Broodstock management and hormonal manipulations of fish reproduction

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Broodstock management and hormonal manipulations of fish reproduction**ABSTRACT**

Control of reproductive function in captivity is essential for the sustainability of commercial aquaculture production, and in many fishes it can be achieved by manipulating photoperiod, water temperature or spawning substrate. The fish reproductive cycle is separated in the growth (gametogenesis) and maturation phase (oocyte maturation and spermiation), both controlled by the reproductive hormones of the brain, pituitary and gonad. Although the growth phase of reproductive development is concluded in captivity in most fishes --the major exemption being the freshwater eel (*Anguilla spp*), oocyte maturation (OM) and ovulation in females, and spermiation in males may require exogenous hormonal therapies. In some fishes, these hormonal manipulations are used only as a management tool to enhance the efficiency of egg production and facilitate hatchery operations, but in others exogenous hormones are the only way to produce fertilized eggs reliably. Hormonal manipulations of reproductive function in cultured fishes have focused on the use of either exogenous luteinizing hormone (LH) preparations that act directly at the level of the gonad, or synthetic agonists of gonadotropin releasing hormone (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. After hormonal induction of maturation, broodstock should spawn spontaneously in their rearing enclosures, however, the natural breeding behaviour followed by spontaneous spawning may be lost in aquaculture conditions. Therefore, for many species it is also necessary to employ artificial gamete collection and fertilization. Finally, a common question in regards to hormonal therapies is their effect on gamete quality, compared to naturally maturing or spawning broodfish. The main factors that may have significant consequences on gamete quality --mainly on eggs-- and should be considered when choosing a spawning induction procedure include (a) the developmental stage of the gonads at the time the

hormonal therapy is applied, (b) the type of hormonal therapy, (c) the possible stress induced by
70 the manipulation necessary for the hormone administration, and (d) in the case of artificial
insemination, the latency period between hormonal stimulation and stripping for *in vitro*
72 fertilization.

74 Keywords: broodstock management, induced spawning, oocyte maturation, spermiation

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1. INTRODUCTION

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The number of aquatic species currently under domestication efforts is rising rapidly, due to the development of commercial aquaculture (Duarte et al., 2007). One of the prerequisites for domestication and the establishment of a sustainable aquaculture industry is the capacity to control reproductive processes of fish in captivity, and to acquire high quality seed (*i.e.*, eggs and sperm) for grow-out of the marketable product. Although many cultured fishes today fulfil this condition, there are important species whose aquaculture industries depend almost exclusively on the collection of juveniles or adults from the wild. Such species include the very popular freshwater eel (*Anguilla* spp.), the Japanese yellowtail and greater amberjack (*Seriola* spp.), some groupers (*Epinephelus* spp.) and the bluefin tuna (*Thunnus* spp.) (Ottolenghi et al., 2004).

Reproduction of fish in captivity can be controlled by environmental manipulations, such as photoperiod, water temperature or spawning substrate. However, the ecobiology of some fishes is not well known, or it is impractical or even impossible to simulate the required environmental parameters for natural reproductive performance (*i.e.* spawning migration, depth, riverine hydraulics, etc.). In these instances, use of exogenous hormones is an effective way to induce reproductive maturation and produce fertilized eggs. Furthermore, in all cultured fishes, hormonal manipulations may be used as management tools to enhance the efficiency of egg production, increase spermiation and facilitate hatchery operations. Finally, hormonal therapies may be employed to induce gamete maturation and enable artificial collection in order to implement inter-specific hybridization, chromosome set manipulation or artificial fertilization for genetic selection programmes.

Broodstock management involves all the appropriate measures taken by the aquaculturist to enable a captive group of fish to undergo reproductive maturation and spawning, and produce fertilized eggs. As indicated above, this management may involve only manipulation of environmental conditions or it may include the use of exogenous hormones. The type of hormones, administration protocols and gamete acquisition procedures may vary depending on the reproductive biology of each cultured species, and a thorough understanding of the endocrine control of gametogenesis, final maturation and spawning is essential for the appropriate management of the species (See other manuscripts in this special issue).

2. GAMETOGENESIS AND FINAL MATURATION

Similar to other vertebrates, the reproductive cycle of fish is separated into two major phases (Fig. 1). The proliferation, growth and differentiation of the gametes constitute the first phase (spermatogenesis and vitellogenesis), while the maturation and preparation of the oocytes and spermatozoa for release and insemination constitutes the second phase (spermiation and oocyte maturation). With very few exceptions --notably the European eel (*Anguilla anguilla*) (van Ginneken and Maes, 2005), spermatogenesis and vitellogenesis usually take place in cultured fishes without significant problems, when optimal rearing conditions have been applied (Buchet et al., 2008; Okumura et al., 2003). The most common reproductive dysfunction in males is reduced sperm volume and diminished quality, whereas unpredictable occurrence or failure of oocyte maturation (OM), and hence ovulation or spawning (Mylonas et al., 2004a; Mylonas et al., 2004c; Mylonas and Zohar, 2001a), is commonly observed in females (Berlinsky et al., 1997; Billard, 1989; Vermeirssen et al., 1998; Vermeirssen et al., 2000). Therefore, hormonal therapies usually address problems related to diminished sperm production during the spawning season and the failure of OM in cultured fishes.

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124 Spermatogenesis and spermiation

The gametogenic process in the males is separated into two phases (Fig. 1).

126 Spermatogenesis is the first phase and it includes the proliferation of the spermatogonia, the
multiplication of the spermatocytes I with multiple mitotic divisions, the production of
128 spermatocytes II with meiotic division and their differentiation to spermatids (Fig. 2). The
process is completed with the production of flagellated spermatozoa, i.e. spermiogenesis
130 (reviewed by Billard, 1986; Schulz and Miura, 2002; Vizziano et al., 2008). The spermatozoa are
released in the sperm ducts during the second phase of the male reproductive cycle, i.e.
132 spermiation, which occurs during the spawning season. Sperm is ejaculated spontaneously by
the fish and with the exception of catfishes (Mansour et al., 2004; Viveiros et al., 2002), it can
134 also be expressed easily from the testes after application of gentle abdominal pressure (i.e.,
stripping). Spermiation and ejaculation can be synchronized with female spawning via
136 pheromonal communications (Stacey, 2003).

Spermatogenesis may be continuous in species showing a tubular testis type or
138 discontinuous in species showing a lobular testis type, which is the most frequent among
teleostean fish (Billard, 1986; Schulz and Miura, 2002; Vizziano et al., 2008). Also,
140 spermatogenesis and spermiation may be temporally separated and during the spawning season
the testes may contain exclusively spermatozoa (Billard, 1986; Malison et al., 1994). In most
142 species, however, there is significant overlap between the two processes, with both
spermatogenesis and spermiation taking place during the spawning season (Jackson and
144 Sullivan, 1995; Mylonas et al., 2003a; Rainis et al., 2003), and it has been shown in the gilthead
sea bream (*Sparus aurata*) that the spermatogonia and Sertoli cell proliferation activity is not

146 blocked by the presence of spermatozoa (Chaves-Pozo et al., 2005), in contrast to fish with
synchronous spermatogenesis.

148 Usually, males show a longer period of spermiation, which encompasses the spawning
season of females by a few months, and they can fertilize eggs of several females in the wild. In
150 addition, a female may spawn with more than one male, either by spawning with many males on
one occasion or by spawning with different males on successive occasions (Petersson and Järvi,
152 2001). These male and female behaviors secure the reproductive success of an individual and
favor the maintenance of genetic variability within a wild population. The same process could
154 be tentatively achieved in fish farms by *in vitro* fertilization of stripped eggs with a pool of
sperm obtained from different males or by having several males and/or females in the same tank,
156 when natural reproduction is possible. The latter is the chosen broodstock management method.

As mentioned above, spermatogenesis is completed with the process of spermiogenesis, an
158 important step for the differentiation of the haploid spermatids into flagellated spermatozoa and
during which the morphology of the species-specific spermatozoon is determined (Billard et al.,
160 1986; Pudney, 1995; Schulz and Miura, 2002; Vizziano et al., 2008). In mammals, spermiation
is known as a complex process by which elongated spermatids undergo their final maturation
162 and are released from supporting Sertoli cells into the tubule lumen, which is open at both ends
(Beardsley and O'Donnell, 2003). This process has been described at the morphological level,
164 but its control remains poorly understood, although it is known that it requires the actions of
both gonadotropins (follicle stimulating hormone, FSH and luteinizing hormone, LH), as well as
166 intratesticular testosterone (T) (Saito et al., 2000). In fish, spermiation corresponds to the
release of the spermatozoa from the spermatocysts into the sperm ducts. At the same time
168 production of seminal fluid is observed (LaFleur and Thomas, 1991). At least in some species,
this process may be associated with the acquisition of fertilizing capacity of the spermatozoa,

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(i.e., capacitation) within the sperm ducts (Schulz and Miura, 2002). In viviparous fish species, spermatozoa may be packed in spermatozeugma or spermatophores, which can be formed within the spermatocysts or in the sperm ducts. In the latter case the spermatozeugmata are released during spermiation into the ducts and transported unmodified to the copulatory organ (Fishelson et al., 2007). Similar to mammals, there is a close morphological and functional intercellular communication between Sertoli cells and germ cells during fish spermatogenesis (De Montgolfier et al., 2007; Loir et al., 1995). At the end of the male fish reproductive cycle, junctions formed by spermatids with Sertoli cells are weak, but finger-like projections may be observed between spermatozoa present in the tubule lumen of rainbow trout (*Oncorhynchus mykiss*) testis (Loir et al., 1995), although in some fishes spermiation is associated with the degeneration of at least some of the Sertoli cells (Schulz et al., 2005b).

From a morphological standpoint, fish spermiation is characterized by the rupture of the spermatocysts and release of the spermatozoa in the sperm ducts. This is followed by the production of seminal fluid, causing 'hydration' of the testes (Schulz and Miura, 2002). In some species, especially the Siluriform catfishes, seminal vesicle secretions contribute to the seminal fluid and participate in prolongation and stabilization of sperm viability (Chowdhury and Joy, 2007). During spermiation, the produced milt (i.e., seminal fluid containing suspended spermatozoa) may be collected by stripping. In most cases, hydration of the milt is enough to allow the spermatozoa to be stripped and usable for fertilization (Vermeirssen et al., 2000). However, as mentioned earlier, in some fishes stripping of semen is difficult because of anatomical reasons (Viveiros et al., 2001).

Therefore, most spermiation induction methods employed in aquaculture are not designed to induce spermatogenesis, which is a long process lasting many days or weeks, but mainly to induce spermiogenesis and production of seminal fluid which allows a greater number of the

194 spermatozoa released from the spermatocysts to be "washed out" of the testes (Mylonas et al.,
1997b; Mylonas et al., 1998a). Methods to induce spermiation are required in some situations
196 when this process is blocked completely. This could be the case for poorly domesticated fish or
when farming conditions are not appropriate with a species ecobiology. Spermiation control
198 may be also needed in order to synchronize the production of sperm with female ovulation, for a
good management of spawners. Finally, sperm needs to be available at the right time and with
200 the required quantity and quality when either large breeding plans are managed for genetic
selection purposes or to cryopreserve enough quantity of sperm, in order to preserve interesting
202 genetic resources.

204 **Vitellogenesis and oocyte maturation (OM)**

For the purpose of hormonal manipulations for the induction of OM, ovulation and
206 spawning, fish are separated into two classifications: fish that spawn only once during the
reproductive season (synchronous and single-batch group-synchronous) and those who spawn
208 multiple times (multiple-batch group-synchronous and asynchronous) (Tyler and Sumpter,
1996). Synchronous ovarian development is characteristic of semelparous fishes such as the
210 Pacific salmon (*Oncorhynchus* spp) and freshwater eels, which reproduce only once in their
lifetime. Single-batch group-synchronous fishes (Fig. 3A) reproduce only once during every
212 annual reproductive period. Multiple spawning fishes, as the term implies, produce multiple
spawns during every reproductive period (Fig. 3B). These spawns may be numerous and
214 regular, *e.g.* daily or every other day for a period of 3-4 months (Mylonas et al., 2004b; Papadaki
et al., 2008; Zohar et al., 1995); or can be few and irregular in their timing, *e.g.* 3-7 spawns with
216 an inter-spawn period of between 3-10 days (Carrillo et al., 1995; Forniés et al., 2001; Marino et

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al., 2003). Egg batch fecundity and egg size may decline with subsequent spawning events
(Kjesbu et al., 1996; Mugnier et al., 2000; Mylonas et al., 2003b).

The type of strategy employed for oogenesis by different fishes has important implications in their broodstock management in aquaculture. For example, for synchronous fishes that spawn once in their lifetime (*e.g.*, freshwater eels and Pacific salmon), sacrificing the fish and collecting their artificially matured gametes directly from the broodfish is a very effective and efficient method for producing fertilized eggs. Obviously the same approach could not be used for fish that reach reproductive maturation at a late age and are expected to have a long reproductive life (*e.g.*, tunas, amberjacks or groupers). Similarly, stripping of eggs and artificial insemination may be used in annually spawning synchronous females to obtain the total amount of available eggs from each female, but the same may not be achieved with multiple spawning fishes that mature and ovulate only a small fraction of their total annual fecundity at every spawning event. Also, annually spawning synchronous fishes have a much more confined reproductive season (*e.g.*, a few weeks) and the production of eggs over an extended period of time to cover grow-out production needs, requires the establishment of many stocks exposed to different photothermal manipulations in order to shift the spawning period (Bromage et al., 2001). Whereas two or three stocks of photoperiodically manipulated gilthead sea bream will be adequate to provide a hatchery with eggs for 9-12 months a year (Zohar and Mylonas, 2001b), for a similar production, six to eight stocks of European sea bass (*Dicentrarchus labrax*) must be maintained, resulting in a higher facility and management cost (Carrillo et al., 1993). Finally, the choice of hormonal therapy for the induction of maturation (mainly OM in the females) may also depend on whether the fish is a synchronous or asynchronous spawner (see later section).

