ELSEVIER

Aquaculture 197 (2001) 99–136

Aquaculture

www.elsevier.nl/locate/aqua-online

Endocrine manipulations of spawning in cultured fish: from hormones to genes

Yonathan Zohar [a,*], Constantinos C. Mylonas [b]

[a] *Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 E. Pratt Street, Baltimore, MD 21202, USA*

[b] *Institute of Marine Biology of Crete, P.O. Box 2214, Iraklion, Crete 71003, Greece*

Received 1 October 2000; accepted 31 December 2000

Abstract

Almost all fish reared in captivity exhibit some form of reproductive dysfunction. In females, there is often failure to undergo final oocyte maturation, ovulation and spawning; while in males milt production may be reduced and of low quality. These dysfunctions are due to the fact that fish in captivity do not experience the conditions of the spawning grounds, and as a result there is a failure of the pituitary to release the maturational gonadotropin, luteinizing hormone (LH). Reproductive hormones have been utilized since the 1930s to stimulate reproductive processes and induce ovulation/spermiation and spawning. The first methods employed freshly ground pituitaries collected from reproductively mature fish, which contained gonadotropins (mainly LH) and induced steroidogenesis and gonadal maturation. Eventually, purified gonadotropins became available, both of piscine and mammalian origin, e.g., carp or salmon gonadotropin, and human chorionic gonadotropin. In the 1970s, spawning induction methods begun employing the newly discovered gonadotropin-releasing hormone (GnRH), which induces the secretion of the fish's own gonadotropin from the pituitary, thereby overcoming the endocrine failure observed in captive broodstocks. Development of highly potent, synthetic agonists of GnRH (GnRHa) constituted the next generation of hormonal manipulation therapies, and created a surge in the use of hormones to control reproductive processes in aquaculture. The most recent development is the incorporation of GnRHa into polymeric sustained-release delivery systems, which release the hormone over a period of days to weeks. These delivery systems alleviate the need for multiple treatments and induce (a) long-term elevation in sperm production and (b) multiple spawning in fish with asynchronous or multiple-batch group-synchronous ovarian physiology. Based on the recent discovery of GnRH multiplicity in fish and the increasing understanding of its functional significance, new GnRH agonists can be designed for more potent, affordable and physio-

* Corresponding author. Tel.: +1-410-234-8803; fax: +1-410-234-8896.
E-mail addresses: zohar@umbi.umd.edu (Y. Zohar), mylonas@imbc.gr (C.C. Mylonas).

0044-8486/01/$ - see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0044-8486(01)00584-1

logically-compatible spawning induction therapies. Future strategies for improved spawning manipulations will be based on understanding the captivity-induced alterations in the GnRH system, and on new approaches for their repair at the level of GnRH gene expression and release.
© 2001 Elsevier Science B.V. All rights reserved.

Keywords: Induced spawning; GnRH; GnRH genes; Gonadotropin; Delivery systems; Broodstock management

---

1. Introduction

Aquaculture has been practiced in some societies for many centuries, but the transition from the low-input, ranching approach to the more intensive, industrialized methods has been done only during the last few decades (Kirk, 1987). Therefore, aquaculture is still far from the sophistication of livestock and poultry production, especially in regards to genetic improvement (Forster, 1999). One of the most serious bottlenecks in the development of commercial aquaculture, and a prerequisite for our ability to implement genetic improvement programs, is the control of reproductive processes of fish in captivity. Acquisition of seedstock from the wild (i.e., larvae or fry, or gametes from gravid broodstock) during the seasonal spawning period is unreliable and unpredictable, and thus inappropriate for the industrialization of aquaculture. Once reproduction can be controlled in culture, a steady supply of fish can be produced by off-season spawning (Bromage and Roberts, 1995), and genetic manipulations can be employed to enhance their growth, survival and flesh quality characteristics (Thorgaard, 1995).

Unfortunately, many fish exhibit reproductive dysfunctions when reared in captivity. Most commonly, females fail to undergo final oocyte maturation (FOM), and thus ovulation and spawning (Zohar, 1988, 1989a,b; Peter et al., 1993), while males produce small volumes of milt, or milt of low quality (Billard, 1986, 1989). Manipulations of various environmental parameters, such as temperature, photoperiod, salinity, tank volume and depth, substrate vegetation, etc. can often improve the reliability of spawning (Zohar, 1989a; Munro et al., 1990; Yaron, 1995). However, in some species hormonal treatments are the only means of controlling reproduction reliably. Over the years, a variety of hormonal approaches have been used successfully (Donaldson and Hunter, 1983; Peter et al., 1988, 1993; Zohar, 1988, 1989a,b; Tucker, 1994). These methods began with the crude use of ground pituitaries from mature fish—containing gonadotropin (GtH)—which were injected into broodstock to induce spawning (Houssay, 1930). Today, various synthetic, highly potent agonists of the gonadotropin-releasing hormone (GnRHa) are available (Peter et al., 1993; Zohar, 1989a,b; Crim and Bettles, 1997), as well as sustained-release delivery systems for their controlled administration (Crim et al., 1988b; Zohar et al., 1990b, 1994; Zohar, 1996; Mylonas et al., 1995a). These methods have contributed significantly to the development of more reliable, less species-specific methods for the control of reproduction of captive broodstocks.

This review examines the reproductive dysfunctions of fish reared in captivity, describes the evolution of the current methods for the hormonal control of spawning in

cultured fish and discusses the future directions of this field. The present manuscript is meant to complement a series of reviews on the subject of spawning induction published earlier (Pickford and Atz, 1957; Fontaine, 1976; Lam, 1982; Donaldson and Hunter, 1983; Zohar, 1988, 1989a,b; Crim and Bettles, 1997; Peter and Yu, 1997).

2. Reproductive dysfunctions of cultured fish

Similar to most wild animals held in captivity, many fish of commercial interest to the aquaculture industry exhibit reproductive dysfunctions. These dysfunctions probably result from the combination of captivity-induced stress (Sumpter et al., 1994; Pankhurst and Van der Kraak, 1997) and the lack of the appropriate "natural" spawning environment (Zohar, 1989a,b; Yaron, 1995; Battaglene and Selosse, 1996; Ohta et al., 1997). Reproductive problems often diminish over the years, after many generations of fish are produced and reared in culture. A good example of such a "domestication" effect is the gilthead seabream (*Sparus auratus*). When the culture of this Mediterranean species began in the early 1970s, the only way a significant number of eggs could be obtained was through exogenous hormone manipulations (Gordin and Zohar, 1978; Zohar and Gordin, 1979; Zohar et al., 1984), and even then strip-spawning was required. As time progressed and more generations of captive fish were produced, the reliance of the industry on hormonal manipulations was significantly reduced. Today, gilthead seabream spawn daily for more than 3 months during the regular spawning season, and hormonal manipulations are used only for non-responsive fish reared under artificial photothermal conditions (Barbaro et al., 1997). Similarly, early in the development of domestic striped bass (*Morone saxatilis*) broodstocks, all females were found to be arrested at the vitellogenesis stage of ovarian development, and instead of undergoing FOM and spawning, their oocytes became atretic and were reabsorbed (Woods and Sullivan, 1993). In more recent years, female striped bass initiating FOM are observed more often, although hormonal treatments are still employed for the induction and synchronization of spawning (Sullivan et al., 1997; Mylonas et al., 1998b).

Reproductive problems are usually more serious in female broodstocks, and can be classified in three types. The first and most severe problem is exemplified by the freshwater eel (genus *Anguilla*), which fail completely to undergo vitellogenesis and spermatogenesis when maintained in captivity. In nature, the European eel (*Anguilla anguilla*) undergoes gonadal development during an 18-month migration from its riverine habitats to the depths of the Sargasso Sea, where gonadal maturation and spawning takes place. It is believed that because the eels are not allowed to undergo this migration, they do not progress through gonadal development in captivity (Fontaine, 1975). Another fish with a similar problem is the Mediterranean amberjack (*Seriola dumerili*), a species of great interest to the aquaculture industry (Abellan and Basurco, 1999). Most of the broodstocks, which are currently available, were developed from wild-caught juveniles. After a few years in captivity, these fish appear to initiate the process of vitellogenesis, but only a very small percentage of females develop oocytes more than 200–300 μm in diameter (G. Marino, personal communication). These oocytes are obviously too immature to be induced to ovulate using exogenous hormones.

Therefore, the fish undergo atresia and reabsorb their eggs at the end of the spawning season. Finally, stocks of grey mullet (*Mugil cephalus*) maintained exclusively in seawater also seem to have a dysfunction during the process of vitellogenesis, which becomes arrested at very early stages (de Monbrison et al., 1997).

The second type of reproductive dysfunction in female broodstock is the absence of final oocyte maturation. Vitellogenesis appears to progress normally in the above-mentioned fish, but at the onset of the spawning season the post-vitellogenic oocytes fail to undergo FOM and ovulation, and become atretic (Tucker, 1994; Berlinsky et al., 1997; Larsson et al., 1997; Mylonas et al., 1997b,e). This is the most common type of reproductive problem encountered in aquaculture and a large body of research has accumulated on the development of hormonal manipulations for the induction of FOM, ovulation and spawning of a multitude of fish species.

The third type of reproductive dysfunction observed in cultured females is the absence of spawning at the end of the reproductive cycle. In species exhibiting this problem, the oocytes undergo normal vitellogenesis, FOM and ovulation in response to the appropriate physiological and environmental stimuli, but the ovulated eggs are not released to the water. These eggs may have one of two fates. In salmonids, the eggs are retained in the abdominal cavity and are reabsorbed over the following months (Bromage et al., 1992). Females of some marine species, like the striped bass (personal observations), common dentex (*Dentex dentex*) (M. Pavlides, personal communication) and white grouper (*Epinephelus aeneus*) (Hassin et al., 1997) may release these eggs at some later time after ovulation (hours to days) even in the absence of any breeding behaviour. Obviously, in these fish the eggs must be stripped manually from the females and fertilized artificially. Strip spawning, however, must occur within a specific window of time after ovulation, since ovulated eggs become "overripe" and can not be fertilized. Depending on the fish species and water temperature, overripening occurs within a few weeks, hours or even minutes. At low temperatures in salmonids, for example, eggs can be maintained in the abdominal cavity for at least a week after ovulation without a significant reduction in fertility, eye-up or hatching success (Sakai et al., 1975; Springate et al., 1984). In species like the catfish (*Clarias macrocephalus*), turbot (*Scophthalmus maximus*) and cod (*Gadus morua*) fertility is maintained for up to 10 h after ovulation (reviewed by Kjørsvik et al., 1990). However, in fish like the striped bass (Stevens, 1966) and the white bass (*M. chrysops*) (Mylonas et al., 1996) loss of fertility occurs in less than 1 h. Since females from a particular broodstock do not undergo FOM and ovulation in synchrony in most species, monitoring fish for ovulation may last for many weeks and is very labour intensive. In addition, it risks damaging the broodstock both due to the stripping process itself, but also due to the multiple handling and sampling that are required prior to stripping, in order to determine the time of ovulation. Finally, multiple handling prior to ovulation can have detrimental effects on the process of FOM and on the quality of the eggs.

With the exception of freshwater eels, the reproductive dysfunctions observed in male cultured broodstocks are limited to captivity-induced reductions in milt production or milt quality. For example, well-spermiating striped bass males placed in small spawning tanks with individual, post-vitellogenic females exhibit a significant reduction in the amount of expressible milt produced (Mylonas et al., 1998a). Also, yellowtail flounder

(*Pleuronectes ferrugineus*) and turbot produce less than 1 ml of milt per fish (Suquet et al., 1992; Clearwater and Crim, 1998), while certain strains of Atlantic salmon (*Salmo salar*) selected genetically for fast growth, produce less than 0.1 ml of milt per kg body weight (Zohar, 1996). Especially in broodstock captured from the wild during the spawning season and moved to captivity, males produce milt with non-motile sperm (Berlinsky et al., 1997), or milt of high viscosity which does not mix well with water and fails to fertilize the eggs (Vermeirssen et al., 1998, 2000). Another problem is that milt production is often inadequate to support artificial insemination procedures in commercial hatcheries, because males are repeatedly handled and stripped of their milt, in order to fertilize the eggs from many females as they undergo FOM and ovulation at different times during the course of the spawning season.

3. Hormonal induction of ovulation and spawning

Most research and development efforts on the use of hormones to control finfish reproductive cycles in aquaculture have focused on the induction of FOM, ovulation, spermiation and spawning in fish that do not complete these processes in captivity. However, hormonal manipulations have important applications in commercial aquaculture, even for fish that do undergo FOM and spermiation spontaneously in captivity. For example, in many salmonid hatcheries, ovulation is induced with hormones in order to synchronize and optimize egg collection and fry production, thereby minimizing the handling and stress to the fish, and reducing labor requirements (Donaldson et al., 1981; Zohar et al., 1990b, 1994; Goren et al., 1995). In Pacific salmon (genus *Oncorhynchus*), heavy mortalities occur prior to FOM and ovulation, especially when broodstock are maintained in saltwater (Sower et al., 1982). Hormonal manipulations can advance maturation and ovulation by a few weeks, thus reducing losses due to pre-spawning mortality (Goren et al., 1995). Another application of hormonal manipulations is for the collection of gametes for inter-specific hybridization via artificial fertilization, since different species do not usually spawn together when placed in tanks. Finally, development of genetic selection programs often requires artificial fertilization, and hormonal manipulations can be used to enable proper maturation and timely collection of gametes. Therefore, hormonal manipulations for the induction of ovulation, spermiation and spawning will continue to play an important role in commercial broodstock management, even after various fish species become properly “domesticated”.

Various hormones of the brain–pituitary–gonad axis have been used to induce final maturation of the gonads in cultured fishes. Key to the development of current and future spawning induction technologies is the basic understanding of the effect of confinement on the endocrine system that governs FOM, ovulation, spermiation and spawning.

3.1. Level of hormonal failure in cultured fish

The lack of FOM, ovulation and spawning in cultured fish results from the fact that captive broodstocks do not experience the environmental conditions of the spawning grounds. Many of the commercially important cultured fish migrate hundreds to

thousands of kilometers to reach the environmental niches where conditions are optimal for the survival of their offspring. During this migration, the fish experience multiple environmental changes, e.g., water salinity, chemistry, temperature, depth and substrate. These combined changes trigger the endocrine processes that result in FOM and ovulation, leading to successful spawning (Lam, 1983; Stacey, 1984). In the absence of such environmental triggers, captive broodstocks become arrested in advanced stages of vitellogenesis, followed by follicular atresia (Zohar, 1989a). Exposing captive broodstocks to the environmental changes experienced in nature has met with some success in inducing spawning in freshwater species (Sinha et al., 1974; Stacey et al., 1979). However, it is impractical for most species to simulate the complex conditions of a spawning ground in commercial hatcheries. Overcoming the absence of a spawning ground may be helped by understanding the hormonal failure that results from the lack of the environmental conditions permissive for FOM, ovulation, spermiation and spawning.

Early success using injected extracts of pituitaries, harvested from captive mature fish, to induce ovulation and spawning in females that otherwise undergo atresia (see Section 3.2 below), suggested that pituitaries of captive broodstock contain the hormones required to trigger spawning. Further studies demonstrated that in captive female gilthead seabream, levels of LH in the pituitary increased steadily with the progress of vitellogenesis and the approach of the spawning season (Zohar, 1988; Elizur et al., 1995). However, after vitellogenesis was completed and pituitary LH levels were maximal, LH levels in the plasma remained undetectable and oocytes underwent atresia. On the other hand, in females that spawned spontaneously, FOM and ovulation were preceded by a distinct surge of LH in the plasma (Zohar, 1988). These data further indicated that in captive fish failing to ovulate, the hormone responsible for triggering FOM and ovulation—i.e., LH—is produced and accumulates in the pituitary, but is not released into the bloodstream. Consequently, LH does not reach the target organ, the ovaries, to trigger final oocyte maturation.

The most solid evidence of the effect of captivity on the brain–pituitary–gonad axis came from a series of studies in striped bass, comparing levels of hormones of the reproductive axis in wild fish captured on their spawning grounds to cultured fish (Mylonas et al., 1997b, 1998b; C. Steven and Y. Zohar, unpublished data). These studies showed that in wild females a plasma LH surge accompanied FOM and ovulation, but plasma LH levels in cultured females remained low and unchanged, while vitellogenic oocytes underwent atresia. However, levels of LH and its mRNA in the pituitary did not differ between wild and captive females. Considered together, the above results strongly suggest that the failure of many cultured fish to undergo FOM, ovulation and spawning in captivity is the result of a lack of LH release from the pituitary to the circulation. This conclusion is further corroborated by the success of gonadotropin-releasing hormone (GnRH) therapy to induce LH release, ovulation and spawning in a multitude of fish (See Section 3.3 below).

3.2. *Use of gonadotropin (GtH) preparations*

Long before the technology to measure fish hormones became available and the level of the hormonal failure leading to the lack of ovulation and spawning was understood,

endocrine manipulations had been used to induce cultured fish to ovulate and spawn. The first report on the ability of exogenous hormones to induce FOM and ovulation in fish was made by Houssay (1930). In studying the gonadotropic functions of the pituitary gland in vertebrates, he injected female fish with ground pituitaries from another species and observed that the females underwent ovulation. Professor B.A. Houssay, an Argentinean, was the co-recipient of the 1947 Nobel Prize in Physiology or Medicine, for his later work on the role of the pituitary on glucose metabolism. His work on the existence in fish of a pituitary gonadotropic hormone resulted in the development of hormonal manipulation methods for the induction of spawning in fish, starting with the "hypophysation" technique.

