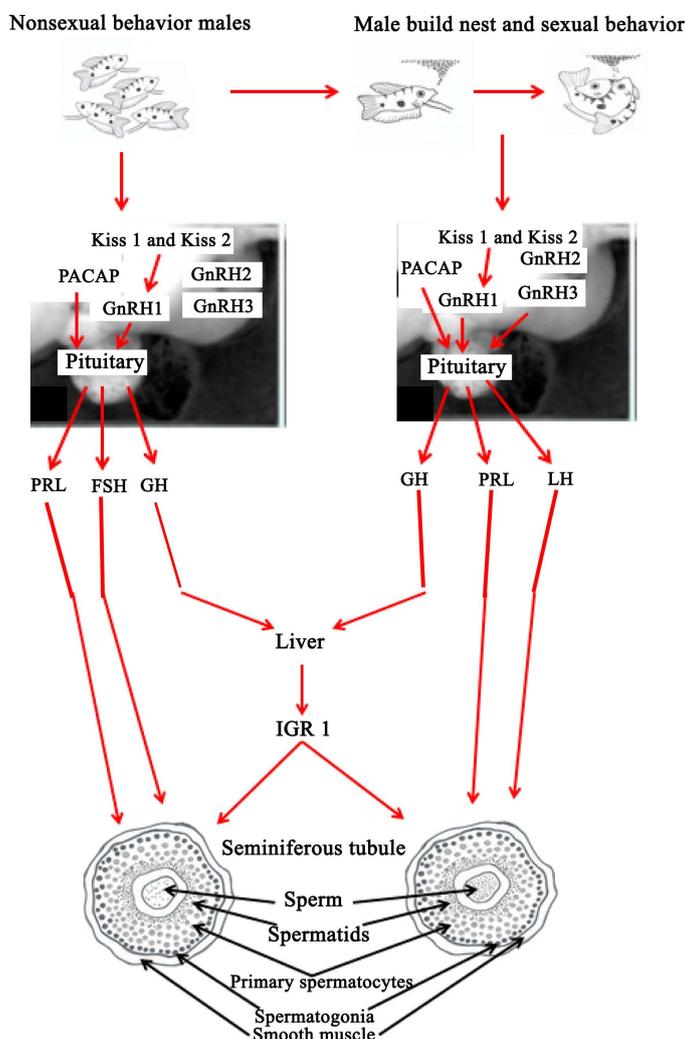
**Figure 2.** Sexual behavior of male blue gourami during the reproductive cycle. (1) The male builds a nest. (2 and 3) Male courts female under the nest. (4) Fertilization takes place and the fertilized eggs float up and stick to the bubble nest. (5) The male guards the eggs in the nest. (6) The male further protects the fry immediately after hatch while they swim in the nest area.

as fish density and pheromones [1] [5]. It has been suggested that the male blue gourami [28] is found in one of two stages after maturation: nonreproductive and reproductive, dependent on conditions that affect the brain and are controlled by the BPG axis. When the males are found in relatively high densities, there is no sexual behavior (or nest building) [5] [10] (Figure 1). Studies of the transcription of many genes in the BPG axis compared to their transcription during spermatogenesis in the testis have indicated which hormones are involved in spermatogenesis. Based on these results, a qualitative model of the two different male stages, dependent upon their environment, is suggested (Figure 1, Figure 3). In the first stage, males are present at high densities, and no sexual behavior or nest building occurs. In the second stage, males are present at low densities and they build nests and become territorial. This situation gives us the opportunity to examine the changes in genes and hormones occurring in the blue gourami brain and BPG axis [28] under these two distinct conditions, mainly GH [22] and PRL [23]; in the plasma and testis,  $E_2$ , T and 17, 20P [24] [25] [26]; and in the liver, VTG and IGF1 [22].

Gene transcription and hormone secretion along the BPG axis are involved in spermatogenesis in the testis during male reproduction. These are affected by environment [29], sexual behavior [5] [10], pheromones and kisspeptin (KISS1 and 2) [30], gonadotropin-releasing hormone (GnRH1 and 3) [16] [17], follicle-stimulating hormone (FSH) [19] [20], luteinizing hormone (LH) [19] [21], pituitary adenylate cyclase-activating polypeptide (PACAP) [18] and its related peptide (PRP) [18], growth hormone (GH) [22], and prolactin (PRL) [18]. FSH and LH act on the ovary to synthesize the steroids  $17\beta$ -estradiol ( $E_2$ ), testosterone (T) and  $17\alpha$ ,  $20\beta$ -dihydroxy-4-pregnen-3-one (17, 20P) [24] [25] [26]; and on the liver to synthesize vitellogenin (VTG) and insulin-like growth factor 1 (IGF1) [22].

#### 4. Discussion

The brain of the male blue gourami is affected by different parameters, and the effects of each individual parameter are difficult to distinguish. The suggested model in Figure 3 shows only the genes encoding hormones that change with sexual behavior. This model suggests that GnRH1 controls spermatogenesis via



**Figure 3.** Gene transcription and hormone secretion along the BPG axis involved in spermatogenesis in the testis during male reproduction. These are affected by environment [29], sexual behavior [5] [10], pheromones and kisspeptin (KISS1 and 2) [30], gonadotropin-releasing hormone (GnRH1 and 3) [16] [17], follicle-stimulating hormone (FSH) [19] [20], luteinizing hormone (LH) [19] [21], pituitary adenylate cyclase-activating polypeptide (PACAP) [18] and its related peptide (PRP) [18], growth hormone (GH) [22], and prolactin (PRL) [23]. FSH and LH act on the ovary to synthesize the steroids  $17\beta$ -estradiol ( $E_2$ ), testosterone (T) and  $17\alpha$ ,  $20\beta$ -dihydroxy-4-pregnen-3-one ( $17, 20P$ ) [24] [25] [26]; and on the liver to synthesize vitellogenin (VTG) and insulin-like growth factor 1 (IGF1) [22].

the BPG axis at both stages: before sexual behavior and during sexual behavior [17]. It controls the change from spermatogonia to spermatids through FSH, 11KT and  $E_2$ . GnRH3 has a stronger effect via the BPG axis on the change from spermatids to sperm through LH, 11KT and 17P. However, it is very difficult to separate the functions of the hormones controlling the various stages of spermatogenesis that occur continually in blue gourami, as in many Labyrinthici fishes. Testis histology clearly shows the different stages of the sperm cells: spermatogonia, primary spermatocytes, spermatids and sperm [17] [20] in the

mature males before and during sexual behavior. The only difference was found in the amount of sperm, which increased during sexual behavior. In the model (Figure 3), we suggest that at this stage, GnRH3 affects LH.

The model suggests that in addition to the BPG axis, the BPLB axis also affects spermatogenesis. PACAP transcription is significantly higher in mature vs. juvenile males [20] [30]. It is suggested that this is because the BPLB axis involvement in spermatogenesis (from spermatogonia to sperm) affects GH and IGF1, but not sperm release into the water during sexual behavior. In male-derived cells from the pituitary gland, the GHRH-like peptide PRP significantly increases GH transcription [30]. PACAP transcription was found to be higher in reproducing males *in vivo*, and GH mRNA level was the same in juvenile, mature and reproducing males [31]. Many published studies support the notion that the somatotrophic axis and liver, via GH and IGF1, also modulate reproduction directly and indirectly, along with the BPG axis in both males and females [32]. This situation was found with the hormones involved in spermatogenesis as described in the qualitative model suggested in the present study (Figure 3).

### Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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