Open Research Journal of Fisheries and Aquaculture
Vol. 1, No. 1, July 2013, PP : 01-05
Available online at http://www.scitecpub.com/Journals.php

RESEARCH ARTICLE

THE AYU: EFFECT OF ISRAELI AQUACULTURE ON GROWTH, GONAD DEVELOPMENT, AND GENE TRANSCRIPTION OF βFSH, β LH AND GH

Gad Degani [1, 2] *and Avshalom Hurvitz [1],

[1]MIGAL – Galilee Technological Center, Kiryat Shmona 11016, Israel; [2]School of Science and Technology, Tel-Hai Academic College. * Corresponding Author: Prof. Gad Degani, MIGAL – Galilee Technological Center, Kiryat Shmona 11016, Israel, Tel.: 972-4-6953544, Fax: 972-4-6944980, E-mail: gad@migal.co.il

SUMMARY

The ayu (*Plecoglossus altivelis*) is a teleost with a one-year life cycle, and following reproduction, the fish usually die. In this study, we describe the ayu, which has been adapted to Israeli aquacultural conditions. The ayu ovaries were examined at vitellogenesis, their β FSH, β LH and growth hormone cDNAs cloned, and the expression of these genes in females at vitellogenesis, measured. Ayu ovaries are of the group-synchronic type and have two distinct batches of oocytes. A low gonad somatic index (GSI) percentage was noted in the small females, as opposed to a high variation in the medium and large females. cDNAs encoding the β subunits of gonadotropin hormones, FSH and LH, and the growth hormone (GH) from the pituitary glands of ayu, were cloned. The similarity between the β subunits of FSH and LH was only 32%, and the deduced amino acid sequences of both ayu LH cDNAs were very similar to their salmonid counterparts. The GH sequence showed an 81% and 75% similarity to the *O. tshawyscha* nucleotide and amino acid sequences, respectively. The expression study demonstrated that vitellogenic females expressed both β FSH and β LH, an expression pattern common to the teleost, with group-synchronic ovary development.

Keywords: ayu, gonad development, gonadotropin transcription, Israeli aquaculture.

INTRODUCTION

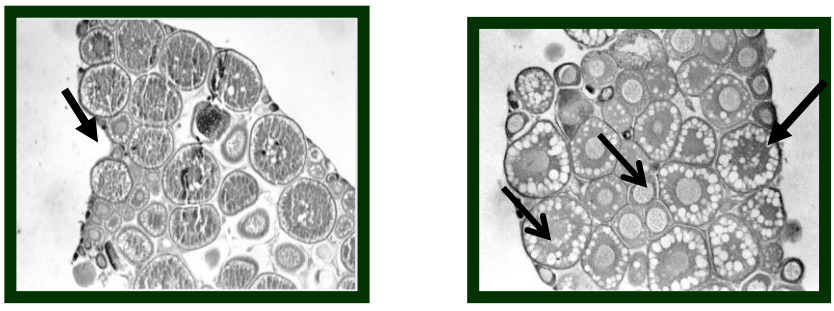
Gonadotropins (GtHs) are glycoproteins, consisting of two non-covalently bound subunits, α and β. Today, the existence of two distinct GtHs, FSH and LH, in the pituitary gland of teleosts, is well accepted. It is believed that FSH regulates gametogenesis, since it promotes the production of 17α estradiol and the incorporation of vitellogenin into the oocytes (Sekine, Saito, Itoh, Kawauchi. & Itoh 1989; Rodriguez, Suzuki, Peter & Itoh 1993). LH, on the other hand, is known to be involved in oocyte maturation and ovulation (Prat, Sumpter & Tyler 1996). This hormone is probably responsible for the final maturation of oocytes (FOM), as it is more active than FSH in stimulating the release of the maturation-inducing factor produced by post-vitellogenic oocytes (Nagahama 1994). The α and β subunits of teleost FSH and LH are encoded by separate genes (Jackson, Goldberg, Ofir, Abraham & Degani 1999; Kajimura, Yoshiura, Suzuki & Aida 2001).

Growth hormone (GH) is a 22 kDa single-chain polypeptide. Together with prolactin, placental lactogen and somatolactin, it forms a family of related polypeptide hormones, whose sequences seem to have evolved from a common ancestor (Niall, Hogan,, Sauer, Rosenblum & Greenwood 1971). GH has been studied extensively, and the GH cDNA nucleotide sequences of many teleosts are available (Ayson, de Jesus, Amemiya, Moriyama, Hirano & Kawauchi, 2000). The influence of GH on fish reproduction has been reviewed by Le Gac, Blaise, Fostier, Le Bail, Loir, Mourot. & Weil (1993). The use of a molecular biological approach has made it possible to examine changes in the mRNA levels of gonadotropins during the gonadal cycle.

