Biological Measurements and Hormonal Profile in Female Gilthead Sea Bream (*Sparus Aurata*) In Response to Human Chorionic Gonadotropin (HCG) Injection

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Abstract

Water temperature and photoperiod only or with HCG injection are the most important factors driving sexual maturation in fish. This study investigates the possibility of induced spawning of Gilthead Sea bream in captivity by exposing to program of water temperature and artificial photoperiod with or without HCG injection. A total number of 60 Gilthead Sea Bream were collected and kept in indoor fiber circular holding tanks filled with salt water, under controlled photoperiods and temperature. They were divided into two groups. The first group was exposed to water temperature 21.5 to 18 ℃ and artificial photoperiod programs (control). The second group was exposed to water temperature 21.5 to 18 ℃ and artificial photoperiod programs and injected with single dose of HCG. The experiment conducted for 4 months. After the end of the third and fourth months, and after 24 and 48 hours after HCG injection, K factor, gonadal weight, gonadosomatic index (GSI %), fecundity and egg diameter were measured and blood samples were collected. The Results of gonadal weight, fecundity, egg diameter, LH and progesterone levels were increased in injected group when compared to control while the results of GSI, and FSH showed significant decreased ($P<0.05$) when compared to control. It could be concluded that HCG injection in single dose (1000 IU/Kg BW) aiding with water temperature and artificial photoperiod programs induced increasing the maturation of gilthead sea bream in captivity.

Key words: Gilthead Sea bream, HCG, Water Temperature, Photoperiod, *Sparus aurata*

Introduction:

The Gilthead Sea bream (*Sparus aurata*) is an important commercial species in the Egyptian coasts of Mediterranean Sea and the most relevant marine species in

Mediterranean aquaculture ***(FAO, 1999)***.

Unfortunately, some dysfunction form of reproduction was exhibited in most fish when reared in captivity condition. Some female species often fail to undergo final oocyte maturation (FOM) stage and do not spawn, while males exhibited reduced production or low quality of milt ***(Mylonas and Zohar, 2001 and Yousefian and Mousavi, 2011).*** Fish Reproduction in captivity can be controlled by environmental manipulations and exogenous hormones ***(Yaron, 1995 and Zohar and Mylonas, 2001).*** Hormonal treatment may be used for management as a tool to enhance the efficiency of increase spermiation, egg production and facilitate hatchery operations ***(Mylonas, et al., 2010).*** Gilthead seabream spawn daily for more than 3 months during the regular spawning season, and hormonal treatment are used only for non-responsive fish reared under artificial photothermal conditions ***(Zohar and Mylonas, 2001).*** Development of gonads and maturation in fishes are known to be regulated by the HPG axis and other environmental factors, remarkably as temperature, photoperiod, nutrition, water flow and spawning substrata, are also involved in this regulation process ***(Li, et al., 2015).*** HCG has been widely used since the 1930s to induce ovulation and spawning in different species of aquacultured fish that are already sexually mature ***(Sahoo et al., 2007 and Minar et al., 2012).***

The present study aimed to investigate the possibility of induced spawning of Gilthead Sea bream in captivity by exposing to program of water temperature and artificial photoperiod with or without HCG injection.

Materials and methods

A total number of 60 (36 male and 24 female) Gilthead Sea Bream *S. aurata* broodstocks were collected from Manzala Lake, Damiatta Governorate and Suez Canal Company for Fish Farming and Aquaculture at Ismailia Governorate, Egypt. Average body weight for males was 290.84 g and 355.87 g for females. Average total length for males was 26.56 cm and 27.35 cm for females. The collected fish were transported to Fish Farming and Technology institute, Suez Canal University, Ismailia Governorate, Egypt. Fish were kept in indoor fiber circular holding tanks with maximum capacity of 3 m^3 (1.7 m diameter and 1.4 m high) filled with salt water and under controlled photoperiods and temperature.

Broodstocks were divided into two groups (three replicates/group). The first group (control) continued 30 fish (18 male and 12 female). They were exposed to water temperature and artificial photoperiod programs as shown in table 1. The second group (treated) continued 30 fish (18 male and 12 female). They were

exposed to water temperature and artificial photoperiod programs and injected with single dose (1 ml HCG kg^{-1} bw (1000 IU HCG / kg bw**))** of HCG (Pregnyl, Argent labs., Redmond WA Washington, United States 98052) when water temperature become 19 and 18 ℃.