3.2.1. Hypophysation and piscine pituitary extracts

Hypophysation, the use of ground pituitaries and pituitary extracts to induce spawning in fish, started in the late 1930s in Brazil (Von Ihering, 1937; Fontenele, 1955), followed by work in the United States (Hasler et al., 1940; Ball, 1954; Palmer et al., 1954) and Japan (Migita et al., 1952). Collection of pituitaries for hypophysation was done from reproductively mature broodstock, either males or females (Fontenele, 1955). It was found that pituitaries collected during the spawning season were more efficacious in inducing spawning. This was later shown to be the result of increased accumulation of GtH (primarily LH) in the pituitary prior to, and during, the spawning season (Zohar, 1989a,b). The collected pituitaries were stored in alcohol or dehydrated in acetone, and were ground in physiological saline prior to injection to the recipient fish. Initially, a ratio of one pituitary from a donor fish for a recipient fish of similar weight was used in the case of males, whereas the ratio was 1.5:1 in the case of females (Fontenele, 1955). However, this approach was not always effective, due to variations in size and GtH content of pituitaries from fish of similar weight (Yaron, 1995). In some cases, the sex of the donor and recipient fish was also matched (Palmer et al., 1954). The appropriate amount of ground pituitaries was divided in two to four doses and given every few hours (Fontenele, 1955) or every few days (Palmer et al., 1954). In more recent applications of the hypophysation method, treatment has been standardized to a small priming dose (10–20% of total) and a larger resolving dose given 12–24 h apart. Effective doses range from 2 to 10 mg of pituitary per kg body weight of the recipient fish (Table 1) (Thalathiah et al., 1988; Parauka et al., 1991; Kucharczyk et al., 1997).

Use of ground pituitaries, however, is associated with various drawbacks, the most important ones being (a) the great variability in pituitary LH content (b) the administration of additional hormones present in the pituitary that may adversely affect the physiology of the treated fish, and (c) the potential for transmission of diseases from donor fish to recipient broodstocks. The LH content of the pituitary varies according to the weight, sex and age of donor fish, the time of the year the fish were sacrificed, and the period of time elapsed from the death of the fish to the collection and preservation of the pituitary (Yaron, 1995). Therefore, the effectiveness of the approach relies heavily on the pituitary collection procedure and the expertise of the hatchery manager. Following the successful usage of ground pituitaries and pituitary extracts for the control of fish reproduction, but realizing their drawbacks mentioned above, efforts were later directed towards the acquisition of purified or partially purified preparations of LH. The

Table 1
Representative applications of hormonal manipulations for the induction of final maturation and spawning of male (M) and female (F) broodstock using various gonadotropin (GtH) preparations

Species	Common name	Sex	Treatment[a]	Reference
Abramis brama	bream	M/F	PiT, hCG	(Kucharczyk et al., 1997)
Acanthopagrus latus	yellowfin porgy	F	hCG	(Leu and Chou, 1996)
Acipenser oxyrhynchus	Gulf of Mexico sturgeon	F	hCG	(Parauka et al., 1991)
Anguilla japonica	Japanese eel	M	PiT, hCG	(Miura et al., 1991b)
		F	pGtH	(Ohta et al., 1997)
Anoplopoma fimbria	sablefish	F	pGtH	(Solar et al., 1987)
Argyrosomus hololepidotus	mulloway	M/F	hCG	(Battaglene and Talbot, 1994)
Catostomus commersonii	white sucker	M/F	PiT	(Ball, 1954)
Clarias batrachus	Asian catfish	F	PiT	(Zonneveld et al., 1988)
Clupea harengus	Pacific herring	F	pGtH	(Kreiberg et al., 1987)
Cnesterodon decemmaculatus	(unknown)	F	PiT	(Houssay, 1930)
Ctenopharyngodon idella	grass carp	F	PiT, hCG	(Thalathiah et al., 1988)
Cyprinus carpio	common carp	F	PiT	(Kime and Dolben, 1985)
Epinephelus striatus	Nassau grouper	F	hCG	(Head et al., 1996)
Esox lucius	pike	M/F	Pit, pGtH	(Billard and Marcel, 1980)
Lutjanus analis	mutton snapper	M/F	hCG	(Watanabe et al., 1998)
Lutjanus argentimaculatus	Mangrove red snapper	M/F	hCG	(Emata et al., 1994)
Heterobranchus longfilis	catfish	F	hCG	(Legendre et al., 1996)
Macquaria novemaculeata	Australian bass	M/F	hCG	(Battaglene and Selosse, 1996)
Micropterus salmoides	largemouth bass	M/F	hCG	(Mayes et al., 1993)
Morone chrysops	white bass	F	hCG	(Kohler et al., 1994)
M. saxatilis	striped bass	F	hCG	(Rees and Harrell, 1990)
Mugil cephalus	grey mullet	F	pGtH, hCG	(Kuo, 1995)
Oncorhynchus kisutch	coho salmon	F	pGtH	(Hunter et al., 1981)
O. nerka	blueback salmon	M/F	PiT	(Palmer et al., 1954)
Prochilodus spp.	curimata	M/F	PiT	(Fontenele, 1955)
Rachydentron canadum	cobia	F	hCG	(Caylor et al., 1994)
Semotilus atromaculatus	creek chub	M/F	PiT	(Ball, 1954)
Siganus guttatus	rabbitfish	F	hCG	(Ayson, 1991)
Solea solea	common sole	F	hCG	(Ramos, 1986a)
Stizostedion vitreum	walleye	F	hCG	(Malison and Held, 1996)
Takifugu rubripes	tiger puffer	F	PiT, hCG	(Miyaki et al., 1992)

[a]Human chorionic gonadotropin (hCG); ground pituitaries or pituitary extracts of vertebrate or piscine origin (PiT), purified piscine GtH (pGtH).

LH preparations of piscine origin are obtained from pituitaries of mature broodstock using chromatographic separation. Their potency may be standardized by in vitro or in vivo bioassays, and by immunoassays. Purified preparations of salmon and carp gonadotropins have been commercially available for some time (Donaldson, 1973; Yaron, 1995). However, due to species specificity of fish LH, the commercially available gonadotropins were useful only for phylogenetically-related fish species.

Extensive reviews of the early studies inducing fish to undergo ovulation, spermiation and spawning using pituitary extracts and purified piscine LH have been published by

Donaldson (1973), Lam (1982) and Donaldson and Hunter (1983). Since then, more species have been added to the list, including the copper redhorse (*Moxosoma hubbsi*) (Branchaud and Gendron, 1993), the Gulf of Mexico sturgeon (*Acipenser oxyrhynchus desotoi*) (Parauka et al., 1991), the tiger puffer (*Takifugu rubripes*) (Miyaki et al., 1992) and the bream (*Abramis brama*) (Kucharczyk et al., 1997). The hypophysation technique, although primitive in its approach, is still used today, especially in developing countries or remote areas where access to expensive purified hormones is limited. Pituitaries from mature fish may be readily available on-site or from local slaughterhouses, and their acquisition and storage does not require sophisticated equipment and expensive facilities. For example, the induction of spawning in Asian carps using locally obtained pituitaries from bighead carp (*Aristichthys nobilis*) and silver carp (*Hypophthalmichthys molitryx*) is equally successful and costs many times less than using purified LH from salmon (Thalathiah et al., 1988).

3.2.2. Human chorionic gonadotropin (hCG)

Although the problem of standardization of potency was solved with the purification of LH from fish pituitaries, availability remained restricted and the cost very high. Consequently, in the early 1970s researchers began experimenting with mammalian GtHs, especially human chorionic gonadotropin (hCG) which is purified from the urine of pregnant females (Katzman and Doisy, 1932). Human chorionic gonadotropin is readily available in clinical grade preparations throughout the world and the potency of all preparations is calibrated according to international standards. Consequently, hCG has been employed in spawning induction trials of many species in culture today (Lam, 1982; Donaldson and Hunter, 1983).

Unlike LH preparations of piscine origin, hCG is often given in a single dose, which ranges between 100 and 4000 international units (IU) per kg body weight. The effectiveness of hCG after a single treatment is probably due to this GtH's relatively long retention time in circulation (Ohta and Tanaka, 1997). This is not related to the fact that it is a heterologous hormone for fish, since in humans it also has a significantly longer half-life in circulation, compared to the gonadotropins of pituitary origin—i.e., follicle stimulating hormone (FSH) and LH (Fontaine et al., 1984). In cobia (*Rachycentron canadum*) a single and relatively low dose of hCG (275 IU kg^{-1}) was enough to induce ovulation in fish with post-vitellogenic oocytes (Caylor et al., 1994). A single treatment with 500 IU hCG kg^{-1} was also effective in inducing ovulation in cultured, as well as wild, Australian bass (*Macquaria novemaculeata*) (Battaglene and Selosse, 1996). The mangrove red snapper (*Lutjanus argentimaculatus*) also spawned after a single injection of male and female broodfish, but required a much higher dose of 1500 IU hCG kg^{-1} (Emata et al., 1994). As with other hormonal treatments, males require a two to four times lower dose of hCG for the induction of spermiation and spawning (Battaglene and Talbot, 1994; Tamaru et al., 1996; Watanabe et al., 1998). Characteristic of the long half-life of hCG in circulation and its prolonged effect on gonadal maturation is the induction of spermatogenesis and spermiation of Japanese eel (*A. japonica*) after a single injection of hCG (Miura et al., 1991a).

One of the often mentioned, though not yet fully proven, drawbacks of using hCG (as well as heterologous piscine LHs) in inducing spawning in fish, is that of refractoriness

to treatment in subsequent spawning seasons. Gonadotropins are large peptides and when fish are treated with heterologous piscine LHs or hCG they may develop antibodies against them (Lam, 1982; Donaldson and Hunter, 1983; Zohar, 1989a). When the same treatment is applied in subsequent years the fish develop an immune response and the injected GtH is immuno-neutralized. The result may be that significantly higher doses are required to induce spawning, or that the treatment is completely ineffective, necessitating the early retirement of otherwise productive broodstock. One of the few published reports examining the antigenicity of hCG in fish was carried out in the goldfish (*Carassius auratus*) and silver carp (Van der Kraak et al., 1989). This study concluded that even after multiple injections of hCG in saline or Freund's adjuvant, no detectable concentrations of hCG antibodies were present. Using an immunoassay, the development of specific antibodies to hCG in response to an hCG treatment was studied in striped bass (Y. Zohar and S. Wehage, unpublished data), since both cultured and wild-caught broodstocks are still treated with this hormone in order to induce ovulation and spawning. Significant titres of hCG antibodies were observed in the circulation of striped bass as soon as 17 days after a single injection with 500 IU kg^{-1} of hCG in saline solution (Fig. 1). Levels of hCG antibodies peaked a month post-injection, and remained elevated for at least 60 days. A second hCG injection at 60 days induced further elevation of hCG antibodies. These results clearly demonstrate the strong immunogenic nature of hCG in striped bass, and suggest that fish indeed develop an immune protection to hCG. More studies are required to document the temporal pattern

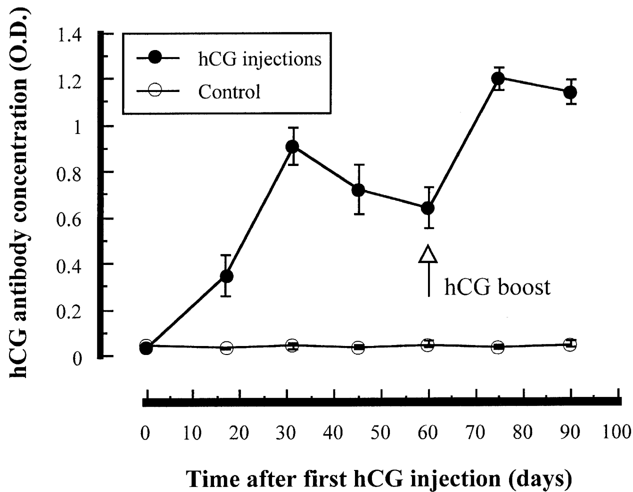

Fig. 1. Mean titers (±s.e.m.) expressed as optical density (OD) of hCG antibodies in the plasma of striped bass at different times after injection with hCG ($n = 4$) or saline ($n = 6$). Two-year-old striped bass weighing (450 g) were held in 2.2 m^3 tanks at 18°C. On days 0 and 60, one group was injected intra-muscularly with 500 IU kg^{-1} body weight of hCG (Sigma) dissolved in 0.9% saline, while a control group was injected with saline alone (0.5 ml kg^{-1}). The concentration of hCG antibodies in the plasma was measured using an hCG non-competitive, solid phase ELISA. The mean anti-hCG titers were significantly elevated in response to the hCG injections (two-way ANOVA, $P \leq 0.0001$). (Y. Zohar and S. Wehage, unpublished).

of the immune response and the duration of the immune memory. Nevertheless, hatchery managers should keep in mind the possibility that their broodstock may develop immune refractoriness to hCG, and likely to other gonadotropic preparations, if fish are treated repeatedly.

There is one situation in which hCG be preferred over GnRHa. The advantage of hCG is that it acts directly at the level of the gonad and does not require the existence of LH stores or activation of the pituitary gonadotropes. Occasionally, GnRHa is not effective or requires a long time to elicit a response. A long latency period between treatment and spawning may result in pre-spawning mortalities, due to stress induced by the capture of gravid wild broodstock, or the transport of cultured broodstock from outdoor ponds/cages to the hatchery. Under these circumstances, hCG may be more appropriate because it acts much faster, via direct stimulation of the gonad, in inducing FOM, spermiation and spawning (Hodson and Sullivan, 1993).

3.3. Use of gonadotropin-releasing hormone (GnRH) and agonists (GnRHa)

Not long after the discovery in the early 1970s of a hypothalamic hormone in mammals, which controls the release of LH (LH-RH or mammalian GnRH) (Schally, 1978), scientists began experimenting with the application of this new hormone in inducing spawning in cultured fish. Indeed, treating mature female carp (*Cyprinus carpio*) with hypothalamic extracts or native GnRH, Breton and Weil (1973) induced a surge of GtH release from the pituitary. With the later development of the first highly potent, synthetic agonists for GnRH (Fujino et al., 1972), work shifted to the use of various GnRHas, which were an order of magnitude more potent than the native peptide. The first studies in female broodstocks indicated that GnRH and GnRHa were effective in inducing ovarian development, FOM and ovulation in doses ranging from 1 to 15 mg GnRH kg^{-1}, or 1 to 100 μg GnRHa kg^{-1} (reviewed in Donaldson and Hunter, 1983; Zohar, 1989a,b). The efficacy of GnRH and its agonists confirmed the hypothesis that in fish with reproductive dysfunctions, the pituitary is still able to synthesize and release the endogenous gonadotropins, given the appropriate treatment and dose.

The use of GnRH peptides for spawning induction therapies has important advantages over the use of GtH preparations. First, GnRH and its agonists are small decapeptides that do not trigger an immune response and can be used again in subsequent spawning seasons with no reduction in their efficacy. Second, by inducing the release of the endogenous LH, the GnRH "repairs" the endocrine disruption that results in the failure of captive fish to undergo FOM, ovulation and spawning. Also, GnRH acts at a higher level of the hypothalamus–pituitary–gonad axis. Consequently, GnRH can provide a more balanced stimulation of reproductive events and, presumably, a better integration of these events with other physiological functions, by directly or indirectly affecting the release of other hormones necessary for successful FOM, spermiation and spawning. Such hormones include prolactin (Weber et al., 1995), growth hormone (Marchant et al., 1989; Peter et al., 1990; Le Gac et al., 1993) and thyroid hormones (Sullivan et al., 1989). A third advantage of GnRHa, is that it can be synthesized and obtained in pure form, and thus does not carry the risk of transmitting diseases (a very serious concern for the use of piscine pituitary extracts). Finally, because of the structural similarity of

the GnRHs among many fish species (Sherwood et al., 1994), the use of GnRHs, unlike the use of gonadotropins, is generic and the same GnRHa has been successfully applied to a wide range of fish species.

Although the administration of native GnRHs induces an immediate surge of GtH in circulation in many fish species, the amplitude of such a surge is too low, and its duration too short to trigger FOM, ovulation and spawning (Omeljaniuk et al., 1987; Crim et al., 1988b; Zohar, 1988; Zohar et al., 1989a). This short-lived effect of the native GnRHs was shown to be the result of a very quick degradation of the injected GnRHs by endopeptidases located in the pituitary, liver and kidney of the injected fish (Zohar et al., 1990a). These enzymes cleave the GnRH decapeptide specifically between positions 5–6 and 9–10, resulting in smaller, inactive fragments. By substituting the position 6 residue of the native GnRH with a dextrorotatory (D) amino acid, and the position 10 amino acid with an ethylamide group, GnRH agonists (GnRHa) are synthesized that are resistant to enzymatic degradation (Goren et al., 1990; Weil et al., 1991, 1992) and are thus cleared from the fish circulation much slower than the native GnRHs (Gothilf and Zohar, 1991). Therefore, the GnRHa remains in the circulation much longer and stimulates a stronger and more prolonged GtH release from the pituitary compared to native GnRHs. Due to their modified polarity and tertiary structure, some of these GnRHas also exhibit increased binding affinity to the pituitary GnRH receptors (De Leeuw et al., 1988; Habibi et al., 1989; Pagelson and Zohar, 1992). The combination of increased resistance to enzymatic cleavage and enhanced receptor binding affinity results in GnRHas which are 30–100 times more potent than the native GnRHs in inducing LH release, in terms of both the amplitude and the duration of the LH surge (Peter et al., 1988; Zohar et al., 1989a,b; Crim and Bettles, 1997). Due to the above-mentioned benefits associated with a GnRHa-based technology, these super-active GnRHas have quickly replaced other hormones in spawning induction therapies.