The presence of large amounts of yolk in the fish egg is an efficient way of making available the necessary components to sustain the developing embryo and pre-larvae until the

opening of the mouth and exogenous feeding. Therefore, the sequestration of yolk precursors into the oocyte during oogenesis (*i.e.*, vitellogenesis) is a key process for successful reproduction and the production of healthy progeny in aquaculture. Vitellogenesis is accompanied by an important growth of the oocyte due to the uptake of yolk precursor proteins, mostly vitellogenin (Vtg) and putatively very low-density lipoproteins (Babin et al., 2007). Vitellogenin, a bulky and complex calcium-binding phospho-glycoprotein synthesized by the liver, is selectively sequestered by the growing ovarian follicles via specific receptors (VtgRs) clustered in endocytic clathrin-coated pits giving rise to the formation of Vtg-containing coated vesicles that move into the peripheral oolema (Hiramatsu et al., 2006). Vesicles fuse with lysosomes leading to the formation of the multivesicular bodies (MVB), which increase in size and are gradually transformed into yolk granules and then into large yolk globules (Le Menn et al., 2007). Besides, MVBs contain lysosomal enzymes, such as Cathepsin D that cleave Vtg into the yolk polypeptides. The participation of this protease and, to some extent, Cathepsin B in Vtg processing has been shown both in freshwater and marine fish (see review by Cerdá et al., 2007).

As in other vertebrates, beginning at the N terminus, the precursor of a complete teleost Vtg molecule consists of a signal peptide, a lipovitellin heavy chain (LvH), a phosvitin (Pv), a lipovitellin light chain (LvL) and a von Willebrand factor type D domain (Vwfd) that in teleosts is cleaved into a β' -component (β' -c) and C-terminal coding region [NH_2 -(LvH-Pv-LvL- β' -CT)- COO^-]. Once assembled, Vtg is glycosylated and phosphorylated post-translationally, and secreted as dimers to the plasma (Finn, 2007). The presence of multiple Vtg genes has been confirmed in various teleosts (Finn, 2007; Hiramatsu et al., 2006; Sawaguchi et al., 2006) and the existence of a noticeably large number of complete Vtg genes was verified in rainbow trout and zebrafish (*Danio rerio*). In the former, the identity of the genes encoding Vtg was very high and, thus, the translated products were possibly very similar (see Hiramatsu et al., 2002 for

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references). Nevertheless, a novel Vtg characterized by a missing phosvitine domain was shown to be present among the zebrafish Vtg genes. This novel “incomplete” Vtg was designated as phosvitin-less form (PvIVtg; NH₂-LvH-LvL-COOH) and was also identified in two tilapia species. Recently, three forms of vitellogenin, VtgA, VtgB and VtgC (PvIVtg) have been identified in many fishes (see Amano et al., 2008; Hiramatsu et al., 2006; Sawaguchi et al., 2006). In general, it appears that members of higher teleost taxa (*Paracanthopterygii* and *Acanthopterygii* spp) express both VtgA and VtgB; additionally VtgC seems to be widely represented among teleosts (Matsubara et al., 2003).

As in other oviparous vertebrates, in teleosts the enzymatic cleavage of Vtg gives rise to the typical suit of yolk proteins that include the lipovitellin (Lv), the phosvitin (Pv) and the β' -c. The Lv is a highly lipidated yolk protein consisting of two polypeptides, the Lv heavy-chain (LvH) and the Lv light chain (LvL). The Pv is a smaller protein in which more than half of its amino acid residues are contained in highly phosphorylated polyserine domains that confer Vtg its calcium-binding properties. The β' -c is the third yolk protein that usually contains neither lipid nor phosphorus (Hiramatsu et al., 2006; Hiramatsu et al., 2002). Lipovitellin appears to serve mainly as nutritional source of amino acids and lipids to the developing embryo, whereas Pv provides the necessary minerals required for its skeletal and metabolic functions. Until now, no physiological or nutritive functions have been attributed to the β' -c or C-terminal peptide (Hiramatsu et al., 2006).

At the end of vitellogenesis, when the accumulation of the necessary yolk proteins and mRNAs for embryonic development has been completed, hormonal stimulation allows the oocytes to undergo OM. After oocyte maturation, ovulation takes place and meiosis is reactivated and completed upon fertilization (Kinsey et al., 2007). During OM, drastic morphological changes are observed in the oocyte together with progression of meiosis. The

most noticeable features, depending on the species, are lipid droplet coalescence and yolk
 290 globule coalescence which result in the clarification of the oocyte's cytoplasm, migration of the
 nucleus (germinal vesicle, GV) to the periphery of the oocyte and dissolution of the nucleus
 292 membrane (GV breakdown, GVBD), and a dramatic increase in volume due to water uptake
 (Cerdá et al., 2007). The GV is visible under the microscope after some chemical processing,
 294 and disappears when GVBD takes place.

In addition to the initial processing of Vtg upon sequestration into the growing oocyte,
 296 which is achieved mainly by Cathepsin D, a second phase of much more intense proteolysis of
 the yolk proteins takes place during OM. The enzymes responsible for this second proteolysis
 298 have been identified in few species; Cathepsin L seems to be the responsible in gilthead sea
 bream, whereas Cathepsin B-like protease seems to be involved in barfin flounder (*Verasper*
 300 *moseri*) (reviewed by Hiramatsu et al., 2006). In addition to its obvious role in providing free
 aminoacids (FAAs) for the developing embryo and larva, the intense proteolysis of the Vtg-
 302 derived yolk proteins is important also for the hydration of the oocyte (Cerdá et al., 2007). This
 is because the produced FAAs and other organic osmolytes play a very important role in
 304 increasing the osmotic pressure of the oocyte's cytoplasm, driving an aquaporin-mediated water
 uptake by the maturing oocyte. A limited hydration may also occur in freshwater fish
 306 possessing benthic eggs (Milla et al., 2006). However, this second proteolysis is particularly
 important in marine fish possessing pelagic eggs and exhibiting a noticeable hydration at OM
 308 (Matsubara et al., 1999). In these teleosts, VtgA and VtgB, and their derivative yolk proteins are
 thought to play distinct roles in regulating oocyte hydration (Finn, 2007; Finn et al., 2002;
 310 Sawaguchi et al., 2006). A dual Vtg system responsible of a specific physiological mechanism
 of egg buoyancy was firstly described in barfin flounder (Matsubara et al., 1999). During OM
 312 most VtgA-derived yolk proteins are cleaved into FAAs but the heavy chain of Lv derived from

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VtgB (LvHB) remains greatly intact. Thus, the ratio of VtgA and VtgB accumulated during vitellogenesis regulates the yield of FAAs during OM, which generate a specific osmotic gradient responsible for oocyte hydration and regulation of egg buoyancy. The remaining LvHB serves as nutrient source for late embryo developmental stages (Matsubara et al., 1999). This seems to be a common situation among marine pelagophil fishes, which spawn pelagic eggs (Amano et al., 2008; Finn, 2007; Hiramatsu et al., 2006; Sawaguchi et al., 2006). Up to date, no molecular alteration of VtgC has been verified during OM in any teleost, thus it does not contribute to the OM-associated production of FAAs. Thus the importance of Vtg multiplicity is obvious because of its participation in critically important physiological reproductive events of marine teleosts, including hydration of mature oocytes, buoyancy and generation of the necessary nutrient stocks for embryo and larvae (Amano et al., 2008).

In conclusion, both vitellogenesis and OM are essential events of female reproductive physiology, in which the multiple Vtg scheme plays an important physiological role in the provision of the required nutrients for the embryo and larval development, as well as the accomplishment of proper egg buoyancy. Improperly hydrated eggs usually do not develop into viable larvae and those with inadequate yolk supplies give rise to very poor survival of the progeny (Brooks et al., 1997; Unuma et al., 2005). Thus, it is essential to undertake fundamental studies in aquacultured teleost species, since both processes have important implications in the quantity and the quality of offspring obtained for the intensive production of these species.

Endocrine control of gametogenesis and final maturation

Gametogenesis (spermatogenesis and vitellogenesis) and final maturation (spermiation and OM) are regulated by a cascade of hormones along the brain-pituitary-gonad (BPG) axis (Fig. 1). In this axis, the secretion of the pituitary gonadotropins FSH and LH is controlled by

the brain via the stimulatory action of the GnRHs (Peter and Yu, 1997; Yu et al., 1997), which
 338 are the primary neuropeptides regulating reproduction, acting as integrators of external
 information (*e.g.*, environment, temperature, water fall and social interactions). Dopamine (DA)
 340 in some fishes exerts a negative effect on the functions of GnRH on the pituitary gonadotrophs
 (Chang and Jobin, 1994). The FSH and LH are released into the bloodstream to act on the
 342 gonad, where they stimulate the synthesis of the sex steroid hormones (androgens, estrogens and
 progestogens), which are the ultimate effectors of gonadal development.

344 Hormonal regulation of fish spermatogenesis and spermiation has been described
 previously (Billard et al., 1990; Schulz and Miura, 2002; Vizziano et al., 2008; Watanabe and
 346 Onitake, 2008) and an updated review can be found in this special issue (Schulz et al., 0000).
 Testicular spermatogenesis, as well as spermiation, is regulated by pituitary FSH and LH
 348 secretion through the action of the sex steroid hormones, as well as other growth factors. Before
 the onset of spermatogenesis, spermatogonial stem cell renewal seems to be regulated by E₂
 350 acting on Sertoli cells (Miura and Miura, 2003). The androgen 11 keto testosterone (11KT) is
 the major regulator of spermatogenesis, while the maturation inducing steroid (MIS) regulates
 352 sperm capacitation and spermiation (Miura and Miura, 2003). Both steroids are synthesized by
 the somatic Leydig cells of the testes, after GtH stimulation. The LH is mainly involved in the
 354 stimulation of androgen production in Leydig cells, whereas FSH seems to exert more complex
 functions in the male testes, stimulating androgen production from the Leydig cells, as well, but
 356 also regulating Sertoli cell activity during spermatogenesis. Although the regulatory
 mechanisms of FSH are mostly unknown, possible functions of FSH in the testes include the
 358 stimulation of Sertoli cell proliferation and differentiation, and the synthesis of certain growth
 factors that act as autocrine and paracrine factors involved in Sertoli cell proliferation and
 360 differentiation and germ cell development (Schulz and Miura, 2002).

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The onset of spermatogenesis is marked by the switch from spermatogonial self-renewal to spermatogonial proliferation through a number of mitotic divisions that is species specific in fishes, a process controlled by the secretion of pituitary GtHs (mainly FSH) (Schulz and Miura, 2002). The FSH acts on Sertoli cells and stimulates 11KT biosynthesis, which in turn regulates the full process of spermatogenesis, mediated also by growth factors (*e.g.*, insulin-like growth factor I, IGF-I or activin B) secreted by the Sertoli cells. In males, FSH levels are high at early spermatogenesis, increase to maximum levels during the rapid testicular growth phase and then decline after spawning. On the other hand, LH is low during early spermatogenesis, increases during spermiogenesis and peaks during the spawning season (Gomez et al., 1999; Mateos et al., 2003; Miwa et al., 1994; Mylonas et al., 1997c), when LH induces a shift in the steroidogenic pathway of the testes leading to the production of the MIS. The MIS is synthesized in the spermatozoa by the activity of 20 β -hydroxysteroid dehydrogenase, converting 17 α -hydroxyprogesterone synthesized in Leydig cells (Asahina et al., 1990; Barry et al., 1990). In response to the production of MIS there is activation of specific enzymes that increase seminal plasma pH, which in turn induces spermatozoa capacitation (Alavi and Cosson, 2005; Clearwater and Crim, 1998; Miura et al., 1992; Woolsey and Ingermann, 2003). In males, androgen production (T and 11KT) remains high through the entire spawning period, even while MIS levels are high, since spermatogenesis, spermiogenesis and spermiation occur concurrently.

In females, a predominant role has been suggested for FSH during vitellogenesis in fishes with synchronous ovarian development. On the other hand, in fish with asynchronous ovarian development the role of FSH in vitellogenesis is less clear and a possible function has been ascribed also to LH. This is due, partly, because of the parallel fluctuations of FSH β and LH β transcripts during ovarian growth of these species (see review by Rosenfeld et al., 2007) and partly because the gonadotropic control of vitellogenesis is relying on the follicular production

of E₂ and it has been shown that both FSH and LH are able to stimulate its synthesis *in vitro*.

386 However, a differential regulation of the two gonadotropins could occur also at the receptor
level. Thus, in the Atlantic halibut (*Hippoglossus hippoglossus*), which shows an asynchronous
388 oogenesis, FSH receptors but not LH receptors were expressed in the smallest follicles
corresponding to the stages of primary growth and vitellogenesis (Kobayashi et al., 2008). Also,
390 it has been shown in the European sea bass, that homologous FSH stimulates the release of E₂ by
ovarian fragments in a dose- and time-dependent manner (Molés et al., 2008). In addition, this
392 stimulation was greater in females in early and mid vitellogenesis, coinciding with the time of
high expression levels of FSH receptor (FSHR) in the ovarian follicles (Luckenbach et al., 2008;
394 Rocha et al., 2008). Also, a specific role of FSH on stimulating cytochrome P-450 aromatase
activity and mRNA expression *in vitro*, the enzyme catalyzing the conversion of T to E₂, has
396 been demonstrated in brown trout (*Salmo trutta*) follicles from vitellogenic ovaries (Montserrat
et al., 2004). Furthermore, FSH seems to stimulate Vtg incorporation into follicles of rainbow
398 trout (Jalabert, 2005). Gonadotropic stimulation of the ovary during the period of vitellogenesis
induces steroidogenesis in a two-cell biosynthetic process, in which the outer theca layer
400 synthesizes T that is transported into the granulosa cells and converted to E₂. During
vitellogenesis, E₂ regulates oocyte development and the synthesis of Vtg and other yolk related
402 proteins by the liver. In addition to the gonadotropic and E₂ control of vitellogenesis, it has been
suggested that other hormones (*e.g.*, T) and paracrine factors could cooperate in the Vtg uptake
404 by the growing follicles (reviewed by Hiramatsu et al., 2006; Jalabert, 2005; Polzonetti-Magni et
al., 2004).

