Chapter 7
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Studies on Oocyte Development in Blue Gourami (*Trichogaster trichopterus*) Fry Involves Several Stages

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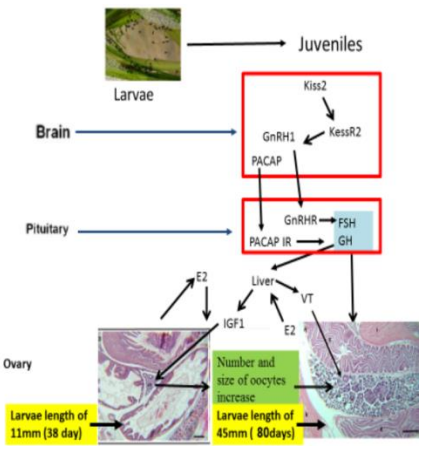
ABSTRACT

We investigated the early stages of ovarian development and the levels of estradiol-17β (E2) and gonadal CYP19 (bgCYP19a) in blue gourami fish (*Trichogaster trichopterus*). Samples of larvae were collected at 38, 55, and 80 days after hatching for histological examination. Ovary size and number of oocytes were smaller in 38-day larvae than in 80-day larvae. All oocytes were in the perinuclear stage on day 38, but some of them had entered vitellogenesis by day 80. The mRNA expression of bgCYP19a was measured by real-time PCR in tissues from 38- and 80-day larvae. There was no significant change in expression level between the two time points. However, E2 levels were significantly higher in 80-day vs. 38-day larvae.



Graphical Abstract: Hormone regulators involved in ovarian development before vitellogenesis in blue gourami. The model includes receptors—GnRHR, PACAP IR, and Kiss2R, and hormones—E2, FSH, GH, IGF1, and VT.

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1. INTRODUCTION

Research into the reproductive physiology of aquarium fish has gained momentum due to their growing economic significance. *Trichogaster trichopterus*, commonly known as blue gourami, is a male-dependent fish that exhibits multiple spawning and asynchronous breeding patterns [1]. Belonging to the family Anabantidae, this species is a member of the suborder Labyrinthici of the order Perciformes, which is characterized by the presence of a labyrinth—an air-filled breathing cavity—located above the gills under the operculum, enabling the fish to survive in low-oxygen environments. The labyrinth is a wrinkled tissue that enhances oxygen uptake by providing a larger surface area. These fish also have accessory organs that aid in gaseous interchange, also facilitating their survival in water with low oxygen levels. The male Anabantidae fish is known to be territorial and protective of its bubble nest. During the breeding season, the male wraps around the female as she lays her eggs. He then collects the eggs in his mouth and spits them into the nest. This behavior has been observed in various studies by Degani [2,3] and Forselius [4]. The blue gourami fish has a unique advantage in terms of controlling the later stages of oogenesis. In females, ovarian development initiates at 3 months of age, and vitellogenesis concludes at 5 months for fish living in dense populations. At this stage, the female fish is ready to reproduce. Oocyte maturation and ovulation occur only when the female is isolated with one male. The well-defined separation of vitellogenic and maturation stages in this species makes it a good model for studying the hormonal control of oogenesis [5]. Oogenesis in mature female blue gourami has been extensively described [6], and the hormones that regulate this process have been thoroughly studied along the brain-pituitary-gonadal (BPG) axis. Degani et al. [7] conducted a study to determine the relative mRNA levels of kisspeptin 2 (Kiss2) and Kiss receptors (Kiss1r [GPR54] and Kiss2r) in the brains of female blue gourami. The researchers collected brain tissue from females before vitellogenesis (previtellogenic) and mature females during vitellogenesis. Statistically significant differences were observed between Kiss2 and Kiss2r mRNA levels in juvenile vs. mature females. However, no significant difference was found in the mRNA levels of Kiss1r between previtellogenic and vitellogenic females. Our analysis of the transcription levels of Kiss1r, Kiss2r, and Kiss2 mRNA revealed significant differences between the genes in both juvenile and mature females. We proposed a qualitative model that demonstrates the role of Kiss2 in regulating the primary stages of oogenesis in vitellogenesis.