The ayu (*Plecoglossus altivelis*) belongs to the Osmeriformes order Plecoglossidae family, which is a small, anadromous and smelt-like fish, closely related to salmonids. This fish is found throughout Japan and in rivers along the coasts of China, Korea, Taiwan and northwards into Manchuria. Ayu is a typical amphidromous fish, generally with a single breeding season during an annual life span (Tachihara and Kimura 1988). Adults spawn in the spring on the lower riverbanks. After spawning, some adults die, while others return to the sea. The hormonal profile during oocyte maturation and ovulation has already been described (Hirose, Nagahama & Adachi, 1983; Hirose, Adachi & Nagahama 1985). The FSH and LH gene expressions of ayu have been studied by Yashida, Nagae, Ito & Soyano (2001) from Japan, who found that the FSH was detected during early vitellogenesis and spermatogenesis, and LH during the late phase of gonadal development. The aim of the present study was to examine the growth and ovary development, as well as the transcription of the β FSH, β LH and GH genes, in ayu females adapted to Israeli conditions of aquaculture.

MATERIALS AND METHODS

Female ayu (*Plecoglossus altivelis*), maintained and bred at Kibbutz Dan in northern Israel, were used in this study. The larvae and fry were grown in containers measuring 2 x 2 x 0.5 m at a temperature of 16°C and under a light regime of 12L 12D. The fish were fed rotifers and algae during the first 20 days, and *Artemia salina* during the subsequent two weeks. Following this period, the fish were fed artificial food (Nihon Nosan Kogyo K.K, Japan). Females found in advanced stages of the gonadal cycle and weighing 83.4 ± 29.89 g were used in this study. For classification of gonadal development, ovaries were collected and processed for histology. The pieces of ovary were fixed in Bouin and processed until they were embedded in paraffin. Histological sections of 2-5 μm were obtained by using a Reichert-Jung (Austria) microtome. The trichrome of Mallory was used to stain the sections (Humason 1979).The cDNA cloning and mRNA expression of β FSH, βLH and GH was carried out as described in detail (Jackson et al., 1999 and Goldberg, Jackson., Yom-Din & Degani 2004). For expression studies, the pituitaries were collected and processed for measurements of mRNA levels by RT-PCR. Statistical analysis of the differences between the β FSH, β LH and GH mRNA levels was analyzed by the ANOVA and t-test.



A B

Figure 1: Histological section of ayu ovary. A contains young oocytes before vitellogenesis and B contains more developed oocytes as well as young ones.

RESULTS

Fig. 1 illustrates the gonadal morphology of the ayu female. Ayu ovaries are of the group-synchronic type with two distinct batches of oocytes. The first contains young oocytes before vitellogenesis, and the second contains more developed oocytes as well as young ones. All of the females used in this study had started vitellogenesis. Small females (50-70 g) were in the early vitellogenesis stage, i.e., although they had started vitellogenesis, many oocytes were in the pre-vitellogenic stage. Medium (70 to 100 g) and large females (over 100 g) were in the advanced vitellogenesis stage, i.e., a large batch of vitellogenic oocytes was revealed in the ovaries of these females. The relationship between the GSI percentage and body weight is shown in Fig. 2a, and the relationship between the weight and the GSI percentage, in Fig. 2b. A low GSI percentage was noted in the small females, as opposed to a high variation in the medium and large females.

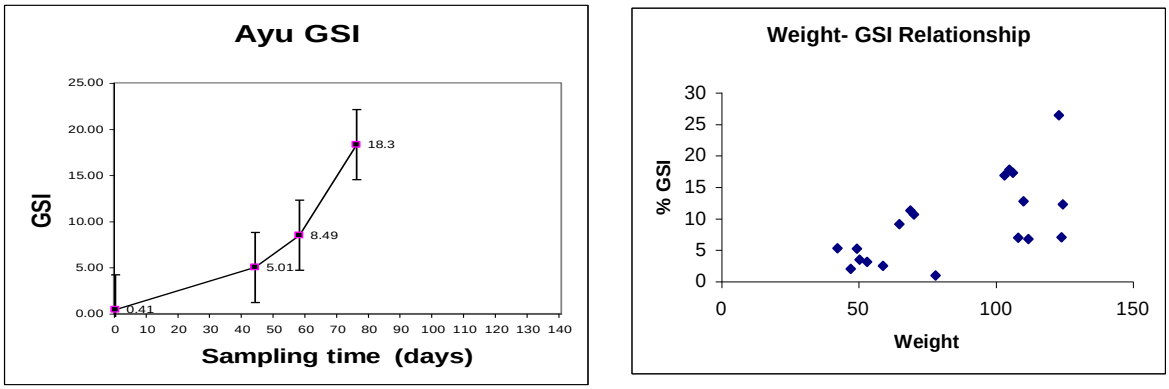


Figure 2: The relationship between the GSI percentage and body weight under aquacultural conditions in northern Israel.