406 At the conclusion of vitellogenesis, OM is triggered by the action of LH on the follicle
cells, which synthesize and secrete the maturation inducing hormone (MIH) or maturation
408 inducing steroid (MIS) (Nagahama et al., 1994; Suwa and Yamashita, 2007). In salmonids

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(*Onchorhynchus* and *Salmo* spp.), and a few freshwater and marine fishes the MIS is the
 410 progestin 17,20 β -dihydroxy-4-pregnen-3-one (17,20 β P). In some other marine species, a
 derivative of 17,20 β P the 17 α ,20 β ,21-trihydroxy-4-pregnen-3-one (20 β S) has been described to
 412 act as MIS (King et al., 1995; Schulz and Miura, 2002; Thomas et al., 1995). Both 17,20 β P and
 20 β S are acting as MIH in European sea bass (Asturiano et al., 2000), striped bass (*Morone*
 414 *saxatilis*) (Mylonas et al., 1997c) and red seabream (*Pagrus major*) (see review by Suwa and
 Yamashita, 2007) (Fig. 4). The MIS binds to specific receptors on the oocyte plasma membrane
 416 and the signal received in the oocyte surface is transduced to the cytoplasm to finally result in
 the formation and activation of the maturation-promoting factor (MPF), which is responsible for
 418 the resumption of meiosis and completion of oocyte maturation (Nagahama et al., 1994).
 Although in some species both FSH and LH have been implicated in the process (Jalabert,
 420 2005), it is generally admitted that oocyte maturation is a two stage LH-dependent event, with
 some paracrine factors also being involved (Bobe et al., 2008). The first stage is referred to as
 422 oocyte maturational competence (OMC) and consists on the acquisition by the follicles of the
 ability to produce and by the oocyte to respond to MIH (Patiño et al., 2001). The second stage
 424 comprises the period of actual production of MIH and resumption of oocyte meiosis (Patiño and
 Sullivan, 2002).

3. OPTIMAL CONDITIONS FOR REPRODUCTION IN CAPTIVITY

The first step in proper broodstock management is the identification of the optimal
 430 conditions required for a species to undergo reproductive maturation and produce gametes of
 good quality. The various factors involved during gametogenesis and having impact on gamete
 432 quality are examined elsewhere in this special issue (Bobe and Babiak 0000). This section

focuses on environmental factors that need to be controlled in order to obtain normal spawning.

Data on the eco-biology of each species of interest in its natural environment may be very useful in creating adequate culture conditions, leading to reproductive maturation and spawning.

Environmental factors may be used during gametogenesis to manipulate fish spawning time in order to get viable gametes on a year-round basis (Bromage et al., 2001; Chemineau et al., 2007). Such manipulations may affect reproductive performance (King and Pankhurst, 2007; Pankhurst and Thomas, 1998). In some cases, a proper control of environmental factors may be enough to obtain natural spawning from cultured fishes, as in the African catfish (*Clarias gariepinus*) (El Naggar et al., 2006; Okumura et al., 2003). Besides, employing optimal environmental conditions reduces stress, which may be enhanced by the spawning induction process itself (Mousa and Mousa, 2006).

3.1. Environment

Spawning induction efficiency may depend greatly on water temperature. Within the range of physiological temperatures, higher temperatures usually speed up the process without any adverse effects. Outside this range, higher temperature is unfavourable and may affect spawning success and progeny quality. Thus, in Arctic charr (*Salvelinus alpinus*), which spawns naturally at a very low temperature, a DA inhibition of LH release may occur at 10°C (Gillet et al., 1996). High temperature may also delay the ovarian response of rainbow trout to GtH, by modifying its steroidogenic pattern (Pankhurst and Thomas, 1998). It is also known that temperature may modulate steroid conjugation and thus active free steroid concentration (Kime, 1979). On the contrary, in species spawning in warmer water, such as the black sea bass (*Centropomus striata*), low temperature may delay this response (Cerdá et al., 1996), and in common carp (*Cyprinus carpio*) the interval between hormone administration and initial egg release was

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negatively correlated with water temperature within the range of 20-26°C (Drori et al., 1994).

However, a deleterious effect of too high temperature was observed in grass carp (*Ctenopharyngodon idella*), by acting at various levels of the brain-pituitary-gonad endocrine axis (Glasser et al., 2004).

Salinity is another environmental parameter that can influence reproductive function.

Because freshwater is limiting and transferring large broodstock is costly and cumbersome, anadromous species such as salmon, which normally spawn in fresh water, are usually maintained in seawater during the reproductive season. However, ovulation may be partially blocked in seawater and follicle hydration is disturbed (Sower and Shreck, 1982), a dysfunction that may be related to the loss of hypo-osmoregulatory ability in mature fish (Uchida et al., 1997). In such case, a short transfer to freshwater may be enough to overcome this blockage (Haffray et al., 1995). On the other hand, in many euryhaline species spawning induction can be successful at various salinities (Haddy and Pankhurst, 2000; Lee et al., 1992). Thus, in the black bream (*Acanthopagrus bucheri*), GnRHa treatment induced ovulation at salinities ranging from 5‰ to 35‰ (Haddy and Pankhurst, 2000). However, a 17,20β-dihydroxy-4-pregnen-3-one plasma level increase was detectable only at 20‰, and the number of ovulations and fertilization success were lowest in fish held at 5‰.

Tank size, water volume and/or depth and stocking density have been shown to influence reproductive success in some cultured fishes. Spontaneous spawning behaviour usually requires moderate to large holding volumes and low stocking densities in most fishes, including the greenback flounder (*Rhombosa tapirina*) (Pankhurst and Fitzgibbon, 2006). In the Nile tilapia (*Oreochromis niloticus*), a low stocking density and water-flow rate is favourable for spontaneous spawning and good egg quality (Tsadik and Bart, 2007). However, the domestication process facilitates spontaneous spawning in captivity as stressed by Zohar and

Mylonas (2001a) for the gilthead seabream. Except in species that require substrate for their spawning, optimizing tank size and water depth should be enough in most fishes to facilitate normal breeding behaviour and obtain successful spawning (Okumura et al., 2003; Okumura et al., 2002), although the impact of such factors may be more, or less significant depending on the species (Buchet et al., 2008; Ibarra-Castro and Dunca, 2007). Even a large pelagic species such as yellowfin tuna (*Thunnus albacares*) may spawn in a relatively small tank (Wexler et al., 2003). In fact, such factors are difficult to analyze by themselves, because tank size is correlated to water volume and depth, and may also influence water flow rate and quality (*i.e.*, dissolved oxygen).

3.2. Social factors

Social interactions have major consequences on spontaneous spawning, both in male and female fish, and attention has been especially paid to pheromones (Stacey, 2003), with goldfish (*Carassius auratus*) being a leading model of hormonal sex pheromone function (Appelt and Sorensen, 2007). However, applications of breeding behaviour and its pheromonal control to broodstock management remain limited (Hong et al., 2006).

In several practical trials for spawning induction, a male to female sex ratio equal or higher to 1 is preferred (Haddy and Pankhurst, 2000; Meseda and Samira, 2006; Pavlidis et al., 2004). In the spotted rose snapper, no significant difference was observed between male to female sex-ratios of 1:1, 1:3 or 1:5 (Ibarra-Castro and Dunca, 2007). Also, spawning may be more efficient in groups than in single pairs both, for spontaneous spawning (El Naggar et al., 2006) and after spawning induction (Forniés et al., 2003). However rigorous experiments evaluating properly the optimal sex ratio for cultured broodstocks are rarely published.

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5. MONITORING REPRODUCTIVE MATURATION

Accurate evaluation of the stage of reproductive maturation is a prerequisite for the success of hormonal induction of OM and spermiation, so that the type of the necessary hormonal treatment can be determined and the time of administration chosen. Treatments given to immature individuals or to adult fish too early in the reproductive cycle are ineffective or inefficient. The most common, practical and perhaps reliable method of determining stage of reproductive maturation in fish is the acquisition of milt by gentle abdominal pressure (Fig. 5A) in the males, although in some species this is not possible (Viveiros et al., 2002), and the biopsying of developing oocytes from the ovary with the use of a catheter in the females (Fig. 5B).

The stage of ovarian maturation is usually determined by (a) measuring the mean or maximum oocyte diameter (Garcia, 1989; Mylonas et al., 2004c; Shiraishi et al., 2005), (b) determining the position of the nucleus (germinal vesicle) (Billard et al., 1995b; Kagawa et al., 2005; Lutes et al., 1987; Mylonas et al., 1995b; Yaron, 1995) or (c) identifying the onset of coalescence of the lipid droplets (Fauvel et al., 1999; Mylonas et al., 2003b; Mylonas et al., 1997d). The stage of testicular maturation may not be evaluated using testicular biopsies during spermatogenesis, and is usually limited to the period of spermiation, when milt can be obtained using abdominal pressure (Billard et al., 1995a; Rurangwa et al., 2004). The stage of spermiation is evaluated based on the ease and/or amount of milt released after abdominal pressure, using subjective evaluation scales. For example, a spermiation index may be established on a subjective scale from 0 to 3, with 0 = no sperm released, 1 = only a drop of sperm released after multiple stripping attempts, 2 = sperm easily released after the first

stripping attempt, and 3 = copious amounts of sperm flowing with the slightest abdominal pressure (Mylonas et al., 2003a).

Other methods for evaluating stage of reproductive maturation include the determination of plasma levels of Vtg in females, or sex steroid hormones in both males and females, as these change dramatically and reliably during the different stages of gametogenesis and maturation. However, these methods are more invasive and may have a process time of hours to days, depending on the method of quantification. Recently, non-invasive methods have been developed for measuring female specific proteins or sex steroid hormones in skin mucus (Hiramatsu et al., 2006; Kishida et al., 1992; Schulz et al., 2005a). Such methods are better suited for large fish that are difficult to handle or very prone to stress, such as the bluefin tunas (*Thunnus* spp) (Corriero et al., 2007).

6. REPRODUCTIVE DYSFUNCTIONS

The reproductive dysfunctions observed in culture range from the complete absence of reproductive development observed in freshwater eel (Kagawa et al., 2005; Palstra et al., 2005; van Ginneken and Maes, 2005), to the absence of only gamete release (*i.e.*, spawning) observed in cultured salmonids (Bromage et al., 1992). However, the most common dysfunctions include the production of lower quantity of milt and/or sperm during the spermiation period and the failure to undergo OM at the completion of vitellogenesis (Mañanos et al., 2008; Mylonas and Zohar, 2001b; Mylonas and Zohar, 2007; Zohar and Mylonas, 2001b).

6.1 Males

As mentioned earlier, reproductive dysfunctions of captive fishes are not restricted to females, since males may produce a reduced amount of milt and of lower quality, even though

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they do undergo complete spermatogenesis and spermiation in captivity (Mañanos et al., 2008; Mylonas and Zohar, 2001b; Mylonas and Zohar, 2007; Zohar and Mylonas, 2001b). A reduced amount of milt production represents a serious problem for those species in which hatchery production is based on artificial fertilization and the acquisition of gametes by manual stripping. Production can be limited by difficulties in acquisition of adequate milt from male breeders and may necessitate the use of a much higher number of male breeders than if spawning could occur spontaneously. On the other hand, for species that spawn spontaneously in the tank, the production of highly viscous milt reduces the rapid dispersal of the spermatozoa and thus reduces the sperm fertilization capacity (Vermeirssen et al., 2000). Lower plasma levels of LH during the spermiation period have been suggested as the cause of the reduced amount of milt produced by some fishes (Mañanos et al., 2002; Mylonas and Zohar, 2001a). The amount of LH in the pituitary or the ability of the pituitary to synthesize LH in response to treatment with exogenous GnRHa 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.

6.2 Females

The simplest reproductive problem in cultured fishes is observed in cultured salmonids, which do undergo vitellogenesis, OM and ovulation, but fail to spawn their eggs and milt when reared in captivity (Bromage and Cumaranatunga, 1988; Zohar, 1989), probably due to a loss of the spawning behaviour caused by domestication, or the lack of the appropriate spawning substrate to place eggs. This reproductive dysfunction in salmonids is not causing great problems to the industry, as the ovulated eggs remain viable in the abdominal cavity for many days to a few weeks, and can be obtained easily by stripping and fertilized artificially (Craig and Harvey, 1984; Sakai et al., 1975). In fact, this characteristic may be advantageous for a

commercial hatchery, particularly if used in conjunction with hormone-based synchronization protocols (see later), as it allows (a) collection of eggs from a number of females that have ovulated at different times during the course of a week and (b) fertilization with selected sperm. In other species, however, the need to collect the eggs by stripping is a serious limitation, as the time of ovulation must be predicted with accuracy, as over-ripening may take place in minutes or hours after ovulation (reviewed in Bromage, 1995).

