4

Controlling fish reproduction in aquaculture

C. Mylonas, Hellenic Center for Marine Research, Greece; and Y. Zohar, University of Maryland Biotechnology Institute, USA

doi: **

Abstract: Industrial aquaculture is a new activity to most parts of the world and is looking for ways to establish a reliable and controlled system for the provision of seed stock for grow-out operations. Control of reproductive function can be achieved, in many fish species, by manipulating photoperiod, water temperature and spawning substrate. The reproductive cycle is separated in two phases – i.e., growth and maturation – which may be controlled by different reproductive hormones at the level of the pituitary and gonad. Although the first phase of reproductive development is concluded in captivity, the second stage of the reproductive cycle – i.e., oocyte maturation (OM) and ovulation in females, and spermiation in males – may require the employment of exogenous hormonal therapies. In some species, these hormonal manipulations are used only as a management tool to enhance the efficiency of egg production and facilitate hatchery operations, but in other fishes exogenous hormones are the only way to produce fertilized eggs at an industrial level. The reproductive cycle is controlled by the interactions of the hormones of the brain–pituitary–gonad axis. From the brain, gonadotropin releasing hormones (GnRHs) travel along neural axons and stimulate the gonadotroph cells of the pituitary to produce and secrete the two gonadotropins (GtH) follicle stimulating hormone (FSH) and luteinizing hormone (LH), which, in turn, act at the level of the gonad to induce steroidogenesis and the production of the androgens, estrogens and progestagens, which are the final effectors of reproductive function. Hormonal manipulations of reproductive function in cultured fishes have focused on the use of either exogenous LH preparations that act directly at the level of the gonad, or synthetic GnRH agonists (GnRHa) that act at the level of the pituitary to induce release of the endogenous LH stores, which, in turn act at the level of the gonad to induce steroidogenesis and the process of OM and spermiation.

Key words: reproduction, induced spawning, final oocyte maturation, spermiation.
4.1 Introduction

As an industrial agricultural activity, aquaculture is quite new to most parts of the world, with the exception of the extensive carp culture (family Cyprinidae) in Asia and the more recent intensification of salmonid production (Oncorhynchus and Salmo spp) in Europe and North America. In essence, it is only since the 1970s that a truly worldwide industry has developed, lately focusing on marine fishes (Kirk, 1987), and this industry is looking for ways to establish a reliable and controlled system for the provision of seed stock for grow-out operations. Control of reproductive function can be achieved, in many fish species, by manipulating photoperiod, water temperature and spawning substrate. However, the conclusion of the final stage of the reproductive cycle – i.e., oocyte maturation and ovulation in females, and spermiation in males – may require the employment of exogenous hormonal therapies. In some species, these hormonal manipulations are used only as a management tool to enhance the efficiency of egg production and facilitate hatchery operations, but in other fishes exogenous hormones are the only way to produce fertilized eggs at an industrial level. This chapter provides a brief description of the reproductive biology of fishes, followed by a description of the major problems encountered in culture, and the hormonal methods developed in the last few decades to address these dysfunctions. Some consideration is also given to future trends in the spawning induction technologies.

4.2 The fish reproductive cycle and its control

The reproductive cycle is separated in two phases – i.e., growth and maturation – which may be controlled by different reproductive hormones at the level of the pituitary and gonad (Fig. 4.1). In females, the first phase includes the growth of the primary oocytes and the accumulation of the yolk precursor, vitellogenin (vtg), in their cytoplasm (Fig. 4.2). At the completion of this phase, which is also referred to as vitellogenesis, the process of oocyte maturation (OM) includes both cytoplasmic and nuclear events that prepare the oocyte for its expulsion from the ovarian follicle (ovulation), its release to the environment during spawning and its fertilization by a single spermatozoon. In males, the growth phase is referred to as spermatogenesis and includes the mitotic proliferation of the spermatagonia into primary spermatocytes, their meiotic division into secondary spermatocytes and their differentiation to spermatids and flagellated spermatozoa. The process of maturation, better known as spermiation, includes the increase in seminal fluid production and the capacitation of the spermatozoa, which are now able to undergo forward motility once released in the water during spawning.

The reproductive cycle is controlled by the interactions of the hormones of the brain–pituitary–gonad axis (Fig. 4.1). From the brain, gonadotropin releasing hormones (GnRHs) produced in specific neuroendocrine cells
(Gothilf et al., 1996, 1997; Holland et al., 2001) travel along neural axons and are released immediately at synapses with the gonadotropic cells of the pituitary gland (Yaron et al., 2003). The synthesis and release of the GnRHS is controlled by environmental and nutritional parameters in such a way that reproduction takes place under optimal conditions (Yu et al., 1997). In response to GnRH stimulation, the gonadotrophs produce and secrete the two gonadotropins (GtH) follicle stimulating hormone (FSH) and luteinizing hormone (LH), which, in turn, act at the level of the gonad to induce steroidogenesis (Rosenfeld et al., 2007) and the production of the androgens, estrogens and progestagens, which are the final effectors of reproductive function.

In addition to the primary GnRH stimulatory system, neurons secreting dopamine (DA) exert an inhibitory action on both the brain (GnRH synthesis and release) and pituitary (down-regulation of GnRH stimulation pathways) (Peter et al., 1993; Peter and Yu, 1997; Yaron et al., 2003; Dufour et al., 2005; Levavi-Sivan et al., 2004). As a result, DA inhibits both basal LH secretion and GnRH-stimulated LH secretion from the pituitary. Although a dopaminergic inhibition on LH release has been demonstrated in all vertebrates, its intensity and temporal action may differ greatly among fishes. A strong dopaminergic inhibition of reproduction has been demonstrated in salmonids, cypriids, silurids, tilapia (Oreochromis spp.), freshwater eel (Anguilla anguilla) and grey mullet (Mugil cephalus) (Saligaut et al., 1999; Silverstein et al.,...

![Fig. 4.1](image-url) Schematic representation of the reproductive axis in fish, its major components and phases, and its environmental and endocrine control.
Fig. 4.2 Microphotographs of histological sections from ovaries. (A) Primary oocytes (po) of striped bass, having a centrally located germinal vesicle (gv) and peripheral nucleoli. (B) Oocytes at various stages of vitellogenesis from Atlantic bluefin tuna. Vitellogenic oocytes have various amounts of lipid droplets (l) and yolk vesicles (y). (C) Vitellogenic oocyte of white bass containing small numbers of lipid droplets. (D) Vitellogenic oocyte of striped bass with a very large percentage of lipid droplets. The periphery is occupied by cortical alveoli (ca). (E) Vitellogenic oocyte of American shad (Alosa sapidissima) showing no lipid droplets. (F) Oocyte of striped bass undergoing GV migration and lipid coalescence. (G) Oocyte of striped bass at the GV breakdown stage. (H) Ovulated egg from striped bass. Photographs are not in the same scale.
Controlling fish reproduction in aquaculture 113

1999; Yaron et al., 2003; Vidal et al., 2004). In contrast, a DA inhibitory system seems to be very weak or absent in most marine fishes (Copeland and Thomas, 1989; Zohar et al., 1995; Kumakura et al., 2003a; Prat et al., 2001).

