Effects of acute versus sustained administration of GnRHa on GtH release and ovulation in the rainbow trout, *Oncorhynchus mykiss*

Bernard Breton^a, Claudine Weil^a, Elisabeth Sambroni^a and Yonathan Zohar^b

^aLaboratoire de Physiologie des Poissons, I.N.R.A. Campus de Beaulieu, F35042 Rennes, Cedex, France

^bNational Center for Mariculture, I.O.L.R., Eilat, Israel

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ABSTRACT

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The effects of different forms of GnRHa (sustained vs. acute) on the stimulation of gonadotropin secretion and the rate of ovulation were investigated in the rainbow trout, *Oncorhynchus mykiss*. A biodegradable sustained release form of DTrp⁶ LH-RH induced a progressive rise in the gonadotropin plasma levels in a dose-dependent manner. The stimulation of gonadotropin secretion was maintained for 3 weeks. All fish ovulated within 5 days after injection using a high dosage (50 µg/kg body weight) whereas with the lower dosages (25 and 12.5 µg/kg body weight) 100% ovulation was reached after 8 days. In contrast, acute injections of GnRHa (20 µg/kg body weight) induced a transient stimulation of gonadotropin secretion. Ovulation rates were first accelerated within 8–10 days and then remained stable but they did not reach 100% 26 days after the injection. At that time the rate of ovulation in the control group had increased to 70%. These results are discussed, along with a comparison of variability of egg quality with treatments.

INTRODUCTION

The synchronization and advancement of the final stages of oocyte maturation have become increasingly important in salmonid fish farming, mainly due to the economics of broodstock management. Indeed, the spawning period in salmonids often extends over 1.5 months, as in the rainbow trout, or longer in the Atlantic salmon, and in some cases induction and control of ovulation become necessary. This is especially true at summer-spawning strains of rainbow trout which ovulate when the water temperature generally exceeds 14–15°C, leading to a rapid loss of egg fertility. In these fish it might be important to fertilize the eggs as soon as ovulation occurs after synchronization of the fish, in order to avoid egg ageing at such a high temperature. This

is also the case in fish kept in sea water where ovulation is often blocked. In salmonids, artificial synchronization of spawning was first obtained by Jala-bert et al. (1978) using a combined treatment with gonadotropin (GtH) and 17α -hydroxy- 20β -dihydroprogesterone. More recently, this has been achieved using gonadotropin-releasing hormone analogues (GnRHa) as in the coho salmon *Oncorhynchus kisutch* (Donaldson et al., 1981; Van der Kraak et al., 1983; Fitzpatrick et al., 1984), or the rainbow trout (Crim et al., 1983a) but generally repeated injections of the peptide were required and this is not always compatible with fish farming practice. Advancement and synchronization of spawning were also obtained in rainbow trout after a combined injection of LH-RHa and pimozone, but this results in poor egg quality and a low survival at the eyed stage, which may have been due to a toxic effect of pimozone (Billard et al., 1984).

A biological characteristic of salmonid species is the length of their gametogenesis under gonadotropin control (Breton et al., 1983). Thus it could be hypothesized that advancement and synchronization of ovulation would require a more prolonged and controlled stimulation of GtH secretion compared to that required in other species (for review see Peter et al., 1986; Zohar, 1988). This has been achieved using cholesterol pellet LH-RH analogue implants in the rainbow trout (Crim et al., 1983a), the Atlantic salmon (Crim et al., 1983b; Crim and Glebe, 1984), and the milkfish (Marte et al., 1988; Tamura et al., 1988). Besides the high amount of GnRH needed in such implants, the use of cholesterol could be questionable as far as it is a steroid precursor and is not degradable. This is of great importance in such species with a relatively low fecundity, broodfish being generally reconditioned after spawning either to be marketed or reused as spawners the following year. Sustained administration of GnRHa via biodegradable delivery systems was shown to stimulate continuous elevated GtH levels and to efficiently induce ovulation and spawning in the gilthead seabream, *Sparus aurata* (Zohar, 1988; Zohar et al., 1989).