The hormone Kiss1 regulates the hypothalamic-pituitary-gonadal (HPG) axis and acts on the caudal hypothalamus. It also appears to affect the receptors of gonadotropin-releasing hormone (GnRH). In blue gourami, the GnRH1 hormone controls vitellogenesis, while GnRH3 regulates oocyte maturation and ovulation. Both of these hormones affect gonadotropins, specifically follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which in turn regulate the reproductive system of blue gourami [8]. Degani's research in 2016 and 2017

demonstrated that gonadotropins play a crucial role in regulating the production of various steroids during oogenesis in blue gourami. Specifically, the hormone FSH is responsible for controlling the production of 17β-estradiol (E2). Degani [9,10] showed that levels of the hormone testosterone (T) increase significantly in the plasma during vitellogenesis. Moreover, Degani and Boker [11] and Degani [12] revealed that 17α,20β-dihydroxy-4-pregnen-3-one (17,20P) levels increase during the oocyte maturation and ovulation stages in blue gourami. Despite several studies on the oogenesis of mature female blue gourami, research on the period of gonadal development before the maturation of ovarian development is scarce. This critical phase of gonadal differentiation is well-documented in different fish species [13-15] and is regulated by various genes and steroids. In blue gourami, cytochrome P450 aromatase (CYP19) in the gonads influences gonadal differentiation in the ovary, as observed by Ezagouri et al. [16], and consistent with findings in other fish species [14].

The objective of this study is to investigate the crucial phase of gonadal differentiation leading to the development of the ovary and characterize the onset of ovarian development in blue gourami fry.

2. MATERIALS AND METHODS

2.1 Fish Maintenance and Growth

For this study, we utilized both male and female blue gourami (*T. trichopterus*) that were bred and maintained at MIGAL laboratories located in northern Israel. The fish were raised in containers with dimensions of 2 x 2 x 0.5 m and were kept at a temperature of 27^{o}C, with a light cycle of 12 h light/12 h dark [17]. The fish were fed an artificial diet with a protein content of 45% and fat content of 7%, which was supplemented with live food such as *Artemia salina*. Details of the breeding and larval growth procedures have been previously outlined by Degani [18,19], and Degani and Gur [20]. Upon hatching, the larvae were allowed to develop for several days before being fed twice a day with a mixture of dry food and yeast which contained 52% protein and 5.8% fat (Miloubar, Oshrat, Israel). After 1 month, the fry were fed solely on dry food, with 48% protein and 6% fat, at approximately 5% of the fish's body weight. In addition, a mixed meat supplement was included in the feed, consisting of 77% turkey heart, 13% chicken liver, 1% vitamins and minerals, and 3% *Tubifex*. The larvae were collected 5 days after hatching and every 2 days thereafter for 2 months for whole body analysis and histological examination during the 80 days of growth. The larvae were sampled on 38, 55 and 80 days after hatching. For sampling, each fish was anesthetized in a clove oil bath (0.25 mg/l), and length was recorded using a binocular microscope. All of the larvae's bodies (n = 7) at different ages (38, 55 and 80 days) were stored in RNALater buffer (Ambion Inc., Austin, TX) and then used for expression studies. Total RNA was extracted using the RNeasy_Total RNA Kit (QIAGEN, Alameda, CA) according to the manufacturer's recommendations [16].

2.2 Real-time PCR Detection of Ovary Aromatase (bgCYP19a) Expression and Estradiol-17β (E2) level

Real-time PCR was used to detect CYP19a in blue gourami ovaries, using the method detailed in Ezagouri et al. [16]. The process involved cloning cDNA from all blue gourami larvae, sequencing, and analyzing the data. Total RNA was extracted from the larvae and used to synthesize first-strand cDNA using the Superscript System (Invitrogen, Carlsbad, CA). Gene-specific primers were designed to amplify, clone and sequence cDNA internal fragments for bgCYP19a, based on conserved DNA sequences and identified through multiple sequence alignment of several larvae aromatase cDNAs, as detailed in Ezagouri et al. [16].

