REVIEW ARTICLE

The control of reproduction in finfish species through GnRH treatments

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Paper received April 26, 2005; accepted September 3, 2005

ABSTRACT

Fish in captivity can show some dysfunctions, at different levels, in the physiological processes of reproduction, due to the lack of synthesis or release of gonadotropins (GtHs) by hypophysis. As a consequence, a worsening of quality and quantity of spawned gametes, or a lack of egg and sperm spawning, can be observed. The farmers can act on fish reproductive cycle manipulating the environmental parameters of rearing, the diet, the genetics or using GnRH treatments. Nowadays, they are used mainly GnRH, synthesized in laboratory as analogues. These releasing factors, naturally produced by hypothalamus, let to overcome the technological and biological limits of the "traditional" hormonal treatments with hCG, being more effective, cheaper and easily available on market. This article makes a historical survey of the conditioning treatments for fish reproduction and also considers the future perspectives of these treatments, examining the topics that research will have to focus, in order to make these treatments common worldwide, in any hatchery and for each farmed species of finfish.

Key Words: Fish species, Reproductive cycles, GnRHa, GnRHA releasing systems.

RIASSUNTO
IL CONTROLLO DELLA RIPRODUZIONE NELLE SPECIE ITTICHE MEDIANTE I TRATTAMENTI CON GNRH

I pesci in cattività possono manifestare, a vari livelli, delle disfunzioni nei processi fisiologici della riproduzione, a causa della mancata sintesi o liberazione delle gonadotropine (GtHs) da parte dell'ipofisi. Di conseguenza si può osservare un peggioramento quali-quantitativo dei gameti emessi, o nei casi più severi una mancata emissione di uova e sperma. Gli allevatori possono intervenire sul ciclo riproduttivo dei pesci manipolando i parametri ambientali, la dieta, il corredo genetico oppure ricorrendo ai trattamenti con gonadotropine (GnRH). Attualmente si usano prevalentemente GnRH, sintetizzati in laboratorio, sotto forma di analoghi. Questi fattori di rilascio, naturalmente prodotti dall'ipotalamo, permettono di superare i limiti tecnologici e biologici dei trattamenti precedenti, dimostrandosi più efficaci, più economici e di più facile reperimento sul mercato. Il presente articolo compie un excursus storico dei trattamenti di condizionamento per la riproduzione dei pesci e considera anche le prospettive future di questi trattamenti, esaminando gli aspetti sui quali la ricerca scientifica dovrà concentrarsi, per renderli comuni ovunque, in ogni avannotteria o schiuditoio e per ogni specie ittica allevata.

Parole chiave: *Specie ittiche, Cicli riproduttivi, GnRHa, Sistemi di rilascio di GnRHa.*

Introduction

The prospects for the further development of aquaculture will be based also on the management of aquatic organisms reproduction (Pillay, 1990). The rearing of many finfish species still rely on the capture of eggs or juveniles in the wild. The management of the technologies for gamete production in captivity is an essential step for aquaculture that would ensure the growth to this sector (Bromage, 1992). Besides, farms need a continuous supply of fry during the whole year, that necessarily means a continuous yield of gametes by broodstocks. Another important step in fish reproduction is to optimise the management and farming techniques of broodstocks, also through the synchronisation of males and females. This brings lower costs and simplification of the collection and incubation of spawned gametes (Zohar and Mylonas, 2001). Unfortunately, even if many finfish species can be considered domesticated, several problems still exist in their reproduction and these need specific interference by farmer. In fact, broodstocks are particularly sensitive to farming conditions and show dysfunctions in the physiological process of reproduction, at different levels (Carillo et al, 1999). The lack of natural environmental stimuli, and the unavoidable stress by captivity, often cause the lack of the final oocyte maturation (FOM) and of ovulation in females, inhibiting also their natural spawning. In males, instead, a decrease of the seminal liquid volume and a worsening of its quality can be observed (Rainis *et al.*, 2003). The decrease of the total amount of spawned milt can become a limiting factor for commercial hatcheries, where large productions can be obtained only handling many spawners. The possibility of collecting and storage of milt without further manipulations and seasonal dependency is very useful for the spread of induced spawning in aquaculture (Mylonas *et al.*, 1997). Furthermore, in this way, they are possible inter-specific hybridisation between species, which would normally ripen in different periods of the year (Mylonas and Zohar, 2001).