In most other fishes, the reproductive dysfunction often observed in culture is that fish undergo vitellogenesis during the reproductive period, but fail to undergo OM and, as a result, there is no ovulation and no spawning of eggs (Agulleiro et al., 2006; Barbaro et al., 2002; Berlinsky et al., 1996; Berlinsky et al., 1997; Chen, 2005; Duncan et al., 2003; Fauvel et al., 2008; Ibarra-Castro et al., 2004; Larsson et al., 1997; Marino et al., 2003; Mugnier et al., 2000; Mylonas et al., 2007; Mylonas et al., 2004a; Mylonas and Zohar, 2001a; Yang and Chen, 2004). The endocrine cause of the failure of female fish to undergo OM has been identified to be a dysfunctional release of LH from the pituitary at the end of vitellogenesis. In striped bass, for example, comparison of plasma levels of reproductive hormones between cultured fish that fail to undergo OM and wild fish captured on their spawning grounds showed that a plasma LH surge accompanied OM and ovulation in wild females, but in females reared in captivity plasma LH levels remained low at the end of vitellogenesis (Mylonas et al., 1997c; Mylonas et al., 1998b; Mylonas and Zohar, 2001a). 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

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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 GnRHs. 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).

7. HORMONAL THERAPIES

Based on the evidence that the failure of cultured fishes to undergo full spermiation and OM is the result of diminished LH release from the pituitary, manipulations of reproductive function have focused first on the use of exogenous LH preparations that act directly at the level of the gonad, and more recently on GnRHa --with or without DA-- that releases the endogenous LH stores from pituitary (Fig. 6). Endogenous LH, in turn, acts at the level of the gonad to induce steroidogenesis and the process of OM and spermiation.

Luteinizing hormone preparations include (a) homogenates and purified extracts from the pituitary of mature fish during the reproductive season --most commonly of carp and salmonids-- that contain high amounts of LH, and (b) purified human Chorionic Gonadotropin (hCG) that has very strong LH activity (Donaldson and Hunter, 1983; Lam, 1982; Zohar, 1989; Zohar and Mylonas, 2001b). Pituitary homogenates were the first type of exogenous hormonal treatments used by aquaculturists for the induction of maturation and spawning (Fontenele, 1955; Houssay, 1930; Von Ihering, 1937). Today, preparations of carp and salmon pituitary extracts (CPE and SPE, respectively) are purified to various extends, and are available in commercial products with their activity being calibrated using bioassays (Donaldson, 1973; Yaron, 1995). Human CG has

624 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
626 standardized bioactivity. Unlike LH preparations of piscine origin, hCG is often effective in a
single dose, presumably due to its long half-life in circulation (Ohta and Tanaka, 1997). This is
628 not 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
630 (Ludwig et al., 2002). Recently, an hCG preparation has been approved for commercial
utilization in commercial aquaculture (CHORULON™, Intervet International bv, The
632 Netherlands).

With the discovery and commercial synthesis of various agonists of GnRH (GnRHa) for
634 human medicine (Schally, 1978; Ulloa-Aguirre and Timossi, 2000), their use for spawning
induction therapies in fish increased rapidly, due to their important advantages over LH
636 preparations. Firstly, GnRHa treatments are not as species-specific as LH ones, due to the high
structural similarity of native GnRHs among fishes (Lethimonier et al., 2004) Secondly, being of
638 synthetic nature, GnRHas do not pose a disease transmission threat, as CPE and SPE may do.
Thirdly, GnRHas are acting at a higher level of the brain-pituitary-gonad axis and stimulate the
640 release of the endogenous GtHs (LH and FSH) as well as other pituitary hormones that may be
important to reproductive functions (Cyr and Eales, 1996; Le Gac et al., 1993; Negatu et al.,
642 1998; Weber et al., 1995), and thus provide for a better integration of reproductive processes.
The only approved GnRHa for use in commercial aquaculture is Azagly-nafarelin
644 (GONAZON™, Intervet International bv, The Netherlands).

As mentioned earlier, in some fishes there is a strong inhibition of basal and GnRH-
646 stimulated release of LH by DA. Therefore, administration of DA antagonists (*e.g.*,
domperidone, pimozide, reserpine or metoclopramide) prior to the treatment with GnRHa

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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 antagonist treatment are used mostly in cyprinids (Kaminski et al., 2004; Mikolajczyk et al., 2003; Mikolajczyk et al., 2004; Yaron, 1995), catfishes (Brzuska, 2001; Silverstein et al., 1999; Wen and Lin, 2004) and mullets (Aizen et al., 2005; Arabaci and Sari, 2004; Glubokov et al., 1994).

Almost from the first spawning induction experiment in cultured fishes, it was recognized that long-term administration of the hormone would result in improved efficacy (Fontenele, 1955). This is because OM and spermiation often require a prolonged hormonal treatment, given in multiple injections (Carrillo et al., 1995; Dabrowski et al., 1994; Mylonas et al., 1992; Pankhurst et al., 1996; Slater et al., 1994). Such repetitive handling may be stressful and damaging to the brood fish and in situations where the broodfish are very large (*e.g.*, 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. As a result, a variety of hormone-delivery systems have been developed during the last 20 years for use in cultured fishes (Mylonas and Zohar, 2001b). Although a delivery system for LH has also been reported (Sato et al., 1995), the ones employed extensively in aquaculture contain exclusively GnRHa. The GnRHa delivery system may be prepared in the form of implantable cylindrical pellets of cholesterol (Weil and Crim, 1983) or Ethylene-Vinyl Acetate (EVAc) (Mylonas et al., 2007; Zohar, 1996), or in the form of injectable biodegradable microspheres using co-polymers of lactic acid and glycolic acid (LGA) or a co-polymer of fatty acid dimer and sebacic acid (Fad-sa) (Barbaro et al., 2002; Breton et al., 1990; Chang et al., 1995; Mylonas et al., 1997b; Mylonas et al., 1995a; Mylonas and Zohar, 2001a; Zohar, 1988). Although the solid implantable GnRHa-delivery systems are easier to use by aquaculturists, the microspheric delivery systems have the

advantage of being biodegradable and of being able to use the same preparation to treat fish with large variation in size. Upon application, the GnRHa delivery systems release GnRHa for periods from 1 to 5 wks, depending on the preparation (Crim et al., 1988; Mañanos et al., 2002; Mylonas et al., 1998b; Mylonas and Zohar, 2001b; Zohar, 1996).

7.1 Spermatogenesis and spermiation

As mentioned earlier, with the exception of the freshwater eels, cultured male fishes do undergo spermatogenesis and spermiation in captivity, but often produce milt of lesser quantity or quality. So, in essence, the objective of hormonal therapies in male cultured fishes is primarily to increase seminal fluid production and secondarily to enhance completion of spermatogenesis (spermiogenesis and spermiation). Due to the long-term nature of the process of spermatogenesis and spermiation, which takes months to weeks (Schulz and Miura, 2002), long-term hormonal therapies with GnRHa-delivery systems have proven more effective in enhancing milt production compared to acute treatments with either LH preparations or GnRHs (Fig. 7). In the rabbitfish (*Siganus guttatus*), for example, milt production increased significantly 24 h after GnRHa injection, but returned to pre-treatment levels 48 h later (Garcia, 1991). In carp, (*Cyprinus carpio*), daily injections of GnRHa induced a sustained elevation of sperm production for 5 d by, but milt volume decreased below pre-treatment levels 3 d after the treatment was interrupted (Takashima et al., 1984). In the winter flounder (*Pleuronectes americanus*) a single GnRHa 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 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). The results indicate that for the induction of a

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sustained increase in milt production, especially in individuals that are stripped spawned during the reproductive season, a long-term hormonal therapy is necessary, either through the use of multiple injections or controlled-release delivery systems.

Many different GnRHa-delivery systems have been used to enhance spermiation in cultured fishes, and the first applications were in salmonid fishes such as Atlantic salmon (*Salmo salar*) (Weil and Crim, 1983; Zohar, 1996), rainbow trout (Breton et al., 1990), coho salmon (*O. kisutch*) (Goren et al., 1995) and chinook salmon (*O. tshawytscha*) (Solar et al., 1995). In the European seabass, treatment with GnRHa-delivery systems resulted in increased milt production for 28-35 d, compared to 7 d only when a single injection of GnRHa was given (Mañanos et al., 2002; Sorbera et al., 1996). Also in the striped bass, GnRHa-delivery systems induced increases in milt production for 14 to 20 d (Mylonas et al., 1997b; Mylonas et al., 1998a). GnRHa implants have also been used in Atlantic halibut to enhance the quality of the sperm, which is extremely viscous and exhibits very little spermatozoa motility towards the end of the spawning season (Vermeirssen et al., 2003), in starry flounder (*Platichthys stellatus*) to increase milt volume and sperm density (Moon et al., 2003) and in greenback flounder (*Rhombosolea tapirina*) to increase sperm volume (Lim et al., 2004). Still, in some species simple injections of GnRHa or LH preparations have been employed for the successful enhancement of spermiation, including the Siberian sturgeon (*A. baerii*) (Williot et al., 2002), the sterlet (*Acipenser ruthenus*) (Rzemieniecki et al., 2004), the precocious European sea bass (Schiavone et al., 2006) and the minnow (*Rhynchocypris oxycephalus*) (Park et al., 2002).

7.2 Oocyte maturation and ovulation

In females, the hormonal therapies employed in aquaculture may be classified to (a) those for the stimulation/completion of vitellogenesis, so that the oocytes undergo/complete

vitellogenesis and can then undergo OM and ovulation in response to another hormonal therapy; and (b) those for the induction of OM and ovulation alone (Mañanos et al., 2008). Since the process of vitellogenesis is a long one, lasting for weeks to months, such therapies are cumbersome, expensive and not used very often, with the exception of the freshwater eel (Kagawa et al., 2005; van Ginneken and Maes, 2005). In addition, as mentioned earlier, vitellogenesis is usually completed in most captive reared fishes. Therefore, most of the hormonal therapies for the control of reproduction in female fishes are focused on the induction of OM and ovulation.

Due to the significant differences both in biology and management, hormonal treatments may be different in species with synchronous ovarian development (single-time and single-batch group-synchronous) and asynchronous ovarian development (multiple-batch group-synchronous and asynchronous) (Tyler and Sumpter, 1996). A single or double GnRHa injection protocol may be effective in synchronous fish (Mylonas et al., 1992), which have all their oocytes developed at the same stage of maturation (Fig. 3A), but GnRHa-delivery systems may be more effective in achieving maximum fecundity in asynchronous species with a long reproductive season (Barbaro et al., 1997; Berlinsky et al., 1996; Larsson et al., 1997; Mugnier et al., 2000; Zohar et al., 1995). Also, if required, strip spawning and artificial insemination (see later) is a good alternative to tank spawning in synchronous fishes, but will result in very poor fecundity in asynchronous species, since the fish mature and ovulate only part of their total season production of vitellogenic oocytes (Fig. 3B), and the stripping process may damage the remaining oocytes.

7.2.1 *Synchronous oogenesis*

The use of CPE, SPE and hCG in spawning induction therapies in synchronous fishes, together with information on doses and treatment protocols has been published in previous

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744 reviews (Donaldson, 1973; Donaldson and Hunter, 1983; Lam, 1982; Mañanos et al., 2008;
Zohar and Mylonas, 2001a). Recent examples of the use of LH preparations include the
746 European catfish (*Silurus glanis*), where 4 mg Kg⁻¹ CPE induced OM and ovulation, though in a
smaller percentage of females compared to a combined GnRHa/DA antagonist treatment
748 (Brzuska, 2001). In the catfish “cachara” (*Pseudoplatystoma fasciatum*) from Brasil, CPE and
hCG were both effective in inducing OM and ovulation (Leonardo et al., 2004). In the Japanese
750 catfish (*S. asotus*), a single injection of 10,000 IU Kg⁻¹ hCG induced OM and ovulation
(Kumakura et al., 2003). Also, a single injection of hCG at 1000 or 2000 IU Kg⁻¹ was effective
752 in inducing OM and ovulation in the spotted sea bass (*Lateolabrax maculatus*) (Lee and Yang,
2002). Finally, in ocellated puffer (*Takifugu ocellatus*), both single and double injections of 6
754 mg Kg⁻¹ CPE 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
756 hCG were effective in inducing ovulation (Zakes and Szczepkowski, 2004).

The aquaculture production of sturgeon (*Acipenser* spp.) relies exclusively on the
758 production of eggs via hormonal induction of OM and ovulation, whereas fertilization is
undertaken artificially. Sturgeon females are evaluated for the completion of vitellogenesis and
760 the extent of the migration of the nucleus by surgical removal of oocytes from the ovary and
their *in vitro* processing (Conte et al., 1988; Williot et al., 1991). The selected mature females
762 may be given sturgeon pituitary extract, CPE, or more recently GnRHa (Burtsev et al., 2002;
Chebanov and Billard, 2001; Webb et al., 1999; Williot et al., 2002; Williot et al., 2001; Zhuang
764 et al., 2002), usually in a priming and a resolving injection spaced 10-24 h apart, and ovulation is
accomplished 24-50 h afterwards. Single treatments with CPE have also been reported to be
766 effective (Williot et al., 2005).