2.3 Histological Examination

Histological samples of larvae during growth and gonadal development were prepared according to previously published methods [5,6] with some modifications to accommodate the specific requirements of this study. Briefly, the larvae were sedated using 0.2 g/l of ethyl m-aminobenzoate methanesulfonate (MS222), and then transferred to 8% formic acid for 48 h. The samples were examined under a light microscope and subjected to a series of incubation and washing steps using different concentrations of ethanol (70%, 96%, and 100%) over several hours, with fresh solutions added at specified intervals. The samples were subsequently incubated in xylene (Frutarom, Haifa, Israel), washed twice for 45 min, and then incubated overnight in a 1:1 mixture of paraffin and xylene at 60°C to facilitate penetration of paraffin into the tissue. The following day, the samples were washed twice with 100% paraffin, 1 h each time, and then incubated overnight at 60°C. The larvae were embedded in both paraffin and glycol methacrylate. For paraffin embedding, 6-µm thin sections were cut using a microtome and stained with Mallory trichrome or PAS as previously described [5].

3. RESULTS

Figs.1–3 present the gonadal development of juvenile female specimens. Fig. 1 shows the histology of 11-mm long juvenile females at the age of 38 days. Prior to this stage, it is challenging to discern the gonads using a light microscope due to their underdeveloped state. However, in the 38-day-old larvae, the ovary can be distinguished, and the presence of primary oocytes at perinuclear stages, as well as the kidney, gut, liver, and muscle, can also be identified (Fig. 1), as previously reported [5]. In female larvae that were 22 mm long and 55 days old, the number of oocytes present in the perinuclear stage increased, and their diameter grew beyond 50 µm (Fig. 2). Similarly, in female larvae that were 45 mm long and 80 days old, multiple oocytes at the beginning of vitellogenesis could be observed (Fig. 3), in agreement with previous findings [5,6]. The expression of bgCYP19a mRNA was examined in larval tissues at 38 and 80 days posthatch by RT-PCR (Fig. 4). bgCYP19a was expressed at both ages, with a relatively high level of mRNA expression observed in the larvae at these stages, as previously reported [6]. The difference in bgCYP19a mRNA

expression between the two ages was not significant (Fig. 4). However, the E2 level in the larval tissues 38 days posthatch was significantly lower compared to 80 days posthatch (Fig. 5).

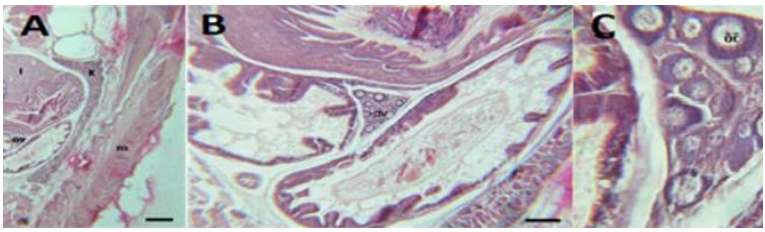

Fig. 1. Histological section of a juvenile female with a standard length of 11 mm (38 days old). (A) Ovary, kidney, gut, liver, and muscle (ov, k, g, l, m, respectively) at a magnification of 190 µm. (B) Close-up view of the ovary (ov) at a magnification of 71 µm. (C) Oocyte (oc) at the perinuclear stage, with a diameter of 20 µm

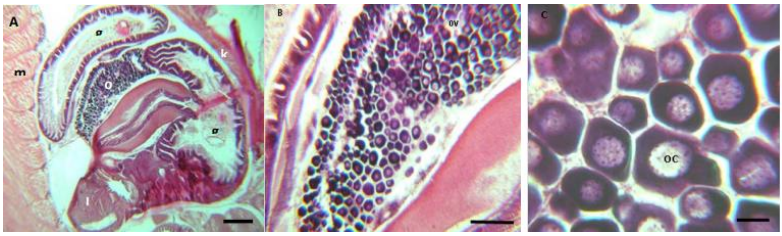

Fig. 2. Histological section of a juvenile female with a standard length of 22 mm (55 days old). (A) Ovary, kidney, gut, liver, and muscle (ov, k, g, l, m, respectively) at a magnification of 320 µm. (B) Close-up view of the ovary (ov) at a magnification of 121 µm. (C) Oocyte (oc) at the perinuclear stage, with a diameter of 20 µm