Fish farmers can interfere at several stages, to overcome the problems of reproduction in finfish and to improve the quali-quantitative production of the gametes: modifying the environmental variables (as water temperature, water salinity, photoperiod and light intensity) to mimic natural conditions, changing volume and depth of the rearing tanks, fish stocking density, putting vegetable supports in the tank, or through the diet (Ballestrazzi *et al.*, 2003), the genetics or, in some cases, using specific molecules active on the endocrine systems.

The treatment with specific molecules is a suitable solution to overcome the reproductive "blocks" in finfish farmed species and let control reproduction, significantly sharing in the technological and commercial *sophistication* of aquaculture (Zohar and Mylonas, 2001).

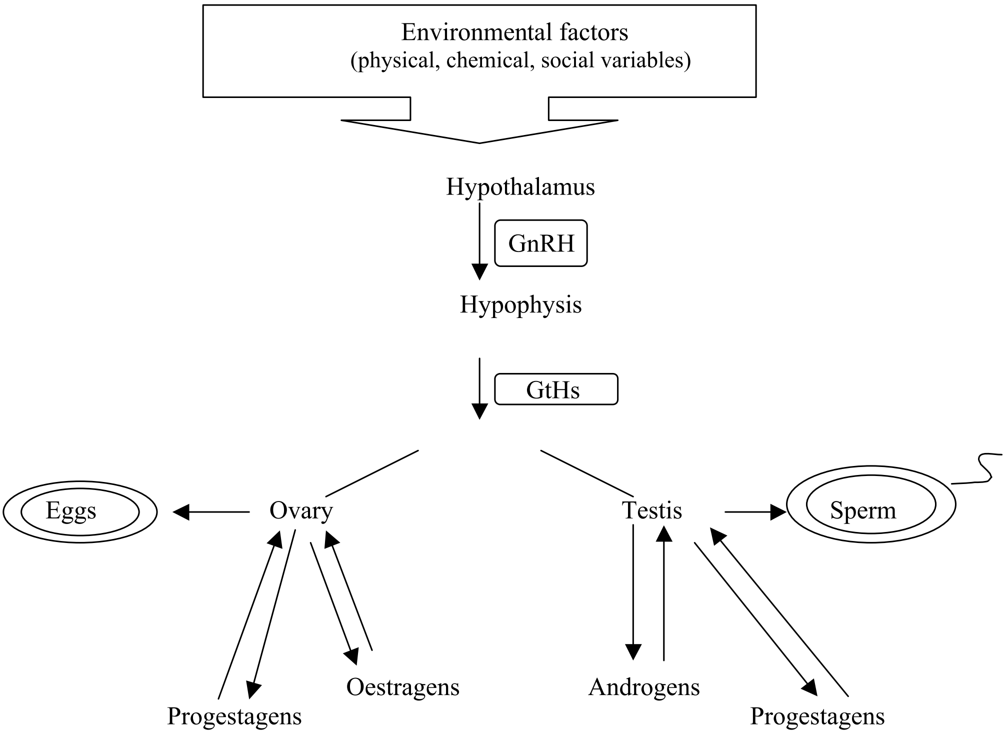
The reproductive cycles in fish

The endocrine control system of the reproduction in finfish is based on the hypothalamus-hypophysis (pituitary gland) -gonads axis, similarly to mammals (Figure 1).

Hypothalamus produces the gonadotropin-releasing factor (GnRH) which act on the pituitary gland. This gland controls synthesis and release of the gonadotropic hormones (GtHs), whose role is to lead the gonads (ovaries and testicles) to produce the gametes. The pituitary gland produces also dopamine, which, on the contrary, has an inhibiting effect on the process.