Thus, in the present work we have studied the efficiency of sustained administration of GnRHa via a biodegradable system in stimulating GtH release and inducing ovulation in the rainbow trout as compared to acute injections of GnRHa. In most studies with salmonids, GnRHa has been injected using high dosages, thus greatly affecting the cost of treatment which must be as low as possible in such low fecundity species. In this study, therefore, the effect of delivering several dosages of GnRH has also been studied.

MATERIALS AND METHODS

Virgin two-year-old rainbow trout (average weight 2.3 kg), originating from a commercial fish farm, were kept in 4-m² circular tanks supplied with running water ($14 \pm 1^\circ\text{C}$). Experiments were carried out at the beginning of the

breeding season, when less than 10% of the population had already ovulated naturally.

The females (160) were placed into one of eight groups ($n=20$ /group) in which all the animals were treated but only seven were submitted to repeated blood samplings. After anaesthesia in 2-phenoxyethanol (0.3 ml/l), blood samples were taken from a caudal vessel with an heparinized syringe before treatments and 1, 3, 6, 9, 24 h and 2, 5, 8, 12, 20 and 26 days thereafter. Treatments and samplings started at 9 a.m. Blood samples were centrifuged for 15 min at 2000g and the plasma was kept frozen until determination of their GtH $_2$ concentration by RIA (Breton et al., 1978). GtH $_2$ is the maturational gonadotropin according to the nomenclature proposed by Kawauchi et al. (1989).

Ovulations were checked twice a week by stripping the females. In some newly ovulated females from each group, except Group 8, batches of 300–400 eggs were sampled and fertilized with a mixture of sperm collected from 10 males. Fertilizations were done in a sperm diluent (D.I.A. 532) at a concentration of 10^{-3} sperm. After hardening, the eggs were transferred to incubation trays, and incubated at 10°C until the eyed stage, at which the fertilization rates were determined.

The following treatments were accomplished by intramuscular injection, the injected volume being 200 μ l/kg body weight.

Group 1: saline control.

Group 2: sustained release DTrp 6 LH-RH (triptoreline DCI) 50 μ g/kg b.w.

Group 3: sustained release DTrp 6 LH-RH (triptoreline DCI) 25 μ g/kg b.w.

Group 4: sustained release DTrp 6 LH-RH (triptoreline DCI) 12.5 μ g/kg b.w.

Group 5: acute injection DTrp 6 LH-RH 20 μ g/kg b.w.

Group 6: acute injection [DAla 6 Pro 9 Net] LH-RH 20 μ g/kg b.w.

Group 7: acute injection [DArg 6 Pro 9 Net] sGnRH 20 μ g/kg b.w.

Group 8: acute injection [DTrp 6 Pro 9 Net] sGnRH 20 μ g/kg b.w.

DTrp 6 LH-RH used for sustained release was a commercial microencapsulated form, in a polyglycolic–polylactic biodegradable matrix, obtained from Ipsen/Beaufour laboratory. It was used as a suspension in a physiological saline solution containing 0.1% Tween 20. DTrp 6 LH-RH was also obtained from the Ipsen/Beaufour laboratory. [DAla 6 Pro 9 Net] LH-RH was purchased from Sigma (St. Louis, MO). Salmon GnRH analogues were synthesized according to the solid phase method (Merrifield, 1963). Their purity was further controlled by reverse phase HPLC. Peptidic contents were estimated as being approximately 80%.

Results were analysed using the Mann–Whitney *U*-test to compare the GtH plasma level between the different groups, and Student's *t*-test to compare the rates of fertilization.

RESULTS

GtH₂ blood plasma levels

In the control group, mean GtH₂ levels remained stable throughout the experimental period (6.2 ± 3.2 ng/ml at the maximum on day 26; Fig. 1A). In fish submitted to the sustained release form of LH-RH, no rapid increase in blood plasma GtH₂ concentrations was observed (Fig. 1B, C, D). Twenty-four hours after injection in all the groups which received this treatment blood plasma had increased slowly. The increments became significant as early as 3 h in Group 2 receiving $50 \mu\text{g/kg}$ b.w. (10.7 ± 2.59 vs. 5.24 ± 1.44 ng/ml, Fig. 1B), in Group 3 receiving $25 \mu\text{g/kg}$ b.w. (8.61 ± 2.33 vs. 3.48 ± 2.05 ng/ml, Fig. 1C) and in Group 4 receiving the smallest dose, $12.5 \mu\text{g/kg}$ b.w. (13.06 ± 4.54 vs. 5.33 ± 2.83 ng/ml, Fig. 1D). The increase was more pronounced from day 2 to day 8. Maximum GtH₂ blood plasma levels were significantly higher than levels measured during the first 24 h after injection; 69.44 ± 15.2 ng/ml in Group 2; 53.19 ± 10.68 ng/ml in Group 3, and 32.47 ± 10.31 ng/ml in Group 4 (Fig. 1B, C, D). This maximum was reached earlier in Group 2 which received the highest dose, whereas in Groups 3 and