Of the multitude of super-active GnRHas tested in fish (Peter et al., 1985; Crim et al., 1988a; Zohar, 1989a; Zohar et al., 1989a), the GnRHas most commonly utilized in spawning induction are the [D-Ala^6,Pro^9,NEthylamide]-mammalian GnRH ([D-Ala^6, Pro^9,NEt]-mGnRH) and the [D-Arg^6,Pro^9,NEthylamide]-salmon GnRH ([D-Arg^6,Pro^9, NEt]-sGnRH). These two agonists are equipotent in the gilthead seabream (Zohar, 1989a,b; Zohar et al., 1989a), landlocked salmon (Crim et al., 1988a) and striped bass (Mylonas and Zohar, unpublished data), whereas [D-Arg^6,Pro^9,NEt]-sGnRH is more potent in inducing GtH release in the Chinese loach (*Paramisgurnus dabryanus*) (Lin et al., 1991), rainbow trout (*Oncorhynchus mykiss*) (Crim et al., 1988a) and goldfish (Peter et al., 1985). Since the [D-Ala^6,Pro^9,NEt]-mGnRH analogue is employed in various applications in human medicine (Barbieri, 1992; Emons and Schally, 1994) it is widely available and less expensive than the fish agonist [D-Arg^6,Pro^9,NEt]-sGnRH. Consequently, [D-Ala^6,Pro^9,NEt]-mGnRH is the most widely investigated GnRHa in inducing FOM, ovulation and spermiation in fish (Table 2). The use of GnRHas in aquaculture has indeed revolutionized broodstock management and, as predicted almost three decades ago (E.M. Donaldson, FAO World Conference on Aquaculture, Kyoto, Japan, 1976; unpublished), it has become a practical and often indispensable tool for hatchery managers. Administration of GnRHa has been done mostly via injections in saline or in the form of sustained-release delivery systems. Some work has been carried out in the

Table 2
Representative applications of hormonal manipulations with agonists of gonadotropin-releasing hormone (GnRHa) for the induction of final maturation and spawning of male (M) and female (F) broodstocks

Species	Common name	Sex	GnRHa[a]	Other treatment[b]	Reference
Anoplopoma fimbria	sablefish	F	A		(Solar et al., 1987)
Aristichthys nobilis	bighead carp	F	A		(Ngamvongchon et al., 1988)
Carassius auratus	goldfish	F	A, T	Pim	(Sokolowska et al., 1984)
Chanos chanos	milkfish	F	A, R		(Marte et al., 1987)
Clarias batrachus	Asian catfish	F	L	Dom	(Alok et al., 1995)
Clarias macrocephalus	catfish	F	A	Pim	(Tan-Fermin et al., 1997)
Clupea harengus	Pacific herring	F	A		(Kreiberg et al., 1987)
Cynoscion nebulosus	spotted seatrout	F	A		(Thomas and Boyd, 1988)
Cyprinus carpio	common carp	F	R	Dom, Met	(Drori et al., 1994)
		M	A		(Takashima et al., 1984)
Dicentrarchus labrax	European seabass	F	A		(Alvariño et al., 1992)
Epinephelus striatus	Nassau grouper	F	A		(Watanabe et al., 1995)
Lates calcarifer	barramundi	F	A		(Garcia, 1996)
Mugil cephalus	grey mullet	F	A	PiT	(Lee et al., 1987)
Mylopharyngodon piceus	black carp	F	A	Res, Pim	(Peter et al., 1988)
Heteropneustes fossilis	catfish	F	L		(Alok et al., 1993)
Hypophthalmichthys molitrix	silver carp	F	A	Pim	(Lin et al., 1986)
Oncorhynchus kisutch	coho salmon	F	A		(Fitzpatrick et al., 1984)
O. mykiss	rainbow trout	F	T		(Breton et al., 1990)
O. nerka	sockeye salmon	M/F	A		(Slater et al., 1995)
Oreochromis spp.	tilapia hybrid	F	A	Dom, Met	(Gissis et al., 1991)
Parabramis pekinensis	bream	F	A	Pim	(Lin et al., 1986)
Paramisgurnus dabryanus	loach	F	A, R	Dom, Pim	(Lin et al., 1991)
Perca flavescens	yellow perch	M/F	A		(Dabrowski et al., 1994)
Salmo salar	Atlantic salmon	M	A		(Weil and Crim, 1983)
S. trutta	brown trout	F	A		(Mylonas et al., 1991)
		F	A	Pim	(Billard et al., 1984)
Salvelinus fontinalis	Arctic charr	F	R		(Gillet et al., 1996)
Sciaenops ocellatus	red drum	F	A		(Thomas and Boyd, 1989)
Solea solea	common sole	F	A		(Ramos, 1986b)
Sparus aurata	gilthead seabream	F	A		(Zohar et al., 1989b)
Stizostedion vitreum	walleye	F	A		(Malison and Held, 1996)

[a] A = D-Ala^{6}Pro^{9}NEt-mGnRH; L = D-Lys^6-sGnRH; R = D-Arg^{6}Pro^{9}NEt-sGnRH; T = D-Trp^6-mGnRH.
[b] Combination treatment with a dopamine antagonist (Pim = pimozide, Dom = domperidone, Met = metoclopramide, Res = reserpine) or a pituitary extract (PiT).

early 1990s examining the potential of oral delivery of GnRHa (Thomas and Boyd, 1989; Solar et al., 1990; McLean et al., 1991; Sukumasavin et al., 1992), but although the results showed some promise, this method has not been studied adequately.

Dopamine (DA) antagonists have often been used in combination with a GnRHa in spawning induction therapies (Peter et al., 1993). Dopamine in some fish acts at the level of the pituitary by inhibiting the basal release of GtH and attenuating the action of GnRH on the gonadotrophs (Chang and Peter, 1983; Chang et al., 1984). In spawning

induction therapies using a combination of GnRHa and DA antagonist, injection of GnRHa is given in two doses and a DA antagonist (e.g., domperidone, pimozide, reserpine or metoclopramide) is given in a single dose at the time of the first GnRHa injection. Administration of the DA antagonist at this time is believed to remove the inhibition on the gonadotrophs and enhance the stimulatory effect of the second GnRHa on GtH release. Dopaminergic inhibition has been decisively demonstrated to be present only in cyprinid fishes and the African catfish (Trudeau and Peter, 1995), and appears to be absent in most commercially important marine fish (Copeland and Thomas, 1989; King et al., 1994; Zohar et al., 1995a,b). Also, the intensity of DA inhibition appears to change over the course of the reproductive cycle and although in the goldfish it is stronger during the spawning season (Peter et al., 1986; Trudeau and Peter, 1995), in other fish it may be minimal during this stage (Linard et al., 1995). As a result, spawning induction protocols using a combined GnRHa-DA antagonist treatment have been used mostly in cyprinid fishes (Peter et al., 1988, 1993; Yaron, 1995).

Recently, aromatase inhibitors (AI) have been examined for their potential to induce FOM and spermiation. In female coho salmon (*O. kisutch*), AI inhibit the conversion of testosterone (T) to 17β-estradiol (E_2) in the ovary, and enable the necessary increase in 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P) production for the induction of FOM and ovulation (Afonso et al., 1999). In male fish, where aromatase activity has not been identified in the gonads, the induction of spermiation observed after AI treatment was probably via stimulation of the GnRH–GtH axis, by the decreased conversion of T to E_2 (Afonso et al., 2000). Treatment of both male and female coho salmon with AI resulted in a significant synchronization of ovulation and spermiation, while a small degree of advancement was also observed. Further research should elucidate the central functions of aromatization in fish, and demonstrate the commercial applicability of this approach to salmon, as well as other cultured species.

3.4. Sustained-release delivery systems for GnRHa

Almost from the first experiments using pituitary extracts for spawning induction, it was recognized that administration of the hormone in a sustained fashion would improve the efficacy of the procedure (Fontenele, 1955). The multiple treatments that are often necessary for a successful response present various problems to the hatchery manager. First, repetitive handling of broodstock requires substantial labor, time and monitoring. Especially in situations where the broodfish are kept outdoors, in ponds or cages, it is difficult, very time consuming, and labor intensive to crowd, capture, anaesthetize and inject the fish with hormones, frequently while hatchery personnel are exposed to the elements of nature. Secondly, repetitive handling is stressful to the fish and can often result in pre-spawning mortalities, or at the very least it can adversely affect the progression of FOM. After hormonal treatment of striped bass, for example, failure to predict the exact time of ovulation with one or two biopsy samplings may result in reduction of egg quality after stripping (unpublished observations).

As mentioned earlier, GnRHas were produced in order to resist enzymatic degradation when injected into the fish, thus resulting in a more prolonged stimulation of GtH

release, compared to the native GnRH peptide (Van der Kraak et al., 1983; Zohar et al., 1989b, 1990a, 1995a,b; Goren et al., 1990). It was expected that a single injection of GnRHa would be adequate to induce FOM, spermiation and spawning (Donaldson and Hunter, 1983). However, even the GnRHa most resistant to enzymatic degradation has a half-life of only 23 min in vivo, compared to 5 min for the native sGnRH (Gothilf and Zohar, 1991). Due to this still short residence time of GnRHa in circulation, the LH surge induced by a single GnRH injection is very short, ranging from a few hours to a few days (Zohar, 1988; Zohar et al., 1989a, 1995a; Crim et al., 1988b; Harmin and Crim, 1993). In most species studied, this duration is too short to induce complete FOM, ovulation and spawning, rendering the single GnRHa injection rather inefficient for inducing spawning. For example, two injections of GnRHa are necessary to induce ovulation in the walleye (*Stizostedion vitreum*) (Pankhurst et al., 1986), yellow perch (*Perca flavescens*) (Dabrowski et al., 1994), European seabass (*Dicentrarchus labrax*) (Carrillo et al., 1995), the Asian bass barramundi (*Lates calcarifer*) (Almendras et al., 1988) and most salmonids (Donaldson and Hunter, 1983; Mylonas et al., 1992; Slater et al., 1994).

Moreover, in some species or under certain conditions, for instance if the water temperature is very low or the fish are not in a very advanced stage of gonadal development, even two injections of GnRHa are not enough to induce ovulation. A good example of the ineffectiveness of the double injection protocol to induce ovulation is the striped bass, a species that completes vitellogenesis in culture but rarely undergoes FOM (Fig. 2). Under ambient water temperature (7–18°C), two GnRHa injections spaced 3 days apart resulted in elevations of plasma GnRHa for at least 7 days in post-vitellogenic females. Plasma LH, E_2 and T increased during the same time, following the profile of the injected GnRHa. Once GnRHa was cleared from the circulation, plasma LH decreased significantly and plasma E_2 and T returned to pre-treatment levels. There was no plasma elevation of 17,20β,21-trihydroxy-4-pregnen-3-one (17,20β,21-P), the maturation-inducing steroid in striped bass (Sullivan et al., 1997), and although 60% of the females initiated the early stages of FOM, none progressed further than the peripheral germinal vesicle stage. Obviously, more than two consecutive GnRHa injections are necessary to support a slow FOM in striped bass, indicating that long-term GnRHa-delivery systems can be important tools in controlling maturation of this species.

3.4.1. Types of GnRHa-delivery systems

Over the last 20 years, a variety of GnRHa-delivery systems have been developed and tested in cultured fishes for the control of FOM, ovulation and spermiation (Table 3). The first such delivery system was prepared using cholesterol and was tested in Atlantic salmon (Weil and Crim, 1983). Varying the percentage of the cholesterol in the matrix with the addition of cellulose can produce "slow" and "fast" releasing implants (Carolsfeld et al., 1988; Sherwood et al., 1988). The fast implants release GnRHa for days and can maintain high plasma GtH levels for at least 8 days, whereas the slow implants release for weeks and can maintain elevated plasma GtH levels for at least 8 weeks (Crim et al., 1983b). Cholesterol implants are prepared as solid, cylindrical pellets (3 mm in diameter) and are implanted intramuscularly using an implanter or a scalpel. This GnRHa-delivery system is easy to fabricate and relatively inexpensive, but the

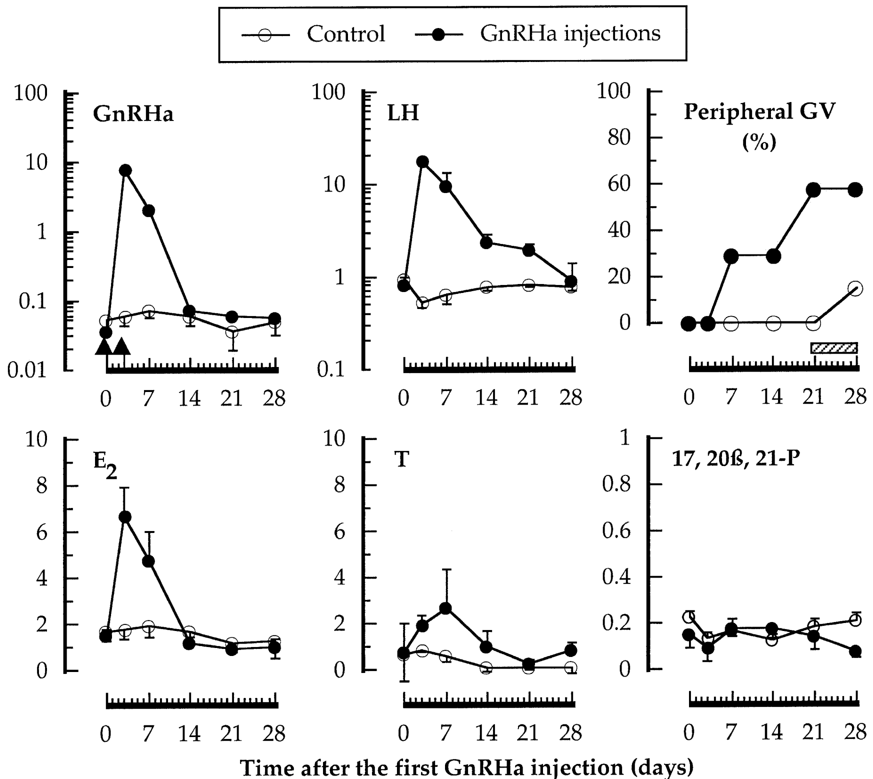

Fig. 2. Mean (±s.e.m.) plasma hormone levels (ng ml^{-1}) and cumulative % oocyte maturation of cultured striped bass ($n = 7$) after two injections (arrows) of GnRHa (15 μg kg^{-1}) or saline during the spawning season (April). Fish (4–8 kg) were maintained in a flow-through system supplied with Chesapeake Bay water (4–2 g l^{-1} salinity; 7–18°C) and had a mean oocyte diameter of 850 (±20) μm at the start of the experiment. With the exception of plasma 17,20β,21-P, all hormone profiles were significantly changed by the GnRHa treatment (ANOVA, $P \leq 0.05$). The horizontal hatched bar indicates the time when atretic oocytes could be seen in the biopsies. Most GnRHa-injected females reached the peripheral GV stage, but only one ovulated within the duration of the study.

GnRHa release from the pellets seems to be extremely variable (Carolsfeld et al., 1988), probably because each implant is prepared individually (Sherwood et al., 1988).

The next type of GnRHa-delivery system was fabricated in the form of microspheres (5–200 μm in diameter), using co-polymers of lactic acid and glycolic acid (LGA). This biodegradable material was originally used for surgical sutures, and was later evaluated as a drug-delivery matrix (Lewis, 1990). The microspheres are produced by the double emulsion, solvent evaporation method (Okada et al., 1994a,b), where the GnRHa is dissolved in microscopic water droplets, which are in turn entrapped in a matrix of LGA. For treatment, the microspheres are suspended in a viscous vehicle and are injected into the muscle using an 18-gauge needle. The release from the microspheres is immediate and can last for a few months, depending on the ratio of lactic acid:glycolic acid and the length, i.e., molecular weight, of the polymer (Chasin and Langer, 1990).

Table 3
Representative applications of sustained-release GnRHa-delivery systems for the induction of final maturation and spawning of male (M) and female (F) broodstocks

Species	Common name	Sex	Delivery system[a]	Reference
Alosa sapidissima	America shad	M	EVAc	(Mylonas et al., 1995b)
Chanos chanos	milkfish	F	Osmo	(Marte et al., 1987)
		F	Chol	(Lee et al., 1986)
Dicentrarchus labrax	European seabass	M	EVAc, Fad-sa	(Sorbera et al., 1996)
Epinephelus aeneus	white grouper	F	EVAc	(Hassin et al., 1997)
Lates calcarifer	barramundi	M/F	Osmo, Chol	(Almendras et al., 1988)
Morone chrysops	white bass	F	EVAc	(Mylonas et al., 1996)
		M	Fad-sa	(Mylonas et al., 1997a)
M. saxatilis	striped bass	F	EVAc	(Mylonas et al., 1998b)
		F	Chol	(Hodson and Sullivan, 1993)
		M	EVAc, Fad-sa	(Mylonas et al., 1997c)
Mugil cephalus	grey mullet	F	EVAc, Fad-sa	(de Monbrison et al., 1997)
Oncorhynchus kisutch	coho salmon	F	EVAc	(Zohar et al., 1990b)
O. mykiss	rainbow trout	F	LGA	(Breton et al., 1990)
		F	Chol	(Crim et al., 1983b)
Paralichthys lethostigma	Southern flounder	F	Chol	(Berlinsky et al., 1996)
Plecoglossus altivelis	ayu	F	Met	(Hirose et al., 1990)
Pleuronectes americanus	winter flounder	F	Chol	(Harmin and Crim, 1992)
Pleu. ferrugineus	yellowtail flounder	F	Fad-sa	(Larsson et al., 1997)
		M	Fad-sa	(Clearwater and Crim, 1998)
Salmo salar	Atlantic salmon	M/F	Chol	(Crim et al., 1983a)
		M/F	Fad-sa	(Mylonas et al., 1995a)
		M	EVAc	(Zohar, 1996)
Sparus aurata	gilthead seabream	F	EVAc, LGA	(Zohar et al., 1990b)
		F	LGA	(Barbaro et al., 1997)
Pagrus major	red seabream	M/F	Chol	(Matsuyama et al., 1995)
P. pagrus	red porgy	F	Fadsa	Present study
Takifugu rubripes	tiger puffer	F	Chol	(Chuda et al., 1997)
Umbrina cirrosa	shi drum	F	Fad-sa	(Mylonas et al., 2000)

[a]Chol = cholesterol/cellulose; EVAc = poly[ethylene–vinyl acetate]; Fad-sa = poly[fatty acid dimer-sebasic acid]; LGA = poly[lactide-glycolide]; Met = poly[methacrylate]; Osmo = osmotic pump.

Most of the LGA preparations used for spawning induction in fish are commercially available preparations (Zohar, 1988; Zohar et al., 1990b; Breton et al., 1990; Chang et al., 1995; Barbaro et al., 1997), which are used for fertility control in humans and livestock (Favero et al., 1995; Barbieri and Hornstein, 1999) or treatment of gonadotropin-related tumors in humans (Emons and Schally, 1994). Recently, LGA delivery systems for GnRHa have been developed specifically for fish, thereby taking into account the low body temperature of fish relative to mammals. These GnRHa-delivery systems have been used successfully to induce FOM and ovulation in the gilthead seabream (Zohar et al., 1995a), Pacific and Atlantic salmon (Mylonas et al., 1993; Zohar, 1996), and various other cultured fishes. Another form of biodegradable microspheres has been produced using polyanhydride co-polymers, specifically a co-polymer of fatty acid dimer and sebasic acid (Fad-sa). These microspheres are also produced

using the double emulsion, solvent evaporation process, and can release GnRHa for at least 8 weeks (Mylonas et al., 1995a). The greatest advantage of biodegradable, microspheric delivery systems is that the same preparation can be used to treat fish varying in size from a few grams to many kg. This can be done because the microspheres are suspended in vehicle and are administered on a volume to weight basis. Also, since over time the microspheres degrade to their monomer constituents, which are all natural products, e.g., lactic acid, glycolic acid, sebasic acid, broodstock retired from production can be consumed for food without any concerns of harmful residual chemicals.