Two types of GtHs, different for structure and chemical role, were identified in the teleosts: GtH I (or FSH), involved in the initial stages of gametogenesis (vitellogenesis and spermatogenesis), and GtH II (or LH), which sets FOM, spermiogenesis and spermiation (Mylonas *et al.*, 1996). GtH II increases in plasma just before spermiation and FOM, determining the switch from the steroidogenic and androgenic production (Testosterone, T and 11-Keto-Testosterone, 11-KT, in males and Oestradiol-17ß and Oestrone, in females) to the progestinic production (like 17α-20ß-dihydroxy-4pregnen-3-one, 17α20ßP and 17-hydroxyprogesterone) respectively in the interstitial testicular cells and the ovarian theca cells. The progestagens cause the FOM in females, while regulate the spermiation in males (stimulating the production of seminal fluid, the spermatozoa maturation) and influence the sexual behaviour (Pankhurst and Thomas, 1998). Most of the milt hydration process is GtHs-dependent, because these hormones stim-

Figure 1. Endocrinologic control of reproduction in finfish



ulate spermiation and increase the total volume of spawned milt (Pankhurst, 1994). Many of the problems observed in finfish reproduction (lack of FOM, of ovulation, of spermiation and/or of spontaneous deposition of the gametes) are the result of the failed emission of GtH II by hypophysis (Rainis *et al.*, 2003).

Hormonal treatments of broodstock

Since the '30ies, several sources of hormones were tested for inducing and settling fish reproduction, supplying fresh extracts of the pituitary gland of sexually ripen fish, naturally rich in GtHs *(Hypophysation)* (Crim and Glebe, 1990). However, these treatments showed several problems: costs and difficult availability of the products, high health risks for fish, variability of the product quality, possible interference of the hormone administered with the endocrine path of the animal. Besides, they were active only on fish phylogenetically close to the donors. For this reason, GtHs were replaced with human chorionic gonadotropin (hCG), that were characterised by a wide availability on the market and a higher chemical purity, ensuring a better efficacy.

The following step in fish reproduction was the replacement of GtHs or hCG with synthetized analogues of gonadotropin-releasing hormone (GnRHa). The synthetized analogues of GnRH are more effective than the native pure forms, as they are synthetized in laboratory to resist better to the enzymatic cleavage and with a higher affinity to the receptors of the pituitary gland. Treatments with GnRHa aim to stimulate the production and release of GtH by hypophysis. The advantages by using GnRHa instead of hCG, are due to the fact that these synthetized hormones are more effective, biologically safer (since they are made in laboratory, without risk for disease transmission), relatively cheaper and easily available. Furthermore, since they are chemically formed by small peptides, they do not interfere with the immune system of the animal and do not cause allergic reactions or resistance after subsequent (repeated) treatments. The results obtained by Sorbera *et al.*, (1996) and

Rainis *et al.*, (2003) suggest that GnRHa act on spermiation in a physiological way, even if the process is super-activated. In the following figures it can be observed that these treatments can cause multiple spawning in females (Figure 2) and extend the reproductive season in males, without affecting sperm quality (Figures 3, 4, and 5). In fact, GnRH probably stimulates also the release of other pituitary hormones involved in the reproductive cycle, like Growth Hormone (GH), Thyroid Stimulating Hormone (TSH) and Somatolactine (Mylonas *et al.*, 1998). The action of GnRHa takes place at a higher level of the animal endocrine pathway, stimulating the reproductive events in a more balanced and integrated way, with the other physiological functions correlated to the reproduction cycle (Mylonas *et al.*, 1996). In some species (common carp, *Cyprinus carpio* and American catfish, *Ictalurus punctatus*) GnRHa treatment is joined with the administration of dopamine antagonists, to neutralise the inhibiting effects of dopamine on hypothalamus. However, the administration of gonadotropins is still considered a practical solution, when a quick, even short lasting, reaction of the animals is required or if unriped fish (with insufficient levels of GtH II or with the unactivated receptors of the pituitary gland) are to be treated (Mylonas and Zohar, 2001).