Fig. 1. The effects of a sustained release form of DTrp⁶ LH-RH on blood plasma GtH levels in rainbow trout. Values are mean $\pm$ s.e.m. ($n=7$). A, Vehicle (control); B, $50 \mu\text{g/kg}$ body weight; C, $25 \mu\text{g/kg}$ body weight; D, $12.5 \mu\text{g/kg}$ body weight. DTrp⁶ LH-RH vs. vehicle, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

4 GtH₂ levels peaked at days 5 and 8, respectively. There was also a dose-response relationship between the injected quantity of sustained release form of LH-RH and the maximum GtH levels reached; levels were significantly higher in fish treated with 50 µg/kg ($P < 0.05$). Thereafter the GtH₂ levels decreased, but remained significantly higher 20 days post-delivery than at the beginning of the experiment ($P < 0.05$).

In fish receiving an acute administration of GnRHa, the GtH₂ blood plasma levels increased more rapidly (Fig. 2), being significantly elevated from 1 h after injection in Groups 5, 6 and 7 which received DTrp⁶ LH-RH, [DAla⁶Pro⁹Net] LH-RH, and [DArg⁶Pro⁹Net] sGnRH, respectively (Fig. 2A, B, C). [DArg⁶Pro⁹Net] sGnRH was the most potent to induce in vivo GtH₂ release, peaking at 60 ng/ml after 3 h (Fig. 2C). The mammalian GnRH analogues were less potent (Fig. 2A, B); [DTrp⁶Pro⁹Net] sGnRH was the least potent, inducing a weak increase in plasma GtH₂ levels, peaking 9 h after treatment ($P < 0.05$) (Fig. 2D).

In taking the day of ovulation as origin, it can be seen that there was no variation in the plasma GtH₂ levels in the control group (Fig. 3A). In groups receiving the sustained release form of GnRH, ovulations took place when

Fig. 2. The effects of acute injection of GnRHa on blood plasma GtH levels in rainbow trout (20 µg/kg body weight). Values are mean $\pm$ s.e.m. ($n=7$). A, DTrp⁶ LH-RH; B, [DAla⁶Pro⁹Net] LH-RH; C, [DArg⁶Pro⁹Net] sGnRH; D, [DTrp⁶Pro⁹Net] sGnRH. GnRHa vs. vehicle, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

Fig. 3. Evolution of the blood plasma gonadotropin levels taking the day of ovulation as origin in fish treated with a sustained release form of DTrp⁶ LH-RH. Values are mean $\pm$ s.e.m. ($n=4-7$). A, Vehicle (control); B, 50 $\mu\text{g/kg}$ body weight; C, 25 $\mu\text{g/kg}$ body weight; D, 12.5 $\mu\text{g/kg}$ body weight.

Fig. 4. Evolution of the blood plasma gonadotropin levels taking the day of ovulation as origin in fish treated with an acute injection of GnRH. A, DTrp⁶ LH-RH; B, [DAla⁶Pro⁹Net] LH-RH; C, [DArg⁶Pro⁹Net] sGnRH. Values are mean $\pm$ s.e.m. ($n=4-7$).

GtH levels were maximal (Fig. 3B, C, D). In groups treated with an acute injection of GnRH α , GtH levels increased from 8 to 5 days prior to ovulation, but always declined to basal levels following ovulation (Fig. 4A, B, C).

Ovulation rates

In the control group the cumulative percent of ovulation increased regularly from day 0 to day 26, reaching 69% at the end of the experiment (Fig. 5A).