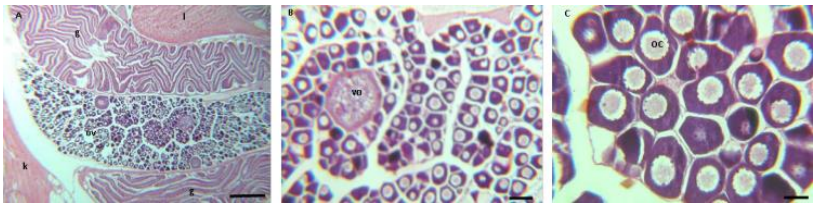

Fig. 3. Cross-sectional view of an 80-day-old female larva that measures 45 mm in length. (A) Ovary (ov), kidney (k), gut (g), and liver (l), scale bar of 360 µm. (B, C) Magnified views of specific structures in the larva. (B) Vitellogenic oocyte (vo), scale bar of 45 µm. (C) Oocyte (oc) at the perinuclear stage, scale bar of 20 µm

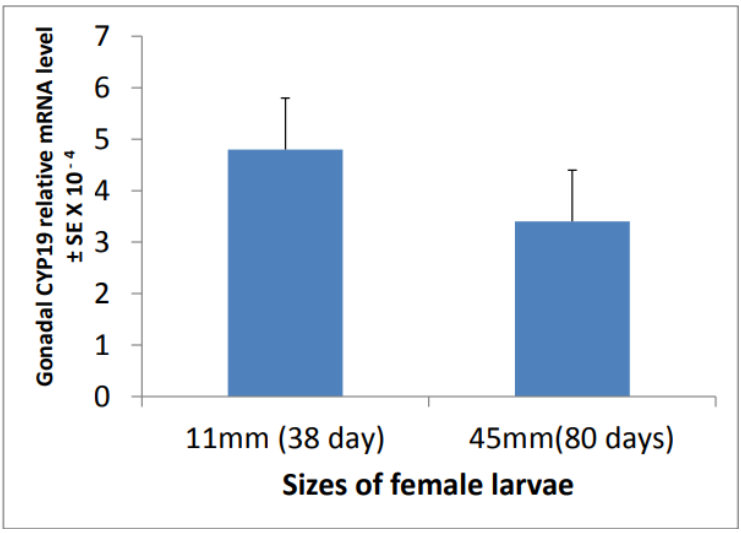

Fig. 4. Mean and standard error of bgCYP19a mRNA level in the gonads of blue gourami larvae normalized to *18S*

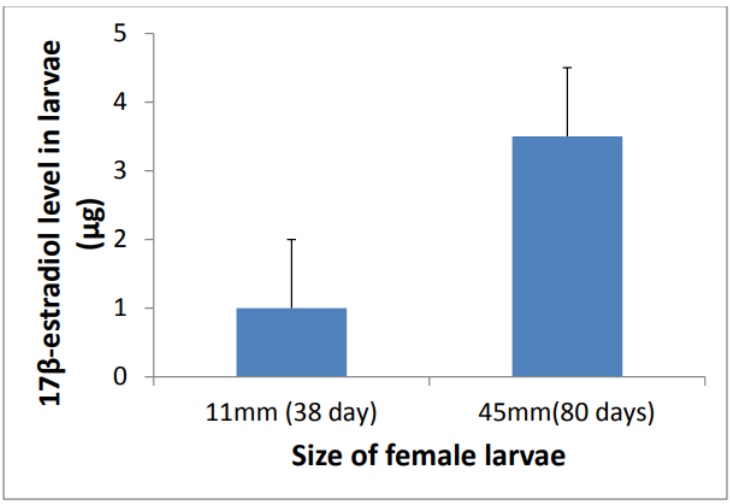

Fig. 5. 17β-estradiol (E2) level in larvae. Each histogram represents the average of five independent measurements (mean ± SE)