During the last ten years, various systems of GnRHa administration were tested in farmed finfish (Nagahama, 1994; Zohar, 1988; Crim and Bettles, 1997; Zohar and Mylonas, 2001; Rainis *et al.*, 2003). First trials were made with injections, a method still very common. However, the increase of GtH II in plasma is limited, after a single GnRHa injection, because the hormone is quickly catabolised by enzymes and removed from blood after 10-23 minutes (Gothilf and Zohar, 1991). For this reason, a single injection is not sufficient, and two or more administrations are necessary to assure the right dose (Weil and Crim, 1983; Takashima *et al.*, 1984; Harmin and Crim, 1993). Furthermore, being necessary to do the injection fairly close to the sexual maturity, it is also necessary to check quite often the animals, to find the right moment of the treatment. Several treatments and fish handlings are therefore ineffectual, from the practical point of view, and concur to increase the mortality by stress, typical of fish during reproductive maturity (Mylonas and Zohar, 2001). On males, instead, a single GnRHa injection has a positive effect on spermiation, only for few days. This so short stimulation can not be useful if females ripen and spawn eggs in synchrony or just a single time during season. However, also in the case of females ripening their gametes several times a year, a bigger and extended milt production is required, to make efficient the induced spawning.

GnRHa releasing-systems

The recent development of the reproductive technologies resulted in the GnRHa incorporation in a polymeric controlled releasing system. This system lets control the releasing rate of the hormone in blood during a period of days or weeks and simultaneously to extend the preserve of the chemical for an indefinite time (years). From a chemical point of view, the new product is compound by the GnRHa diluted in an inert matrix, that dissolves after administration, releasing the biologically active molecule. The compounds used as matrix can be: cholesterol and cellulose, lactic acid and glycolic acid, or co-polymers of dimer fatty acid and sebasic acid, or acetate of ethylene and vinyl (EVAc). The rate of release of GnRHa by matrix can be easily controlled, varying the molecular weight of the polymer, the percent of loading of the inert matrix, the type (hydrophobic or hydrophilic) and the form of the matrix (sphere or disk).

The EVAc system seems more suitable, also because it is easy to prepare and can be stored for years at –20°C, ensuring a steadily efficacy. The administering of EVAc can be based on injections of microspheres suspended in a liquid or with an intramuscolar solid device. All these controlled releasing-systems allow to overcome technical and biological limits of the injections.

In males, the GnRHa implants increase the production of seminal fluid and extend the reproductive season. No decrease of density, motility and fertility of male gametes was observed respect injections, since this last method of administration causes only a temporarily limited peak of GtH II, that is responsible of the increased production of seminal liquid, but contemporarely of a decrease in sperm density (Piper *et al.*, 1982; Forniés *et al.*,

Figure 2. Effects of treatments with GnRHa on relative fecundity and multiple spawning of European sea bass females (Forniés *et al.*, 2001).