Water temperatures program ranged from 21.5 to 18˚C by using chiller systems and artificial photoperiod program was applied by using neon lamps (Table 1). Light intensity in the range of 600 lux measured by Light meter (YK-10LX, Taiwan), which was changed every 15 days as well as water temperature at a rate of half degree until it reaches the target temperature 19 and 18 ℃. Large windows should be avoided to prevent direct sunlight rolling on the tanks by covering with black sheet.

After the end of the third and fourth months 2 female fish from each replicate were netted from the first group. After 24 and 48 hours from HCG injection (second group), 2 female fish from each replicate were netted. Fish netted were anaesthetized with tricaine methane sulfonate (Ms-222, dose:100 mg/L, Argent Lab. Inc. Philippines) ***(Popovic et al., 2012)***.

The Fulton's condition factor (k) were measured according to the following formula

K = $\frac{BW \times 100}{L^3}$ (BW: Body weight, L: Total Length) **(Hastings and Dickie, 1972)**

Gonadal weight and GSI were calculated according to the following formula:

GSI = $\frac{Gonad\ weight}{Total\ Body\ weight} \times 100$ (***Albertine-Berhaut, 1973)***.

Fecundity were calculated according to the following formula:

Fecundity = $\frac{no.of\ eggs \times gonad\ weight}{weight\ of\ gonad\ sample}$ ***(Hunter et al., 1992).***

30 – 50 oocytes were separated from the ovarian tissues and placed in saline solution (0.9 % NaCl) then, taken on slide to measure oocyte diameter that was measured to the nearest 0.01µm by using ocular micrometer on the binocular microscope at a power magnification of 4X.50 and the percentage occurrence were plotted against the oocyte diameter class interval in order to confirm the ovulation pattern ***(Amein, 1996).***

Fasting blood samples were quickly withdrawn by heparinize syringe (5000 IU, Amoun Pharmaceutical Co.) from heart puncture. Plasma was separated and stored at −80 ℃ for subsequent analysis for hormonal determination. FSH, LH, Estradiol (E2) and Progesterone levels were determined by using commercial assay ELISA kits as manufactories instructions.

Table 1*: water temperature and artificial photoperiod programs throughout the experiment:*

Day No.	Water Temperature °C	Artificial Photoperiod program	
		Day hours	Night hours
1 - 15	21.5	11	13
16 - 30	21	10:45	13:15
31 - 45	20.5	10:30	13:30
46 - 60	20	10:15	13:45
61 - 75	19.5	10:30	13:30
76 - 90	19	10:45	13:15
91 - 105	18.5	11	13
106 - 120	18	12	12

Results

The effect of water temperature and artificial photoperiod programs with and without HCG injection on K factor, gonadal weight, GSI, fecundity and egg diameter of female gilthead sea bream:

In the present investigations, the highest value of k factor of female gilthead sea bream was 1.98 % at the third month at temperature 19 °C after 48 hours from injection in treated group (G_2), while, the lowest value was recorded after the fourth month at temperature 18 °C in G_2 (Fig.1).

Gonadal weight and GSI were significantly ($P<0.05$) higher after the fourth month in both control and treated groups (24 and 48 hours) at 18 °C when compared to the values during the third month at 19 °C (Fig. 2).

Egg diameter showed highest significant ($P<0.05$) value (0.59 mm) after 48 hours from HCG injection at 18 °C when compared to the other values. At 19 °C during the third month control group and 24 hours after injection exhibited significant ($P<0.05$) lower values when compared to the other (Fig. 3).

Fecundity was significantly ($P<0.05$) higher after the fourth month in both control and treated groups (24 and 48 hours) at 18 °C when compared to the values during the third month at 19 °C (Fig. 4).

The effect of water temperature and artificial photoperiod programs with and without HCG injection on E2, progesterone, FSH and LH levels of female gilthead sea bream:

The peak levels of E2 was recorded at water temperature 18 °C after 48 hours from injection (11.13±0.34 pg/ml) in treated group while progesterone recorded highest level at water temperature 18 °C after 24 hours from injection (0.77±0.19

ng/ml) as shown in (Fig.5). There is no significant
difference between the levels of FSH during third and fourth months at temperature 19 and 18 °C. The highest significant level of LH was recorded after 24- and 48-hours injection as well as control group at water temperature 18 °C after the fourth month when compared to groups at temperature 19 °C (Fig. 6).