In fish treated with the sustained release form of DTrp⁶ LH-RH, in all groups the ovulation rates were significantly increased as early as day 5. They reached 100% on day 8 in the group receiving the highest dose (50 μ g/kg) and on day 12 in the other two groups (Fig. 5A).

There was no difference between the rates of ovulation in the control group and Group 8 receiving an acute 20- μ g injection of [DTrp⁶Pro⁹Net] sGnRH, although this compound slightly stimulated the GtH secretion (Fig. 3B). In the other groups (5, 6 and 7), the acute injection of GnRH α induced a rapid increase in ovulation rates which plateaued on day 8 (Fig. 5B). At the end of the experiment (day 26) not all fish had ovulated.

Fig. 5. Cumulated percentage of ovulation in rainbow trout treated by different forms of GnRH α . A, Sustained release form of DTrp⁶ LH-RH: ○—○ control; □—□ DTrp⁶ LH-RH 50 μ g/kg body weight; △—△ DTrp⁶ LH-RH 25 μ g/kg body weight; ●—● DTrp⁶ LH-RH 12.5 μ g/kg body weight. B, Acute injection of GnRH α : ○—○ control; □—□ DTrp⁶ LH-RH 20 μ g/kg body weight; △—△ [DAla⁶Pro⁹Net] LH-RH 20 μ g/kg body weight; ●—● [DArg⁶Pro⁹Net] sGnRH 20 μ g/kg body weight; ■—■ [DTrp⁶Pro⁹Net] sGnRH 20 μ g/kg body weight.

TABLE 1

Mean values of fertilization rates according to the treatments (see Material and Methods)

Group:	1	2	3	4	6	7	8
$\bar{X}$	53	35	24	27	40	35	24
s.e.m.	31	32	20	21	34	19	17
<i>n</i>	5	7	8	9	6	7	7

The fertilization rates are given in Table 1. No significant differences were observed with respect to fertilization rate between groups.

DISCUSSION

From the results it appears that both modes of GnRHa administration, either sustained or acute, were able to stimulate gonadotropin secretion and ovulation in rainbow trout. Since the sustained release form of DTrp⁶ LH-RH stimulated GtH secretion for a long time (around 3 weeks), it can be hypothesized that the peptide was continuously released from the biodegradable matrix over this period, and that contrary to mammals this continuous infusion of GnRH did not induce any desensitization of the pituitary gland. It is also clear that a low dose of sustained release form of GnRH, i.e. 12.5 µg/kg, was sufficient to induce a significant increase in plasma GtH levels, similar in amplitude but more prolonged than that obtained with the same peptide administered in an acute injection of 20 µg/kg. Further work is necessary to determine whether it is possible to decrease the amount of sustained release form able to induce a prolonged stimulation of the gonadotropin secretion and ovulation.

Among the analogues used in acute treatments the most active was [DArg⁶Pro⁹Net] sGnRH which also induced a prolonged stimulation of GtH secretion, but which returned to basal levels after 5 days. The superactivity of this analogue to stimulate GtH secretion has already been described in the case of the goldfish (Peter et al., 1985; Sokolowska et al., 1985), and although its activity in salmonids has been mentioned by Crim et al. (1988), this is the first demonstration that it is more active than others in salmonids.

The shape of the gonadotropin signal preceding ovulation differed significantly according to type of treatment. Acute treatment induced a transient increase in gonadotropin secretion around 8 to 4 days before ovulation. The sustained release form of GnRHa also induced an increase in gonadotropin levels 4 days before ovulation but the high GtH levels were maintained during and after ovulation. This may explain, in part, the difference in action of the two kinds of treatments to induce ovulation.

Indeed, according to the form of GnRH used, the ovulation rates were quite

different. With the sustained release form, 100% of fish ovulated from 5 days (50 $\mu\text{g/kg}$) to 12 days (12.5 $\mu\text{g/kg}$) after injection. After an acute treatment there was first a stimulation of the ovulation rates, but thereafter they never reached 100%, even 26 days after the beginning of the experiment. Thus the sustained release form was more potent in inducing ovulation than the acute treatment. As already mentioned, this was possibly linked to the gonadotropin signals induced by these two forms. The transient stimulation of GnRH α could induce ovulation only in fish which were close to ovulation whereas after sustained treatment there was a continuous gonadotropic stimulation starting around 6–8 days prior to ovulation which might have accelerated all the final stages of maturation. Sustained administration of GnRH α via biodegradable implants was shown to induce elevated GTH levels for at least 10 days in the female gilthead seabream, *Sparus aurata*, and to substantially increase the proportion of spawning females as compared to a single injection of the hormone (Zohar, 1988).