The similarity between the β subunits of FSH and LH was only 32%. It was found that the deduced amino acid sequences of both ayu LH cDNAs were very similar to their salmonid counterparts. The GH sequence showed an 81% and 75% similarity to the nucleotide and amino acid sequences of *O. tshawyscha*, respectively. The cDNA cloning and sequence analyses of β FSH , β LH and GH have been registered in the Genbank (FSH: AY148427; GH: AY148493).

We utilized the cloned cDNAs to measure variations in the levels of their respective mRNAs in the pituitary gland at the maturation stage. The measurements were conducted by RT-PCR, as described above. The rationale behind the use of this method was that under proper conditions, the amount of a specific amplification product is linearly proportional to the concentration of its corresponding mRNA. The amplification of β FSH cDNA produced a 492 base pair (bp) product and that of β LH cDNA, a 576 bp product. The amplification of the internal standard (the cDNA of 18S rRNA) generated a 430 bp product. The identity of each PCR product was confirmed by DNA sequencing. Females found in the three weight groups expressed β FSH. A significant increase in β FSH expression was noted in females in the advanced vitellogenesis stage (large- and medium-sized females), as compared to females in the early vitellogenesis stage (Fig. 3A).

A unique pattern of expression of β LH was detected only in small females (less than 55 g) in the early vitellogenesis stage, and in large females (over 120 g) in the advanced vitellogenesis stage expressing the gene. Medium-sized females did not express any β LH. Large females in the advanced vitellogenesis stage expressed more β LH in comparison to the small females, but the difference was not significant. The relationship between LH expression and the gonadal vitellogenesis stage is shown in Figure 3B. No fish were found to express GH.

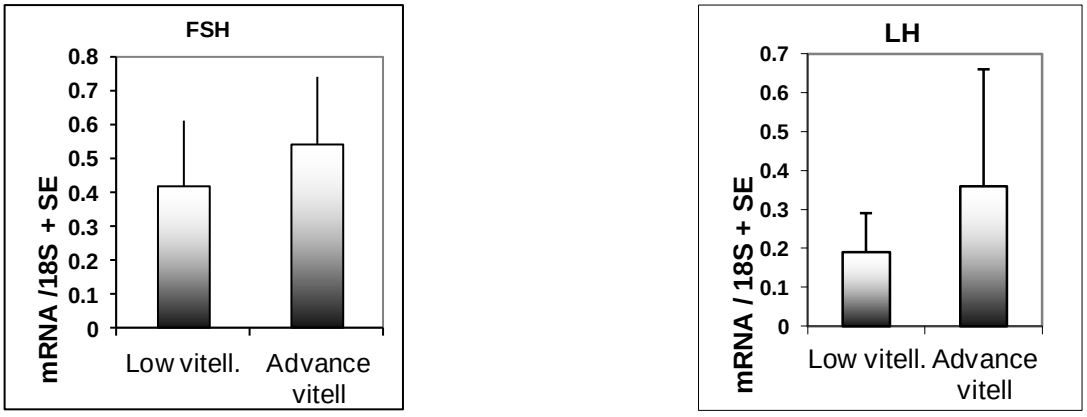


Figure 3: The β FSH and β LH mRNA expression in females found in the advanced vitellogenesis stages (large- and medium-sized females), as compared to females in the early vitellogenesis stage.

DISCUSSION

By histological means, we were able to classify the gonadal stages of the ayu females. All females were found to be in the vitellogenesis stage. The GSI corresponded to the histological classification of the ovaries, i.e., females found in the early vitellogenesis stage showed lower GSI than females found in the advanced vitellogenesis stage, but there was no clear correlation between body size and GSI among the large females. Ayu populations are characterized by a large variation in adult body size (Iguchi & Hino 1996), which influences the reproductive pattern; large females usually reproduce once, while small females may reproduce twice during the same breeding season (Iguchi 1996).

We were able to measure the mRNA level of β FSH and β LH of the females. The results showed that small-, medium- and large-sized females expressed β FSH. Small, early vitellogenic females and large females, found to be in the advanced vitellogenesis stage, expressed mainly LH. This unique expression of β LH could be related to the dual pattern of spawning within the population or it can signify for a yet un-identified role for this GTH at the early vitellogenesis stage.