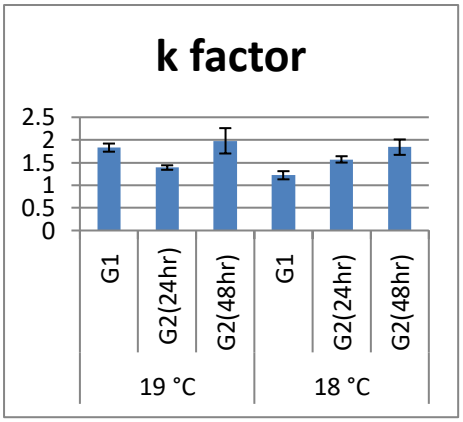

Fig.1: The effect of artificial photoperiod, water temperature and hormonal treatments on condition factor (K) of female Gilthead sea bream S. aurata

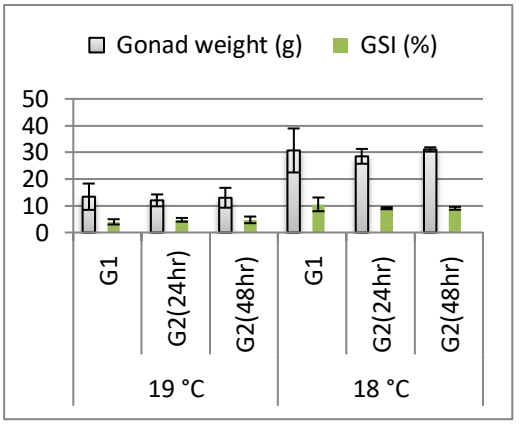

Fig.2: The effect of artificial photoperiod, water temperature and hormonal treatments on gonadal weight and GSI of female Gilthead sea bream *S. aurata*

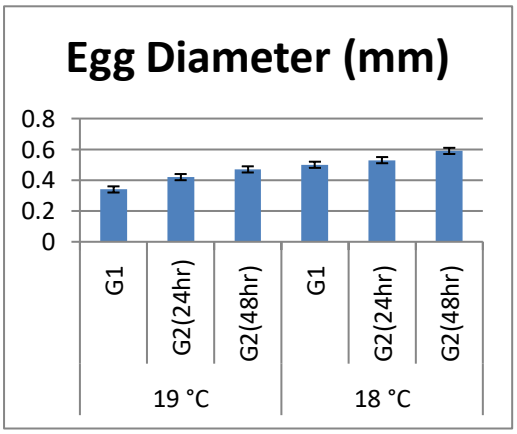

Fig.3: The effect of artificial photoperiod, water temperature and hormonal treatments on Egg Diameter of female

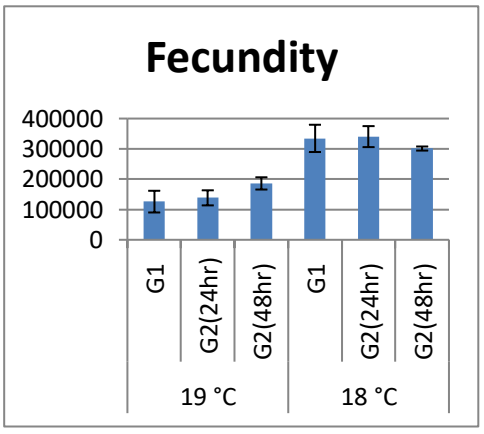

Fig.4: The effect of artificial photoperiod, water temperature and hormonal treatments on fecundity of female Gilthead sea bream *S.*

Discussion

Condition factor doesn't relate the feeding condition of the adult stage but includes the state of gonadal development based on fat reserves during the spawning period ***(Vazzoler and Vazzoler, 1965).*** In the present study K factor was peaked in G_2 after the third month at water temperature 19 °C after 48 hours from HCG injection when spawning season approach that related to body weight (280.63±44.23 g) and shorter length (24.33±1.96 cm). This increasing in K factor may be attributed to the development of gonads to be matured. These results are agreed with ***Chaoui et al., (2006) and Oudjane et al., (2017).***

There is normally a gradual increase in the condition factor during the reproductive period (G_2 after the third month at water temperature 19 °C and after 48 hours from HCG injection) then normalization occurs immediately afterwards in G_1 after the fourth month at temperature 18 °C where exhibited lowest value (1.22±0.09).