Although the results obtained with our sustained release form of GnRH and those obtained with cholesterol pelleted [DTrp⁶Pro⁹Net] LH-RH are comparable (Crim and Glebe, 1984), it appears that our preparation using a polylactic polyglycolic and biodegradable matrix was more efficient in inducing 100% ovulation within 8 days instead of 16 and using 10-fold less hormone. It might be that this kind of biodegradable matrix allows better protection and release of the peptide.

As in earlier work in the same species (Crim et al., 1983a) the fertilization rates were rather low in the treated groups, but not significantly different from the control. Although the effect of the treatments cannot be rejected, the use of virgin fish as well as the temperature (14–15°C) could have reduced egg quality or induced a rapid decrease of egg fertility ability. In other salmonids, such as the coho salmon, *Oncorhynchus kisutch*, LH-RH α induced spawning without modifying egg quality (Fitzpatrick et al., 1984).

From the present experiments and previous work with salmonids, it appears that salmonids do not need a combined treatment using GnRH α and a dopamine receptor antagonist such as pimozide or domperidone. Thus in these species the dopaminergic inhibitory tone on GnRH action at the pituitary level is not as important as in other species and especially in cyprinids (Peter et al., 1986). This does not justify the recent dichotomy (Crim and Peter, 1988) made between freshwater species, in which the combined treatment would be the best method to induce spawning, and marine species, in which implantation of GnRH α in long-term release form would be the best way. In our opinion each species represents a special case for which every parameter, including the nature, form and dosage of peptide, the stage of maturity of recipient animals, the temperature of treatment, etc., might be defined in order to obtain high ovulation and fecundity rates.

In conclusion, the use of a biodegradable sustained release form of GnRH α

seems to be a good alternative to induce spawning in salmonids and certainly in other species. It avoids repeated injections and, being biodegradable, the problem of residues for marketing the fish. The price for such a treatment was around \$0.8/kg. It was made using a commercial preparation especially developed for human therapy, which requires a very high-grade purity not always necessary for veterinary use. More work is still necessary to determine the conditions of use, especially in order to decrease the cost of such a treatment by lowering the dose and maybe developing a special formulation for fish use including the nature of the analogue and of the matrix.

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REFERENCES

- Billard, R., Reinaud, P., Hollebecq, M.G. and Breton, B., 1984. Advancement and synchronization of spawning in *Salmo gairdneri* and *S. trutta* following administration of LH-RHA combined or not with pimozide. *Aquaculture*, 43: 57-66.
- Breton, B., Prunet, P. and Reinaud, P., 1978. Sexual differences in salmon gonadotropin. *Ann. Biol. Anim. Biochim., Biophys.*, 18: 754-765.
- Breton, B., Fostier, A., Zohar, Y., Le Bail, P.Y. and Billard, R., 1983. Gonadotropine glycoproteique et oestradiol-17 β pendant le cycle reproducteur chez la truite fario (*Salmo trutta*) femelle. *Gen. Comp. Endocrinol.*, 49: 220-231.
- Crim, L.W. and Glebe, B.D., 1984. Advancement and synchrony of ovulation in Atlantic salmon with pelleted LH-RH analog. *Aquaculture*, 43: 47-56.
- Crim, L.W. and Peter, R.E., 1988. Induced spawning of Asian fishes. *Proceedings of a fish breeding workshop: concluding remarks. Aquaculture*, 74: 165-166.
- Crim, L.W., Sutterlin, A.N., Evans, D.M. and Weil, C., 1983a. Accelerated ovulation by pelleted LH-RH analogue treatment of spring spawning rainbow trout (*Salmo gairdneri*) held at low temperature. *Aquaculture*, 35: 299-307.
- Crim, L.W., Evans, D.M. and Vickery, B.H., 1983b. Manipulation of the seasonal reproductive cycle of the landlocked Atlantic salmon (*Salmo salar*) by LH-RH analogues administered at various stages of gonadal development. *Can. J. Fish. Aquat. Sci.*, 40: 61-67.
- Crim, L.W., Nestor, J.J. and Wilson, C.E., 1988. Studies of the biological activity of LH-RH analogs in the rainbow trout, landlocked salmon and the winter flounder. *Gen. Comp. Endocrinol.*, 71: 372-388.
- Donaldson, E.M., Hunter, G.A. and Dye, H.M., 1981. Induced ovulation in coho salmon (*Oncorhynchus kisutch*). I. Preliminary study of the use of LH-RH and two high potency LH-RH analogues. *Aquaculture*, 26: 129-141.
- Fitzpatrick, M.S., Suzumoto, B.K., Schreck, C.B. and Oberbillig, D., 1984. Luteinizing hor-