4.2.1 Vitellogenesis, oocyte maturation and ovulation

At the onset of vitellogenesis, the ovigerous lamellae of the ovary contain nests of primary oocytes (Fig. 4.2a), which are arrested at prophase I (Guraya, 1986; Wallace and Selman, 1990; Selman et al., 1993). After a period referred to as primary growth, or previtellogenesis, during which the appearance of the ovarian follicle (i.e., the granulosa and theca layers) takes place, vitellogenesis, or the secondary growth, begins (Fig. 4.2b). Vitellogenesis is a hormone-dependent process and its immediate effector is the estrogen 17β-estradiol (E2), produced from the androgen testosterone (T) by the ovarian follicle in a two-cell process involving both the theca and granulosa cell layers (Nagahama, 1994). Regulation of steroidogenesis and E2 production at this time is controlled by the pituitary GtH, in some species by FSH and in others by LH (Rosenfeld et al., 2007). As the name implies, the major characteristic of vitellogenesis is the production of vtg, which takes place in the liver, and its sequestration in a pinocytosis-mediated process into the developing oocyte (Mommsen and Walsh, 1988; Tyler and Sumpter, 1996). At the start of vitellogenesis, the oocytes may be 150–250 μm in diameter (Fig. 4.2b) and, depending on fish species, at the end of the process the post-vitellogenic oocytes may have a diameter of 550 μm, as in shi drum (Umbrina cirrosa) (Mylonas et al., 2004a), 850 μm, as in striped bass (Morone saxatilis) (Mylonas et al., 1997e), 1400 μm, as in wreckfish (Polyprion americanus) (Fauvel et al., 2007), or up to 4 mm, as in Salmo and Oncorhynchus species (Bromage et al., 1992). Once sequestered into the oocytes, vtg is stored in the yolk globules (or granules), until the process of oocyte maturation. Another type of nutrients accumulating into the growing vitellogenic oocytes are the lipids. Depending on the species of fish, lipids may be present in the form of triglycerides, phospholipids or wax esters (Lund et al., 2000), and the amount and type of lipid class dominating the cytoplasm determines the presence (Fig. 4.2b, c and d) or absence (Fig. 4.2e), and size and number of lipid droplets (Fig. 4.2c and d) (Mylonas et al., 1997e, 2004a; Corriero et al., 2007). Thus, at the end of vitellogenesis the oocyte has a large cytoplasm filled completely with yolk globules and, in most cases, lipid droplets, a centrally located nucleus (named germinal vesicle, GV), and thick zona radiata (the future chorion) and follicular layers (Fig. 4.2c and d).

Oocyte maturation is the second phase of the gametogenic cycle in females, and it is regulated by an LH surge from the pituitary and the production of the maturation inducing hormone (MIH) by the ovarian follicle (Nagahama et al., 1994; Suwa and Yamashita, 2007). The MIH is a
progestagen, and in most fishes it is 17α,20β-dihydroxy-progesterone (DHP), whereas in others it is 17α,20β,21-trihydroxy-progesterone (20β-S). Oocyte maturation begins with the resumption of meiosis, which has been arrested in prophase I, and the migration of the GV to the animal pole of the oocyte (Fig. 4.2f), underneath the micropyle (Mylonas et al., 1997b). At the same time, and depending on fish species, coalescence of the lipid droplets takes place, resulting in the production of a single lipid droplet (Fig. 4.2g) (Mylonas et al., 1997e). As OM progresses, the GV membrane dissolves (germinal vesicle breakdown, GVBD), chromosomes condense and the first polar body is expelled from the oocyte. During this time, there is a chemical modification of the yolk, with the proteolysis of the vtg and the production of its major components, lipovitellin, phosvitin and b'-component, and free amino acids (FAA) (Cerdá et al., 2007), which is apparent in the coalescence of the yolk globules and the change in staining properties of the yolk (Fig. 4.2g and h) (Mylonas et al., 1997e). Depending on whether the eggs produced are pelagic or benthic (i.e., positively or negatively buoyant), the extent of this proteolysis varies and results in the increase in the osmotic pressure of the oocyte, which drives a drastic uptake of water (Cerdá et al., 2007) with a many-fold increase in size. After hydration, the follicular wall ruptures and the oocyte is ovulated (Fig. 4.2h) into the ovarian cavity, in most fishes, or in the abdominal in others (e.g., Salmonidae, Acipenseridae).

4.2.2 Spermatogenesis and spermiation

Spermatogenesis is the process of mitotic proliferation of the spermatogonia, their meiotic division into haploid spermatocytes, and their transformation into flagellated spermatozoa (Schulz and Miura, 2002). During mitotic proliferation, each spermatogonium goes through a species-specific number of divisions, which ranges between five and 15. During these divisions all daughter cells maintain direct cytoplasmic bridges between them; they are all contained within an individual spermatocyst, and undergo simultaneous development and maturation from spermatogonia to spermatocytes I and II, spermatids and finally flagellated spermatozoa (Fig. 4.3). Once spermatogenesis is completed, the Sertoli cells that surround each spermatocyst rupture and the spermatozoa are released into the testicular lumen. The presence and relative abundance of spermatozoa with the above maturation stage gametes is used as an indication of the degree of testicular development in fish.

With the rupture of the spermatocyst starts the period of spermiation, during which the spermatozoa undergo maturation (capacitation) as they move along the tubular lumen of the testes and are gathered in the efferent ducts. Capacitation is the process by which the spermatozoa acquire the capacity for motility once released in the water, and thus the ability to fertilize the eggs (Billard et al., 1995; Miura and Miura, 2003). At the same
Fig. 4.3  Microphotographs of histological sections from testes of European sea bass at various stages of maturation. (A) Before the onset of the reproductive season, containing only spermatogonia (sg). (B) At the early stage of spermatogenesis in November, containing spermatocyst with spermatocytes I and II (sc) and spermatids (st), including some spermatozoa (sz). (C) In December when spermatiation begins, showing the extensive rupture of the mature spermatocysts and the aggregation of spermatozoa in the tubules. (D) In January at the onset of the females spawning season, with the tubules containing almost exclusively spermatozoa. (E) Spent testes at the end of the season, showing residual spermatozoa, new spermatogonia and hypertrophied somatic cells. Photographs are not in the same scale.

time, the testes produce large volumes of seminal fluid, in which the spermatozoa are transported and released to the environment during spawning. During the period of spermatiation, gentle abdominal pressure can result in the release of milt (seminal fluid with spermatozoa) in most fishes, and the quality of the milt (density, percentage motility and survival) can be evaluated (Suquet et al., 1994; Billard et al., 1995; Fauvel et al., 1999).