The effects of hormonal administration under artificial photoperiod and water temperature programs were clearly observed on gonadal weight, GSI, fecundity and egg diameter. The highest GSI value of female gilthead sea bream was 10.53 % in G_1 after the fourth month at water temperature 18 °C. The fecundity and egg diameter could be measured from the third sample where the eggs were distinguishable by naked eye and could be separated. The largest egg diameter was obtained (0.590 mm) of treated groups at water temperature 18 °C at the fourth month after 48 hours from HCG injection when compared to 24 hours after HCG injection either in temperature 18 or 19 °C. The highest fecundity was recorded significantly ($P<0.05$) after the fourth month at water temperature 18 °C when compared to other groups at water temperature 19 °C. These results of GSI was in agreement with ***Chaoui, et al. (2006)*** who recorded that, the GSI value of wild gilthead sea bream was 14 %. Concerning egg diameter. ***FAO (1999)*** reported that the minimum oocyte diameter of gilthead sea bream was 500 µm while ***Wahbi et al., (2017)*** recorded that, the fertilized eggs diameter in case of HCG injection was (850±50µm) after 54 hours.

The current results showed numerical increase in FSH after fourth month at water temperature 18 °C that reached the peak value (1.16 miu/ml) just before spawning under the effect of artificial photoperiod and water temperature programs. Increasing FSH levels in female might have a role on procedures of oogenesis, primary and secondary oocytes and undergo vitellogenesis with yolk granules ***(Rainis et al., 2003 and Yousefian and Mousavi, 2011).***

LH levels in females begin to increase till reach the peak after the

fourth month 0.52 miu/ml in G_1 (artificial photoperiod and water temperature group) and 0.57 miu/ml in G_2 (artificial photoperiod, water temperature and HCG group) after 48 hours of injection. LH in female is involved in FOM and slightly out of stimulation of maturation inducing hormone production. These results were agreed with ***Rainis et al. (2003) and Yousefian and Mousavi (2011)*** who demonstrated that, GTH II is involved in FOM and increase in plasma just before FOM in female and determining the switch from the steroidogenic and androgenic production.

Progesterone in female at G_1 (artificial photoperiod and water temperature group) released under the effect of LH where it reached the peak after the fourth month at 18 ℃ (0.56 ng/ml). In G_2 (artificial photoperiod, water temperature and HCG group) progesterone reached the highest level (0.77 ng/ml) after the fourth month at 18 ℃ after 24 hours from HCG injection which might be released under the effect of HCG injection. Progesterone has a very necessary role for FOM and ovulation successfully ***(Rainis et al., 2003)***. FSH and LH were released into the bloodstream acting on the gonad, where they stimulate the synthesis of the sex steroid hormones (androgens, estrogens and progestogens), which are the ultimate effectors on gonadal development ***(Yu et al., 1997 and Mylonas et al., 2010).***

The effects of hormonal administration in females under artificial photoperiod and water temperature programs in G_1 and under artificial photoperiod, water temperature programs and HCG injection in G_2 were clearly affect on gonadal weight, GSI, fecundity and egg diameter.

Results confirmed that gonadal development, maturation stages and the changes in reproductive cycle in gilthead sea bream broodstocks may be controlled by artificial photoperiod and water temperature programs which triggered HPG axis to start and complete the reproductive cycle only or with HCG injection ***(Li, et al., 2015 and Franz and Manfred, 2012).*** Increasing in gonadal weight and GSI observed in this study may be attributed to increasing the level of measured FSH and LH. These data demonstrated that artificial photoperiod and water temperature program only were enhance the gonadal maturation, however with HCG injection have the greater effect on maturation. These results largely confirmed by the findings of ***(Badran, 2015)*** who reported that under artificial photoperiod and water temperature program for 4 months gilthead sea bream reached to final maturation and released eggs but in different quality and quantity. The best result was recorded in fish group which exposed to 18° C then to 19° C.