The last type of GnRHa-delivery system used for spawning induction is prepared in the form of a solid, monolithic implant, using a non-degradable co-polymer of Ethylene and Vinyl Acetate (EVAc) (Rhine et al., 1980a). In this delivery system, the GnRHa is mixed with an inert bulking agent, and the mixture is entrapped by the EVAc matrix. Upon application, the inert matrix dissolves, carrying with it the active compound (Brown et al., 1986), in this case the GnRHa. The release kinetics of the EVAc delivery system can be controlled by varying the molecular weight of the polymer, the percent loading of the inert matrix, the type of matrix (hydrophobic vs. hydrophilic), the shape of the implants (sphere vs. disk) or by applying a coating to the implant (Rhine et al., 1980a,b; Hsu and Langer, 1985; Langer, 1991). The EVAc implants developed for fish release GnRHa for periods from 2 to 5 weeks (Zohar et al., 1990b; Zohar, 1996; Mylonas et al., 1998b). The EVAc implants are fabricated as disks 2 mm in diameter and are administered intramuscularly using an implanter. Unlike the biodegradable microspheres and similar to the cholesterol pellets, EVAc delivery systems have a long shelf-life and can maintain their effectiveness for up to 3 years if stored desiccated at −20 C.

3.4.2. Induction of FOM, ovulation and spawning

Treatment of females with GnRHa-delivery systems during the post-vitellogenesis period induces significant elevations in plasma LH, which are maintained until some time after ovulation (Crim et al., 1988b; Zohar, 1988, 1996; Zohar et al., 1990b). The type of LH profile obtained in response to sustained treatment with GnRHa seems to vary between species. For example, in striped bass (Mylonas et al., 1998b) and white bass (Mylonas et al., 1997b), even though plasma GnRHa is high and constant in the plasma for 10–14 days after treatment with GnRHa-delivery systems, plasma LH increases gradually during this period, with a sharp rise during ovulation (Fig. 3). This profile is identical to the one observed in wild striped bass during the spawning period (Mylonas et al., 1997d). This observation may be explained by a changing sensitivity of the pituitary to stimulation by GnRH (Peter et al., 1987), perhaps reflecting the ability of the pituitary to release the appropriate amount of LH according to the stage of gonadal development.

Another possible profile after treatment with a GnRHa-delivery system is that LH may increase in the plasma immediately after implantation and then is maintained at high levels throughout the period when the fish are undergoing FOM and spawning. As a result, plasma LH profiles parallel those of the GnRHa released from the delivery systems (Zohar et al., 1990b; Zohar, 1996). This profile is representative of the gilthead

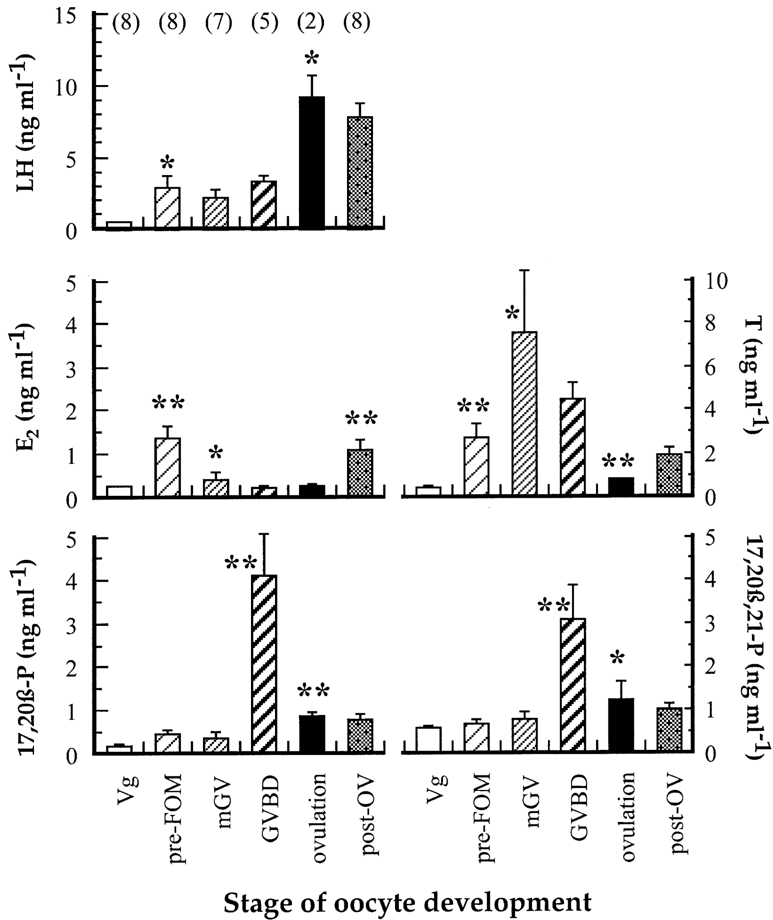

Fig. 3. Mean (± s.e.m.) plasma hormone levels of cultured female white bass at various stages of final oocyte maturation (FOM), induced by treatment with a GnRHa-implant (EVAc; 50 μg kg^{-1}). Data from individual fish were pooled according to maturation stage (sample sizes are shown in parentheses) as follows: after GnRHa treatment but before the onset of FOM (pre-FOM), germinal vesicle migration (mGV), after GV breakdown (GVBD) and after ovulation (post-OV). Sample means that were significantly different from the previous maturation stage are indicated by asterisks (ANOVA, DNMR; $^{*}P \leq 0.05$, $^{**}P \leq 0.01$) (from Mylonas et al., 1997b).

seabream, which undergoes repeated daily cycles of FOM, ovulation and spawning (Zohar et al., 1995a). Constantly elevated LH in the plasma is not the natural profile, however, since female gilthead seabream undergoing daily spawnings without the use of external hormones have fluctuating LH levels (Gothilf et al., 1997). Plasma LH peaks 8 h before ovulation and decreases significantly (by 50%) 4 h after ovulation. Nevertheless, treatment of gilthead seabream with various types of GnRHa-delivery systems induces the characteristic daily cycle of FOM, ovulation and spawning for many weeks, which is observed in spontaneously spawning broodstocks (Zohar et al., 1995a).

Compared to the untreated controls, the fish produce higher numbers of eggs of similar quality, i.e., fertilization and hatching success (Barbaro et al., 1997).

One of the earliest applications of GnRHa-delivery systems have been in the synchronization of ovulation of cultured salmonids, especially Atlantic salmon (Crim et al., 1983b; Crim and Glebe, 1984; Zohar et al., 1990b). Since salmonids ovulate but do not spawn in culture conditions, their eggs must be stripped artificially. Synchronization of ovulation makes egg collection easier and more efficient for the hatchery, and limits repeated and stressful checking of the females in order to determine the time of ovulation. A GnRHa-delivery system can be administered up to 6 weeks prior to the anticipated date that females in a particular population ovulate, and between 80% and 100% of the fish can be expected to ovulate within 2 weeks after treatment (Crim et al., 1983a; Crim and Glebe, 1984; Zohar et al., 1990b; Goren et al., 1995). The efficacy of the delivery systems in inducing ovulation weeks before the natural time in salmonids has led to the use of this method in the Pacific salmon industry for preventing egg losses due to pre-spawning mortalities. Use of GnRHa-delivery systems does not compromise the quality of the eggs, as there are no differences in fertilization and hatching success between eggs obtained from treated or naturally ovulating fish (Zohar et al., 1990b; Breton et al., 1990; Goren et al., 1995; Haraldsson et al., 1993; Mylonas et al., 1993).

GnRHa-delivery systems have shown great potential in inducing multiple spawnings in marine species with asynchronous or multiple-batch group-synchronous ovarian development. In these fish, treatment with a single injection of GnRHa may initiate FOM and spawning, assuming the fish are in a very advanced stage of ovarian development, but once the GnRHa is cleared from the circulation, most of the fish cease spawning. Further spawnings require another GnRHa injection. For example, the European sea bass spawns three to four times during the annual reproductive season, which lasts 4–6 weeks (Carrillo et al., 1995). Treatment with two GnRHa injections over a 12 h period induces ovulation (Carrillo et al., 1995), but for further ovulations to occur more GnRHa injections must be administered. The sustained administration of GnRHa via EVAc implants or LGA microspheres induces the typical 3–4 spawns (Forniés et al., 2000). Another example is the gilthead seabream, in which only 20% of the fish treated with a single GnRHa injection initiate the normal daily cycle of spawning, whereas more than 80% of the females given GnRHa-delivery systems undergo daily cycles of FOM, ovulation and spawning for many weeks (Zohar, 1988; Zohar et al., 1995a; Barbaro et al., 1997). A less dramatic, but still significant effect of the GnRHa-delivery systems, is the induction of daily spawnings in the Mediterranean red porgy (*Pagrus pagrus*). Treatment with a GnRHa injection resulted in an increase in the amount of fertile eggs produced 3 days after treatment, but much higher numbers of eggs were produced after day 3 in response to treatment with a microspheric GnRHa-delivery system (Fig. 4). A greater number of eggs was produced daily until day 31 after treatment.

Similarly, encouraging results have been obtained recently with a great number of flatfishes. Development of cultured broodstocks has only recently begun, thus most of the eggs produced for farming purposes are obtained from wild broodstocks. For example, in yellowtail flounder (*Pleu. ferrugineus*) treatment with either EVAc or Fad-sa GnRHa-delivery systems induced almost three times as many spawns compared

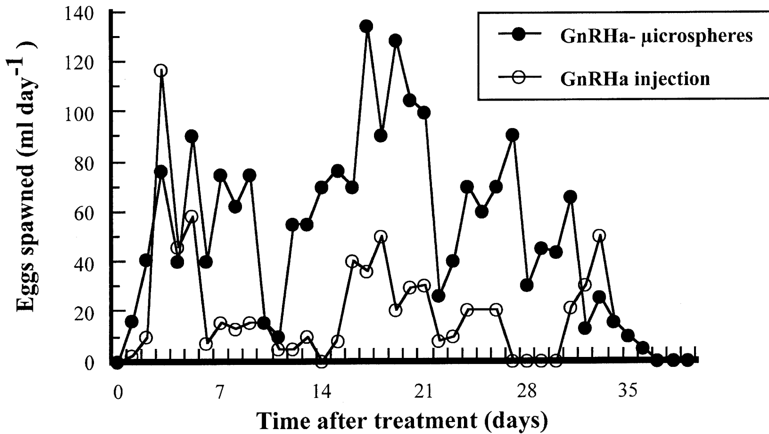

Fig. 4. Daily egg production (ml tank^{-1}) of red porgy (*Pagrus pagrus*) in response to treatment with GnRHa in the form of a single injection (15 μg kg^{-1}) or a sustained-release delivery system (Fad-sa microspheres; 50 μg kg^{-1}). The experiments were carried out at the Meneou Marine Aquaculture Research Station, Fisheries Department, Republic of Cyprus during the Spring spawning season of 1997. Each tank contained five females and seven males, of mean (±S.D.) weight of 1.4 (±0.3) and 1.1±(0.2) kg, respectively.

to controls, producing significantly larger amounts of eggs and of greater quality (Larsson et al., 1997). Also, in mature summer flounder (*Paralichthys dentatus*) obtained from the wild, a single treatment with cholesterol pellets loaded with GnRHa induced eight consecutive ovulations in fish with oocytes > 500 μm (Berlinsky et al., 1997). Fish treated with carp pituitary extract (CPE) required three to five daily injections to ovulate and produced only a maximum of five ovulations, whereas untreated control fish did not show any signs of FOM. Finally, although wild North Sea plaice (*Pleu. platessa*) do spawn when moved to captivity, GnRHa released from coconut oil or methacrylate resin increased significantly the amount of eggs spawned during a 20 day period (Scott et al., 1999).

3.4.3. Enhancement of spermiation

Long-term treatment with GnRHa via delivery systems has proven effective in enhancing milt production in fish, and offers certain advantages over the use of acute treatments with either GtH preparations or GnRHas. First, single injection treatments often induce a transient elevation of seminal plasma production, with a much smaller increase in spermatozoa production (Clemens and Grant, 1965; Garcia, 1991). The result is that more milt is produced, but with a lower spermatocrit (Takashima et al., 1984; Garcia, 1991). This "thinning" effect is desirable in situations where, due to maintenance in captivity, fish produce milt with abnormally high spermatocrit (> 85%). However, in most cases, a simple increase of seminal fluid does not constitute a successful hormonal treatment. For example, in hatcheries producing hybrid striped bass, male white bass are stripped repeatedly of their milt over the course of a few days, in order to fertilize eggs obtained from striped bass females ovulating at different times. White bass are many times smaller than the striped bass females and produce a

relatively small volume of milt (Mylonas et al., 1997a). Because of their small size, and probably also due to the repeated handling, fish run out of milt after 2–3 days, referred to as "drying up" (Bayless, 1972). Treatment of fish with an hCG injection restores milt release, but the milt is extremely thin and contains mostly seminal fluid. Treatment with GnRHa-delivery systems, however, increases milt production significantly, without any decrease in sperm density, motility or fertilizing ability of the spermatozoa (Mylonas et al., 1997a).

In some cases, however, production of milt with very high sperm density can be a serious problem in cultured fish, especially in flatfish broodstocks. For example, in the North Sea plaice, milt from wild fish during the spawning season has a spermatocrit of < 60% and flows readily upon stripping. On the contrary, male fish maintained in captivity produce milt with abnormally high spermatocrit, in excess of 85%, which is also very viscous and sticky (Vermeirssen et al., 1998). This milt does not mix well with water and, obviously, cannot fertilize eggs. A similar observation was made in captive Atlantic halibut (*Hippoglossus hippoglossus*), where GnRHa-delivery systems induced thinning of the milt, making it appropriate for use in artificial fertilization procedures. Conversely, milt from control fish had very high viscosity and spermatocrit (Vermeirssen et al., 2000).

The second, and most serious, problem of single GnRHa injections is that they do not stimulate a long-term elevation of sperm production. A single injection of GnRHa induces an increase in sperm production lasting only for a few days. For example, in the rabbitfish (*Siganus guttatus*) milt production increased significantly 24 h after GnRHa injection, but returned to pre-treatment levels 48 h later (Garcia, 1991). Sustained elevation of sperm production was maintained for 5 days in carp by daily injections of GnRHa, but 3 days after the treatment was interrupted, milt volume decreased below pre-treatment levels (Takashima et al., 1984). In the winter flounder (*Pleu. americanus*) a single injection did not increase milt production, whereas two injections given 24 h apart induced a significant increase in total expressible milt (Harmin and Crim, 1993). Finally, in the Atlantic salmon, significant increases in the amount of expressible milt were produced by repeated injections of GnRHa every 2 days (Weil and Crim, 1983). The above results underscore the need for a sustained presence of GnRHa in the fish's circulation, in order to induce long-term increases in sperm production.

The potential of GnRHa-delivery systems to induce spermiation was examined initially in salmonids. Cholesterol pellets, EVAc implants, and LGA and Fad-sa microspheres have all been used successfully to advance the onset of spermiation or increase the amount of expressible milt in Atlantic salmon (Weil and Crim, 1983; Mylonas et al., 1995a; Zohar, 1996), brown trout (*S. trutta*) (Goren et al., 1995), rainbow trout (Breton et al., 1990), chinook salmon (*O. tshawystcha*) (Solar et al., 1995), coho salmon (*O. kisutch*) (Zohar et al., 1990b; Goren et al., 1995) and sockeye salmon (*O. nerka*) (P. Swanson, W. Dickhoff and Y. Zohar, unpublished data). Successful treatments with GnRHa-delivery systems have also been documented in temperate basses of the Moronidae family. For example, in the European seabass, a single injection of GnRHa induced significant increases in milt production for 7 days only, but treatment with two different types of GnRHa-delivery systems resulted in elevations of milt production for 28–35 days (Fig. 5). The sustained increase of milt

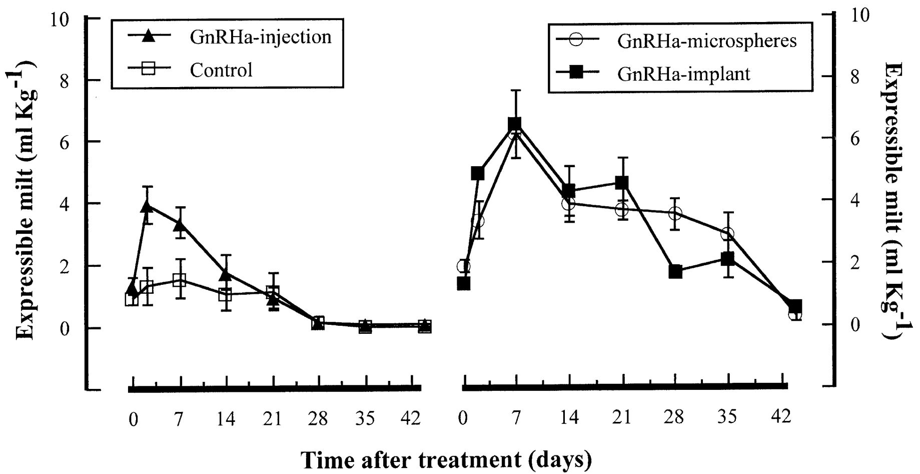

Fig. 5. Mean (±s.e.m.) total expressible milt production of European seabass (*Dicentrarchus labrax*) treated with GnRHa in various ways. Fish ($n = 8$) were treated with either a single GnRHa injection (25 μg kg^{-1}), GnRHa microspheres (Fad-sa; 50 μg kg^{-1}) or a GnRHa implant (EVAc; 50 μg kg^{-1}). Fish treated with GnRHa produced significantly higher amounts of milt than controls for the first 7 days after treatment (ANOVA, DNMR, $P \leq 0.05$). Moreover, fish treated with GnRHa-delivery systems continued to produce higher amounts of milt for up to 35 days after treatment (ANOVA, DNMR, $P \leq 0.05$). (from Sorbera et al., 1996).

production was not associated with decreases in sperm density or sperm motility (Sorbera et al., 1996). Similarly, in the striped bass both GnRHa-delivery systems induced immediate and long-term increases in milt production, lasting for 14 to 20 days (Mylonas et al., 1997c, 1998a), while spermatozoa production was maintained at high levels. As observed in females, the release of GnRHa from the delivery systems induced a sustained elevation of LH in the plasma (Weil and Crim, 1983; Mylonas et al., 1997c), which was then followed by increases in 17,20β-P or 11-ketotestosterone (Mylonas et al., 1997a, 1998a; Vermeirssen et al., 1998).