The general view of the involvement of pituitary GTHs in the male reproductive cycle, as with the female, is that FSH controls mainly the early stages of gametogenesis (Ohta et al., 2007), while LH regulates the process of spermatiation (Schulz and Miura, 2002). Again, the final effectors of the GTHs on reproductive function are the gonadal steroids. Estrogens seem to induce the mitotic proliferation of the spermatogonia prior to the onset of...
gametogenesis (Miura and Miura, 2003). Then androgens (T and 11-keto T, 11-KT) regulate the whole process of spermatogenesis, to the production of flagellated spermatozoa (Miura et al., 1991b; Schulz and Miura, 2002). The maturation of the spermatozoa and the acquisition of motility capacity are controlled by DHP, the same progestagen that acts as an MIH in the female. This action involves the spermatozoa themselves, in a receptor-mediated process that results in an increase in seminal plasma pH (Miura and Miura, 2003).

4.2.3 Spawning

Spawning is the release of the mature gametes (eggs and capacitated spermatozoa) to the external environment (in the vast majority of fishes), in order to produce the zygote. As spermatozoa lose their motility within seconds or minutes after release from the testes and contact with the water (Billard et al., 1995) and eggs absorb water resulting in the closure of the micropyle (Cerdá et al., 2007), spawning must be extremely synchronous in order to result in fertilized eggs. Therefore, fish employ both breeding rituals and pheromones in order to signal to the other species their readiness for spawning (Liley, 1983; Stacey, 1984; Stacey et al., 1994; Zabala et al., 1997; Kobayashi et al., 2002). Spawning may take place in pairs (e.g., flounder, catfish), single females with a group of males (e.g., Salmonidae, Moronidae), or large groups of males and females (e.g., Sparidae, Thunnidae). Also, the eggs produced may be pelagic, demersal or may stick to each other and various substrates, such as rocks and vegetation. All these characteristics must be known for each fish of interest and be evaluated by the aquaculturist, in order to achieve the optimum results in terms of egg fecundity and fertilization success.

4.3 Reproductive strategies and dysfunctions in captivity

Being the largest vertebrate class with more that 27000 species and a very long evolutionary history (Nelson, 2006), fishes exhibit an amazing diversity in reproductive biology and strategies. For the purpose of broodstock management and hormonal manipulations for the induction of OM, ovulation and spawning in aquaculture operations, female fish may be separated into two classifications: synchronous spawners (synchronous and single-batch group-synchronous) and asynchronous spawners (multiple-batch group-synchronous and asynchronous) (Tyler and Sumpter, 1996). Synchronous spawners reproduce once in their lifetime or once during an annual reproductive season, and their ovary contains a single, uniform population of developing oocytes during the reproductive season (Fig. 4.4a). On the other hand, asynchronous spawners reproduce multiple times during every reproductive period. These spawns may be numerous and regular, e.g. daily or every other day for a period of 3–4 months; or can be few and irregular in
their timing, e.g. 3–7 spawns with an inter-spawn period of between 3 and 10 d. The ovaries of these species contain oocytes at all stages of development during the reproductive season (Fig. 4.4b), and different batches of oocytes mature during each OM, ovulation and spawning event.

In terms of the males, the situation is somewhat simpler. Spermatogenesis and spermiation may be temporally separated, with spermiation occurring after the conclusion of spermatogenesis, and during the spawning season the testes may contain exclusively spermatozoa (Billard, 1986; Malison et al., 1994). In the majority of species, however, there is significant overlap between the two processes, with both spermatogenesis and spermiation taking place during the spawning (Jackson and Sullivan, 1995; Mylonas et al., 2003; Rainis et al., 2003). Therefore, management of male reproduction in captivity and induction of spermiation utilizes very similar methods.

Reproductive dysfunctions of captive fishes are often restricted to the females, since males do undergo complete maturation in captivity, albeit at times possibly producing a reduced amount of milt and of lower quality (Mylonas and Zohar, 2001a, 2007; Zohar and Mylonas, 2001b; Mañanos et al., 2008). The simplest reproductive problem in females is observed in salmonids (Onchorhynchus and Salmo spp.), which do undergo vitellogenesis, OM and ovulation, but fail to spawn their eggs when reared in captivity (Bromage and Cumaranatunga, 1988; Zohar, 1989), probably due to the

![Fig. 4.4 Microphotographs of histological sections from ovaries of striped bass, a synchronous fish (A), and of bluefin tuna, an asynchronous fish (B). Photographs are not in the same scale.](image-url)
lack of the appropriate spawning substrate to place their eggs. The most common reproductive dysfunction in captivity is the failure of OM upon completion of vitellogenesis. As a result there is no ovulation and no spawning of eggs (Berlinsky et al., 1996, 1997; Larsson et al., 1997; Mugnier et al., 2000; Mylonas and Zohar, 2001b; Barbaro et al., 2002; Duncan et al., 2003; Marino et al., 2003; Ibarra-Castro et al., 2004; Mylonas et al., 2004a; Yang and Chen, 2004; Chen, 2005; Agulleiro et al., 2006; Fauvel et al., 2007; Mylonas et al., 2007).

The failure of captive females to undergo OM is due to dysfunctional release of LH from the pituitary. In striped bass, for example, the levels of various reproductive hormones were compared between cultured fish that fail to undergo OM during the spawning season and wild fish captured on their spawning grounds (Mylonas et al., 1997d, 1998b; Steven et al., 2000; Mylonas and Zohar, 2001b). In wild females, a plasma LH surge was observed during OM and ovulation, but in females reared in captivity plasma LH levels remained low at the end of vitellogenesis. However, LH was synthesized and stored in the pituitary during vitellogenesis, since levels of LH and its mRNA in the pituitary did not differ between wild and captive females, demonstrating that the problem is one of lack of release and not synthesis in captivity. In addition, mRNA levels of the pituitary receptor for the GnRH most relevant to pituitary LH synthesis were similar between wild and captive females. This suggests that the disruption in LH release from the pituitaries of captive fish is not due to a dysfunction in pituitary responsiveness, but may be related to the control of pituitary function by the reproductive brain. In fact, differences were observed between wild and captive females undergoing OM, when comparing the pituitary content of the endogenous GnRHas. The GnRH mRNA levels within the brain, however, were similar between the two groups, indicating that the altered pituitary content of GnRH in captive fish may be a result of altered release from the hypothalamus, rather than deficient synthesis (Steven, 2000; Steven et al., 2000).