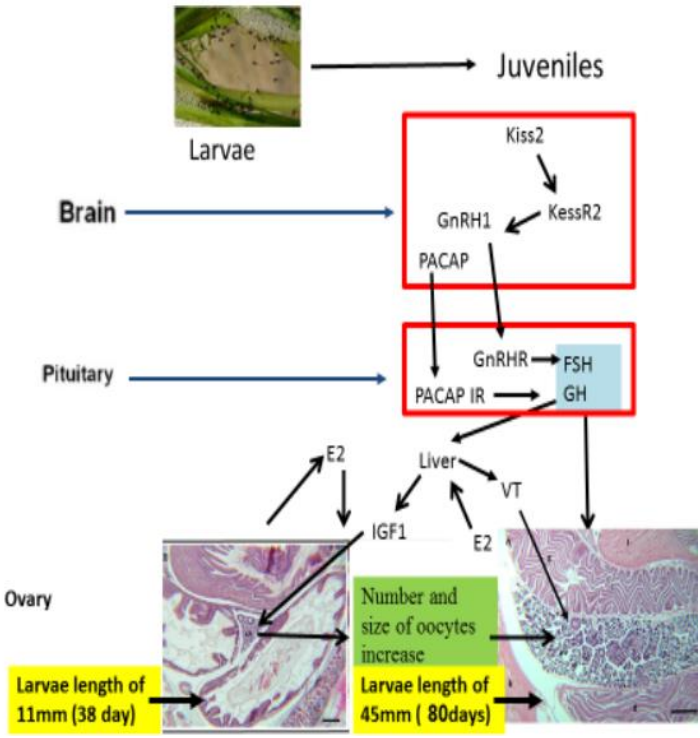

Fig. 6. Proposed qualitative model outlining the hormone regulators involved in ovarian development before vitellogenesis in blue gourami. The model includes receptors GnRHR, pituitary adenylate-cyclase-activating polypeptide (PACAP) IR, and Kiss2R, as well as hormones E2, FSH, growth hormone (GH), insulin-like growth factor (IGF1), and vitellogenin (VT). The model is based on Degani [21,22], Degani and Boker [23], Jackson et al. [5], Degani et al. [12,18,21,22], Mananos et al. [24], and Levy and Degani [25,26]. The proposed model provides a better understanding of the mechanisms involved in regulating oocyte development before vitellogenesis in blue gourami

4. DISCUSSION

The present study describes ovarian development in blue gourami larvae. At the age of 38 days, only small previtellogenesis ovaries were observed; at the age of 80 days, vitellogenesis was about to begin. Vitellogenesis is an important stage in fish reproduction, especially in economically valuable species, as it is controlled by steroids affected by FSH that contribute to gonad differentiation. However, this period varies in different fish species. In this study, the expression of bgCYP19a mRNA was high before vitellogenesis and slightly decreased when

vitellogenesis began, which is consistent with the findings of Ezagouri et al. [16], who reported that bgCYP19a plays a significant role in the regulation of vitellogenesis in blue gourami. They found that bgCYP19a mRNA levels were high at the start of vitellogenesis and decreased significantly as the process advanced. This was explained by the fact that the process of vitellogenesis halts and waits for maturation, which is dependent on male behavior [16]. Moreover, the level of E2 in the ovaries of larvae was low after 38 days of growth, but increased at 80 days of growth, just before vitellogenesis initiation. This finding is consistent with a previous report by Degani [2]. According to Degani et al. [12], the transcription of Kiss2 and Kiss receptors (Kiss2r, Kiss1r) in the brain, and of GnRH1, 2 and 3 [12] indicates the effect of FSH and LH in controlling oogenesis [10] through steroids. The process of larval growth and gonad development before the synthesis and accumulation of vitellogenin in oocytes is complex, because not only is the BPG axis involved, but the somatic axis also plays a crucial role. In blue gourami species, the somatic axis, which includes PACAP [25], growth hormone (GH) [27], and insulin-like growth factor (IGF1) [9], is involved in oogenesis. This interaction has also been observed in other fish species, such as the Russian sturgeon (*Acipenser gueldenstaedtii*) [13,28-30,12,20], European eel (*Anguilla anguilla*) [23,14], and others [15]. We suggest a qualitative model for ovarian development before vitellogenesis (Fig. 6).