The cloned ayu GH cDNA should prove useful in studies of mRNA expression. Recent studies on GH functions have confirmed other physiological effects, in addition to the well-established growth-promoting effects. The participation of GH in reproduction has been studied in several teleosts by various approaches, including gene expression (Goldberg et al., 2004). In this study, no GH expression was detected in maturing and mature females. Many fish continue to grow after reaching maturity. This pattern apparently does not occur in the ayu, which probably stop investing in growth once the gonad developmental stage has begun.

REFERENCES

[1] Ayson, F.G., de Jesus, E.G., Amemiya, Y., Moriyama, S., Hirano, T & Kawauchi, H. 2000. Isolation, cDNA cloning, and growth promoting activity of rabbitfish (Siganus guttatus) growth hormone. General and Comparative Endocrinology 117: 251-259.

[2] Goldberg, D., Jackson, K., Yom-Din, S. & Degani, G. 2004. Growth hormone of Trichogaster trichopterus: cDNA cloning, sequencing and analysis of mRNA expression during oogenesis. Journal of Aquaculture in the Tropic 19 : 215-229.

[3] Hirose, K., Adachi, S. & Nagahama, Y.1985. Changes in plasma steroid hormone levels during sexual maturation in the ayu, Plecoglossus altivelis. Nippon Suisan Gakkaishi 51 : 399-403.

[4] Hirose, K., Nagahama, Y. & Adachi, S. 1983. Changes in serum concentrations of gonadotropin, 17a-hydroxyprogesterone, and 17a, 20b-dihydroxy-4-pregene-3-one during synthetic LH-RH- Induced final oocyte maturation and ovulation in ayu, Plecoglossus altivelis. Nippon Suisan Gakkaishi 49 : 33-40.

[5] Humason, G. L. 1979. Animal Tissue Technique, 4th ed., San Francisco; WH Freeman & Co.

[6] Jackson, K., Goldberg, G., Ofir, M., Abraham, M. & Degani, G. 1999. Blue gourami (Trichogaster trichopterus) gonadotropic b subunits (I & II) cDNA sequences and expression during oogenesis. Journal of Molecular Endocrinology 23 : 177-187.

[7] Iguchi, K.1996. Size-specific spawning pattern in ayu, Plecoglossus altivelis. Ichthyological Research 43: 193-198.

[8] Iguchi, K. & Hino, T. 1996. Effect of competitor abundance on feeding territoriality in a grazing fish, the ayu Plecoglossus altivelis. Ecological Research 11 .

[9] Kajimura, S., Yoshiura, Y., Suzuki, M. & Aida K. 2001. cDNA cloning of two gonadotropin beta subunits (GTH-Ibeta and -IIbeta) and their expression profiles during gametogenesis in the Japanese flounder (Paralichthys olivaceus). General and Comparative Endocrinology 122: 117-129.

[10] Le Gac, F., Blaise, O., Fostier, A., Le Bail, P. Y., Loir, M., Mourot, B. & Weil, C. 1993.

[11] Growth hormone (GH) and reproduction: a review. Fish Physiology and Biochemistry 11 219-2312.

[12] Nagahama, Y. 1994. Endocrine regulation of gametogenesis in fish. The International of Development Biology : 38, 217-229.

[13] Niall, H. D., Hogan, M. L., Sauer, R., Rosenblum, I. Y. & Greenwood, F. C. 1971. Sequences of pituitary and placental lactogenic and growth hormones: evolution from a primordial peptide by gene reduplication. Proceedings of the National Academy of Sciences U S A 68 : 866-870.

[14] Prat, F., Sumpter, J. P. & Tyler, C.R. 1996. Validation of radioimmunoassays for two salmon gonadotropins (GTH I and GTH II) and their plasma concentrations throughout the reproductivecycle in male and female rainbow trout (Oncorhynchus mykiss). Biology of Reproduction 54: 1375-1382.

[15] Rodriguez, J. N., Suzuki, K., Peter, R. E. & Itoh, H. 1993. Effects of common carp gonadotropin I and II on vitellogenin synthesis and uptake and 17b estradiol secretion in goldfish, Carassius auratus. French Journal of Physiology 11: 34-41.

[16] Sekine, S., Saito, A., Itoh, H., Kawauchi, H. & Itoh, S. 1989. Molecular cloning and sequence analysis of chum salmon gonadotropin cDNAs. Proceeding of the National Academy of Sciences USA 86 : 8645-8649.

[17] Tachihara, K. & Kimura, S. 1988. Some notes on the over-winter ayu Plecoglossus altivelis in Lake Ikeda, Kagoshiima Prefecture. Nippon Suisan Gakkaishi 54 : 1107-1113.

[18] Yashida, D. Nagae, M. , Ito, F. & Soyano, K. 2001. Molecular cloning of cDNAs encoding pituitary glycoprotein hormone , FSH and LH subunits in ayu, Plecoglossus altivelis Zoological Science 18 : 929-936.