Similarly in males, lower plasma levels of LH during the spawning period have been suggested as the cause of the reduced amount of milt produced by some fishes (Mylonas and Zohar, 2001b; Mañanos et al., 2002). As with females, the amount of LH in the pituitary or the ability of the pituitary to synthesize LH in response to treatment with exogenous GnRHas is not affected in these fishes, suggesting that again the reproductive dysfunction in the males may be identified in the brain control of GtH synthesis and/or release.

4.4 Hormonal therapies for the control of reproduction

Based on the accumulating evidence that the failure of fishes to undergo OM and full spermiation in captivity is the result of diminished LH release
from the pituitary, manipulations of reproductive function in cultured fishes have focused on the use of either exogenous LH preparations that act directly at the level of the gonad, or GnRHa that acts at the level of the pituitary to induce release of the endogenous LH stores (Fig. 4.5). Endogenous LH, in turn, acts at the level of the gonad to induce steroidogenesis and the process of OM and spermiation.

### 4.4.1 Gonadotropin preparations

Gonadotropin preparations include pituitary homogenates and pituitary extracts (PE) that contain LH (as well as other pituitary hormones), purified piscine LH or purified human chorionic gonadotropin (hCG), which has very strong LH activity (Lam, 1982; Donaldson and Hunter, 1983; Zohar, 1989; Zohar and Mylonas, 2001b). The main advantage of GtH preparations is that they act directly at the level of the gonad. Pituitary homogenates were the first type of exogenous hormonal treatments used by aquaculturists for the induction of maturation and spawning (Houssay, 1930; Von Ihering, 1937; Fontenele, 1955). Today, preparations of carp pituitary extract (CPE) and purified salmon GtH are available commercially and are used worldwide. These purified PE are more effective than the earlier pituitary homogenates, since they are purified to various extents and their activity is usually calibrated using bioassays (Yaron, 1995; Donaldson, 1973). Nevertheless, they still maintain the disadvantages of risking pathogen transmissions, as well as a high degree of species...
specificity, due to the significant differences in the primary structure of fish
GtH (Rosenfeld et al., 2007). Treatments with pituitary homogenates and
PE are usually split into a smaller priming dose (10–20% of total) and a
larger resolving dose given 12–24 h apart (Thalathiah et al., 1988; Parauka
et al., 1991; Kucharczyk et al., 1997; Chen, 2005).
Human CG has also been used extensively in hormonal manipulation of
reproduction in fishes, as it has been available throughout the world for
some time now, and it is purified and of clinical grade and standardized
bioactivity. Unlike GtH preparations of piscine origin, hCG is most often
effective in a single dose (100 and 4000 IU Kg⁻¹), presumably due to its
long residence time in circulation (Ohta and Tanaka, 1997). This is unre-
related to its heterologous nature in fish, since it has been shown to have a
significantly longer half-life compared to the pituitary GtHs both in fish
(Fontaine et al., 1984) and humans (Ludwig et al., 2002). Recently, an
hCG preparation has been approved for commercial utilization in com-
mercial aquaculture (CHORULON™, Intervet International bv, The
Netherlands).

4.4.2 Gonadotropin-releasing hormone agonists
The use of GnRHAs for spawning induction therapies has important advan-
tages over the use of GtH preparations. Firstly, being of synthetic nature,
GnRHAs do not pose a disease transmission threat, as pituitary homoge-
nates or extracts may do. Secondly, GnRHAs treatments are less species-
specific than GtH ones, due to the high structural similarity of native
GnRHs among fishes (Lethimonier et al., 2004). Thirdly, and perhaps most
importantly, GnRHAs stimulate the release of the endogenous GtHs and
other necessary pituitary hormones (Le Gac et al., 1993; Weber et al., 1995;
Cyr and Eales, 1996; Negatu et al., 1998), and thus they provide for a better
integration of reproductive processes by acting at a higher level of the
brain–pituitary–gonad axis. Although hundreds of different GnRHAs are
available, the only approved GnRHa for use in commercial aquaculture
is Azagly-nafarelin (GONAZON™, Intervet International bv, The
Netherlands), which has so far been shown to be efficacious only in salmo-
ids (Haffray et al., 2005).
As mentioned earlier, in some fishes there is a strong inhibition of basal
and GnRH-stimulated release of LH by DA. Therefore, administration of
DA antagonists prior to the treatment with GnRHa removes the inhibition
on the gonadotrophs and enhances the stimulatory effect of GnRHa on
LH release. Currently, hormonal manipulations of reproduction using
a combined GnRHa/DA treatment are used mostly in cyprinids
(Yaron, 1995; Mikolajczyk et al., 2003, 2004; Kaminski et al., 2004), cat-
fishes (Silverstein et al., 1999; Brzuska, 2001; Wen and Lin, 2004)
and mullets (Glubkov et al., 1994; Arabaci and Sari, 2004; Aizen et al.,
2005). There are several DA antagonists available in the market that
proved to be useful for hormone treatments in aquaculture (i.e., domperidone, pimozide, reserpine and metoclopramide); these are usually administered as a liquid solution injected prior to, or at the same time as the GnRHa treatment.

4.4.3 Sustained-release delivery systems

It was recognized almost from the first spawning induction experiment (Fontenele, 1955), that sustained administration of the hormone would result in improved efficacy. This is because the process of OM and spermiation often requires a prolonged treatment with exogenous hormones, given in multiple injections (Mylonas et al., 1992; Dabrowski et al., 1994; Slater et al., 1994, Carrillo et al., 1995, Pankhurst et al., 1996). Such repetitive handling of broodstock requires substantial labor, time and monitoring, and in situations where the broodfish are very large (groupers, amberjacks or tunas) or kept outdoors – in ponds or cages – it is very time-consuming and labor-intensive to crowd, capture, anaesthetize and inject the fish with hormones.