One of the very first applications of GnRHa in aquaculture included the synchronization of
 768 OM and ovulation in salmonids, as a tool for enhancing broodstock management operations and
 reducing pre-spawning mortalities (Breton et al., 1990; Crim and Glebe, 1984; Donaldson et al.,
 770 1981). Treatment with GnRHa is usually given <2 weeks before the onset of natural maturation
 of the broodstock, and is given in the form of two injections ($10\text{-}100\text{ }\mu\text{g Kg}^{-1}$) spaced 3 days
 772 apart or in a single application of a GnRHa-delivery system ($10\text{-}50\text{ }\mu\text{g Kg}^{-1}$). Both the two
 GnRHa injection (Mylonas et al., 1992; Sullivan et al., 1989; Van Der Kraak et al., 1985) and
 774 GnRHa-delivery system protocols (Breton et al., 1990; Crim et al., 1983; Crim and Glebe, 1984;
 Goren et al., 1995) induce ovulation in 100% of the population within 10-14 days after
 776 treatment. Single or multiple injections of GnRHa have also been used extensively in other
 fishes with synchronous ovarian development. In the two-injection protocols, GnRHa is given
 778 in a priming dose (5-10%) and a resolving dose (95-90%). If a DA antagonist is also used, it is
 administered together with the priming dose. For example, in the ocellated puffer both a single
 780 and double injections of $50\text{ }\mu\text{g Kg}^{-1}$ GnRHa were effective in inducing OM and ovulation (Chen,
 2005), while similar results were obtained using 2-4 injections of GnRHa in the bullseye puffer
 782 (*Spoeroides annulatus*) (Duncan et al., 2003). In the grey mullet (*Mugil cephalus*), two
 injections of $30\text{ }\mu\text{g Kg}^{-1}$ GnRHa together with 15 mg Kg^{-1} of the DA antagonist metoclopramide
 784 were very effective in inducing spawning within 24 h (Aizen et al., 2005). Similarly, two
 injections of $20\text{ }\mu\text{g Kg}^{-1}$ GnRHa with 5 mg Kg^{-1} of the DA antagonist pimozide induced
 786 ovulation in 95% of treated common carp (Mikolajczyk et al., 2004). Two injections of GnRHa
 in combination with a DA antagonist have been used successfully also in the koi carp (*Cyprinus*
 788 *carpio*) (Arabaci et al., 2004), lake mullet (*Chalcalburnus tarichi*) (Arabaci and Sari, 2004) and
 wild catfish (*Silurus asotus*) (Wen and Lin, 2004). Finally, a single injection of $20\text{ }\mu\text{g Kg}^{-1}$
 790 GnRHa induced ovulation in tench (*Tinca tinca*) (Rodríguez et al., 2004).

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GnRHa-delivery systems have also been shown to be very effective in inducing OM, ovulation and spawning in fishes with synchronous ovarian development (Mylonas and Zohar, 2001b; Mylonas and Zohar, 2007). Whereas a GnRHa-delivery system induced spawning in the yaqui catfish (*Ictalurus pricei*), combined sGnRHa/DA antagonist or catfish PE treatments were ineffective (Mylonas and Zohar, 2001b). In the tiger puffer (*T. rubripes*), GnRHa-delivery systems ($400 \mu\text{g Kg}^{-1}$) induced ovulation after 18 and 10 d in fish with small (800-900 μm) and large (900-1000 μm) mean oocyte diameter, 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 (Brzuska and Bialowas, 2002).

7.2.3 Asynchronous oogenesis

In fish with asynchronous ovarian development, such as the greater ambejack, GnRHa-delivery systems have been used preferentially to injections for the induction of multiple OM and ovulation cycles (Fig. 8). For example, GnRHa-delivery systems induced two consecutive spawns within 3 d in white bass (*M. chrysops*) (Mylonas et al., 1997a) and greater amberjack (*Seriola dumerili*) (Mylonas et al., 2004c), five spawns in 7 d in the barramundi (*Lates calcarifer*) (Almendras et al., 1988), five ovulations in 2 weeks in striped trumpeter (*Latris lineate*) (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 in 10 d in the dusky grouper (*E. marginatus*) (Marino et al., 2003). The above species are considered to have a multiple-batch group-synchronous ovarian development, and are able to produce a few spawns in irregular intervals during the annual reproductive season. Still, the greatest potential of sustained-release GnRHa-delivery systems is in the induction of OM in asynchronous fishes with daily --or almost

daily-- ovulation/spawning frequency. Some examples, include the red porgy (*Pagrus pagrus*),
 816 red seabream (*P. major*) and gilthead seabream (*Sparus aurata*), which have an asynchronous
 mode of ovarian development and are capable of undergoing OM and spawning on a 24-h cycle
 818 for periods of a few months (Mylonas et al., 2004b; Watanabe and Kiron, 1995; Zohar et al.,
 1995). While a single injection of GnRHa in the gilthead seabream induced only 20% of the
 820 broodstock to undergo daily spawning, a GnRHa-delivery system induced daily spawning in
 >70% of the broodstock. Similar results have been obtained with the other two species
 822 (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
 824 the number of ovulations per spawning season (Barbaro et al., 2002; Berlinsky et al., 1996;
 Larsson et al., 1997).

826 Different GnRHa-delivery systems have been used also to induce multiple spawns in
 various flatfishes, which often do not mature spontaneously in captivity. In the greenback
 828 flounder (*Rhombosolea tapirina*), for example, GnRHa-delivery systems induced daily
 ovulations (Poortenaar and Pankhurst, 2000), and in wild-caught summer flounder (*Paralichthys*
 830 *dentatus*) GnRHa implants induced daily ovulations for 8 d (Berlinsky et al., 1997). Moreover,
 in fish maintained in captivity for more than a year, the same treatment induced not only
 832 ovulation, but also spontaneous spawning (Watanabe et al., 1998). Similarly in turbot
 (*Scophthalmus maximus*), treatment with a GnRHa-delivery system induced multiple ovulations
 834 in all 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
 836 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

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(Larsson et al., 1997). The same two GnRHa-delivery systems have also induced daily spawnings for up to 2 weeks in the Senegal sole (*Solea senegalensis*) (Agulleiro et al., 2006).

The most recent success of the GnRHa-delivery systems has been in the induction of OM, ovulation and spawning of viable eggs in wild-caught Atlantic bluefin tuna (*Thunnus thynnus*) reared in sea cage for a period of 1 to 3 years (Mylonas et al., 2007) and in Southern bluefin tuna (*T. maccoyii*) reared in land-based tanks (M. Deichmann, Clean Seas Tuna Ltd, personal communication). GnRHa administration was done underwater in free swimming fish, since it is not possible to anaesthetizing such large (60-120 Kg) bluefin tunas (Mylonas et al., 2007). The use of the same GnRHa-delivery systems has resulted in the induction of 4 consecutive spawnings 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).

850

8. ARTIFICIAL INSEMINATION

Ideally, broodstock should be able to spawn spontaneously in their rearing enclosures (tanks, ponds or cages), even if maturation has been induced hormonally. This allows the fish to express their normal breeding behaviour, release their gametes in synchrony -- thus, producing eggs of high fertilization success, and undergo multiple spawnings in fish with asynchronous ovarian development -- thus, resulting in high seasonal fecundity. Eggs may be collected from the spawning enclosures with various methods, including passive egg collectors fitted on the surface overflow (Liu et al., 2000; Zohar et al., 1995) or active collection with dip nets inside a sea cage (Masuma, 2006; Sawada et al., 2005) for pelagophil fish (*i.e.*, with pelagic, buoyant eggs), or with special containers or mats for fish spawning demersal or adhesive eggs (Huner and Dupree, 1984; Piper et al., 1982).

860

862 However, the natural breeding behavior followed by spontaneous spawning may be lost
in aquaculture conditions, and hormonal induction of OM and spermiation does not ensure
864 spontaneous spawning of the fish –*i.e.*, the timely and synchronous release of both gametes,
necessary for the production of fertilized eggs. This may be due to inappropriate tank size, lack
866 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 many species it is also
868 necessary to employ artificial gamete collection and fertilization, using strip spawning (Billard et
al., 2004; Bromage et al., 1992; Hurvitz et al., 2007; Manning and Crim, 1998; Marino et al.,
870 2003; Suquet et al., 1995; Williot et al., 2005; Yaron, 1995). In addition, artificial insemination
methods are worth developing for management reasons. Some inter-species hybrids exhibit
872 valuable traits for aquaculture, but these could not be produced by natural, spontaneous mating
(Bartley et al., 2001; Paspatis et al., 1999). The development of biotechnologies such as sex
874 control or polyploidy production depends on gamete acquisition and manipulation (Gomlesky,
2003). Finally, the different mating designs required for genetic improvement programs require
876 to cross a large number of specific groups or individuals, and often at the same time and in the
same conditions (Dupont-Nivet et al., 2006), which can be performed only using *in vitro*
878 fertilization. Such fish genetic improvement is more and more associated with genetic resource
preservation, in order to help maintain genetic variability within a fishfarm stock or
880 dissemination of valuable traits (Chao and Liao, 2001).

Artificial insemination methods have been described adequately in previous articles
882 (Alavi et al., 2007; Billard, 1988; Billard et al., 1995a; Scott and Baynes, 1980) and very little
has changed in recent years. Briefly, ovulated eggs and mature sperm (*i.e.*, milt) are obtained in
884 separate dry containers using abdominal pressure or surgery, preventing any water and urine
contamination. In the earliest applied 'dry' method, milt and eggs were mixed thoroughly and

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886 insemination was achieved in the ovarian fluid, before adding culture water -- either fresh or
marine water, depending on species. The later used "wet" method employed culture water
888 immediately after mixing of the milt with the eggs, and insemination was achieved in culture
water. In natural spawning, spermatozoa are usually immotile in the milt and forward motility is
890 initiated by dilution with the culture water. Sperm motility lasts for only a very short period of
time (Cosson, 2007; Scott and Baynes, 1980; Suquet et al., 1994), thus water or urine
892 contamination during sperm stripping should be avoided, as it may activate sperm before mixing
with the eggs.

894 Spermatozoa face many challenges during insemination (Molony and Sheaves, 2001)
and various ways to improve fertilization success have been examined for application in
896 artificial fertilization techniques. One approach is the dilution of milt with an immobilization
solution prior to mixing it with the eggs (Linhart et al., 1987), at which time fertilization is
898 initiated with an activating solution. Such activating solutions are chosen in order to optimize
the percentage and duration of spermatozoa motility, and the composition of these solutions
900 must be adjusted to each fish species taking into account several factors, such as the qualitative
ionic composition, osmotic pressure, and pH of the milt (Alavi and Cosson, 2005; Alavi and
902 Cosson, 2006; Cosson, 2004). However, to our knowledge, there is no means to supply extra-
energy to fish spermatozoa for swimming. The metabolism of fish spermatozoa is species-
904 specific and there is variation among species in the relative importance of ATP pools vs ATP
synthesis to support motility (Burness et al., 2005; Mansour et al., 2003).

906 Another parameter which needs to be optimized for artificial insemination protocols is
the sperm: egg ratio, which is very variable among fishes (Alavi et al., 2007; Suquet et al.,
908 1995), and the evaluation of the type of sperm motility and velocity needed for the success of
fertilization (Cosson, 2007; Martínez-Pastor et al., 2008; Rurangwa et al., 2004), although sperm

910 motility has not always been associated well with fertilization success (Cruz-Casallas et al.,
2005). The variability in fertilization success of fish spermatozoa raised the question of sperm
912 competition, when pools of sperms from different broodfish are used to maintain genetic
variability in the offsprings, and several studies have been performed to analyze relationships
914 between spermatozoa characteristics and offspring genotypes. Spermatozoa velocity has been
shown to be the primary determinant of sperm competition success in Atlantic salmon (*Salmo*
916 *salar*) (Gage et al., 2004) but the relation is opposite in Atlantic cod (*Gadus morhua*) (Rudolfsen
et al., 2008). However, even after equalizing sperm number for *in vitro* fertilization with a pool
918 of males in carp (*Cyprinus carpio*), sperm motility, initial sperm concentration and sperm
velocity could not explain all the variability in number of sired offspring (Kaspar et al., 2007).
920 Further studies are needed to understand other underlying mechanisms of sperm competition
(Stoltz and Neff, 2006), including those able to explain interaction effects with eggs from
922 different females (Rudolfsen et al., 2008).

In regards to the acquisition of eggs for artificial insemination, one of the most
924 important parameters of successful fertilization is the establishment of the time of ovulation
either after natural or hormonally-induced OM. This is because once the eggs are ovulated into
926 the ovarian or abdominal cavity they begin to lose their viability, in a process referred to as over-
maturation or over-ripening (see Billard et al., 1986; Bromage, 1995). This process is
928 temperature-dependent and species-specific and in salmonids it may last for a few weeks
(Springate et al., 1984), in turbot (*Scophthalmus maximus*) for 10-20 h (see Bromage, 1995) in
930 Atlantic halibut and chub mackerel (*Scomber japonicus*) for 4-6 hours (Bromage et al., 1994;
Shiraishi et al., 2005), in groupers of the genus *Epinephelus* for 1-2 h (Tucker, 1994) and in the
932 white bass (*Morone saxatilis*) and Japanese eel (*Anguilla japonica*) it is only a few minutes
(Mylonas et al., 1996; Ohta et al., 1996). Failure to strip the eggs within the appropriate time

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934 interval after ovulation will result in greatly reduced fertilization success. Although it has been
reported that there may be seasonal changes in sperm quality (Alavi et al., 2008; Mylonas et al.,
936 2003a; Papadaki et al., 2008), in general, sperm collection can be undertaken at any time during
the natural spermiation period or after hormonal stimulation. In addition, sperm from most
938 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., 2003a; Papadaki et al., 2008). Therefore, a
940 typical artificial insemination protocol should plan for (a) collection and storage of sperm a few
hours before the expected time of ovulation and (b) stripping of the eggs at the appropriate time
942 after hormonal therapy. This procedure will ensure optimal results in fertilization success.

Once insemination is completed (after 5-10 min) eggs are rinsed with plenty of culture
944 water and are placed in incubators. Eggs of pelagophil fishes are incubated in cylindroconical
containers, which maintain the eggs in constant motion through the use of water upwelling and
946 aeration. Eggs of species with sticky eggs, such as catfishes, cyprinids or sturgeons, may be first
treated to remove the stickiness, using clay, talc suspensions or enzyme solutions (Linhart et al.,
948 2004), or may be incubated as egg masses using "artificial males" (Piper et al., 1982).