5. CONCLUSION

The hormones and receptors that play a role in the development of ovaries in blue gourami before the process of vitellogenesis are known as hormone regulators. These regulators include the receptors GnRHR, PACAP IR, and Kiss2R, as well as the hormones E2, FSH, GH, IGF1, and VT.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Degani G. The effect of temperature, light, fish size and container size on breeding of Trichogaster trichopterus. Israel Journal of Aquaculture. 1989; 41:67-73.
2. Degani G. The effects of human chorionic gonadotropin on steroid changes in Trichogaster trichopterus. Comparative Biochemistry and Physiology Part A: Physiology. 1990;96:525-528.
3. Degani G. The effect of sexual behavior on oocyte development and steroid changes in Trichogaster trichopterus. Copeia. 1993;4:1091-1096.
4. Forselius S. Studies of anabantid fishes. Parts I, II, III. Zoologiska Bidrag Fran Uppsala. 1975;32:593-597.
5. Jackson K, Abraham M, Degani G. Oocyte maturation triggered by the male in a tropical fish Trichogaster trichopterus Journal of Morphology. 1994;220:1-9.

6. Jackson K, Abraham M, Degani G. Cellular events in trichogaster trichopterus adenohypophysis during final oocyte maturation. Indian Journal of Fisherie. 2005;52:1-13.
7. Degani G. Blue Gourami (*Trichogaster trichopterus*) Model for Labyrinth Fish. Laser Pages Publishing, Israel; 2001.
8. Jackson K, Goldberg G, Ofir R, Abraham M, Degani G. Blue gourami (*Trichogaster trichopterus*) gonadotropic subunits (I & II) cDNA sequences and expression during oogenesis. Journal of Molecular Endocrinology. 1999;23:177-187.
9. Degani G. Expression of the growth hormone and insulin-like growth factor 1 genes in the male and female blue gourami (*Trichogaster trichopterus*) at different temperatures. Journal of Asian Scientific Research. 2014;4:413-427.
10. Degani G. Oogenesis control in multi-spawning blue gourami (*Trichogaster trichopterus*) as a model for the Anabantidae family. Internal Journal of Scientific Research. 2016;5:179-184.
11. Degani G. Steroids controlled by various hormones influence oogenesis and spermatogenesis of the model fish, *Trichogaster trichopterus* (Anabantidae, Pallas). In Advances in Sex Steroids, Avid Science Publications, Chapter 1; 2017.
12. Degani G, Alon A, Stoler A, Bercovich D. Evidence of a reproduction-related function for brine Kisspeptin 2 and its receptors in fish (*Trichogaster trichopterus*). International Journal of Zoological Investigations. 2017;3:106-122.
13. Hurvitz A. Degani G, Goldberg D, Yom Din S, Jackson K, Levavi-Sivan B. Cloning of FSHβ, LHβ and glycoprotein α subunits from the Russian sturgeon (Acipenser gueldenstaedtii), β subunits mRNA expression, gonad development and steroid levels in immature fish. General and Comparative Endinocrinology. 2005;140:61-73.
14. Tzchori I, Degani G, Hurvitz A, Moav B. Cloning and developmental expression of the cytochrome P450 aromatase gene (CYP19) in the European eel (*Anguilla angu*illa). General and Comparative Endinocrinology. 2004;38:271-280.
15. Yaron Z, Levavi-Sivan B. Endocrine regulation of fish reproduction. In A.P. Farrell (ed.), Encyclopedia of Fish Physiology: From Genome to Environment, Elsevier. 2011;2:1500-1508.
16. Ezagouri E, Yom-Din S, Goldberg D, Jackson K, Levavi-Sivan B, Degani G. Expression of the two CYP19 (P450 aromatase) genes in the male and female blue gourami (*Trichogaster trichopterus*) during the reproduction cycle General and Comparative Endinocrinology. 2008;159:208-213.
17. Degani G, Boker R. Sensitivity to maturation inducing steroids and gonadotropin in the oocytes of Blue gourami *Trichogaster trichopterus* (Anabantidae, Pallas, 1770). General and Comparative Endinocrinology. 1992;85:430-439.
18. Degani G, Gal E, Vaya J. *In vitro* biosynthesis of steroids in ovary of female *Trichogaster trichopterus* (Pallas, 1770). Comparative Biochemistry and Physiology. 1994;109:715-723.