3.5. Sustained-release delivery systems for GtH

Although GnRHa-delivery systems have proven very effective in inducing FOM, ovulation and spawning in females, and enhancing the production of milt in the males, GnRHa therapies are not successful if used in broodfish which are not at the final stages of gonadal development. For example, no advancement of gonadal development was observed in the Pacific herring (*Clupea harengus pallasi*) when fish were treated in September during the early stages of vitellogenesis and spermatogenesis (Carolsfeld et al., 1988). On the contrary, FOM and spermiation was induced in February when fish were in advanced stages of gonadal development. Similarly, in the southern flounder (*Par. lethostigma*) (Berlinsky et al., 1996) and summer flounder (*Par. dentatus*) (Berlinsky et al., 1997), GnRHa-cholesterol pellets were ineffective in inducing FOM in females in early or mid-vitellogenesis, whereas daily injections of CPE induced oocyte

growth and, subsequently, FOM. In the few species where GnRHa has been shown to enhance vitellogenesis and spermatogenesis, it does so only after these processes are already well under way. A possible reason for the failure of GnRHa treatments to induce gonadal development before the advanced stages of gonadogenesis is that the pituitary is less responsive to GnRHa and, therefore, GtHs are either not synthesized or not released (Crim and Evans, 1983; Breton et al., 1998). The effectiveness of co-treatment of GnRHa with T in inducing GtH synthesis/release and gonadogenesis in some fishes (Lee et al., 1986b; de Monbrison et al., 1997; Henry et al., 1998) suggests that the pituitary must first be exposed to gonadal steroids before GnRHa can stimulate the release of GtH.

Therefore, for stimulating the early stages of gonadogenesis, GtH preparations may be more appropriate than GnRHa. Indeed, it has been well-demonstrated in the Japanese eel, a species that does not undergo any gonadal development in captivity, that treatment with hCG or purified salmon LH can induce complete gonadogenesis (Ohta et al., 1996, 1997). However, for the GtH treatment to be effective, the therapy must be given on a weekly basis over the course of many weeks. This is obviously cumbersome and potentially injurious to the fish. Obviously, a better approach could be the development of a delivery system that releases GtH in a sustained pattern, so that handling and treatment of the fish can be reduced to two or three treatments during the whole reproductive season. Such a delivery system has been developed using an emulsion of lipophilized gelatin (LG) with various lipid anhydrites (Sato et al., 1995, 1997). The LG emulsion was loaded with purified salmon LH (sLH) and was shown to produce a sustained elevation of plasma sLH for 24 days after treatment of Japanese eel. Compared to immature females treated weekly with sLH in saline, females given the LG emulsion attained a higher gonadosomatic index after 9 weeks and developed gonads with oocytes

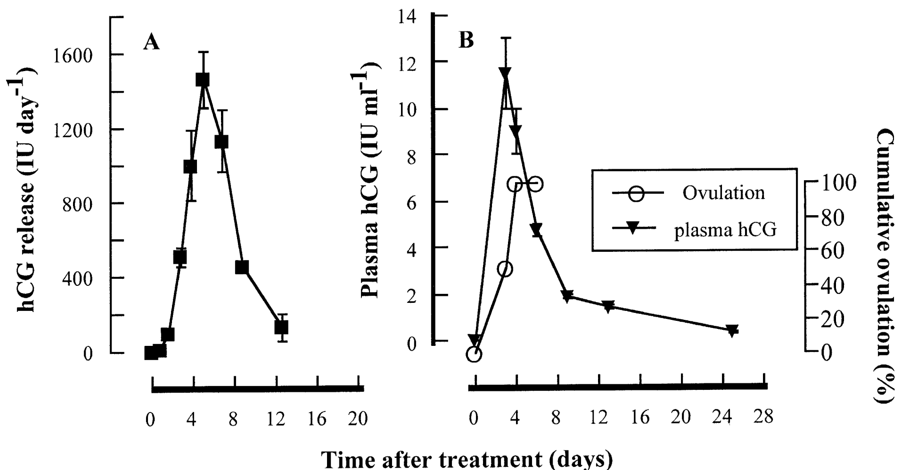

Fig. 6. Mean ($n = 2$) daily in vitro release of hCG from a poly[ortho-acetate] delivery system (A), and plasma hCG levels ($n = 2$) and cumulative % ovulation of striped bass ($n = 5$) treated with this delivery system at a dose of 3600 IU hCG kg^{-1} (B).

at the germinal vesicle migration stage (Sato et al., 1997). At the end of this experiment, females were induced to ovulate successfully, using a combination of sLH and 17,20β-P, the maturation-inducing steroid in the eel.

Another delivery system for GtH is being developed using a biodegradable, poly-orthoester polymer (Seymour et al., 1994), and is loaded with hCG in a powder form (C. Mylonas, Y. Zohar and J. Heller; unpublished data). The delivery system is prepared in the form of a very viscous ointment, which can be injected in the fish using a syringe and a 16-gauge needle. Preliminary data indicate that in vitro release of hCG from this system peaks in 5 days, but significant amounts continue to be released for 13 days (Fig. 6A). The in vivo release kinetics of this delivery system were examined in post-vitellogenic striped bass females. Plasma hCG levels were maximal 3 days after treatment and detectable amounts of hCG were still in the blood after 25 days (Fig. 6B). Final oocyte maturation and ovulation was induced in 100% of the females within 5 days after the treatment. Although further development is required in order to make it appropriate for long-term treatments for the induction of gonadal development, this hCG-delivery system can reduce the required application frequency to once every 3–4 weeks, instead of every week.

4. Next generation of spawning induction therapies

From the available research, it has become clear that the failure of captive (both cultured and wild-caught) fish to undergo FOM, ovulation, spermiation and spawning is the result of the lack of LH secretion from the pituitary. Consequently, most spawning induction related research and development efforts have focused on the use of exogenous GnRHs to trigger the release of LH and the chain of events leading to final gonadal development. However, it is also evident that the endocrine system upstream from the pituitary is impaired in captive fish, which adversely affects the normal functioning of the endogenous GnRH system in captive broodstocks. The next generation of spawning induction technologies will thus be based on understanding the nature of the captivity-induced alterations in the GnRH system, and on developing strategies to correct them. Such an approach requires a thorough fundamental investigation of the GnRH system in cultured species.

Since the first isolation and characterization of a piscine GnRH from the salmon brain (Sherwood et al., 1983), it has become widely accepted that most bony fish possess two forms of GnRH in their brains (reviewed by Sherwood et al., 1994; King and Millar 1995, 1997). These forms are the ubiquitous chicken (c) GnRH II, and a species-specific form: salmon(s) GnRH in salmonids, catfish GnRH in catfish species (Bogerd et al., 1992) and mammalian GnRH in eels and a number of other primitive bony fish (King et al., 1990). More recently, the presence of three native GnRHs has been demonstrated, for the first time in any vertebrate species, in the brain of the gilthead seabream (Powell et al., 1994; Gothilf et al., 1995a,b). Although two of these GnRH forms are those known previously: sGnRH and cGnRH II, the third was characterized as a novel GnRH and named seabream (sb) GnRH. A series of follow up studies demonstrated that the same three GnRHs are present in a number of other perciform fish: striped bass (Gothilf

et al., 1995a), tilapia (Weber et al., 1997), red seabream (Okuzawa et al., 1997), African cichlid and pumpkinseed (Powell et al., 1995), black seabream and striped knifejaw (Senthilkumaran et al., 1999). Therefore, it appears that the presence of three GnRHs is widespread in perciform fish, the largest order of teleosts that contains many species with reproductive dysfunctions in culture. For the three GnRHs, studying the presence in the pituitary (Gothilf et al., 1995a; Weber et al., 1997), localization in the brain (Gothilf et al., 1996; Okuzawa et al., 1997), LH release activity (Zohar et al., 1995a,b) and physiological changes in peptide and mRNA levels (Gothilf et al., 1997; Parhar and Sakuma, 1997; Holland et al., 1998; Senthilkumaran et al., 1999) has led to the conclusion that the novel sbGnRH is the principle endogenous LH releaser and the most physiologically-relevant form to induce FOM, ovulation and spawning in gilthead seabream and other perciform species. This conclusion suggests that sbGnRHa, which was completely overlooked until recently, is the GnRH form that should be targeted in future studies on the effects of confinement on the GnRH system and the failure of cultured fish to spawn in captivity. Moreover, future design of highly potent GnRH agonists for improved and more cost-effective spawning induction therapies should focus on agonists of the most physiologically-relevant GnRH (sbGnRH) or the most potent LH releaser (cGnRH II, Zohar et al., 1995a,b). Since both of these GnRH forms are present in the pituitary of fish undergoing final gonadal development (Holland et al., 1998), and a peak in their synthesis is observed 8 h before ovulation in gilthead seabream (Gothilf et al., 1997), a combined administration of sbGnRH and cGnRH II agonists should also be considered for more efficient induction of spawning.

Studying gene expression and peptide levels of the three GnRH forms in wild striped bass captured on their spawning grounds and in captive broodstocks, a clear effect of confinement on sbGnRH mRNA and peptide levels was recently observed (C. Steven and Y. Zohar, unpublished). These findings suggest that both synthesis and release of the most physiologically-relevant GnRH form is affected in captivity. The three GnRHs and their cDNAs were recently cloned from a number of perciform fishes (Gothilf et al., 1995b, 1996; White et al., 1995; Chow et al., 1998). Understanding the regulation of expression of these genes by environmental and endocrine factors may lead to future, simple strategies to manipulate the expression of specific GnRH genes, thereby overcoming the adverse effect of confinement on the GnRH system, and leading to successful completion of oogenesis and spermatogenesis. Finally, combining our understanding of the hormonal regulation of gametogenesis and of the GnRH genes with recent advancements in gene transfer technology will result, possibly, in the development of transgenic fish with GnRH genes driven by environmentally regulated promoters. Utilization of such fish will enable us to turn on and off the desired GnRH genes, thereby controlling the timing of gonadal development and quiescence.

5. Conclusions

Hormonal manipulations for the induction of FOM, ovulation, spermiation and spawning have made possible the control of reproductive processes of cultured fish, and have contributed significantly to the sophistication and expansion of the aquaculture

industry. Both GtH and GnRHa preparations are effective, but each one has its particular advantages and disadvantages. The recent expansion of the use of GnRHa-delivery systems in spawning induction protocols underscores the potential of this approach to become a universal method for the aquaculture industry. The discovery of multiple GnRHs in fish, the understanding of their roles in regulating final gonadal development, and the recognition that captivity adversely affects GnRH synthesis and release, pave the way for the design of novel, more potent, physiologically-compatible and affordable strategies to manipulate spawning in commercial broodstocks. However, it is important to stress that although endocrine tools can overcome, to a large degree, the "obstacles" that cultured fish have in reproducing successfully in captivity, hatchery managers should still make an effort to understand and replicate the environmental conditions that every species requires in order to undergo FOM, spermiation and spawning.

Acknowledgements

We would like to thank the many scientific and industry collaborators who participated in the development and testing of various hormonal manipulation therapies over the last decade. We would also like to acknowledge the Maryland and Massachusetts Sea Grant Programs, United States Department of Agriculture, National Science Foundation, the US–Israel Binational Agricutural R&D Fund and the Maryland Agricultural Experiment Station for funding various aspects of the research described in this paper.

References

Abellan, E., Basurco, B., 1999. Options Méditerranéennes, No. 24: Marine Finfish Species Diversification; Current Situation and Prospects in Mediterranean Aquaculture. C.I.H.E.A.M, Zaragosa, pp. 1–139.

Afonso, L.O.B., Iwama, G.K., Smith, J., Donaldson, E.M., 1999. Effects of the aromatase inhibitor Fadrozole on plasma sex steroid secretion and ovulation rate in female coho salmon, *Oncorhynchus kisutch*, close to maturation. Gen. Comp. Endocrinol. 113, 221–229.

Afonso, L.O.B., Iwama, G.K., Smith, J., Donaldson, E.M., 2000. Effects of the aromatase inhibitor Fadrozole on reproductive steroids and spermiation in male coho salmon (*Oncorhynchus kisutch*) during sexual maturation. Aquaculture 188, 175–187.

Almendras, J.M., Duenas, C., Nacario, J., Sherwood, N.M., Crim, L.W., 1988. Sustained hormone release: III. Use of gonadotropin releasing hormone analogues to induce multiple spawnings in sea bass, *Lates calcarifer*. Aquaculture 74, 97–111.

Alok, D., Krishnan, T., Talwar, G.P., Garg, L.C., 1993. Induced spawning of catfish, *Heteropneustes fossilis* (Bloch), using D-Lys^6 salmon gonadotropin-releasing hormone analog. Aquaculture 115, 159–167.

Alok, D., Pillai, D., Talwar, G.P., Garg, L.C., 1995. D-Lys^6 salmon gonadotropin-releasing hormone analogue-domperidone induced ovulation in *Clarias batrachus* (L.). Asian Fish. Sci. 8, 263–266.

Alvariño, J.M.R., Zanuy, S., Prat, F., Carrillo, M., Mañanos, E., 1992. Stimulation of ovulation and steroid secretion by LHRHa injection in the sea bass (*Dicentrarchus labrax*): effect of time of day. Aquaculture 102, 177–186.

Ayson, F.G., 1991. Induced spawning of rabbitfish, *Siganus guttatus* (Bloch) using human chorionic gonadotropin (HCG). Aquaculture 95, 133–137.

Ball, R.C., 1954. Use of pituitary material in the propagation of minnows. Prog. Fish-Cult. 17, 108–113.

Barbaro, A., Francescon, A., Bozzato, G., Merlin, A., Belvedere, P., Colombo, L., 1997. Induction of

spawning in gilthead seabream, *Sparus aurata* L., by long-acting GnRH agonist and its effects on egg quality and daily timing of spawning. Aquaculture 154, 349–359.

Barbieri, R.L., 1992. Clinical applications of GnRH and its analogues. Trends Endocrinol. Metab. 3, 30–34.

Barbieri, R.L., Hornstein, M.D., 1999. Assisted reproduction—in vitro fertilization success is improved by ovarian stimulation with exogenous gonadotropins and pituitary suppression with gonadotropin-releasing analogues. Endocr. Rev. 20, 249–252.

Battaglene, S.C., Selosse, P.M., 1996. Hormone-induced ovulation and spawning of captive and wild broodfish of the catadromous Australian bass, *Macquaria novemaculeata* (Steindachner), (Percichthyidae). Aqua. Res. 27, 191–204.

Battaglene, S.C., Talbot, R.B., 1994. Hormone induction and larval rearing of mulloway, *Argyrosomus hololepidotus* (Pisces: Sciaenidae). Aquaculture 126, 73–81.

Bayless, J.D., 1972. Artificial Propagation and Hybridization of Striped bass, *Morone saxatilis* (Walbaum). South Carolina Wildlife and Marine Resources Department, Columbia, pp. 1–186.

Berlinsky, D.L., King, W.V., Smith, T.I.J., Hamilton II, R.D., Holloway Jr., J., Sullivan, C.V., 1996. Induced ovulation of Southern flounder *Paralichthys lethostigma* using gonadotropin releasing hormone analogue implants. J. World Aquacult. Soc. 27, 143–152.

Berlinsky, D.L., William, K., Hodson, R.G., Sullivan, C.V., 1997. Hormone induced spawning of summer flounder *Paralichthys dentatus*. J. World Aquacult. Soc. 28, 79–86.

Billard, R., 1986. Spermatogenesis and spermatology of some teleost fish species. Reprod. Nutr. Dev. 26, 877–920.

Billard, R., 1989. Endocrinology and fish culture. Fish Physiol. Biochem. 7, 49–58.

Billard, R., Marcel, J., 1980. Stimulation of spermiation and induction of ovulation in pike (*Esox lucius*). Aquaculture 21, 181–195.

Billard, R., Reinaud, P., Hollebecq, M.G., Breton, B., 1984. Advancement and synchronization of spawning in *Salmo gairdneri* and *S. trutta* following administration of LRH-A combined or not with pimozide. Aquaculture 43, 57–66.

Bogerd, J., Li, K.W., Janssen-Dommerholt, C., Goos, H.J.Th., 1992. Two gonadotropin-releasing hormones from the African catfish (*Clarias gariepinus*). Biochem. Biophys. Res. Commun. 187, 127–134.

Branchaud, A., Gendron, A.D., 1993. Artificial spawing and rearing of the copper redhorse, *Moxosoma hubbsi* (Teleostei: Catostomidae). Can. Field Nat. 107, 279–282.

Breton, B., Weil, C., 1973. Effets du LH/FSH-RH synthetique et d'extraits hypothalamiques de carpe sur la secretion d'hormone gonadotrope in vivo chez la carp (*Cyprinus carpio* L.). C.R. Acad. Sci. Paris 27 (Serie D), 2061–2064.

Breton, B., Weil, C., Sambroni, E., Zohar, Y., 1990. Effects of acute versus sustained administration of GnRHa on GtH release and ovulation in the rainbow trout, *Oncorhyncus mykiss*. Aquaculture 91, 371–383.

Breton, B., Govoroun, M., Mikolajczyk, T., 1998. GTH I and GTH II secretion profiles during the reproductive cycle in female rainbow trout: relationship with pituitary responsiveness to GnRH-A stimulation. Gen. Comp. Endocrinol. 111, 38–50.

Bromage, N.R., Roberts, R.J., 1995. Broodstock Management and Egg and Larval Quality. Blackwell, Oxford, 424 pp.