9. GAMETE QUALITY

Gamete quality may be defined as the capacity of eggs and sperm to give rise to normal
952 developing embryos and pre-larvae, and not only high fertilization and hatching success or low
mortality (Bonnet et al., 2007). Gamete quality can be a limiting factor in commercial
954 hatcheries, affecting the quantity and quality of the larvae and fry from a given broodstock. The
purpose of this section is not to review the current knowledge of the factors that determine
956 gamete quality in general (see Bobe and Babiak, 0000 in this issue), but to draw the attention to

some considerations relating to the use of hormonal therapies for the induction of OM, ovulation
958 and spermiation.

A common question in regards to hormonal therapies is their effect on egg quality,
960 compared to naturally ovulating or spawning broodfish (Avery et al., 2004; Bonnet et al., 2007;
Papanikos et al., 2003; Slater et al., 1995). These effects may be due to modifications of
962 maternal mRNA and could have delayed consequences on embryonic development (Bonnet et
al., 2007). Hormonal therapies are recommended only if a broodstock is not reproducing
964 normally in captivity, or for management purposes, such as to increase synchronization of
maturation, or to implement inter-specific hybridization or genetic selection programs. In that
966 respect, it makes little practical difference if the resulting egg quality is slightly, yet significant
statistically, reduced compared to naturally spawning, wild populations. Nevertheless,
968 appropriately employed hormonal therapies do not usually have a negative effect on egg quality
(Barbaro et al., 1997; Duncan et al., 2003; Gillet et al., 1996; Haffray et al., 2005; Mugnier et al.,
970 2000) and may enhance egg and sperm quality in some instances (Larsson et al., 1997;
Vermeirssen et al., 2000). Still, further investigations are needed for some species, especially
972 when starting domestication, to improve spawning induction methods (Avery et al., 2004), and,
in some cases, both natural and induced spawning can be used to manage a stock of breeders
974 (Watanabe et al., 2001). The main factors that may have significant consequences on gamete
quality --mainly eggs-- and should be considered when choosing a spawning induction
976 procedure include (a) the developmental stage of the gonads at the time the hormonal therapy is
applied, (b) the type of hormonal therapy, (c) the possible stress induced by the manipulation
978 necessary for the hormone administration, and, (d) in the case of artificial insemination, the
latency period between hormonal stimulation and stripping for *in vitro* fertilization.

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When a hormonal therapy is applied at an early reproductive maturation stage, as it is done in freshwater eels, the aim is to stimulate gametogenesis (Kagawa et al., 2005). However, spawning induction protocols are applied at the end of gametogenesis to induce OM in the females and enhance spermiation in the males. In females, if a hormonal treatment is given too early in the breeding season, when vitellogenesis has not yet been completed in some individuals, the therapy may fail to induce spawning or may give eggs of poor quality (Gardes et al., 2000), whereas on the other hand spawning induction performed too late, at the end of the breeding season may also prove unsuccessful (Carral et al., 2003).

The nature of the hormonal treatment (Denson et al., 2007; Malison et al., 1998; Zohar and Mylonas, 2001b) and the method of its delivery (Barbaro et al., 2002; Gardes et al., 2000; Mylonas and Zohar, 2001b; Szabó, 2001) may also affect egg quality, whereas sperm quality could be less variable due to the type of hormonal treatment (Miranda et al., 2005). A too high level of hormone can also have deleterious effects on egg quality (Gardes et al., 2000; Mylonas et al., 1992), especially in the form of a single injection, whereas hormone-delivery systems give more satisfactory results (Mylonas and Zohar, 2001b). Controlled-release delivery systems for reproductive hormones (mainly GnRHa) produce a long-term elevation in plasma gonadotropins, thus providing a better stimulation of OM and spermiation, resulting in gametes of better quality, especially in fish with asynchronous ovarian development. In addition, hormone-delivery systems are more advantageous than hormone injections in terms of handling stress, as they are effective after a single administration and reduce the excessive and often very damaging handling of the broodfish (Agulleiro et al., 2006; Mugnier et al., 1998). In addition, some hormone delivery systems can be administered underwater in moving fish, in situations where handling and anesthesia is not feasible (Harvey et al., 1988; Mylonas et al., 2007).

Finally, in the case of species that do not spawn spontaneously after ovulation in
1004 captivity and fertilization is achieved artificially after stripping, it has been shown that the
latency period, during which the eggs remain in the ovarian or abdominal cavity after ovulation
1006 and before stripping is directly related to loss of egg quality (Bromage et al., 1994). The latency
period depends on both intrinsic (Wendling et al., 2000) and environmental parameters
1008 (Brzuska, 1999), some of which include species, water temperature (Yaron, 1995), type of
hormone and dose (Wen and Lin, 2004), as well as the history of fish in the preceding period
1010 (*e.g.*, low vs high temperatures) (Tveiten et al., 2001; Van Der Kraak and Pankhurst, 1996) and
the stage of ovarian maturity at the time of the hormone treatment (Matsuyama et al., 1997).
1012 Therefore, the latency period of each species under the specific hormonal induction protocol and
hatchery conditions must be thoroughly examined in order to achieve high spawning success
1014 together with high egg quality.

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1018 **10. REFERENCES**

- 1020 Agulleiro, M. J., Anguis, V., Cañavate, J. P., Martínez-Rodríguez, G., Mylonas, C. C., Cerdá, J.,
2006. Induction of spawning of captive-reared Senegal sole (*Solea senegalensis*) using
1022 different administration methods for gonadotropin-releasing hormone agonist. *Aquaculture*.
257: 511-524.
- 1024 Aizen, J., Meiri, I., Tzchori, I., Levavi-Sivan, B., Rosenfeld, H., 2005. Enhancing spawning in
the grey mullet (*Mugil cephalus*) by removal of dopaminergic inhibition. *Gen. Comp.*
1026 *Endocrinol.* 142: 212-221.
- Alavi, H. S. M., Cosson, J., 2005. Sperm motility in fishes. I. Effects of temperature and pH: a
1028 review. *Cell Biology International*. 29: 101-110.
- Alavi, H. S. M., Cosson, J., 2006. Sperm motility in fishes. (II) Effects of ions and osmolality: a
1030 review. *Cell Biology International*. 30: 1-14.
- Alavi, H. S. M., Psenicka, M., Rodina, M., Pollicar, T., Linhart, O., 2008. Changes of sperm
1032 morphology, volume, density and motility and seminal plasma composition in *Barbus barbus*
(Teleostei: Cyprinidae) during the reproductive season. *Aquat. Living Resour.* 21: 75-80.
- 1034 Alavi, S. M. H., Linhart, O., Cowand, K., Rodina, M., 2007. Fish spermatology: implications for
aquaculture management. In: Alavi, S.M.H., Cosson, J., Coward, K., Rafiee, G., (Eds.), *Fish*
1036 *Spermatology*. Alpha Science Intl, Oxford (UK), pp. 397-461.
- Almendras, J. M., Duenas, C., Nacario, J., Sherwood, N. M., Crim, L. W., 1988. Sustained
1038 hormone release. III. Use of gonadotropin releasing hormone analogues to induce multiple
spawnings in sea bass, *Lates calcarifer*. *Aquaculture*. 74: 97-111.

- 1040 Amano, H., Fujita, T., Hiramatsu, N., Kagawa, H., Matsubara, T., Sullivan, C. V., Hara, A.,
2008. Multiple vitellogenin-derived yolk proteins in Gray mullet (*Mugil cephalus*): Disparate
1042 proteolytic patterns associated with ovarian follicle maturation. Mol. Repro. Dev. 75: 1307-
1317.
- 1044 Appelt, W., Sorensen, P. W., 2007. Female goldfish signal spawning readiness by altering when
and where they release a urinary pheromone. Anim. Behav. 74: 1329-1338.
- 1046 Arabaci, M., Cagiran, H., Sari, M., 2004. Induction of ovulation in ornamental common carp
(Koi, *Cyprinus carpio* L.) using LHRHa ([d-Ser(tBu)⁶, Pro⁹-NEt]-LHRH) combined with
1048 haloperidol and carp pituitary extract. Aqua. Res. 35: 10-14.
- Arabaci, M., Sari, M., 2004. Induction of ovulation in endemic pearl mullet (*Chalcalburnus*
1050 *tarichi*), living in the highly alkaline Lake Van, using GnRHa ([d-Ser(tBu)⁶, Pro⁹-NEt]-
GnRH) combined with haloperidol. Aquaculture. 238: 529-535.
- 1052 Asahina, K., Barry, Y. P., Aida, K., Fusetani, N., Hanyu, I., 1990. Biosynthesis of 17a,20a-
dihydroxy-4-pregnen-3-one from 17a-hydroxyprogesterone by spermatozoa of the common
1054 carp. J. Exp. Zool. 255: 244-249.
- Asturiano, J. F., Sorbera, L. A., Ramos, J., Kime, D. E., Carrillo, M., Zanuy, S., 2000. Hormonal
1056 regulation of the European seabass reproductive cycle: an individualized female approach. J.
Fish Biol. 56: 1155-1172.
- 1058 Avery, T. S., Boyce, D., Brown, J. A., 2004. Mortality of yellowtail flounder, *Limanda*
ferruginea (Storer), eggs: effects of temperature and hormone-induced ovulation.
1060 Aquaculture. 230: 297-311.
- Babin, P. J., Carnevali, O., Lubzens, E., Schneider, W. J., 2007. Molecular aspects of oocyte
1062 vitellogenesis in fish. In: Babin, P.J., Cerdá, J., Lubzens, E., (Eds.), The Fish Oocyte: From
Basic Studies to Biotechnological Applications. Springer, The Netherlands, pp. 39-76.

Broodstock management and hormonal manipulations of fish reproduction

- 1064 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
1066 and its effects on egg quality and daily timing of spawning. *Aquaculture*. 154: 349-359.
- Barbaro, A., Franceson, A., Bertotto, D., Bozzato, G., Di Maria, I., Patarnello, P., Furlan, F.,
1068 Colombo, L., 2002. More effective induction of spawning with long-acting GnRH agonist in
the shi drum, *Umbrina cirrosa* L. (Sciaenidae, Teleostei), a valuable candidate for
1070 Mediterranean mariculture. *J. Appl. Ichthyol.* 18: 192-199.
- Barry, T., Aida, K., Okumura, T., Hanyu, I., 1990. The shift from C-19 to C-21 steroid synthesis
1072 in spawning male common carp, *Cyprinus carpio*, is regulated by the inhibition of androgen
production by progestogens produced by spermatozoa. *Biol. Reprod.* 43: 105-112.
- 1074 Bartley, D. M., Rana, K., Immink, A. J., 2001. The use of inter-specific hybrids in aquaculture
and fisheries. *Rev. Fish Biol. Fish.* 10: 325-337.
- 1076 Beardsley, A., O'Donnell, L., 2003. Characterization of normal spermiation and spermiation
failure induced by hormone suppression in adult rats. *Biol. Reprod.* 68: 1299-1307.
- 1078 Berlinsky, D. L., King, W. V., Smith, T. I. J., Hamilton, R. D., II, Holloway, J., Jr., Sullivan, C.
V., 1996. Induced ovulation of Southern flounder *Paralichthys lethostigma* using
1080 gonadotropin releasing hormone analogue implants. *J. World Aquac. Soc.* 27: 143-152.
- 1082 Berlinsky, D. L., William, K., Hodson, R. G., Sullivan, C. V., 1997. Hormone induced spawning
of summer flounder *Paralichthys dentatus*. *J. World Aquac. Soc.* 28: 79-86.
- 1084 Billard, R., 1986. Spermatogenesis and spermatology of some teleost fish species. *Reprod. Nutr.*
Develop. 26: 877-920.
- Billard, R., 1988. Artificial insemination and gamete management in fish. *Mar. Fresh. Behav.*
1086 *Physiol.* 14, 3-21.
- Billard, R., 1989. Endocrinology and fish culture. *Fish Physiol. Biochem.* 7: 49-58.

- 1088 Billard, R., Christen, R., Cosson, J., Gatty, J. L., Letellier, L., Renard, P., Saad, A., 1986.
Biology of gametes of some teleost species. *Fish Physiol. Biochem.* 2: 115-130.
- 1090 Billard, R., Cosson, J., Crim, L. W., Suquet, M., 1995a. Sperm physiology and quality. In:
Bromage, N.R., Roberts, R.J., (Eds.), *Broodstock Management and Egg and Larval Quality*.
1092 Blackwell Science, Oxford, pp. 25-52.
- Billard, R., Cosson, J., Noveiri, S. B., Pourkazemi, M., 2004. Cryopreservation and short-term
1094 storage of sturgeon sperm, a review. *Aquaculture*. 236: 1-9.
- Billard, R., Cosson, J., Perchec, G., Linhart, O., 1995b. Biology of sperm and artificial
1096 reproduction in carp. *Aquaculture*. 129: 95-112.
- Billard, R., Le Gac, F., Loir, M., 1990. Hormonal control of sperm production in teleost fish.
1098 *Progress in Comparative Endocrinology*. Wiley-Liss, Inc.
- Bobe, J., Babiak, I., 0000. Gamete quality. *Gen. Comp. Endocrinol.* 000: 000-000.
- 1100 Bobe, J., Jalabert, B., Fostier, A., 2008. Oogenesis: post-vitellogenic events leading to a
fertilizable oocyte. In: Rocha, B.G., Arukwe, M.J., Kapoor, A., (Eds.), *Fish Reproduction*.
1102 Science Publishers, Enfield, pp. 1-36.
- Bonnet, E., Fostier, A., Bobe, J., 2007. Microarray-based analysis of fish egg quality after
1104 natural or controlled ovulation. *BMC Genomics*. 8:55.
- Breton, B., Weil, C., Sambroni, E., Zohar, Y., 1990. Effects of acute versus sustained
1106 administration of GnRHa on GtH release and ovulation in the rainbow trout, *Oncorhynchus*
mykiss. *Aquaculture*. 91: 371-383.
- 1108 Bromage, N., Bruce, M., Basavaraja, N., Rana, K., Shields, R., Young, C., Dye, J., Smith, P.,
Gillespie, M., Gamble, J., 1994. Egg quality determinants in finfish: the role of overripening
1110 with special reference to the timing of stripping in the Atlantic halibut *Hippoglossus*
hippoglossus. *J. World Aquac. Soc.* 25: 13-21.