19. Degani G, Gur N. Growth of juvenile *Trichogaster leerii* (Bleeker, 1852) on diets with different protein levels. Aquaculture Research. 1992;23:161-166.
20. Degani G, Hurvitz A, Levavi-Sivan B. Vitellogenin level in the plasma of Russian sturgeon (*Acipenser gueldenstaedtii*) Northern Israel. Journal of Marine Science: Research and Development. 2017;7:1-4.
21. Degani G, Jackson K, Dosoretz C, Plotzky Y. Molecular DNA variation in *Salamandra infraimmaculata* from different habitats. Israel Journal of Zoology. 1999;44:239-246.
22. Degani G, Jackson K, Marmelstein G. The effect of LHRH analogue on sex steroid profiles in female *Trichogaster trichopterus* (Anabantidae, Pallas). Journal of Aquaculture in the Tropics. 1995;10:297-307.
23. Degani G, Tzhori I, Yom-Din G, Goldberg D, Jackson K. Growth differences and growth hormone expression in male and female European eels (*Anguilla anguilla* L.). General and Comparative Endinocrinology. 2003;134:88-93.
24. Mananos E, Zohar Y, Degani G. The relationship between gonadotropin and sexual behavior of male Trichogaster trichopterus (Pallas). Indian Journal of Fisheries. 1997;44:239-246.
25. Levy G, Degani G. Evidence for a reproduction-related function of pituitary adenylate cyclase-activating polypeptide-related peptide (PRP) in an Anabantidae fish. Journal of Molecular Endocrinology 2011;46:1-11.
26. Levy G, Degani G. Involvement of GnRH, PACAP and PRP in the reproduction of Blue Gourami females (*Trichogaster trichopterus*). Journal of Molecular Neuroscience. 2012;48:9730-9738.
27. Goldberg D, Jackson K, Yom-Din S, Degani G. Growth hormone of Trichogaster trichopterus: cDNA cloning, sequencing and analysis of mRNA expression during oogenesis. Journal of Aquaculture in the Tropics. 2004;19:215-229.
28. Hurvitz A, Jackson K, Degani G, Levavi-Sivan B. Use of endoscopy for gender and ovarian-stage determinations in Russian sturgeon (Acipenser gueldenstaedtii) grown in aquaculture. Aquaculture. 2007;270:158-166.
29. Yom-Din S, Hollander-Cohen L, Aizen J, Boehm B, Shpilman M, Golan M, Hurvitz A, Degani G, Levavi-Sivan B. Gonadotropins in the Russian sturgeon: Their role in steroid secretion and the effect of hormonal treatment on their secretion. Plos One. 2016;13:1-23.
30. Yom Din S, Hurvitz A, Goldberg D, Jackson K, Levavi-Sivan B, Degani G. Cloning of Russian sturgeon (*Acipenser gueldenstaedtii*) growth hormone and insulin-like growth factor 1 and their expression in male and female fish during the first period of growth. Journal of Endocrinological Investigation. 2008;31:201-210.

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