Since the 1980s, a variety of hormone-delivery systems, almost exclusively for GnRHa, have been developed for use in a variety of fishes. The first such delivery system was prepared using cholesterol and was tested in Atlantic salmon (Salmo salar) (Weil and Crim, 1983). Cholesterol implants are prepared as solid, cylindrical pellets (3 mm in diameter) and are implanted intramuscularly using an implanter. The next type of GnRHa-delivery system was fabricated in the form of microspheres (5–200 μm in diameter), using copolymers of lactic acid and glycolic acid (LGA) (Okada et al., 1994) or a copolymer of fatty acid dimer and sebacic acid (Fad-sa) (Mylonas et al., 1995). For treatment, the microspheres are suspended in a viscous vehicle and are injected into the muscle (Zohar, 1988; Breton et al., 1990; Chang et al., 1995; Mylonas et al., 1997c; Mylonas and Zohar, 2001b; Barbaro et al., 2002). The greatest advantage of biodegradable, microspheric delivery systems is that the same preparation can be used to treat fish with large variations in size. Also, since over time the microspheres degrade to their monomer constituents, which are all natural products – e.g., lactic acid, glycolic acid or sebacic acid – broodstock retired from production can be consumed for food without any concerns over harmful residual chemicals.

Another type of GnRHa-delivery system used for spawning induction is prepared in the form of a solid implant, using a non-degradable co-polymer of ethylene and vinyl acetate (EVAc) (Zohar, 1996). 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 GnRHa. The EVAc implants are fabricated as disks 2 or 3 mm in diameter and are administered intramuscularly using an implanter (Mylonas et al., 2007), releasing GnRHa
for periods from 2–5 weeks (Zohar, 1996; Mylonas et al., 1998b; Mañanos et al., 2002).

4.5 Induction of oocyte maturation and ovulation

As mentioned earlier, for the purpose of broodstock management and hormonal manipulation of OM and spawning, fish are separated into two classifications: synchronous spawners (single-time and single-batch group-synchronous) and asynchronous spawners (multiple-batch group-synchronous and asynchronous) (Tyler and Sumpter, 1996). These differences in reproductive strategies may necessitate the employment of different approaches in terms of hormonal therapy and egg acquisition. For example, single or double injections of GnRHa in liquid form may be effective in synchronous fish (Mylonas et al., 1992), which have all their oocytes developed at the same stage of maturation, but may not be the best approach to achieve maximum fecundity in asynchronous species with a long reproductive season (Zohar et al., 1995). Also, if required, strip spawning and artificial insemination is a good alternative to tank spawning in synchronous fishes, but will result in very poor fecundity in asynchronous species, since the fish ovulate only part of their total season production of vitellogenic oocytes, and the stripping process may damage the remaining oocytes.

The use of GtH preparations in inducing OM, ovulation and spawning in synchronous fishes has been summarized well in previous reviews, which report also more extensive information on doses and treatment protocols (Donaldson, 1973; Lam, 1982; Donaldson and Hunter, 1983; Zohar and Mylonas, 2001a; Mañanos et al., 2008). Some more recent examples include the European catfish (Silurus glanis), which was successfully induced to ovulate using 4 mg Kg⁻¹ CPE, though in a smaller percentage of females compared to a combined GnRHa/DA treatment (Brzuska, 2001). In the Japanese catfish (Silurus asotus), a single injection of 10000 IU Kg⁻¹ hCG induced OM and ovulation from June to September (Kumakura et al., 2003b). In the Brazilian catfish ‘cachara’ (Pseudoplatystoma fasciatum), both CPE and hCG were effective in inducing ovulation (Leonardo et al., 2004). Also, hCG at 1000 or 2000 IU Kg⁻¹ was effective as a single injection in inducing ovulation in the Korean spotted sea bass (Lateolabrax maculatus) (Lee and Yang, 2002). Finally, in wild-caught ocellated puffer (Takifugu ocellatus), both single- and double-injections of 6 mg Kg⁻¹ PE or 2500 IU Kg⁻¹ hCG were very effective in inducing OM and ovulation (Chen, 2005), and in pikeperch (Sander lucioperca), either single or multiple injections of 200 IU Kg⁻¹ hCG were effective in inducing ovulation (Zakes and Szczepkowski, 2004).

The continued interest in the control of reproduction of the European eel has resulted in improved knowledge on the dysfunctions of this species.
in culture and has identified some blocks in the endocrine axis. The European eel undergoes gametogenesis during its long migration from European rivers to the Sargasso sea, off the coast of North America (Tesch, 2003). In captivity, the absence of this migration results in the complete absence of both oogenesis and spermatogenesis. Recent research has identified dopamine as the brain hormone responsible for the blocking of the reproductive axis and the absence of pubertal development (Vidal et al., 2004). Still, however, the only available practical method for the induction of gametogenesis in the freshwater eel is the weekly administration of fish gonadotropin extracts in the female (Ohta et al., 1997; Sato et al., 1997; Pedersen, 2003; Palstra et al., 2005) and one or two injections of the same gonadotropin or of hCG in the male (Miura et al., 1991a; Ohta et al., 1996; Ohta and Tanaka, 1997). Final oocyte maturation is induced by the administration of the MIS, eggs and sperm are collected by stripping, and fertilization is accomplished artificially (Pedersen, 2004). A very promising new way to induce gametogenesis in the European eel is recently undergoing development (Guido van den Thillart and Herman Spanik, unpublished data), and employs genetically engineered zebrafish cell lines expressing the genes of zebrafish LH and FSH, controlled by a constitutive promoter. Once implanted subcutaneously to silver migrating eels, such cells are shown to produce continuously the two zF-GtHs, thus stimulating gonadogenesis. The development of this method will alleviate the need for multiple injections of heterologous hormones and will probably increase the effectiveness of maturation induction approaches in the freshwater eels.