HCG has powerful effect of LH because it contains high amounts of

LH, and purified HCG that has very strong LH activity for inducing ovulation and spawning ***(Zohar and Mylonas, 2001 and Minar, 2012).*** The effectiveness of HCG after a single treatment was probably due to this GTH's relatively long retention time in circulation ***(Ohta and Tanaka, 1997 and Zohar and Mylonas, 2001)***

Conclusion:

From the present results it could be concluded that HCG injection in single dose (1000 IU/Kg BW) aiding with water temperature and artificial photoperiod programs induced increasing of progesterone levels, eggs diameter, fecundity and maturation stage of gilthead sea bream in captivity.

References

Albertine-Berhaut, J. (1973): Biologie des stades juveniles detéleostéens Mugilidae Mugil auratus Risso 1810, Mugil capito Cuvier 1829 et Mugil saliens Risso 1810: I. Régime alimentaire. Aquaculture 2, 251-266.

Amein, A. (1996): A study of the biology and population dynamics of Litherinus bungus (Forsskal, 1775) in the Gulf of Suez, Egypt, M. Sc. Thesis, Marine Science Department, Faculty of Science, Suez Canal university, Egypt.

Badran M. F. (2015): Studies on Reproduction of Marine Fish. Master thesis, Fac. Agri. Sci. Suez Canal University, 27-48 pp.

Chaoui L., Kara M.H., Faure E. and Quignard J.P. (2006): Growth and Reproduction of the Gilthead Sea bream *Spaurs aurata* in Mellah lagoon (North-eastern Algeria). scien. Mar. 70: 545– 552.

FAO (1999): Manual on Hatchery Production of Sea bass and Sea bream, Roma, Vol 1. pp: 15-20.

Franz, L. and Manfred, K. (2012): The effect of water temperature on gamete maturation and gamete quality in the European grayling (*Thymalus thymallus*) based on experimental data and on data from wild populations. *Fish Physiology and Biochemistry* **38,** 455–467.

Hastings W.H. and Dickie L.M. (1972): Feed formulation and evaluation. J.E. Halver. Fish Nutrition Academic Press, Inc. p 327:374.

Hunter, J. R.; Macewicz, B. J.; Lo, N. C.; Kimbrell, C. A. (1992):
Fecundity, spawning and maturity o f female Dover sole,
Microstomous pacificus, with an evaluation of assumptions andprecision. Fish. Bull. 90, 101–128

Li, G. L., Zhang, M.Z., Deng, S.P., Chen, H.P., and Zhu, C.H. (2015): Effects of temperature and fish oil supplementation on ovarian development and foxl2 mRNA expression in spotted scat Scatophagus argus.
Journal of Fish Biology (2015) 86, 248–260.

Minar, M. H., M. Shamsuddin, M.G.U. Bablu and S.I. Bhuyan (2012): Induced spawning practices of different fishes in the hatcheries of Barisal district, Bangladesh. Trends. Fish, Res., 1: 14- 17.

Mylonas, C. and Zohar, Y. (2001): Use GnRH-delivery systems for the control of reproduction in fish, Reviews in Fish Biology and Fisheries, 10: 463-49.

Mylonas, C. C., Fostier, A. and Zanuy, S. (2010): Broodstock management and hormonal manipulations of fish reproduction. General and Comparative Endocrinology 165 (2010) 516–534.

Ohta, H. and Tanaka, H. (1997): Relationship between serum levels of human chorionic gonadotropin (hCG) And 11- ketotestosterone after a single injection of hCG and induced maturity in the male Japanese eel, *Anguilla japonica*. Aquaculture 153, 123–134.

Oudjane, F., Bourenane, N. and Wafa, T. (2017): Study Biometrique and Length–Weight Relationships in Sea-Bream *Sparus aurata* (Sparidae) of the Two Gulfs, Skikda and Annaba (Northern is Algeria). Journal of Chemical and Pharmaceutical Research, 2017, 9(4):378-381

Popovic N. T., Strunjak-Perovic I., Coz-Rakovac R., Barisic J., Jadan M., Berakovic A. P. and Klobucar R. S. (2012): Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. J. Appl. Ichthyol. 28 (2012), 553–564.

Rainis, S., Mylonas CC., Kyriakou Y, and Divanach P. (2003): Enhancement of spermiation in European sea bass *(Dicentrarchus labrax)* at the end of the reproductive season using GnRHa implants. Aquaculture, 219: 873- 890.