Bromage, N., Jones, J., Randall, C., Thrush, M., Springate, J., Duston, J., Barker, G., 1992. Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (*Oncorhynchus mykiss*). Aquaculture 100, 141–166.

Brown, L., Siemer, L., Munoz, C., Edelman, E., Langer, R., 1986. Controlled release of insulin from polymer matrices: control of diabetes in rats. Diabetes 35, 692–697.

Carolsfeld, J., Sherwood, N.M., Kreiberg, H., Soawer, S.A., 1988. Induced sexual maturation of herring using GnRHa 'quick-release' cholesterol pellets. Aquaculture 70, 169–181.

Carrillo, M., Zanuy, S., Prat, F., Cerda, J., Ramos, J., Mañanos, E., Bromage, N., 1995. Sea bass (*Dicentrarchus labrax*). In: Bromage, N.R., Roberts, R.J. (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell, Oxford, pp. 138–168.

Caylor, R.E., Biesiot, P.M., Franks, J.S., 1994. Culture of cobia (*Rachycentron canadum*): cryopreservation of sperm and induced spawning. Aquaculture 125, 81–92.

Chang, J.P., Peter, R.E., 1983. Effects of pimozide and Des Gly^{10}, (D-Ala^{6}) luteinizing hormone releasing

hormone ethylamide on serum gonadotropin concentrations, germinal vesicle migration and ovulation in female goldfish, *Carassius auratus*. Gen. Comp. Endocrinol. 52, 30–37.

Chang, J.P., Peter, R.E., Crim, L.W., 1984. Effects of dopamine and apomorphine on gonadotropin release from the transplanted pars distalis in goldfish. Gen. Comp. Endocrinol. 55, 347–350.

Chang, C.F., Yueh, W.S., Lee, M.F., Schally, A.V., 1995. A microencapsulated analog of LH-RH accelerates maturation but without stimulating sex reversal in the protandrous black porgy, *Acanthopagrus schlegeli*. Reprod. Nutr. Dev. 35, 339–349.

Chasin, M., Langer, R., 1990. Biodegradable polymers as drug delivery systems. In: Swarbrick, J. (Ed.), Drugs and the Pharmaceutical Sciences, vol. 45, Marcel Dekker, New York, pp. 347–365.

Chow, M.M., Kight, K.E., Gothilf, Y., Alok, D., Stubblefield, J., Zohar, Y., 1998. Multiple GnRHs present in a teleost species are encoded by separate genes: analysis of the sbGnRH and cGnRH-II genes from the striped bass, *Morone saxatilis*. J. Mol. Endocrinol. 21, 277–289.

Chuda, H., Matsuyama, M., Ikeda, Y., Matsuura, S., 1997. Development of the maturation- and ovulation-induction method in cultured tiger puffer *Takifugu rubripes* by hormonal treatments. Bull. Jpn. Soc. Sci. Fish. 63, 728–733.

Clearwater, S.J., Crim, L.W., 1998. Gonadotropin releasing hormone-analogue treatment increases sperm motility, seminal plasma pH and sperm production in yellowtail flounder *Pleuronectes ferrugineus*. Fish Physiol. Biochem. 19, 349–357.

Clemens, H.P., Grant, F.B., 1965. The seminal thinning response in carp (*Cyprinus carpio*) and rainbow trout (*Salmo gairdneri*) after injections of pituitary extracts. Copeia 1965, 174–177.

Copeland, P.A., Thomas, P., 1989. Control of gonadotropin release in the Atlantic croaker (*Micropogonias undulatus*): evidence for lack of dopaminergic inhibition. Gen. Comp. Endocrinol. 74, 474–483.

Crim, L.W., Bettles, S., 1997. Use of GnRH analogues in fish culture. In: Fingerman, M., Nagabhushanam, R., Thompson, M.F. (Eds.), Recent Advances in Marine Biotechnology, Vol. 1: Endocrinology and Reproduction. Oxford and IBH Publishing, New Delhi, pp. 369–382.

Crim, L.W., Evans, D.M., 1983. Influence of testosterone and/or LHRHa on precocious sexual development in the juvenile rainbow trout. Biol. Reprod. 29, 137–142.

Crim, L.W., Glebe, B.D., 1984. Advancement and synchrony of ovulation in Atlantic salmon with pelleted LHRH analog. Aquaculture 43, 47–56.

Crim, L.W., Evans, D.M., Vickery, B.H., 1983a. Manipulation of the seasonal reproductive cycle of the landlocked Atlantic salmon (*Salmo salar*) by LHRH analogues administered at various stages of gonadal development. Can. J. Aquat. Fish. Sci. 40, 61–67.

Crim, L.W., Sutterlin, A.M., Evans, D.M., Weil, C., 1983b. Accelerated ovulation by pelleted LHRH analogue treatment of spring-spawning rainbow trout (*Salmo gairdneri*) held at low temperatures. Aquaculture 35, 299–307.

Crim, L.W., Nestor Jr., J.J., Wilson, C.E., 1988a. Studies of the biological activity of LHRH analogs in the rainbow trout, landlocked salmon, and the winter flounder. Gen. Comp. Endocrinol. 71, 372–382.

Crim, L.W., Sherwood, N.M., Wilson, C.E., 1988b. Sustained hormone release. II. Effectiveness of LHRH analog (LHRHa) administration by either single time injection or cholesterol pellet implantation on plasma gonadotropin levels in a bioassay model fish, the juvenile rainbow trout. Aquaculture 74, 87–95.

Dabrowski, K., Ciereszko, A., Ramseyer, L., Culver, D., Kestemont, P., 1994. Effects of hormonal treatment on induced spermiation and ovulation in the yellow perch (*Perca flavescens*). Aquaculture 120, 171–180.

De Leeuw, R., Van't Veer, C., Smit-Van Dijk, W., Goos, H.J.T., Van Oordt, P.G.W.J., 1988. Binding affinity and biological activity of gonadotropin-releasing hormone agonists in the African catfish, *Clarias gariepinus*. Aquaculture 71, 119–131.

de Monbrison, D., Tzchori, I., Holland, M.C., Zohar, Y., Yaron, Z., Elizur, A., 1997. Acceleration of gonadal development and spawning induction in the Mediterranean grey mullet, *Mugil cephalus*: preliminary studies. Israeli J. Aquacult.—Bamidgeh 49, 214–221.

Donaldson, E.M., 1973. Reproductive endocrinology of fishes. Am. Zool. 13, 909–927.

Donaldson, E.M., Hunter, G.A., 1983. Induced final maturation, ovulation and spermiation in cultured fishes. In: Hoar, W.S., Randall, D.J., Donaldson, E.M. (Eds.), Fish Physiology. Reproduction, vol. IXB, Academic Press, Orlando, FL, pp. 351–403.

Donaldson, E.M., Hunter, G.A., Dye, H.M., 1981. Induced ovulation in coho salmon (*Oncorhynchus kisutch*):

II. Preliminary study of the use of LH-RH and two high potency LH-RH analogues. Aquaculture 26, 129–141.

Drori, S., Ofir, M., Levavi-Sivan, B., Yaron, Z., 1994. Spawning induction in common carp (*Cyprinus carpio*) using pituitary extract or GnRH superactive analogue combined with metoclopramide: analysis of hormone profile, progress of oocyte maturation and dependence on temperature. Aquaculture 119, 393–407.

Elizur, A., Meiri, I., Rosenfeld, H., Zmora, N., Knibb, W.R., Zohar, Y., 1995. Seabream gonadotropins: sexual dimorphism in gene expression. In: Goetz, R., Thomas, P. (Eds.), Reproductive Physiology of Fish. Fish Symposium 95, Austin, Texas, pp. 13–15.

Emata, A.C., Eullaran, B., Bagarinao, T.U., 1994. Induced spawning and early life description of the mangrove red snapper, *Lutjanus argentimaculatus*. Aquaculture 121, 381–387.

Emons, G., Schally, A.V., 1994. The use of luteinizing hormone releasing hormone agonists and antagonists in gynaecological cancers. Hum. Reprod. 9, 1364–1379.

Favero, R.J., Cruz, L.C., Kesler, D.J., 1995. The effect of constant delivery of gonadotropin releasing hormone on fertility of cattle. Drug Dev. Ind. Pharm. 21, 2377–2384.

Fitzpatrick, M.S., Suzumoto, B.K., Schreck, C.B., Oberbilling, D., 1984. Luteinizing hormone-releasing hormone analogue induces precocious ovulation in adult coho salmon (*Oncorhynchus kisutch*). Aquaculture 43, 67–73.

Fontaine, M., 1975. Physiological mechanisms in the migration of marine and amphihaline fish. Adv. Mar. Biol. 13, 241–355.

Fontaine, M., 1976. Hormones and the control of reproduction in aquaculture. J. Fish. Res. Board. Can. 33, 922–939.

Fontaine, Y.A., Dufour, S., Tanguy, G., Khan, I.A., Cedard, L., Leloup-Hatey, J., 1984. Clairances metaboliques de la gonadotropine chorionique humaine (hCG) et de la gonadotropin de carpe (cGtH) chez un poisson teleosteen, l'anguille. Proceedings of the Colloque Physiologie des Poissons. INRA/IFREMER.

Fontenele, O., 1955. Injecting pituitary (hypophyseal) hormones into fish to induce spawning. Prog. Fish-Cult. 18, 71–75.

Forniés, M.A., Mañanos, E., Laureau, S., do Santos, A., Carrillo, M., Mylonas, C.C., Zohar, Y., Zanuy, S., 2000. Different delivery systems for the optimization of the induction of the spawn in sea bass (*Dicentrarchus labrax*). Proceedings of the Aqua 2000, Nice, France, p. 217.

Forster, J., 1999. Aquaculture chickens, salmon: a case study. World Aquacult. 30 (3), 33–40.

Fujino, M., Kobayashi, S., Obayashi, M., Shinagawa, S., Fukuda, T., Kitada, C., Nakayama, R., Yamazaki, I., White, W.F., Rippel, R.H., 1972. Structure-activity relationships in the C-terminal part of luteinizing hormone releasing hormone. Biochem. Biophys. Res. Commun. 49, 863–869.

Garcia, L.M.B., 1991. Spermiation response of mature rabbitfish, *Siganus guttatus* Bloch, to luteinizing hormone-releasing hormone analogue (LHRHa) injection. Aquaculture 97, 291–299.

Garcia, L.M.B., 1996. Bioactivity of stored luteinizing hormone-releasing analogue (LHRHa) in sea bass, *Lates calcarifer* Bloch. J. Appl. Ichthyol. 12, 91–93.

Gillet, C., Breton, B., Mikolajczyk, T., 1996. Effects of GnRHa and pimozide treatments on the timing of ovulation and on egg quality in Arctic charr (*Salvelinus alpinus*) at 5 and 10°C. Aquat. Living Resour. 9, 257–263.

Gissis, A., Levavi-Sivan, B., Rubin-Kedem, H., Ofir, M., Yaron, Z., 1991. The effect of gonadotropin releasing hormone superactive analog and dopamine antagonists on gonadotropin level and ovulation in tilapia hybrids. Israeli J. Aquacult.—Bamidgeh 43, 123–136.

Gordin, H., Zohar, Y., 1978. Induced spawning of *Sparus aurata* (L.) by means of hormone treatments. Ann. Biol. Anim. Biochim. Biophys. 18, 985–990.

Goren, A., Zohar, Y., Fridkin, M., Elhanati, E., Koch, Y., 1990. Degradation of gonadotropin releasing hormone in the gilthead seabream, *Sparus aurata*: I. Cleavage of native salmon GnRH, mammalian LHRH and their analogs in the pituitary. Gen. Comp. Endocrinol. 79, 291–305.

Goren, A., Gustafson, H., Doering, D., 1995. Field trials demonstrate the efficacy and commercial benefit of a GnRHa implant to control ovulation and spermiation in salmonids. In: Goetz, F.W., Thomas, P. (Eds.), Reproductive Physiology of Fish, 1995. Fish Symposium 95, Austin, Texas, pp. 99–101.

Gothilf, Y., Zohar, Y., 1991. Clearance of different forms of GnRH from the circulation of the gilthead seabream, *Sparus aurata*, in relation to their degradation and bioactivities. In: Scott, A.P., Sumpter, J.P.,

Kime, D.E., Rolfe, M.S. (Eds.), Reproductive Physiology of Fish. Fish Symposium 91, Sheffield, pp. 35–37.

Gothilf, Y., Elizur, A., Zohar, Y., 1995a. Three forms of gonadotropin-releasing hormon in gilthead seabream and striped bass: physiological and molecular studies. In: Goetz, F.W., Thomas, P. (Eds.), Reproductive Physiology of Fish, 1995. Fish Symposium 95, Austin, Texas, pp. 52–54.

Gothilf, Y., Chow, M., Elizur, A., Chen, T.T., Zohar, Y., 1995b. Molecular cloning and characterization of a novel gonadotropin-releasing hormone from gilthead seabream (*Sparus aurata*). Mar. Mol. Biol. Biotech. 4, 27–35.

Gothilf, Y., Munoz-Cueto, J.A., Sagrillo, C.A., Selmanoff, M., Chen, T.T., Kah, O., Elizur, A., Zohar, Y., 1996. Three forms of gonadotropin-releasing hormones in a perciform fish (*Sparus aurata*): cDNA characterization and brain localization. Biol. Reprod. 55, 636–645.

Gothilf, Y., Meiri, I., Elizur, A., Zohar, Y., 1997. Preovulatory changes in the levels of three gonadotropin-releasing hormone-encoding messenger ribonucleic acids (mRNAs), gonadotropin β-subunit mRNAs, plasma gonadotropin, and steroids in the female gilthead seabream, *Sparus aurata*. Biol. Reprod. 57, 1145–1154.

Habibi, H.R., Marchant, T.A., Nahorniak, C.S., Van Der Loo, H., Peter, R.E., Rivier, J.E., Vale, W.W., 1989. Functional relationship between receptor binding and biological activity for agonists of mammalian and salmon gonadotropin-releasing hormones in the pituitary of goldfish (*Carassius auratus*). Biol. Reprod. 40, 1152–1161.

Haraldsson, H., Sveinsson, T., Skulason, S., 1993. Effects of LHRHa treatments upon the timing of ovulation and upon egg and offspring quality in Arctic charr, *Salvelinus alpinus* (L.). Aquacult. Fish. Manage. 24, 145–150.

Harmin, S.A., Crim, L.W., 1992. Gonadotropin releasing-hormone analog (GnRH-A) induced ovulation and spawning in female winter flounder, *Pseudopleuronectes americanus* (Walbaum). Aquaculture 104, 375–390.

Harmin, S.A., Crim, L.W., 1993. Influence of gonadotropin hormone-releasing hormone analog (GnRH-A) on plasma sex steroid profiles and milt production in male winter flounder, *Pseudopleuronectes americanus* (Walbaum). Fish Physiol. Biochem. 10, 399–407.

Hasler, A.D., Meyes, R.K., Field, H.M., 1940. The use of hormones for the conservation of muskellunge, *Esox masquinongy immaculatus* Garrard. Copeia 1, 43–46.

Hassin, S., de Monbrison, D., Hanin, Y., Elizur, A., Zohar, Y., Popper, D.M., 1997. Domestication of the white grouper, *Epinephelus aeneus*: 1. Growth and reproduction. Aquaculture 156, 305–316.

Head, W.D., Watanabe, W.O., Ellis, S.C., Ellis, E.P., 1996. Hormone-induced multiple spawning of captive Nassau grouper broodstock. Prog. Fish-Cult. 58, 65–69.

Henry, J.C., McLean, E., Mayer, I., Donaldson, E.M., 1998. Inductions of precocios maturation in masculinized Atlantic salmon by treatment with sustained-release LHRH and testosterone. Aquacult. Int. 6, 261–268.

Hirose, K., Kagawa, H., Yoshida, M., Kumakura, M., Yamanaka, H., 1990. Application of LHRH copolymer pellet for induction of final oocyte maturation and ovulation in ayu *Plecoglossus altivelis*. Bull. Jpn. Soc. Sci. Fish. 56, 1731–1734.

Hodson, R., Sullivan, C.V., 1993. Induced maturation and spawning of domestic and wild striped bass, *Morone saxatilis* (Walbaum), broodstock with implanted GnRH analogue and injected hCG. Aquacult. Fish. Manage. 24, 389–398.

Holland, M.C.H., Gothilf, Y., Meiri, I., King, J.A., Okuzawa, K., Elizur, A., Zohar, Y., 1998. Levels of the native forms of GnRH in the pituitary of the gilthead seabream, *Sparus aurata*, at several characteristic stages of the gonadal cycle. Gen. Comp. Endocrinol. 112, 394–405.

Houssay, B.A., 1930. Accion sexual de la hipofisis en los peces y reptiles. Rev. Soc. Arg. Biol. 106, 686–688.

Hsu, T.T.P., Langer, R., 1985. Polymers for the controled release of macromolecules: Effect of molecular weight of ethylene–vinyl acetate copolymer. J. Biomed. Mater. Res. 19, 445–460.

Hunter, G.A., Donaldson, E.D., Dye, H.M., 1981. Induced ovulation in coho salmon (*Oncorhynchus kisutch*): I. Further studies on the use of salmon pituitary preparations. Aquaculture 26, 117–127.

Katzman, P.A., Doisy, E.A., 1932. Preparation of extracts of the anterior pituitary-like substance of urine of pregnancy. J. Biol. Chem. 98, 739–754.

Kime, D.E., Dolben, I.P., 1985. Hormonal changes during induced ovulation of the carp, *Cyprinus carpio*. Gen. Comp. Endocrinol. 58, 137–149.

King, J.A., Millar, R.P., 1995. Evolutionary aspects of gonadotropin-releasing hormone and its receptor. Cell. Mol. Neurobiol. 15, 5–23.

King, J.A., Millar, R.P., 1997. Coordinated evolution of GnRHs and their receptors. In: Parhar, I.S., Sakuma, Y. (Eds.), GnRH Neurons: Gene to Behavior. Brain Shuppan Publishing, Tokyo, pp. 51–78.

King, J.A., Dufour, S., Fontaine, Y.A., Millar, R.P., 1990. Chromatographic and immunological evidence for mammlian GnRH and chicken GnRH II in eel (*Anguilla anguilla*) brain and pituitary. Peptides 11, 506–514.