The synchronization of ovulation in salmonids was one of the very first applications of GnRHa in aquaculture (Donaldson et al., 1981; Crim and Glebe, 1984; Breton et al., 1990). The treatment is usually given in the form of two injections (10–100 µg Kg⁻¹) spaced 3 d apart or a single application of a GnRHa-delivery system (10–50 µg Kg⁻¹), given around two weeks before the onset of natural maturation of the broodstock. The two-injection (Van Der Kraak et al., 1985; Sullivan et al., 1989; Mylonas et al., 1992) and GnRHa-delivery system protocols (Crim et al., 1983; Crim and Glebe, 1984; Breton et al., 1990; Goren et al., 1995) induce ovulation in 100% of the stock within two weeks after treatment. Single or multiple injections of GnRHa have also been used extensively in other synchronous fishes. When a two-injection protocol is used, GnRHa is given in a priming (5–10 %) and resolving dose (95–98 %), and if a DA antagonist is also used it is given with the priming dose. For example, in the ocellated puffer both single and double injections of 50 µg Kg⁻¹ GnRHa were effective in inducing OM (Chen, 2005), while similar results were obtained using 2–4 injections of GnRHa in the bullseye puffer (Sporoides annulatus) (Duncan et al., 2003). In the grey mullet, two injections of 30 µg Kg⁻¹ GnRHa together with 15 mg Kg⁻¹ metoclopramide were very effective in inducing spawning within 24 h (Aizen et al., 2005). Similarly, two injections of
20 μg Kg⁻¹ GnRHa with 5 mg Kg⁻¹ pimoide induced ovulation in 95 % of treated common carp (*Cyprinus carpio*) (Mikolajczyk *et al*., 2004). Two injections of GnRHa in combination with a DA antagonist have been used successfully also in the koi carp (*Cyprinus carpio*) (Arabaci *et al*., 2004), lake mullet (*Chalcalburnus tarichi*) (Arabaci and Sari, 2004) and wild catfish (*Seriola asorur*) (Wen and Lin, 2004). Finally, a single injection of 20 μg Kg⁻¹ GnRHa induced ovulation in tench (*Tinca tinca*) (Rodríguez *et al*., 2004).

Sturgeon (*Acipenser* spp.) aquaculture for meat and caviar relies exclusively on the use of hormonal spawning induction methods and the artificial fertilization of the obtained eggs. Sturgeon females are evaluated for the completion of vitellogenesis and the extent of the migration of the nucleus (polarization index, PI) by laparoscopic (Hurvitz *et al*., 2007) or surgical removal of oocytes from the ovary and their *in vitro* processing (Williot *et al*., 1991; Conte *et al*., 1988). The selected mature females may be given sturgeon pituitary extract, carp pituitary extract or, more recently, GnRHa (Webb *et al*., 1999; Chebanov and Billard, 2001; Williot *et al*., 2001, 2002; Burtsev *et al*., 2002; Zhuang *et al*., 2002), usually in priming and resolving injections spaced 10–24 h apart, and ovulation is accomplished 24–50 h afterwards. Single treatments with carp pituitary homogenate have also been reported to be effective (Williot *et al*., 2005). Acquisition of eggs is done using caesarian surgery or, more recently, by inserting a scalpel into the abdominal pore and making a small incision at the basal part of the oviducts (Chebanov and Billard, 2001). Sperm can be used fresh or cryopreserved (Billard *et al*., 2004) and the amount of sperm produced may also be enhanced using GnRHa-based hormonal therapies (Williot *et al*., 2002).

The greater efficacy of GnRHa-delivery systems in inducing OM in synchronous fishes has been demonstrated well since the mid-1990s (Mylonas and Zohar, 2001a, 2007). A GnRHa-delivery system was the only hormonal preparation able to induce spawning in the yaqui catfish (*Ictalurus pricei*), whereas combined sGnRHa/DA antagonist or catfish PE treatments were ineffective (Mylonas and Zohar, 2001a). In the tiger puffer (*Takifugu rubripes*), GnRHa-delivery systems (400 μg Kg⁻¹) induced ovulation in 18 and 10 d in fish with mean oocyte diameter of 800–900 μm and 900–1000 μm, respectively (Matsuyama *et al*., 1997). Other examples of applications in synchronous fishes include the bullseye puffer (Duncan *et al*., 2003), cobia (*Rachycentron canadum*) (Kilduff *et al*., 2002), devil stinger (*Inimicus japonicus*) (Takushima *et al*., 2003) and common carp (Bialowas and Bialowas, 2002).

GnRHa-delivery systems have been used preferentially to liquid injections in a variety of asynchronous fishes. For example, GnRHa-delivery systems induced two consecutive spawns within 3 d in white bass (*M. chrysops*) (Mylonas *et al*., 1997b) and greater amberjack (*Seriola dumenili*)
(Mylonas et al., 2004c), five spawns within 7 d in the barramundi (Lates calcarifer) (Almendraas et al., 1988), five ovulations within two weeks in striped trumpeter (Latria lineata) (Morehead et al., 1998), one to four ovulations within 7 d in the black sea bass (Centropristis striata) (Watanabe et al., 2003) and seven ovulations within 10 d in the dusky grouper (E. marginatus) (Marino et al., 2003). The greatest potential, however, of sustained-release GnRHa-delivery systems is in the induction of OM in asynchronous fishes with a daily or almost daily ovulation/spawning frequency. For example, the red porgy (Pagrus pagrus), red seabream (P. major) and gilthead seabream (Sparus aurata) have an asynchronous mode of ovarian development and are capable of undergoing OM and spawning on a 24 h cycle for periods up to four months (Watanabe and Kiron, 1995; Zohar et al., 1995; Mylonas et al., 2004b). A single GnRHa injection in the gilthead seabream induced daily spawning in only 20% of the stock, while a GnRHa-delivery system induced daily spawning in >70% of treated females. Similar results have been obtained with the other two sparids (Matsuyama et al., 1995; Zohar and Mylonas, 2001a). Thus, GnRHa-delivery systems result in significant increases in fecundity, by increasing the number of broodfish undergoing OM, and the number of ovulations per spawning season (Barbaro et al., 2002). The latest success of the GnRHa-delivery systems has been in the induction of OM, ovulation and spawning in cage-cultured Atlantic bluefin tuna (Thunnus thynnus) (Mylonas et al., 2007) and tank-cultured Southern bluefin tuna (T. maccocii) (M. Deichmann, Clean Seas Tuna Ltd, personal communication), which resulted in the production of fertilized eggs and viable larvae. Due to the inability of anaesthetizing bluefin tunas and the great difficulties in handling such large (60–120 Kg) and fast swimming pelagic fishes, GnRHa administration was done underwater in free swimming fish (Mylonas et al., 2007). It is expected that this method will continue to be used as the standard for the induction of spawning in captive-reared Atlantic bluefin tuna, until such time as land-based facilities are build or appropriate sea-cage sites are identified, which can reproduce the optimal environmental conditions necessary for reproductive maturation and spawning (e.g., temperature-photoperiod combination, water quality, etc.). Recently, the use of these GnRHa-delivery systems has induced spawning for four consecutive days in a captive-reared stock at Vibo Valentia, Italy, producing many millions of fertilized eggs, allowing the first larval rearing of Atlantic bluefin tuna in the Mediterranean Sea (G. Demetrio, unpublished data).