Broodstock management and hormonal manipulations of fish reproduction

- 1112 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
1114 rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 100: 141-166.
- Bromage, N., Porter, M., C., R., 2001. The environmental regulation of maturation in farmed
1116 finfish with special reference to the role of photoperiod and melatonin. Aquaculture. 197: 63-
98.
- 1118 Bromage, N. R., 1995. Broodstock management and seed quality - general considerations. In:
Bromage, N.R., Roberts, R.J., (Eds.), Broodstock Management and Egg and Larval Quality.
1120 Blackwell Science, Oxford, pp. 1-24.
- Bromage, N. R., Cumaranatunga, R., 1988. Egg production in the rainbow trout. In: Muir, J.F.,
1122 Roberts, R.J., (Eds.), Recent Advances in Aquaculture. Croom Helm/Timber Press Inc.,
London, pp. 63-138.
- 1124 Brooks, S., Tyler, C. R., Sumpter, J. P., 1997. Egg quality in fish: what makes a good egg? Rev.
Fish Biol. Fish. 7: 387-416.
- 1126 Brzuska, E., 1999. Artificial spawning of herbivorous fish: use of an LHRH-a to induce
ovulation in grass carp *Ctenopharyngodon idella* (Valenciennes) and silver carp
1128 *Hypophthalmichthys molitrix* (Valenciennes). Aqua. Res. 30: 849-856.
- Brzuska, E., 2001. Artificial spawning of European catfish *Silurus glanis* L.: differences
1130 between propagation results after stimulation of ovulation with carp pituitary and Ovopel.
Aquacult. Int. 32: 11-19.
- 1132 Brzuska, E., Bialowas, H., 2002. Artificial spawning of carp, *Cyprinus carpio* (L.). Aqua. Res.
33: 753-765.

- 1134 Buchet, V., Coquard, E., Sévère, A., Barone, H., 2008. Influence of tank volume on
vitellogenesis and spawning performances in sea bass *Dicentrarchus labrax* L. Aqua. Res.
1136 39: 420-426.
- Burness, G., Moyes, C. D., Montgomerie, R., 2005. Motility, ATP levels and metabolic enzyme
1138 activity of sperm from bluegill (*Lepomis macrochirus*). Comp. Biochem. Physiol. A140: 11-
17.
- 1140 Burtsev, I. A., Nikolaev, A. I., Maltsev, S. A., Igumnova, L. V., 2002. Formation of
domesticated broodstocks as a guarantee of sustainable hatchery reproduction of sturgeon for
1142 sea ranching. J. Appl. Ichthyol. 18: 655-658.
- Carral, J. M., Rodriguez, R., Celafda, J. D., Saez-Royuela, A., Aguilera, A., Melendre, P., 2003.
1144 Successful gonadal development and maturation of tench (*Tinca tinca* L.) in small concrete
ponds. J. Appl. Ichthyol. 19:130-131.
- 1146 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
1148 and Egg and Larval Quality. Blackwell Science, Oxford, pp. 138-168.
- Carrillo, M., Zanuy, S., Prat, F., Serrano, R., Bromage, N., 1993. Environmental and hormonal
1150 control of reproduction in sea bass. In: Muir, J.F., Roberts, R.J., (Eds.), Recent Advances in
Aquaculture, volume IV. Institute of Aquaculture, Blackwell Scientific Publications, Oxford,
1152 pp. 43-54.
- Cerdá, J., Fabra, M., Raldúa, D., 2007. Physiological and molecular basis of fish oocyte
1154 hydration. In: Babin, P.J., Cerdá, J., Lubzens, E., (Eds.), The Fish Oocyte: from Basic Studies
to Biotechnological Applications. Kluwer Academic Publishers, Dordrecht, The Netherlands,
1156 pp. 349-396.

Broodstock management and hormonal manipulations of fish reproduction

- Cerdá, J., Selman, K., Wallace, R. A., 1996. Observations on oocyte maturation and hydration in vitro in the black sea bass, *Centropristis striata* (Serranidae). *Aquat. Living Resour.* 9: 325-335.
- 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. Develop.* 35: 339-349.
- Chang, J. P., Jobin, R. M., 1994. Regulation of gonadotropin release in vertebrates: a comparison of GnRH mechanisms of action. In: Davey, K.G., Peter, R.E., Tobe, S.S., (Eds.), *Perspectives in Comparative Endocrinology*. National Research Council of Canada, Ottawa, pp. 41-51.
- Chao, N. H., Liao, I. C., 2001. Cryopreservation of finfish and shellfish gametes and embryos. *Aquaculture*. 197: 161-180.
- Chaves-Pozo, E., Mulero, V., Meseguer, J., Ayala, A. G., 2005. An overview of cell renewal in the testis throughout the reproductive cycle of a seasonal breeding teleost, the gilthead seabream (*Sparus aurata* L.). *Biol. Reprod.* 72:593-601.
- Chebanov, M., Billard, R., 2001. The culture of sturgeons in Russia: production of juveniles for stocking and meat for human consumption. *Aquat. Living Resour.* 14: 375-381.
- Chemineau, P., Malpoux, B., Brillard, J. P., Fostier, A., 2007. Seasonality of reproduction and production in farm fishes, birds and mammals. *Animal*. 1: 419-432.
- Chen, Y. F., 2005. Induced ovulation and embryonic development of ocellated puffer, *Takifugu ocellatus*. *J. Appl. Ichthyol.* 21: 136-140.
- Chowdhury, I., Joy, K. P., 2007. Seminal vesicle and its role in the reproduction of teleosts. *Fish Physiol. Biochem.* 33: 383-398.

- 1180 Clearwater, S. J., Crim, L. W., 1998. Gonadotropin releasing hormone-analogue treatment
increases sperm motility, seminal plasma pH and sperm production in yellowtail flounder
1182 *Pleuronectes ferrugineus*. Fish Physiol. Biochem. 19: 349-357.
- Conte, F. S., Doroshov, S. I., Lutes, P. B., Strange, E. M., 1988. Hatchery manual for the white
1184 sturgeon (*Acipenser transmontanus* Richardson) with application to other North American
Acipenseridae. Cooperative Extension University of California. Division o Agriculture and
1186 Natural Resources.
- Corriero, A., Medina, A., Mylonas, C. C., Abascal, F. J., Deflorio, M., Aragón, L., Bridges, C.
1188 R., Santamaria, C. A., Heinisch, G., Vassallo-Agius, R., Belmonte, A., Fauvel, C., Garcia, A.,
Gordin, H., De Metrio, G., 2007. Histological study of the effects of treatment with
1190 gonadotropin-releasing hormone agonist (GnRHa) on the reproductive maturation of captive-
reared Atlantic bluefin tuna (*Thunnus thynnus* L.). Aquaculture. 272: 675-686.
- 1192 Cosson, J., 2004. The ionic and osmotic factors controlling motility of fish spermatozoa.
Aquacult. Int. 12: 69-85.
- 1194 Cosson, J., 2007. Methods to analyse the movements of fish spermatozoa and their flagella. In:
Alavi, S.M.H., Cosson, J., Coward, K., Rafiee, G., (Eds.), Fish Spermatology. Alpha Science
1196 Intl, Oxford, UK, pp. 63-102.
- Craik, J. C. A., Harvey, S. M., 1984. Egg quality in rainbow trout: the relation between egg
1198 viability, selected aspects of egg composition and time of stripping. Aquaculture. 40: 115-
134.
- 1200 Crim, L. W., Evans, D. M., Vickery, B. H., 1983. Manipulation of the seasonal reproductive
cycle of the landlocked Atlantic salmon (*Salmo salar*) by LHRH analogues administered at
1202 various stages of gonadal development. Can. J. Aquat. Fish. Sci. 40: 61-67.

Broodstock management and hormonal manipulations of fish reproduction

- Crim, L. W., Glebe, B. D., 1984. Advancement and synchrony of ovulation in Atlantic salmon
1204 with pelleted LHRH analog. *Aquaculture*. 43: 47-56.
- Crim, L. W., Sherwood, N. M., Wilson, C. E., 1988. Sustained hormone release. II.
1206 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
1208 juvenile rainbow trout. *Aquaculture*. 74: 87-95.
- Cruz-Casallas, P. E., Lombo-Rodriguez, D. A., Velasco-Santamaria, Y. M., 2005. Milt quality
1210 and spermatozoa morphology of captive *Brycon siebenthalae* (Eigenmann) broodstock. *Aqua*.
Res. 38: 682-686.
- Cyr, D. G., Eales, J. G., 1996. Interrelationships between thyroidal and reproductive endocrine
1212 systems in fish. *Rev. Fish Biol. Fish.* 6: 165-200.
- Dabrowski, K., Ciereszko, A., Ramseyer, L., Culver, D., Kestemont, P., 1994. Effects of
1214 hormonal treatment on induced spermiation and ovulation in the yellow perch (*Perca*
1216 *flavescens*). *Aquaculture*. 120: 171-180.
- De Montgolfier, B., Dufresne, J., Letourneau, M., Nagler, J., Fournier, A., Aude, t. C., Cyr, D.
1218 G., 2007. The expression of multiple connexins throughout spermatogenesis in the rainbow
trout testis suggests a role for complex intercellular communication. *Biol. Reprod.* 76, 2-8.
- Denson, M. R., Jenkins, W. E., Berlinsky, D. L., Smith, T. I. J., 2007. A comparison of human
1220 chorionic gonadotropin and luteinizing hormone releasing hormone analogue for ovulation
1222 induction in black sea bass *Centropristis striata* (Linnaeus, 1758). *Aqua. Res.* 38: 918-925.
- Donaldson, E. M., 1973. Reproductive endocrinology of fishes. *Amer. Zool.* 13: 909-927.
- 1224 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*.
1226 Academic Press, Orlando, Florida, pp. 351-403.

- Donaldson, E. M., Hunter, G. A., Dye, H. M., 1981. Induced ovulation in coho salmon
1228 (*Oncorhynchus kisutch*). II. Preliminary study of the use of LH-RH and two high potency
LH-RH analogues. *Aquaculture*. 26: 129-141.
- 1230 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
1232 metoclopramide: analysis of hormone profile, progress of oocyte maturation and dependence
on temperature. *Aquaculture*. 119: 393-407.
- 1234 Duarte, M., Marbá, N., Holmer, M., 2007. Rapid domestication of marine species. *Science*. 16:
382-383.
- 1236 Duncan, N. J., Rodriguez M. de O., G. A., Alok, D., Zohar, Y., 2003. Effects of controlled
delivery and acute injections of LHRHa on bullseye puffer fish (*Sphoeroides annulatus*)
1238 spawning. *Aquaculture*. 218: 625-635.
- Dupont-Nivet, M., Vandeputte, M., Haffray, P., Chevassus, B., 2006. Effect of different mating
1240 designs on inbreeding, genetic variance and response to selection when applying individual
selection in fish breeding programs. *Aquaculture*. 252: 161-170.
- 1242 El Nagggar, G. O., John, G., Rezk, M. A., Elwan, W., Yehia, M., 2006. Effect of varying density
and water level on the spawning response of African catfish *Clarias gariepinus*: Implications
1244 for seed production. *Aquaculture*. 261: 904-907.
- Fauvel, C., Savoye, O., Dreanno, C., Cosson, J., Suquet, M., 1999. Characteristics of sperm of
1246 captive seabass in relation to its fertilization potential. *J. Fish Biol.* 54: 356-369.
- Fauvel, C., Suquet, M., Sévère, A., Mylonas, C. C., Papandroulakis, N., 2008. Slow-release
1248 GnRHa therapy prevented atresia during vitellogenesis and induced ovulation of captive
wreckfish (*Polyprion americanus*). *Cybio*. 32(2) suppl: 191.

Broodstock management and hormonal manipulations of fish reproduction

- 1250 Finn, R. N., 2007. The maturational disassembly and differential proteolysis of paralogous
vitellogenins in a marine pelagophil teleost: A conserved mechanism of oocyte hydration.
1252 Biol. Reprod. 76: 936-948.
- Finn, R. N., Østby, G. C., Norberg, B., Fyhn, H. J., 2002. *In vivo* hydration in Atlantic halibut
1254 (*Hippoglossus hippoglossus*); proteolytic liberation of free amino acids, and ion transport, are
driving forces for osmotic water influx. J. Exp. Biol. 205: 211-224.
- 1256 Fishelson, L., Gon, O., V., H., Delarea, Y., 2007. Comparative spermatogenesis,
spermatocytogenesis, and spermatzeugmata formation in males of viviparous species of
1258 clinid fishes (Teleostei: Clinidae, Blennioidei). The Anatomical Record. 290: 311-323.
- Fontaine, Y. A., Dufour, S., Tanguy, G., Khan, I. A., Cedard, L., Leloup-Hatey, J., Clairances
1260 metaboliques de la gonadotropine chorionique humaine (hCG) et de la gonadotropin de carpe
(cGtH) chez un poisson teleosteen, l'anguille. Colloque Physiologie des Poissons. INRA and
1262 IFREMER, 1984.
- Fontenele, O., 1955. Injecting pituitary (hypophyseal) hormones into fish to induce spawning.
1264 Prog. Fish-Cult. 18: 71-75.
- Forniés, M. A., Carrillo, M., Mañanos, E., Sorbera, L. A., Zohar, Y., Zanuy, S., 2003. Relative
1266 potencies of the forms of GnRH and their analogs on LH release in sea bass. J. Fish Biol. 63:
73-89.
- 1268 Forniés, M. A., Mañanos, E., Carrillo, M., Rocha, A., Laureau, S., Mylonas, C. C., Zohar, Y.,
Zanuy, S., 2001. Spawning induction of individualised European seabass females
1270 (*Dicentrarchus labrax*) using different GnRH α -delivery systems. Aquaculture. 202: 221-234.
- Gage, M. J. G., Macfarlane, C. P., Yeates, S., Ward, R. G., Searle, J. B., Parker, G. A., 2004.
1272 Spermatozoal traits and sperm competition in Atlantic salmon: relative sperm velocity is the
primary determinant of fertilization success. Current Biology. 14: 44-47.