King, W.V., Thomas, P., Harrell, R.M., Hodson, R.G., Sullivan, C.V., 1994. Plasma levels of gonadal steroids during final oocyte maturation of striped bass, *Morone saxatilis* L. Gen. Comp. Endocrinol. 95, 178–191.

Kirk, R., 1987. A History of Marine Fish Culture in Europe and North America. Fishing News Books, Farnham, England, 192 pp.

Kjørsvik, E., Mangor-Jensen, A., Holmefjord, I., 1990. Egg quality in fishes. Adv. Mar. Biol. 26, 71–113.

Kohler, C.C., Sheehan, R.J., Habicht, C., Malison, J.A., Kayes, T.B., 1994. Habituation to captivity and controlled spawning of white bass. Trans. Am. Fish. Soc. 123, 964–974.

Kreiberg, H., Hunter, G.A., Donaldson, E.M., Clarke, W.G., Baker, I., 1987. Induced ovulation and spermiation in the Pacific herring (*Clupea harengus pallasi*) using salmon pituitary preparations and a synthetic gonadotropin-releasing hormone analogue. Aquaculture 61, 155–161.

Kucharczyk, D., Kujawa, R., Luczynski, M., Glogowski, J., Babiak, I., Wyszomirska, E., 1997. Induced spawning in bream, *Abramis brama* (L.), using carp and bream pituitary extract and hCG. Aquat. Res. 28, 139–144.

Kuo, C.M., 1995. Manipulation of ovarian development and spawning in grey mullet, *Mugil cephalus*. Israeli J. Aquacult.—Bamidgeh 47, 43–58.

Lam, T.J., 1982. Applications of endocrinology to fish culture. Can. J. Aquat. Fish. Sci. 39, 11–137.

Lam, T.J., 1983. Environmental influences on gonadal activity in fish. In: Hoar, W.S., Randall, D.J., Donaldson, E.M. (Eds.), Fish Physiology. Reproduction, Part B, vol. IX, Academic Press, New York, pp. 65–116.

Langer, R., 1991. Polymer implants for drug delivery in the brain. J. Controlled Release 16, 53–60.

Larsson, D.G.J., Mylonas, C.C., Zohar, Y., Crim, L.W., 1997. Gonadotropin releasing hormone-analogue (GnRH-A) advances ovulation and improves the reproductive performance of a cold-water batch-spawning teleost, the yellowtail flounder (*Pleuronectes ferrugineus*). Can. J. Aquat. Fish. Sci. 54, 1957–1964.

Le Gac, F., Blaise, O., Fostier, A., Le Bail, P.Y., Loir, M., Mourot, B., Weil, C., 1993. Growth hormone (GH) and reproduction: a review. Fish Physiol. Biochem. 11, 219–232.

Lee, C.S., Tamaru, C.S., Banno, J.E., Kelley, C.D., Bocek, A., Wyban, J.A., 1986. Induced maturation and spawning of milkfish, *Chanos chanos* Forsskal, by hormone implantation. Aquaculture 52, 199–205.

Lee, C.S., Tamaru, C.S., Miyamoto, G.T., Kelley, C.D., 1987. Induced spawning of grey mullet (*Mugil cephalus*) by LHRH-a. Aquaculture 62, 327–336.

Legendre, M., Linhart, O., Billard, R., 1996. Spawning and management of gametes, fertilized eggs and embryos in Siluroidei. Aquat. Living Resour. 9, 59–80.

Leu, M.-Y., Chou, Y.-H., 1996. Induced spawning and larval rearing of captive yellowfin porgy, *Acanthopagrus latus* (Houttuyn). Aquaculture 143, 155–166.

Lewis, D.H., 1990. Controlled release of bioactive materials from lactide/glycolide polymers. In: Chasin, M., Langer, R. (Eds.), Biodegradable Polymers as Drug Delivery Systems. Marcel Dekker, New York, pp. 1–41.

Lin, H.R., Kraak, G.V.D., Liang, J.-Y., Peng, C., Li, G.-Y., Lu, L.-Z., Zhou, X.-J., Chang, M.L., Peter, R.E., 1986. The effects of LHRH analogue and drugs which block the effects of dopamine on gonadotropin secretion and ovulation in fish cultured in China. In: Billard, R., Marcel, J. (Eds.), Aquaculture of Cyprinids. INRA, Paris, pp. 139–150.

Lin, H.R., Zhou, X.J., Van Der Kraak, G., Peter, R.E., 1991. Effects of gonadotropin-releasing hormone agonists and dopamine antagonists on gonadotropin secretion and ovulation in Chinese loach, *Paramisgurnus dabryanus*. Aquaculture 95, 139–147.

Linard, B., Anglade, I., Bennani, S., Salbert, G., Navas, J.M., Bailhache, T., Pakde, F., Jégo, P., Valotaire, Y., Saligaut, C., Kah, O., 1995. Some insights into sex steroid feedback mechanisms in the trout. In: Goetz,

F.W., Thomas, P. (Eds.), Reproductive Physiology of Fish, 1995. Fish Symposium 95, Austin, Texas, pp. 49–51.

Malison, J.A., Held, J.A., 1996. Reproduction and spawning in walleye (*Stizostedion vitreum*). J. Appl. Ichthyol. 12, 153–156.

Marchant, T.A., Chang, J.P., Nahorniak, C.S., Peter, R.E., 1989. Evidence that gonadotropin-releasing hormone also functions as a growth hormone releasing hormone in the goldfish. Endocrinology 124, 2509–2518.

Marte, C.L., Sherwood, N.M., Crim, L.W., Harvey, B., 1987. Induced spawing of maturing milkfish (*Chanos chanos* Forsskal) with gonadotropin-releasing hormone (GnRH) analogues administered in various ways. Aquaculture 60, 303–310.

Matsuyama, M., Takeuchi, H., Kashiwagi, M., Hirose, K., Kagawa, H., 1995. Induced gonadal development and spawning of immature red sea bream *Pagrus major* with LHRH-a administration in different ways during winter season. Fish. Sci. 61, 472–477.

Mayes, K.B., Rosenblum, P.M., Brandt, T.M., 1993. Raceway spawning of Florida largemouth bass: effects of acclimation time and hormone treatment on spawning success. Prog. Fish-Cult. 55, 1–8.

McLean, E., Parker, D.B., Warby, C.M., Sherwood, N.M., Donaldson, E.M., 1991. Gonadotropin release following oral delivery of luteinizing-releasing hormone and its superactive analogue (des-Gly^{10}[D-Ala^{6}] LHRH ethylamide) to 17β-oestradiol-primed coho salmon, *Oncorhynchus kisutch* (Walbaum). J. Fish Biol. 38, 851–858.

Migita, M., Matsumoto, J., Kinoshita, H., Sasaki, A., Ashikawa, I., 1952. Studies on the hypophyseal hormone of fish: I. Stimulating effect upon ovulation of trout. Bull. Jpn. Soc. Sci. Fish. 17, 25–31.

Miura, T., Yamauchi, K., Nagahama, Y., Takahashi, H., 1991a. Induction of spermatogenesis in male Japanese eel, *Anguilla japonica*, by a single injection of human chorionic gonadotropin. Zool. Sci. 8, 63–73.

Miura, T., Yamauchi, K., Takahashi, H., Nagahama, Y., 1991b. Hormonal induction of all stages of spermatogenesis in vitro in the male Japanese eel (*Anguilla japonica*). Proc. Natl. Acad. Sci. U. S. A. 88, 5774–5778.

Miyaki, K., Tachihara, K., Ebisu, R., Tsukashima, Y., Matsumura, Y., Fujita, S., Hayashita, G., Tabeta, O., 1992. Induction of ovarian maturation of the tiger puffer, *Takifugu rubripes* by gonadotropic hormone injections. Fisheries. Sci. 40, 439–442.

Munro, A.D., Scott, A.P., Lam, T.J., 1990. Reproductive Seasonality in Teleosts: Environmental Influences. CRC Press, Boca Raton, FL, 254 pp.

Mylonas, C.C., Hinshaw, J., Sullivan, C.V., 1991. Induced ovulation of brown trout (*Salmo trutta*) using GnRHa and T_3, and hormone treatment effects on progeny development. In: Scott, A.P., Sumpter, J.P., Kime, D.E., Rolfe, M.S. (Eds.), Proceedings of the Fourth International Symposium on Reproductive Physiology of Fish. Fish Symposium 91, Sheffield, p. 285.

Mylonas, C.C., Hinshaw, J.M., Sullivan, C.V., 1992. GnRHa-induced ovulation of brown trout (*Salmo trutta*) and its effects on egg quality. Aquaculture 106, 379–392.

Mylonas, C.C., Swanson, P., Woods III, L.C., Jonsson, E., Jonasson, J., Stefansson, S., Zohar, Y., 1993. GnRHa-induced ovulation and sperm production in striped bass, Atlantic and Pacific salmon using controlled release devices. Proceedings of the World Aquaculture Congress, Torremolinos, 3–7 June 1993, Spain.

Mylonas, C.C., Tabata, Y., Langer, R., Zohar, Y., 1995a. Preparation and evaluation of polyanhydride microspheres containing gonadotropin-releasing hormone (GnRH), for inducing ovulation and spermiation in fish. J. Controlled Release 35, 23–34.

Mylonas, C.C., Zohar, Y., Richardson, B.M., Minkinnen, S.P., 1995b. Induced spawning of wild American shad, *Alosa sapidissima*, using sustained administration of gonadotropin-releasing hormone analog (GnRHa). J. World Aquacult. Soc. 26, 240–251.

Mylonas, C.C., Magnus, Y., Gissis, A., Klebanov, Y., Zohar, Y., 1996. Application of controlled-release, GnRHa-delivery systems in commercial production of white bass × striped bass hybrids (sunshine bass), using captive broodstocks. Aquaculture 140, 265–280.

Mylonas, C.C., Gissis, A., Magnus, Y., Zohar, Y., 1997a. Hormonal changes in male white bass (*Morone chrysops*) and evaluation of milt quality after treatment with a sustained-release GnRHa-delivery system. Aquaculture 153, 301–313.

Mylonas, C.C., Magnus, Y., Gissis, A., Klebanov, Y., Zohar, Y., 1997b. Reproductive biology and endocrine regulation of final oocyte maturation of captive white bass. J. Fish Biol. 51, 234–250.

Mylonas, C.C., Scott, A.P., Vermeirssen, E.L.M., Zohar, Y., 1997c. Changes in plasma gonadotropin II and sex-steroid hormones, and sperm production of striped bass after treatment with controlled-release gonadotropin-releasing hormone agonist-delivery systems. Biol. Reprod. 57, 669–675.

Mylonas, C.C., Scott, A.P., Zohar, Y., 1997d. Plasma gonadotropin II, sex steroids, and thyroid hormones in wild striped bass (*Morone saxatilis*) during spermiation and final oocyte maturation. Gen. Comp. Endocrinol. 108, 223–236.

Mylonas, C.C., Woods III, L.C., Zohar, Y., 1997e. Cyto-histological examination of post-vitellogenesis and final oocyte maturation in captive-reared striped bass. J. Fish Biol. 50, 34–49.

Mylonas, C.C., Woods III, L.C., Thomas, P., Schulz, R.W., Zohar, Y., 1998a. Hormone profiles of captive striped bass (*Morone saxatilis*) during spermiation, and long-term enhancement of milt production. J. World Aquacult. Soc. 29, 379–392.

Mylonas, C.C., Woods III, L.C., Thomas, P., Zohar, Y., 1998b. Endocrine profiles of female striped bass (*Morone saxatilis*) during post-vitellogenesis, and induction of final oocyte maturation via controlled-release GnRHa-delivery systems. Gen. Comp. Endocrinol. 110, 276–289.

Mylonas, C.C., Georgiou, G., Stephanou, D., Atack, T., Afonso, A., Zohar, Y., 2000. Preliminary data on the reproductive biology and hatchery production of the shi drum (*Umbrina cirrosa*) in Cyprus. In: Basurco, B. (Ed.), Cahiers Options Méditerranéennes. Mediterranean Marine Aquaculture Finfish Species Diversification, C.I.H.E.A.M, Zaragoza, Spain, pp. 303–312.

Ngamvongchon, S., Pawaputanon, O., Leelapatra, W., Johnson, W.E., 1988. Effectiveness of an LHRH analogue for the induced spawning of carp and catfish in northeast Thailand. Aquaculture 74, 35–40.

Ohta, H., 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.

Ohta, H., Kagawa, H., Tanaka, H., Okuzawa, K., Hirose, K., 1996. Milt production in the Japanese eel *Anguilla japonica* induced by repeated injections of human chorionic gonadotropin. Fish. Sci. 62, 44–49.

Ohta, H., Kagawa, H., Tanaka, H., Okuzawa, K., Iimura, N., Hirose, K., 1997. Artificial induction of maturation and fertilization in the Japanese eel, *Anguilla japonica*. Fish Physiol. Biochem. 17, 163–169.

Okada, H., Doken, Y., Ogawa, Y., Toguchi, H., 1994a. Preparation of three-month depot injectable microspheres of leuprorelin acetate using biodegradable polymers. Pharm. Res. 11, 1143–1147.

Okada, H., Yamamoto, M., Heya, T., Inoue, Y., Kamei, S., Ogawa, Y., Toguchi, H., 1994b. Drug delivery using biodegradable microspheres. J. Control. Release 28, 121–129.

Ouzawa, K., Granneman, J., Bogerd, J., Goos, H.J.Th., Zohar, Y., Kagawa, H., 1997. Distinct expression of GnRH genes in the red seabream brain. Fish Physiol. Biochem. 17, 71–79.

Omeljaniuk, R.J., Shih, S.H., Peter, R.E., 1987. In vivo evaluation of dopamine receptor-mediated inhibition of gonadotropin secretion from the pituitary gland of the goldfish, *Carassius auratus*. J. Endocrinol. 144, 449–458.

Pagelson, G., Zohar, Y., 1992. Characterization of gonadotropin-releasing hormone (GnRH) receptors in the gilthead seabream (*Sparus aurata*). Biol. Reprod. 47, 1004–1008.

Palmer, D.D., Burrows, R.E., Robertson, O.H., Newman, H.W., 1954. Further studies on the reactions of adult blueback salmon to injected salmon and mammalian gonadotropins. Prog. Fish-Cult. 17, 99–107.

Pankhurst, N.W., Van der Kraak, G., 1997. Effects of stress on reproduction and growth of fish. In: Iwama, G.K., Pickering, A.D., Sumpter, J.P., Schreck, C.B. (Eds.), Fish Stress and Health in Aquaculture. Cambridge Univ. Press, Cambridge, pp. 73–93.

Pankhurst, N.W., van der Kraak, G., Peter, R.E., 1986. Effects of human chorionic gonadotropin, DES-GLY^{10} (D-ALA^{6}) LHRH-ethylamide and pimozide on oocyte final maturation, ovulation and levels of plasma sex steroids in the walleye (*Stizostedion vitreum*). Fish Physiol. Biochem. 1, 45–54.

Parhar, I.S., Sakuma, Y., 1997. Regulation of forebrain and midbrain GnRH neurons in juvenile teleosts. Fish Physiol. Biochem. 17, 81–84.

Parauka, F.M., Troxel, W.J., Chapman, F.A., McBay, L.G., 1991. Hormone-induced ovulation and artificial spawning of Gulf of Mexico sturgeon (*Acipenser oxyrhynchus desotoi*). Prog. Fish-Cult. 53, 113–117.

Peter, R.E., Yu, K.L., 1997. Neuroendocrine regulation of ovulation in fishes: basic and applied aspects. Rev. Fish Biol. Fish. 7, 173–197.

Peter, R.E., Nahorniak, C.S., Sokolowska, M., Chang, J.P., Rivier, J.E., Vale, W.W., King, J.A., Millar, R.P., 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., Omeljaniuk, R.J., Sokolowska, M., Shih, S.H., Billard, R., 1986. Interactions of catecholamines and GnRH in regulation of gonadotropin secretion in teleost fish. Recent Prog. Horm. Res. 42, 513–547.

Peter, R.E., Habibi, H.R., Marchant, T.A., Nahorniak, C.S., 1987. Vertebrate GnRH: phylogeny and structure–function relationships. Ann. N. Y. Acad. Sci. 519, 299–309.

Peter, R.E., Lin, H.R., Van Der Kraak, G., 1988. Induced ovulation and spawning of culture freshwater fish in China: advances in application of GnRH analogues and dopamine antagonists. Aquaculture 74, 1–10.

Peter, R.E., Habibi, H.R., Chang, J.P., Nahorniak, C.S., Yu, K.L., Huang, Y.P., Marchant, T.A., 1990. Actions of gonadotropin-releasing hormone (GnRH) in the goldfish. In: Epple, A., Scanes, C.G., Stetson, M.H. (Eds.), Progress in Comparative Endocrinology. Wiley-Liss, New York, pp. 393–398.

Peter, R.E., Lin, H.R., van der Kraak, G., Little, M., 1993. Releasing hormones, dopamine antagonists and induced spawning. In: Muir, J.F., Roberts, R.J. (Eds.), Recent Advances in Aquaculture. Blackwell Scientific, Oxford, pp. 25–30.

Pickford, G.E., Atz, J.W., 1957. The Physiology of the Pituitary Gland of Fishes. New York Zoological Society, New York, 613 pp.

Powell, J.F.F., Zohar, Y., Elizur, A., Park, M., Fischer, W.H., Craig, A.G., Rivier, J.F., Lovejoy, D.A., Sherwood, N.M., 1994. Three forms of gonadotropin-releasing hormone characterized from brains of one species. Proc. Natl. Acad. Sci. U. S. A. 91, 12081–12085.

Powell, J.F.F., Fischer, W.H., Park, M., Craig, A.G., Rivier, J.E., White, S.A., Francis, R.C.F., Fernald, R.D., Licht, P., Warby, C., Sherwood, N.M., 1995. Primary structure of a solitary form of gonadotropin-releasing hormone (GnRH) in cichlid pituitary: Three forms of GnRH in brain of cichlid and pumpkinseed fish. Regul. Pept. 57, 43–53.

Ramos, J., 1986a. Induction of spawning in common sole (*Solea solea* L.) with human chorionic gonadotropin (HCG). Aquaculture 56, 239–242.