Finally, GnRHa-delivery systems have been employed with great success in inducing multiple spawnings, often of improved quality compared to the few naturally spawning females, in various flatfishes. For example, GnRHa-delivery systems induced daily ovulations in the greenback flounder (Rhombosolea tapirina) (Poortenaar and Pankhurst, 2000), and in wild-caught summer flounder (Paralichthys dentatus) GnRHa implants induced daily
ovulations for 8 d (Berlinsky et al., 1997), whereas in fish maintained for
more than a year in captivity the same treatment induced not only ovulation
but also tank spawning (Watanabe et al., 1998). Similarly in turbot (Scoph-
thalmus maximus), treatment with a GnRHa-delivery system induced mul-
tiple ovulations in 100% of treated fish compared to 50% of controls
(Mugnier et al., 2000). Also, in the yellowtail flounder (Pleuronectes
ferrugineus) different GnRHa-delivery systems induced an average of
eight consecutive ovulations, compared to three in control fish, resulting
in the production of twice as many eggs and of higher fertilization and
hatching percentage than control females (Larsson et al., 1997). The
same two GnRHa-delivery systems have also induced daily spawnings
for up to two weeks in the Senegal sole (Solea senegalensis), though
with very limited fertilization success (Agulleiro et al., 2006; Guzmán et al.,
2008).

4.6 Induction of spermiation

As mentioned earlier, the dysfunction observed in cultured male fishes is
not the absence of any stage of testicular development, but rather a reduc-
tion in the spermiation process and the production of expressible milt. Due
to the long-term nature of the process of spermatogenesis and spermiation
– as opposed to OM in females – long-term hormonal therapies with
GnRHa-delivery systems have proven more effective in enhancing milt
production compared to acute treatments with either GtH preparations or
GnRHas. 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 d in carp by daily injections of GnRHa,
but 3 d after the treatment was interrupted, milt volume decreased below
pre-treatment levels (Takashima et al., 1984). In the winter flounder (Pleu-
ronectes 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 European
sea bass (Dicentrarchus labrax) a single injection of GnRHa at the end of
the spawning season was effective in maintaining milt volume of stripped
males for only 3 d, compared to 17 d of GnRHa implants (Rainis et al.,
2003). These results underline the need for a long-term hormonal therapy,
in order to induce sustained increases in milt production.

Another disadvantage of single hormone injections is that they usually
induce only a short-lived elevation of seminal plasma production, with a
much smaller increase in spermatozoa production (Clemens and Grant,
1965; Garcia, 1991) and the increase in milt volume is accompanied with
significant reductions in sperm density (Takashima et al., 1984; Garcia,
1991). In white bass, treatment with an hCG injection may restore milt
release in males stripped completely of their milt, but the milt is extremely thin and contains mostly seminal fluid (Bayless, 1972). On the contrary, GnRHa-delivery systems increase milt production significantly, without any decrease in sperm density, motility or fertilizing ability of the spermatozoa (Mylonas et al., 1997a).

Many different GnRHa-delivery systems have been used to enhance spermiation in cultured fishes, beginning with salmonid species such as Atlantic salmon (Zohar, 1996; Weil and Crim, 1983), rainbow trout (Oncorhynchus mykiss) (Breton et al., 1990), chinook salmon (O. tshawytscha) (Solar et al., 1995), coho salmon (O. kisutch) (Goren et al., 1995). GnRHa-delivery systems have also been very effective in basses of the Moronidae family. For example, in the European sea bass at the peak of the spawning season, a single injection of GnRHa induced increases in milt production for 7 d only, whereas treatment with GnRHa-delivery systems resulted in increased milt production for 28–35 d (Mañanos et al., 2002). Also in the striped bass, GnRHa-delivery systems induced long-term increases in milt production, lasting for 14–20 d (Mylonas et al., 1997c; Mylonas et al., 1998a). GnRHa implants have also been used in Atlantic halibut (Hippoglossus hippoglossus) to enhance the quality of the sperm (Vermeirssen et al., 2003), in starry flounder (Platichthys stellatus) to increase milt volume and sperm density (Moon et al., 2003) and in greenback flounder to increase sperm volume (Lim et al., 2004). Still, in some species simple injections of GnRHa of GtH preparations have been employed for the successful enhancement of spermiation, such as the Siberian sturgeon (A. baeri) (Williot et al., 2002), the sterlet (Acipenser ruthenus) (Rzemienicki et al., 2004), the precocious European sea bass (Schiavone et al., 2006) and the minnow (Rhyynchocypris oxycephalus) (Park et al., 2002).

4.7 Spontaneous spawning versus artificial insemination

Hormonal induction of OM and spermiation does not ensure spawning of the fish – i.e., release of their gametes – in a timely and synchronous way so that fertilized eggs are produced. This may be due to inappropriate tank size, lack of bottom substrate for the preparation of a nest or plant substrate for the adhesion of the eggs, and possibly other reasons that are not yet known. Therefore, for some species it is also necessary to employ artificial gamete collection and fertilization, using strip spawning. Such species include all salmonids, sturgences and various carps, groupers and flatfishes. In these situations, it is important to establish with varying degrees of accuracy the time of ovulation after the hormonal treatment. This is because once the eggs are ovulated into the ovarian or abdominal cavity, they begin to lose their viability in a species- and temperature-dependent process that may last from minutes (e.g., Moronidae) to days.
(Salmonidae). Failure to strip the eggs within the appropriate time interval
after hormonal stimulation will result in greatly reduced fertilization
success. The same is not true for sperm collection, which can be done at
any time after hormonal stimulation. In addition, sperm from most fishes
can be maintained viable without the use of cryopreservation or extenders
for many hours (Rainis et al., 2003) to many days (Mylonas et al., 2003;
Papadaki et al., 2008). Therefore, a typical artificial insemination protocol
should plan for (i) collection and storage of sperm a few hours before the
expected time of ovulation and (ii) stripping of the eggs at the appropriate
time after hormonal therapy. This procedure will ensure optimal results in
fertilization success.

4.8 Future trends

Basic studies of fish reproductive physiology and endocrinology, in combi-
nation with functional genomics and modern tools of biotechnology, will
lead to more precise and efficient control of reproduction in farmed fish
and to better supplies of optimal quality seed. It is clear from the present
review that GnRHs are of considerable importance to normal and induced
gametogenesis in farmed fish. Understanding the environmental and endo-
crine regulation of GnRH gene expression is key to developing strategies
for overcoming the GnRH failure that results in the lack of OM, ovulation
and spawning in captive fish. More studies on the functional significance
of GnRH multiplicity will lead to better tailored GnRH-based spawning
induction technologies that will administer or manipulate the relevant,
physiological combination of GnRH forms. In addition, the discovery and
understanding of factors that control the early establishment of the GnRH
system can be used to develop new approaches to induce sterility or preco-
cious puberty in farmed fish. The introduction of zebrafish as a model for
the study of the GnRH system (Steven et al., 2003; Palevitch et al., 2007)
and the recent development of transgenic zebrafish expressing a green
fluorescent protein (GFP) reporter gene under control of the GnRH
promoter (Abraham et al., 2008) provide very powerful tools for
improving our understanding of how the GnRH system is regulated in fish.
Using these tools, simple manipulations of GnRH neuronal development
and GnRH synthesis and release may be developed in the future to
control the onset of puberty and induce oocyte maturation, ovulation and
spawning.