- mone-releasing hormone analogue induces precocious ovulation in adult coho salmon (*Oncorhynchus kisutch*). *Aquaculture*, 43: 67–73.
- Jalabert, B., Breton, B. and Fostier, A., 1978. Precocious induction of oocyte maturation and ovulation in coho salmon (*Oncorhynchus kistuch*). *J. Fish. Res. Board Can.*, 85: 1423–1429.
- Kawauchi, H., Suzuki, K., Itoh, H., Swanson, P., Naito, N., Nagahama, Y., Nozaki, M., Nakai, Y. and Itoh, S., 1989. The duality of teleost gonadotropin. *Fish Physiol. Biochem.*, 7: 29–38.
- Marte, C.L., Crim, L.W. and Sherwood, N.M., 1988. Induced gonadal maturation and rematuration in milkfish: limited success with chronic administration of testosterone and gonadotropin-releasing hormone analogues (GnRH-A). *Aquaculture*, 74: 131–145.
- Merrifield, R.B., 1963. Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. *J. Am. Chem. Soc.*, 85: 2149–2154.
- Peter, R.E., Nahorniak, C.S., Sokolowska, M., Chang, J.P., Rivier, J.E., Vale, W.W., King, J.A. and Millar, R.R., 1985. Structure–activity relationships of mammalian, chicken and salmon gonadotropin releasing hormones in vivo in goldfish. *Gen. Comp. Endocrinol.*, 58: 231–242.
- Peter, R.E., Chang, J.P., Nahorniak, C.S., Omeljanuik, R.J., Sokolowska, M., Shih, S.H. and Billard, P., 1986. Interactions of catecholamines and GnRH in regulation of gonadotropin secretion in teleost fish. *Rec. Prog. Hormone Res.*, 42: 513–548.
- Sokolowska, M., Peter, R.E. and Nahorniak, C.S., 1985. The effects of different doses of pimozide and [DAla⁶, Pro⁹ N-ethylamide] LH-RH (LH-RH A) on gonadotropin release and ovulation in female goldfish. *Can. J. Zool.*, 63: 1252–1256.
- Tamura, C.S., Lee, C.S., Kelley, C.D. and Banno, J.D., 1988. Effectiveness of chronic LH-RH analogue and 17 α -methyltestosterone therapy, administered at different times prior to the spawning season, on the maturation of milkfish (*Chanos chanos*). *Aquaculture*, 70: 159–167.
- Van der Kraak, G., Lin, H.R., Donaldson, E.M., Dye, H.M. and Hunter, G.A., 1983. Effects of LH-RH and des Gly¹⁰ [DAla⁶] LH-RH ethylamide on plasma gonadotropin levels and oocyte maturation in adult female coho salmon (*Oncorhynchus kisutch*). *Gen. Comp. Endocrinol.*, 49: 470–476.
- Zohar, Y., 1988. Gonadotropin releasing hormone in spawning induction in teleosts: basic and applied considerations. In: Y. Zohar and B. Breton (Editors), *Les Colloques de l'INRA: Reproduction in Fish. Basic and Applied Aspects in Endocrinology and Genetics*, INRA, Paris, pp. 47–62.
- Zohar, Y., Goren, A., Tosky, M., Pagelson, D., Leibovitz, D. and Koch, Y., 1989. The bioactivity of gonadotropin releasing hormone and its regulation in the gilthead seabream *Sparus aurata*: in vivo and in vitro studies. *Fish. Physiol. Biochem.*, 7: 59–64.