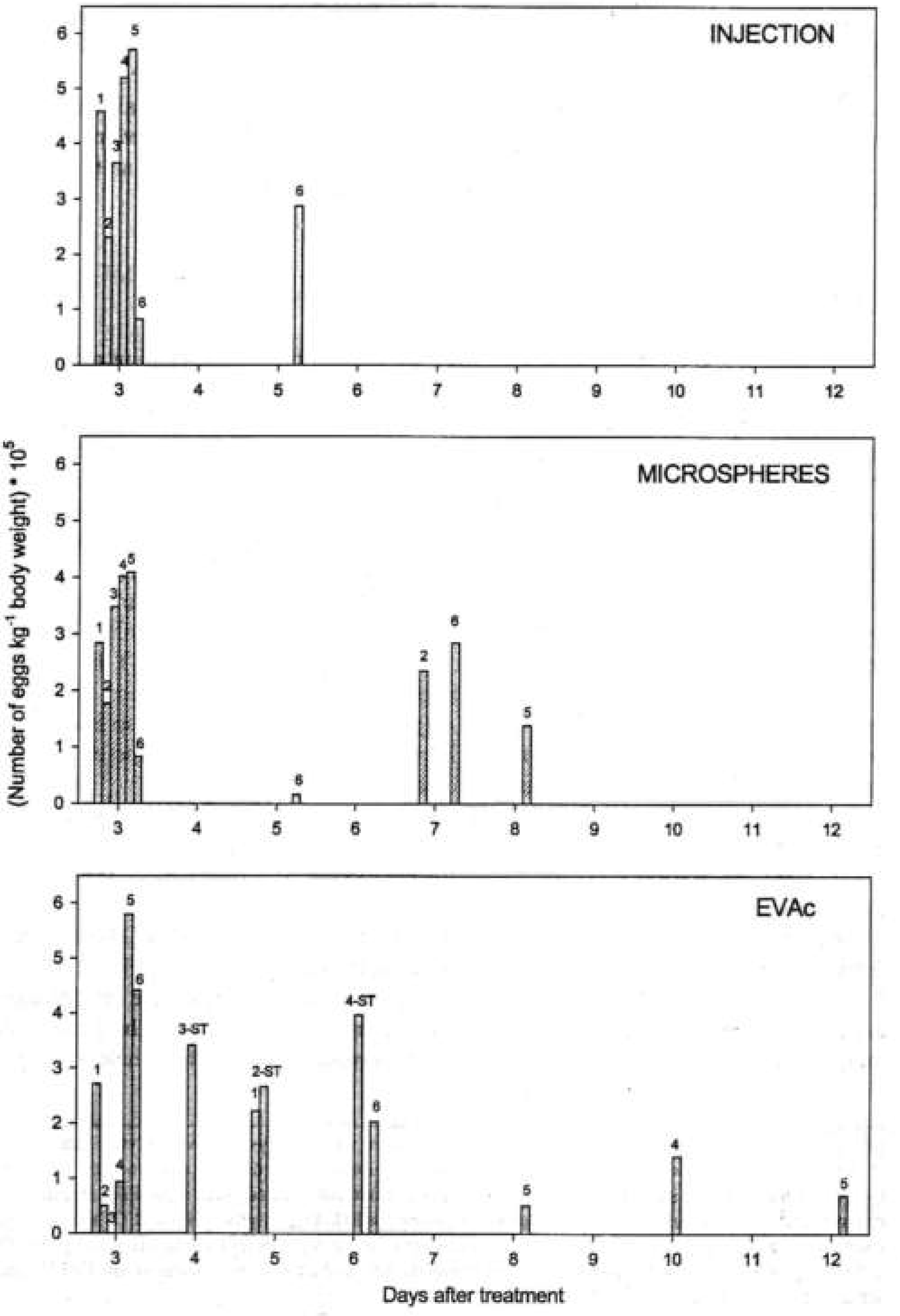
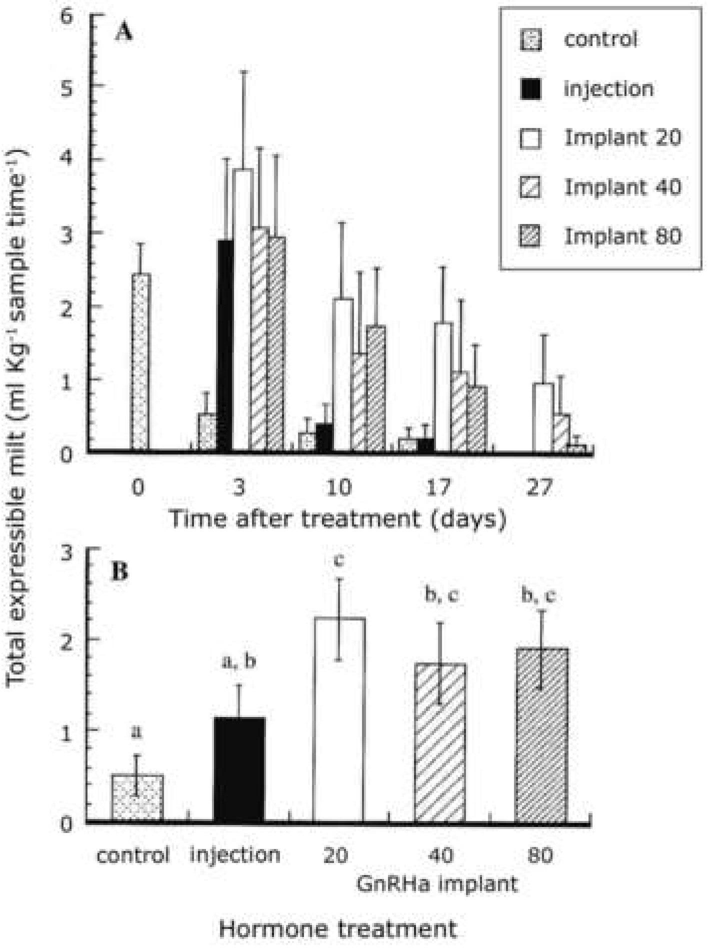