Ramos, J., 1986b. Luteinizing hormone-releasing hormone analogue (LH-RHa) induces precocious ovulation in common sole (*Solea solea* L.). Aquaculture 54, 185–190.

Rees, R.A., Harrell, R.M., 1990. Artificial spawning and fry production of striped bass and hybrids. In: Harrell, R.M., Kerby, J.H., Minton, R.V. (Eds.), Culture and Propagation of Striped Bass and its Hybrids. American Fisheries Society, Bethesda, MD, pp. 43–72.

Rhine, W.D., Hsieh, D.S.T., Langer, R., 1980a. Polymers for sustained macromolecule release: procedures to fabricate reproducible delivery systems and control release kinetics. J. Pharm. Sci. 69, 265–269.

Rhine, W.D., Sukhatme, V., Hsie, D.S.T., Langer, R.S., 1980b. A new approach to achieve zero-order release kinetics from diffusion-controlled polymer matrix system. Controlled Release Bioact. Mater. 1980, 177–186.

Sakai, K., Nomura, M., Takashima, F., Oto, H., 1975. The over-ripening phenomenon of rainbow trout-II changes in the percentage of eyed eggs, hatching rate and incidence of abnormal alevins during the process of over-ripening. Bull. Jpn. Soc. Sci. Fish. 41, 855–860.

Sato, N., Kawazoe, I., Shiina, Y., Furukawa, K., Suzuki, Y., Aida, K., 1995. A novel method of hormone administration for inducing gonadal maturation in fish. Aquaculture 135, 51–58.

Sato, N., Kawazoe, I., Suzuki, Y., Aida, K., 1997. Development of an emulsion prepared with the lipophilized gelatin and its application for hormone administration in the Japanese eel *Anguilla japonica*. Fish Physiol. Biochem. 17, 171–178.

Schally, A.V., 1978. Aspects of hypothalamic regulation of the pituitary gland. Science 202, 18–28.

Scott, A.P., Witthames, P.R., Vermeirssen, E.L.M., Carolsfeld, J., 1999. Prolonged-release gonadotrophin-releasing hormone analogue implants enhance oocyte final maturation and ovulation, and increase plasma concentrations of sulfated C_{21} steroids in North Sea plaice. J. Fish Biol. 55, 316–328.

Senthilkumaran, B., Okuzawa, K., Gen, T., Ookura, T., Kagawa, H., 1999. Distribution and seasonal variations in levels of three native GnRHs in the brain and pituitary of perciform fish. J. Neuroendocrinol. 11, 181–186.

Seymour, L.W., Duncan, R., Duffy, J., Ng, S.Y., Heller, J., 1994. Poly(ortho ester) matrices for controlled release of the antitumour agent 5-fluorouracil. J. Controlled Release 31, 201–206.

Sherwood, N.M., Eiden, L., Brownstein, M., Spiess, J., Reveir, J., Vale, W., 1983. Characterization of a teleost gonadotropin-releasing hormone. Proc. Natl. Acad. Sci. U. S. A. 80, 2794–2798.

Sherwood, N.M., Crim, L.W., Carolsfeld, J., Walters, S.M., 1988. Sustained hormone release. I. Characteristics of in vitro release of gonadotropin-releasing hormone analogue (GnRH-A) from pellets. Aquaculture 74, 75–86.

Sherwood, N.M., Parker, D.B., McRory, J.E., Lescheid, D.W., 1994. Molecular evolution of growth hormone-releasing hormone and gonadotropin-releasing hormone. In: Sherwood, N.M., Hew, C.L. (Eds.), Fish Physiology. Molecular Endocrinology of Fish, vol. XIII, Academic Press, New York, pp. 3–66.

Sinha, V.R.P., Jhingran, V.G., Ganapati, S.V., 1974. A view on spawning of the Indian major carps. Arch. Hydrobiol. 73, 518–526.

Slater, C., Schreck, C.B., Swanson, P., 1994. Plasma profiles of the sex steroids and gonadotropins in maturing female spring chinook salmon (*Oncorhynchus tshawytscha*). Comp. Biochem. Physiol. 109A, 167–175.

Slater, C.H., Schreck, C.B., Amend, D.F., 1995. GnRHa injection accelerates final maturation and ovulation/spermiation of sockeye salmon (*Oncorhynchus nerka*) in both fresh and salt water. Aquaculture 130, 279–285.

Sokolowska, M., Peter, R.E., Nahorniak, C.S., Pan, C.H., Chang, J.P., Crim, L.W., Weil, C., 1984. Induction of ovulation in goldfish, *Carassius auratus*, by pimozide and analogues of LH-RH. Aquaculture 36, 71–83.

Solar, I.I., Baker, I.J., Donaldson, E.M., 1987. Effect of salmon gonadotropin and a gonadotropin releasing hormone analogue on ovarian hydration and ovulation in captive sablefish (*Anoplopoma fimbria*). Aquaculture 62, 319–325.

Solar, I.I., McLean, E., Baker, I.J., Sherwood, N.M., Donaldson, E.M., 1990. Short communication: induced ovulatioin of sablefish (*Anoplopoma fimbria*) following oral administration of desGly^{10}-(D-Ala^{6}) LH-RH ethylamide. Fish Physiol. Biochem. 8, 497–499.

Solar, I.I., Smith, J., Dye, H.M., MacKinlay, D.D., Zohar, Y., Donaldson, E.M., 1995. Induced ovulation of chinook salmon using a GnRHa implant: effect on spawning, egg viability and hormone levels. In: Goetz, F.W., Thomas, P. (Eds.), Reproductive Physiology of Fish, 1995. Fish Symposium 95, Austin, Texas, p. 144.

Sorbera, L.A., Mylonas, C.C., Zanuy, S., Carillo, M., Zohar, Y., 1996. Sustained administration of GnRHa increases milt volume without altering sperm counts in the sea bass. J. Exp. Zool. 276, 361–368.

Sower, S.A., Schreck, C.B., Donaldson, E.M., 1982. Hormone-induced ovulation of coho salmon (*Oncorhynchus kisutch*) held in seawater and fresh water. Can. J. Aquat. Fish. Sci. 39, 627–632.

Springate, J.R.C., Bromage, N.R., Elliot, J.A.K., Hudson, D.L., 1984. The timing of ovulation and stripping and their effects on the rates of fertilization and survival to eying, hatch and swim-up in the rainbow trout (*Salmo gairdneri*). Aquaculture 43, 313–322.

Stacey, N.E., 1984. Control of the timing of ovulation by exogenous and endogenous factors. In: Potts, G.W., Wooton, R.J. (Eds.), Fish Reproduction: Strategies and Tactics. Academic Press, London, pp. 207–222.

Stacey, N.E., Cook, A.F., Peter, R.E., 1979. Spontaneous and gonadotropin-induced ovulation in the goldfish, *Carassius auratus* L.: effects of external factors. J. Fish Biol. 15, 349–361.

Stevens, R.E., 1966. Hormone-induced spawning of striped bass for reservoir stocking. Prog. Fish-Cult. 28, 19–28.

Sukumasavin, N., Leelapatra, W., McLean, E., Donaldson, E.M., 1992. Orally induced spawing of Thai carp (*Puntius gonionotus* Bleeker), following co-administration of Gly^{10} (D-Arg^{6}) sGnRH ethylamide and domperidone. J. Fish Biol. 40, 477–479.

Sullivan, C.V., Bernard, M.G., Hara, A., Dickhoff, W.W., 1989. Thyroid hormones in trout reproduction: enhancement of GnRHa and partially purified salmon GtH-induced ovarian maturation in vivo and in vitro. J. Exp. Zool. 250, 188–195.

Sullivan, C.V., Berlinsky, D.L., Hodson, R.G., 1997. Reproduction. In: Harrel, R.M. (Ed.), Striped Bass and Other Morone Culture. Elsevier, New York, pp. 11–73.

Sumpter, J.P., Pottinger, T.G., Rand-Weaver, M., Campbell, P.M., 1994. The wide-ranging effects of stress in fish. In: Davey, K.G., Peter, R.E., Tobe, S.S. (Eds.), Perspectives in Comparative Endocrinology. National Research Council of Canada, Ottawa, pp. 535–538.

Suquet, M., Omnes, M.H., Normant, Y., Fauvel, C., 1992. Influence of photoperiod, frequency of stripping

and presence of females on sperm output in turbot, *Scophthalmus maximus* (L.). Aquacult. Fish. Manage. 23, 217–225.

Takashima, F., Weil, C., Billard, R., Crim, L.W., Fostier, A., 1984. Stimulation of spermiation by LHRH analogue in carp. Bull. Jpn. Soc. Sci. Fish. 50, 1323–1329.

Tamaru, C.S., Carlstrom Trick, C., FitzGerald, W.J., Ako, H., 1996. Induced final maturation and spawning of the marbled grouper *Epinephelus microdon* captured from spawning aggregations in the Republic of Palau, Micronesia. J. World Aquacult. Soc. 27, 363–372.

Tan-Fermin, J.D., Pagador, R.R., Chavez, R.C., 1997. LHRHa and pimozide-induced spawining of Asian catfish *Clarias macrocephalus* (Gunther) at different times during an annual reproductive cycle. Aquaculture 148, 323–331.

Thalathiah, S., Ahmad, A.O., Zaini, M.S., 1988. Induced spawning techniques practised at Batu Berendam, Melaka, Malaysia. Aquaculture 74, 23–33.

Thomas, P., Boyd, N., 1988. Induced spawning of spotted sea trout, red drum and orangemouth corvina (family: Sciaenidae) with luteinizing hormone-releasing hormone analog injection. Contrib. Mar. Sci. 30, 43–48.

Thomas, P., Boyd, N.W., 1989. Dietary administration of an LHRH analogue induces spawning of spotted seatrout (*Cynoscion nebulosus*). Aquaculture 80, 363–370.

Thorgaard, G.H., 1995. Biotechnological approaches to broodstock management. In: Bromage, N.R., Roberts, R.J. (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell Science, Oxford, pp. 76–93.

Trudeau, V.L., Peter, R.E., 1995. Functional interactions between neuroendocrine systems regulating GtH-II release. In: Goetz, F.W., Thomas, P. (Eds.), Reproductive Physiology of Fish, 1995. Fish Symposium 95, Austin, Texas, pp. 44–48.

Tucker, J.W., 1994. Spawning of captive serranid fishes: a review. J. World Aquacult. Soc. 25, 345–359.

Van der Kraak, G., Lin, H.R., Donaldson, E.M., Dye, H.M., Hunter, G.A., 1983. Effects of LH-RH and des-Gly^{10}-[D-Ala^{6}]-LH-RH ethylamide on plasma gonadotropin levels and oocyte maturation in adult female coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 49, 470–476.

Van der Kraak, G., Pankhurst, N.W., Peter, R.E., Lin, H.R., 1989. Lack of antigenicity of human chorionic gonadotropin in silver carp (*Hypophthalmichthys molitrix*) and goldfish (*Carassius auratus*). Aquaculture 78, 81–86.

Vermeirssen, E.L.M., Scott, A.P., Mylonas, C.C., Zohar, Y., 1998. Gonadotrophin-releasing hormone agonist stimulates milt fluidity and plasma concentrations of 17,20β-dihydroxylated and 5β-reduced, 3α-hydroxylated C21 steroids in male plaice (*Pleuronectes platessa*). Gen. Comp. Endocrinol. 112, 163–177.

Vermeirssen, E.L.M., Shields, R., Mazorra de Quero, C., Scott, A.P., 2000. Gonadotrophin-releasing hormone agonist raises plasma concentrations of progestogens and enhances milt fluidity in male Atlantic halibut (*Hippoglossus hippoglossus*). Fish Physiol. Biochem. 22, 77–87.

Von Ihering, R., 1937. A method for inducing spawning in fish. Prog. Fish-Cult. 34, 15–16.

Watanabe, W.O., Ellis, S.C., Ellis, E.P., Head, W.D., Kelley, C.D., Moriwake, A., Lee, C.S., Bienfang, P.K., 1995. Progress in controlled breeding of Nassau grouper (*Epinephelus striatus*) broodstock by hormone induction. Aquaculture 138, 205–219.

Watanabe, W.O., Ellis, E.P., Ellis, S.C., Chaves, J., Manfredi, C., Hagood, R.W., Sparsis, M., Arneson, S., 1998. Artificial propagation of mutton snapper *Lutjanus analis*, a new candidate marine fish species for aquaculture. J. World Aquacult. Soc. 29, 176–187.

Weber, G.M., Borski, R.J., Powell, J.F.F., Sherwood, N.M., Grau, E.G., 1995. In vivo and in vitro effects of gonadotropin-releasing hormone on prolactin in the tilapia *Oreochromis mossambicus*. Am. Zool. (Abstract), 34, 121A.

Weber, G.M., Powell, J.F.F., Park, M., Fischer, W.H., Craig, A.G., Rivier, J.E., Nanakorn, U., Parhar, I.S., Grau, E.G., Sherwood, N.M., 1997. Evidence that gonadotropin-releasing hormone (GnRH) functions as a prolactin-releasing factor in a teleost fish (*Oreochromis mossambicus*) and primary structures for three native GnRH molecules. J. Endocrinol. 132, 2360–2370.

Weil, C., Crim, L.W., 1983. Administration of LHRH analogues in various ways: effect on the advancement of spermiation in prespawning landlocked salmon, *Salmo salar*. Aquaculture 35, 103–115.

Weil, C., Breton, B., Sambroni, E., Zmora, N., Zohar, Y., 1991. Bioactivity of various forms of GnRH in relation to their resistance to degradation at the pituitary level in the rainbow trout, *Oncorhynchus mykiss*.

In: Scott, A.P., Sumpter, J.P., Kime, D.E., Rolfe, M.S. (Eds.), Reproductive Physiology of Fish. Fish Symposium 91, Sheffield, pp. 51–53.

Weil, C., Breton, B., Sambroni, E., Zmora, N., Zohar, Y., 1992. In vitro bioactivities of various forms of GnRH in relation to their resistance to degradation at the pituitary level in the rainbow trout, *Oncorhynchus mykiss*. Gen. Comp. Endocrinol. 87, 33–43.

White, S.A., Kasten, T.L., Bond, C.T., Adelman, J.P., Fernald, R.D., 1995. Three gonadotropin-releasing hormone genes in one organism suggest a novel role for an ancient peptide. Proc. Natl. Acad. Sci. U. S. A. 92, 8363–8367.

Woods III, L.C., Sullivan, C.V., 1993. Reproduction of striped bass, *Morone saxatilis* (Walbaum), broodstock: monitoring maturation and hormonal induction of spawning. Aquacult. Fish. Manage. 24, 211–222.

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

Zohar, Y., 1988. Gonadotropin releasing hormone in spawning induction in teleosts: basic and applied considerations. In: Zohar, Y., Breton, B. (Eds.), Reproduction in Fish: Basic and Applied Aspects in Endocrinology and Genetics. INRA Press, Paris, pp. 47–62.

Zohar, Y., 1989a. Fish reproduction: its physiology and artificial manipulation. In: Shilo, M., Sarig, S. (Eds.), Fish Culture in Warm Water Systems: Problems and Trends. CRC Press, Boca Raton, pp. 65–119.

Zohar, Y., 1989b. Endocrinology and fish farming: aspects in reproduction growth, and smoltification. Fish Physiol. Biochem. 7, 395–405.

Zohar, Y., 1996. New approaches for the manipulation of ovulation and spawning in farmed fish. Bull. Natl. Res. Inst. Aquacult., Suppl. 2, 43–48.

Zohar, Y., Gordin, H., 1979. Spawning kinetics in the gilthead sea bream after low doses of human chorionic gonadotropin (hCG). J. Fish Biol. 15, 665–670.

Zohar, Y., Billard, R., Weil, C., 1984. La reproduction de la daurade (*Sparus aurata*) et du bar (*Dicentrarchus labrax*): connaissance du cycle sexuel et controle de la gametogenese et de la ponte. In: Barnabe, G., Billard, R. (Eds.), L'Aquaculture du bar et des Sparides. INRA, Paris, pp. 3–24.

Zohar, Y., Goren, A., Tosky, M., Pagelson, G., Leibovitz, D., Koch, Y., 1989a. The bioactivity of gonadotropin-releasing hormones and its regulation in the gilthead seabream, *Sparus aurata*: in vivo and in vitro studies. Fish. Physiol. Biochem. 7, 59–67.

Zohar, Y., Tosky, M., Pagelson, G., Finkelman, Y., 1989b. Induction of spawning in the gilthead seabream, *Sparus aurata*, using [D-Ala^{6}-Pro^{9}NEt]-LHRH: comparison with the use of HCG. Israeli J. Aquacult.—Bamidgeh 41, 105–113.

Zohar, Y., Goren, A., Fridkin, M., Elhanati, E., Koch, Y., 1990a. Degradation of gonadotropin-releasing hormones in the gilthead seabream *Sparus aurata*: II. Cleavage of native salmon GnRH, mammalian LHRH and their analogs in the pituitary, kidney and liver. Gen. Comp. Endocrinol. 79, 306–331.

Zohar, Y., Pagelson, G., Gothilf, Y., Dickhoff, W.W., Swanson, P., Duguay, S., Gombotz, W., Kost, J., Langer, R., 1990b. Controlled release of gonadotropin releasing hormones for the manipulation of spawning in farmed fish. Controlled Release Bioact. Mater. 17, 51–52.

Zohar, Y., Doering, D., Langer, B., 1994. Application of controlled release technology in aquaculture. Controlled Release Bioact. Mater. 21, 110–112.

Zohar, Y., Harel, M., Hassin, S., Tandler, A., 1995a. Gilthead sea bream (*Sparus aurata*). In: Bromage, N.R., Roberts, R.J. (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell, Oxford, pp. 94–117.

Zohar, Y., Elizur, A., Sherwood, N.M., Rivier, J.F., Zmora, N., 1995b. Gonadotropin-releasing potencies of the three native forms of gonadotropin-releasing hormones present in the brain of gilthead seabream, *Sparus aurata*. Gen. Comp. Endocrinol. 97, 289–299.

Zonneveld, N., Rustidja, Viveen, W.J.A.R., Mudana, W., 1988. Induced spawning and egg incubation of the Asian catfish, *Clarias batrachus*. Aquaculture 74, 41–47.