To be successful, exogenous application of GnRHa and other hormonal
preparations in spawning induction therapies must be precisely synchro-
nized with the acquisition of follicular maturational and ovulatory com-
tence. Fish that are treated too early or too late will either not spawn or
spawn unfertilizable or poor quality eggs. In most cases, prediction of
‘readiness’ of the female for spawning induction is determined based on ovarian biopsy and measurement of oocyte diameter and/or microscopic observation of morphological characteristics of the oocytes, such as GV migration or occurrence of atresia. Although it has been standard practice for decades, the ovarian biopsy method is not ideal, as it is stressful to the fish, impossible to conduct on small (such as ornamental fishes) or very large (such as bluefin tuna) species, and not very accurate. The field of spawning induction needs non-invasive and more precise methods to determine the readiness of the female, as well as to predict and optimize the success of the spawning induction treatment. Many hormones and other factors are involved in the process of acquiring ovarian maturational competence, and these may be used as precise indicators of its progress (Patiño et al., 2001). Determining which factors are relevant and best suited as indicators will be greatly facilitated using genomics and proteomics information, and several recent studies have used this approach (Boe et al., 2004; Aegerter et al., 2005; von Schalburg et al., 2005; Bonnet et al., 2007). More work needs to be done in order to fully exploit this approach in fish and develop DNA or molecule microarrays in order to efficiently and comprehensively assess spawning readiness in aquacultured species. In addition, significant effort has been devoted to developing methodologies that measure reproductive indicators, such as steroids and vtg, using mucous or muscle samples (Bridges et al., 2003; Susca et al., 2001). Application of novel and non-invasive sampling modalities, together with the development of fish ‘gene chips’ for reproductive factors, will undoubtedly lead to the future use of such approaches to optimize the timing and enhance the success of spawning manipulation in farmed fish.

Finally, recent research has established a new paradigm for fish reproductive endocrinology. The simplistic partitional view of the brain–pituitary–gonadal axis has been replaced by a more complex and integrated web of endocrine interactions. The multiple brain GnRHS and their receptors were shown to be expressed locally in the pituitary (Mohamed et al., 2005) and gonads (Lin and Peter, 1996; Gray et al., 2002; Uzbekova et al., 2002; Soverchia et al., 2007). In both the ovary and testis of fish, GnRH has been demonstrated to directly affect gamete development and maturation (Habibi et al., 1988; Gazourian et al., 1997; Pati and Habibi, 2000; Soverchia et al., 2007). Likewise, the pituitary gonadotropins were shown to be expressed locally in the gonads, and this expression is regulated by GnRH (Wong et al., 2004). The occurrence of a complete GnRH–GtH–steroid axis within the fish gonads should be considered when hormonally manipulating reproduction and inducing spawning, as its balanced expression may be critical to production of highest quality gametes and embryos. Thus, a more complete understanding of how the entire endocrine–reproductive axis is coordinated in fish is expected to lead to better application of
spawning manipulation protocols and optimization of seed production in aquaculture.

4.9 Sources of further information and advice

Further information in this subject can be obtained in recent reviews (Schulz and Miura, 2002; Miura and Miura, 2003; Mylonas and Zohar, 2007; Mañanos et al., 2008). Information on fish reproduction in general can also be obtained from the website REPROFISH (www.reprofish.eu), a website established currently in Europe, with the objective of acting as a portal for students, scientists and professionals interested in fish reproduction and its control. Through the site, the interested professional can access scientific articles and protocols related to the control of reproduction in fish under culture conditions. Also, articles related to fish reproduction and its control are published in the proceedings of the International Symposium on Fish Reproductive Physiology and the International Symposium on Fish Endocrinology, which take place once every four years. Journals such as Aquaculture, Aquaculture Research, Fish Physiology and Biochemistry, General and Comparative Endocrinology and Biology of Reproduction publish many articles related to fish reproduction.

4.10 References


New technologies in aquaculture


Controlling fish reproduction in aquaculture


FONTENELE, O (1955) Injecting pituitary (hypophyseal) hormones into fish to induce spawning, Prog Fish-Cult, 18, 71–5.


GOREN, A, GUSTAFSON H and DOERING D (1995) Field trials demonstrate the efficacy and commercial benefit of a GnRHα implant to control ovulation and spermiation in salmonids, in Goetz F W and Thomas P (eds), Reproductive Physiology of Fish, Austin, TX, Fish Symposium 95, 99–101.


HARMON, S A and CIRRI L W (1993) Influence of gonadotropin hormone-releasing hormone analog (GnRH-A) on plasma sex steroid profiles and milt production


Controlling fish reproduction in aquaculture


136 New technologies in aquaculture

carpio L.) under laboratory, commercial and natural conditions, Aquaculture, 234, 447–60.


Controlling fish reproduction in aquaculture


Ohta, H and Tanaka H (1997) Relationship between serum levels of human chorionic gonadotropin (hCG) and 11-ketotestosterone after a single injection of hCG and induced maturity in the male Japanese eel, Anguilla japonica, Aquaculture, 153, 123–34.


New technologies in aquaculture


Controlling fish reproduction in aquaculture


Solar, I I, Smith J, Dye H M, Mackinlay D D, Zohar Y and 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 and Thomas P (eds), Reproductive Physiology of Fish, Austin, TX, Fish Symposium 95, 144.


New technologies in aquaculture


VAN DER KRAAK, G, DYE H M, DONALDSON E M and HUNTER G A (1985) Plasma gonadotropin, 17β-estradiol, and 17a,20β-dihydroxy-4-prenugen-3-one levels during luteinizing hormone-releasing hormone analogue and gonadotropin induced ovulation in coho salmon (Oncorhyncus kisutch), Can J Zool, 63, 824–33.


VON HERING, R (1937) A method for inducing spawning in fish, Prog Fish-Cult, 34, 15–16.


Controlling fish reproduction in aquaculture


New technologies in aquaculture