Figure 3. Mean (± SEM) total milt collected from European sea bass at various time (A) and through out the trial, in control and treated fish with GnRHa iniections or implants. Values not sharing common superscript letters are significantly different (P <0.05). (Rainis *et al.*, 2003).



2001). A possible physiological explanation is that probably the GnRHa administration causes a bigger hydration of the spermatic ducts. The higher intra-testicular hydrostatic pressure allows the movement of spermatozoa from the testis to the deferent ducts, causing an increase of the total amount of seminal liquid (Mylonas *et al.*, 1997). In some trials, on Atlantic salmon (*Salmo salar*) and European sea bass (*Dicentrarchus labrax*), it was noted that the increase of the sperm volume was due to an increase in sperm cells production, so it was not only due to a higher hydration (Mylonas *et al.*, 1995). The capacity to cause the increase in the seminal liquid production is strictly correlated with the type of GnRHa releasing-system used. In fact, the capacity to cause the GtH II peak in plasma changes a lot, according as the administration method tested (Mañanos *et al.*, 2002).

In females, the use of GnRHa can improve the quality and quantity of the produced eggs, if compared to those of broodstocks spontaneously spawning (Forniés *et al.*, 2001). Furthermore, it is possible the synchronisation of females, in order to handle simultaneously all the spawners. Encouraging results were obtained in different trials, with females tested, irrespective by their different oocyte maturation type: asynchronous, synchronous or multiple oocytes groups (Pankhurst,

Figure 4. Mean (± SEM) sperm density of milt collected from European sea bass, in control and treated fish with GnRHa iniections or implants. Values not sharing common superscript letters are significantly different (P <0.05). (Rainis *et al.*, 2003).

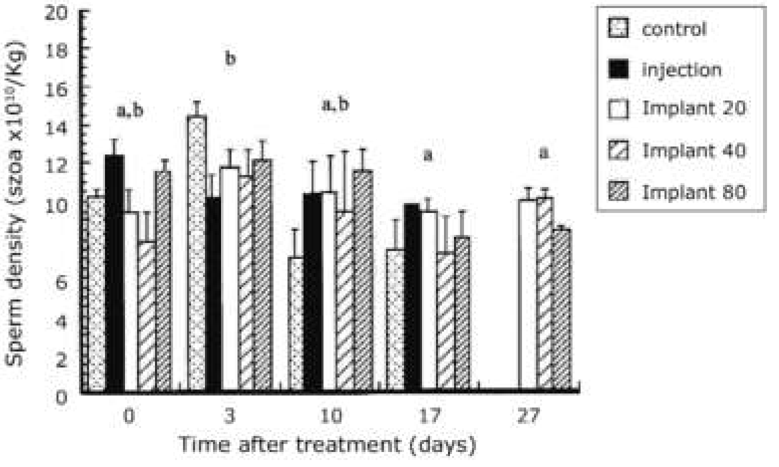
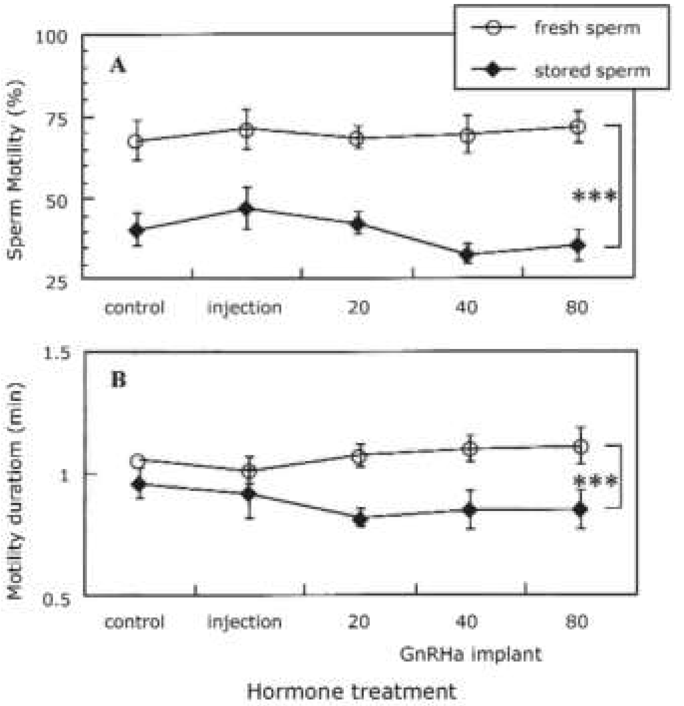