Sahoo, S.K., S.S. Giri, S. Chandra and A.K. Sahu. (2007): Spawning performance and egg quality of Asian catfish Clarias batrachus (linn.) at various doses of human chorionic gonadotropin (HCG) injection and latency periods during spawning induction. Aquaculture, 266: 289-292.

Vazzoler, A.d.M., Vazzoler, G. (1965): Relation between condition factor and sexual development in Sardinella aurita (Cuv. & Val. 1847). Anais da Academia Brasileira de Ciências 37, 353-359.

Wahbi, O. M., El-Greisy, Z. A. and El-Sayed, H. S. (2017): Reproductive Performance of Sea Bream (*Sparus aurata*) Induced with Two Different Hormone Protocols with Respect to the Effect on Gonadotrophic Cells. J. Biol. Sci., 17 (6): 278 – 287, 2017.

Yaron, Z., (1995) : Endocrine control of gametogenesis and spawning induction in the carp. Aquaculture 129: 49–73.

Yousefian, M., and Mousavi, S., E. (2011): The mechanism of reproduction and hormonal function

in finfish species: A Review. Scientific Research and Essays Vol. 6(17), pp. 3561-3570, ISSN 1992-2248 ©2011 Academic Journals.

Yu, K.L., Lin, X.W., da Cunha Bastos, J. and Peter, R.E. (1997): Neural regulation of GnRH in teleost fishes. In: Parhar, I.S., Sakuma, Y. (Eds.), GnRH Neurons: Gene to Behavior. Brain Shuppan, Tokyo, pp. 277–312.

Zohar, Y. and Mylonas, C.C. (2001): Endocrine manipulations of spawning in cultured fish: from hormnes to genes, Aquaculture 197, 99–136.

القياسات الحيوية ومستويات الهرمونات فى اناث اسماك الدنيس نتيجة أستخدام هرمون المشيمة الأدمى

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تعتبر درجة حرارة الماء والاضاءة فقط أو مع حقن هرمون المشيمة الأدمى من أهم العوامل التي تحفز النضج الجنسي في الأسماك. تُظهر هذه الدراسة إمكانية حث اسماك الدنيس على التفريخ فى الاسر عن طريق تعريضها الى برنامج لدرجة حرارة الماء والاضاءة الاصطناعية فقط أو بالاضافة الى استخدام هرمون المشيمة الادمى. تم جمع ٦٠ من أمهات اسماك الدنيس المستخدمة في هذه الدراسة من بحيرة المنزلة بمحافظة دمياط وشركة قناة السويس لتربية الأسماك والأحياء المائية في محافظة الإسماعيلية ، مصر. تم وضع الأسماك في احواض دائرية داخلية مملوءة بالماء المالح تحت برنامج للاضاءة ودرجة الحرارة مقسمة إلى مجموعتين. المجموعة الأولى تعرضت لبرنامج لدرجات حرارة الماء وبرنامج الاضاءة الاصطناعية بينما تعرضت المجموعة الثانية إلى برنامج لدرجة حرارة الماء وبرنامج الاضاءة الاصطناعية بالاضافة الى الحقن بجرعة واحدة من هرمون المشيمة الأدمى. أجريت التجربة لمدة ٤ أشهر وبعد نهاية الشهرين الثالث والرابع وبعد ٢٤ و ٤٨ ساعة من الحقن بالهرمون، تم قياس معامل الحالة ووزن الغدد التناسلية ، الدليل التناسلى، الخصوبة وقطر البيض وجمعت عينات الدم. تم تسجيل زيادة فى وزن الغدد التناسلية ، الخصوبة ، قطر البويضات ومستويات هرمونات LH والبروجسترون في المجموعة المعاملة بالهرمون مقارنة بالكنترول بينما أظهرت نتائج الدليل التناسلى ومستوي هرمون FSH أنخفاضا معنويا بالمقارنة مع الكنترول. وقد خلصت الدراسة الى ان استخدام هرمون المشيمة الأدمى بالأضافة الى برنامج للأضاءة الاصطناعية ودرجة حرارة الماء قد أعطى افضل النتائج لحث زيادة النضج الجنسى فى اسماك الدنيس فى الاسر.

الكلمات الدالة: الدنيس ، هرمون المشيمة الأدمى ، درجة حرارة المياة ، الأضاءة ، التفريخ