Figure 5. Mean (± SEM) % sperm motility (A) and motility duration (B) of milt from European sea bass, in control and treated fish with GnRHa iniections or implants. Motility evaluations were done both in freshly collected milt and after overnight storage at + 4°C without any extender.
(*** : P< 0.001). (Rainis *et al.*, 2003).



1998). However, the efficacy of the treatments changes according to the species, to the type of releasing-system, to the ripening stage of fish and also to the environmental conditions. All these variables can affect, to some extent, the positive effect of the treatment, causing a worsening in egg quality. However a small reduction of the gamete fertility can be accepted in commercial hatcheries, if the management advantages obtained by the synchronisation of broodstocks are considered (Mylonas *et al.*, 1992).

GnRHa releasing-systems were successfully used to stimulate maturity and spawning of the gametes in about 40 different finfish species for females and about 20 species for males, as for instance: Striped Bass (*Morone saxatilis*), White Bass (*M. chrysops*) European sea bass (*Dicentrarchus labrax*), *Ciprinidae* (Gold fish, *Carassius auratus*; Common carp, *Cyprinus carpio*), *Salmonidae* (Atlantic Salmon, *Salmo salar*; Rainbow trout, *Oncorhynchus mykiss*; Red Salmon, *Oncorhynchus nerka*) and *Cianidae* (Milk fish, *Chanos chanos*).

The use of the GnRHa controlled releasing-systems, in experimental protocols for rising sperm production, is potentially an universal method acceptable by any commercial hatchery and for any species (Mylonas, 1996).

Since some years, studies have been developed on alternative methods of administration, as for instance oral administration. The use of GnRHa carried by feed would be very useful in particular situations, as in off-shore cages farming or for animals that do not stand any handling before their spawning. In these cases the difficulties are related to the choice of the right moment of administration, to the contemporarely presence of different sizes of fish in cages or tanks and obviously to the necessity of GnRHa forms not digestible. Another important limit to consider is that most of finfish species naturally decrease their feed intake or stop feeding, when close to the spawning period.

Conclusions

GnRHa replaced the fresh extracts of the pituitary gland of sexually ripen fish as a tool for inducing and settling fish reproduction. The technology progress resulted in the GnRHa incorporation in a polymeric controlled releasing system, that can be based on injections of microspheres suspended in a liquid or with an intramuscolar solid device. All these controlled releasing-systems allow to overcome technical and biological limits of the injections of pure GnRHa.

Next objectives of research, in order to make these treatments more and more effective, should have:

- to establish the mixture and the minimum dose of GnRHa, for different species;
- to define the period (of the ripening season) more suitable for the administration to each species;
- to detect the optimum releasing rate of GnRHa in blood;
- to reduce the laboriousness and the economic cost of the treatment, so that GnRHa could become a routinary method in controlling the reproduction in fish hatcheries;
- to develop and test alternative forms of administration, as oral administration.

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