- 1274 Garcia, L. M. B., 1989. Development of an ovarian biopsy technique in the sea bass, *Lates*
calcarifer. Aquaculture. 77: 97-102.
- 1276 Garcia, L. M. B., 1991. Spermiation response of mature rabbitfish, *Siganus guttatus* Bloch, to
luteinizing hormone-releasing hormone analogue (LHRHa) injection. Aquaculture. 97: 291-
1278 299.
- Gardes, L., Villanove, P., Buchet, V., Fauvel, C., 2000. Induced spawning of red drum,
1280 *Sciaenops ocellatus*: use of multivariate and univariate analysis methods in the search for side
effects of LH-RHa treatments and ovarian development state upon spawn quality. Aquat.
1282 Living Resour. 13:19-27.
- Gillet, C., Breton, B., Mikolajczyk, T., 1996. Effects of GnRHa and pimozide treatments on the
1284 timing of ovulation and on egg quality in Arctic charr (*Salvelinus alpinus*) at 5 and 10°C.
Aquat. Living Resour. 9: 257-263.
- 1286 Glasser, F., Mikolajczyk, T., Jalabert, B., Baroiller, J.-F., Breton, B., 2004. Temperature effects
along the reproductive axis during spawning induction of grass carp (*Ctenopharyngodon*
1288 *idella*). Gen. Comp. Endocrinol. 136: 171-179.
- Glubokov, A. I., Kouril, J., Mikodina, E. V., Barth, T., 1994. Effects of synthetic GnRH
1290 analogues and dopamine antagonists on the maturation of Pacific mullet, *Mugil so-iuy* Bas.
Aquaculture and Fisheries Management. 25: 419-425.
- 1292 Gomez, J. M., Weil, C., Ollitrault, M., Le Bail, P. Y., Breton, B., Le Gac, F., 1999. Growth
hormone (GH) and gonadotropin subunit gene expression and pituitary and plasma changes
1294 during spermatogenesis and oogenesis in rainbow trout (*Oncorhynchus mykiss*). Gen. Comp.
Endocrinol. 113: 413-428.
- 1296 Gomlesky, B., 2003. Chromosome set manipulation and sex control in common carp: a review.
Aquat. Living Resour. 16: 408-415.

Broodstock management and hormonal manipulations of fish reproduction

- 1298 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.
- 1300 In: Goetz, F.W., Thomas, P., (Eds.), Reproductive Physiology of Fish. Fish Symposium 95,
Austin, Texas, pp. 99-101.
- 1302 Haddy, J. A., Pankhurst, N. W., 2000. The effects of salinity on reproductive development,
plasma steroid levels, fertilisation and egg survival in black bream *Acanthopagrus butcheri*.
- 1304 Aquaculture. 188: 115-131.
- Haffray, P., Enright, W. J., Driancourt, M. A., Mikolajczyk, T., Rault, P., Breton, B., 2005.
- 1306 Optimization of breeding of salmonids: Gonazon TM, the first officially approved inducer of
ovulation in the EU. Aquaculture Magazine. March: 52-56.
- 1308 Haffray, P., Fostier, A., Normant, Y., Fauré, A., Loir, M., Jalabert, B., Maisse, G., Le Gac, F.,
1995. Effect of seawater rearing or freshwater transfer on final maturation and gamete quality
- 1310 in Atlantic salmon, *Salmo salar*. Aquat. Living Resour. 8: 135-145.
- Harmin, S. A., Crim, L. W., 1993. Influence of gonadotropin hormone-releasing hormone analog
- 1312 (GnRH-A) on plasma sex steroid profiles and milt production in male winter flounder,
Pseudopleuronectes americanus (Walbaum). Fish Physiol. Biochem. 10: 399-407.
- 1314 Harvey, B., Denny, C., Kaiser, S., Young, J., 1988. Remote intramuscular injection of
immobilising drugs into fish using a laser-aimed underwater dart gun. The Veterinary Record.
- 1316 122: 174-177.
- Hiramatsu, N., Matsubara, T., Fujita, T., Sullivan, C. V., Hara, A., 2006. Multiple piscine
- 1318 vitellogeniins: biomarkers of fish exposure to estrogenic endocrine disruptors in aquatic
environments. Mar. Biol. 149: 35-47.

- 1320 Hiramatsu, N., Matsubara, T., Hara, A., Donato, D. M., Hiramatsu, K., Denslow, N. D.,
Sullivan, C. V., 2002. Identification, purification and classification of multiple forms of
1322 vitellogenin from white perch (*Morone americana*). Fish Physiol. Biochem. 26: 355-370.
- Hong, W.-S., Chen, S.-X., Zhang, Q.-Y., Zheng, W.-Y., 2006. Sex organ extracts and artificial
1324 hormonal compounds as sex pheromones to attract broodfish and to induce spawning of
Chinese black sleeper (*Bostrichthys sinensis* Lacépède). Aqua. Res. 37: 529-534.
- 1326 Houssay, B. A., 1930. Accion sexual de la hipofisis en los peces y reptiles. Rev. Soc. Arg. Biol.
106: 686-688.
- 1328 Huner, J. V., Dupree, H. K., 1984. Methods and economics of channel catfish production, and
techniques for the culture of flathead catfish and other catfishes. In: Dupree, H.K., Huner,
1330 J.V., (Eds.), Third Report to the Fish Farmers. The Status of Warmwater Fish Farming and
Progress in Fish Farming Research. U.S. Fish and Wildlife Service, Washington, D.C., pp.
1332 44-82.
- Hurvitz, A., Jackson, K., Degani, G., Levavi-Sivan, B., 2007. Use of endoscopy for gender and
1334 ovarian stage determinations in Russian sturgeon (*Acipenser gueldenstaedtii*) grown in
aquaculture. Aquaculture. 270: 158-166.
- 1336 Ibarra-Castro, L., Dumas, S., Duncan, N., Gonad development and LHRHa induced spawning in
female rosey spotted snapper *Lutjanus guttatus*. 5th International Symposium on Fish
1338 Endocrinology, Castellon, Spain, 2004.
- Ibarra-Castro, L., Dunca, N. J., 2007. GnRHa-induced spawning of wild-caught spotted rose
1340 snapper *Lutjanus guttatus*. Aquaculture. 272: 737-746.
- Jackson, L. F., Sullivan, C. V., 1995. Reproduction of white perch: the annual gametogenic
1342 cycle. Trans. Amer. Fish. Soc. 124: 563-577.

Broodstock management and hormonal manipulations of fish reproduction

- Jalabert, B., 2005. Particularities of reproduction and oogenesis in teleost fish compared to
1344 mammals. *Reprod. Nutr. Develop.* 45: 261-279.
- Kagawa, H., Tanaka, H., Ohta, H., Unuma, T., Nomura, K., 2005. The first success of glass eel
1346 production in the world: basic biology on fish reproduction advances new applied technology
in aquaculture. *Fish Physiol. Biochem.* 31: 193-199.
- Kaminski, R., Kuszniierz, J., Myszkowski, L., Wolnicki, J., 2004. The first attempt to artificially
1348 reproduce the endangered cyprinid lake minnow *Eupallasella perenurus* (Pallas). *Aquacult.*
1350 *Int.* 12: 3-10.
- Kaspar, V., Kohlmann, K., Vandeputte, M., Rodina, M., Gela, D., Kocour, M., Alavi, H. S. M.,
1352 Hulak, M., Linhart, O., 2007. Equalizing sperm concentrations in a common carp (*Cyprinus*
carpio) sperm pool does not affect variance in proportions of larvae sired in competition.
1354 *Aquaculture.* 272: 204-209.
- Kilduff, P., DuPaul, W., Oesterling, M., Olney, J., Jr., Tellock, J., 2002. Induced spawning of
1356 cobia, *Rachycentron canadum*, and early larval husbandry. *World Aquaculture.* 33: 35-38.
- Kime, D. E., 1979. The effect of temperature on the testicular steroidogenic enzymes of the
1358 rainbow trout, *Salmo gairdneri*. *Gen. Comp. Endocrinol.* 39: 290-296.
- King, H. R., Pankhurst, N. W., 2007. Additive effects of advanced temperature and photoperiod
1360 regimes and LHRHa injection on ovulation in Atlantic salmon (*Salmo salar*). *Aquaculture.*
273: 729-738.
- King, W., V., Ghosh, S., Thomas, P., Sullivan, C. V., 1995. Ovarian receptors for 17 α , 20 β , 21-
1362 trihydroxy-4-pregnen-3-one (20 β -S) in striped bass. In: Goetz, F.W., Thomas, P., (Eds.),
1364 *Reproductive Physiology of Fish*, 1995. Fish Symposium 95, Austin, Texas, pp. 314.

- Kinsey, W. H., Sharma, D., Kinsey, S. C., 2007. Fertilization and egg activation in fishes. In:
1366 Babin, P.J., Cerdá, J., Lubzens, E., (Eds.), The fish oocyte: From Basic Studies to
Biotechnological Applications. Springer, The Netherlands, pp. 397-410.
- 1368 Kishida, M., Anderson, T. R., Specker, J. L., 1992. Induction by β -Estradiol of vitellogenin in
striped bass (*Morone saxatilis*): characterization and quantification in plasma and mucus.
1370 Gen. Comp. Endocrinol. 88: 29-39.
- Kjesbu, O. S., Kryvi, H., Norberg, B., 1996. Oocyte size and structure in relation to blood
1372 plasma steroid hormones in individually monitored, spawning Atlantic cod. J Fish Biol. 49:
1197-1215.
- 1374 Kobayashi, T., Pakarinen, P., Torgersen, J., Huhtaniemi, I., Anderson, O., 2008. The
gonadotropin receptors FSH-R and LH-R of Atlantic halibut (*Hippoglossus hypoglossus*) – 2.
1376 Differential follicle expression and asynchronous oogenesis. General and Comparative
Endocrinology 156 : 595-602. Gen. Comp. Endocrinol. 156: 595-602.
- 1378 Kumakura, N., Sakai, K., Takashima, F., 2003. Reproductive cycle and human chorionic
gonadotropin-induced ovulation in hatchery reared Japanese catfish *Silurus asotus*. Fisheries
1380 Sci. 69: 495-504.
- LaFleur, G. J., Thomas, P., 1991. Evidence for a role of Na^+/K^+ -ATPase in the hydration of
1382 Atlantic croaker and spotted seatrout oocytes during final maturation. J. Exp. Zool. 258: 126-
136.
- 1384 Lam, T. J., 1982. Applications of endocrinology to fish culture. Can. J. Aquat. Fish. Sci. 39: 11-
137.
- 1386 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

Broodstock management and hormonal manipulations of fish reproduction

- 1388 performance of a cold-water batch-spawning teleost, the yellowtail flounder (*Pleuronectes*
1390 *ferrugineus*). Can. J. Aquat. Fish. Sci. 54: 1957-1964.
- 1390 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.
- 1392 Le Menn, F., Cerdá, J., Babin, P. J., 2007. Ultrastructural aspects of the ontogeny and
differentiation of ray-finned fish ovarian follicles. In: Babin, P.J., Cerdá, J., Lubzens, E.,
1394 (Eds.), The Fish Oocyte: from Basic Studies to Biotechnological Applications. Kluwer
Academic Publishers, Dordrecht, The Netherlands, pp. 1-37.
- 1396 Lee, C.-S., Tamaru, C. S., Kelley, C. D., Moriwake, A., Miyamoto, G. T., 1992. The effect of
salinity on the induction of spawning and fertilization in the striped mullet, *Mugil cephalus*.
1398 Aquaculture. 102: 289-296.
- Lee, W.-K., Yang, S.-W., 2002. Relationship between ovarian development and serum levels of
1400 gonadal steroid hormones, and induction of oocyte maturation and ovulation in the cultured
female Korean spotted sea bass *Lateolabrax maculatus* (Jeom-nong-eo). Aquaculture. 207:
1402 169-183.
- Leonardo, A. F. G., Romagosa, E., Borella, M. I., Batlouni, S. R., 2004. Induced spawning of
1404 hatchery-raised Brazilian catfish, cachara *Pseudoplatystoma fasciatum* (Linnaeus, 1766).
Aquaculture. 240: 451-461.
- 1406 Lethimonier, C., Madigou, T., Munoz-Cueto, J.-A., Lareyre, J.-J., Kah, O., 2004. Evolutionary
aspects of GnRH α , GnRH neuronal systems and GnRH receptors in teleost fish. Gen. Comp.
1408 Endocrinol. 135: 1-16.
- Lim, H. K., Pankhurst, N. W., Fitzgibbon, Q. P., 2004. Effects of slow release gonadotropin
1410 releasing hormone analog on milt characteristics and plasma levels of gonadal steroids in
greenback flounder *Rhombosolea tapirina*. Aquaculture. 240: 505-516.

- 1412 Linhart, O., Gela, D., Rodina, M., Kocour, M., 2004. Optimization of artificial propagation in
European catfish, *Silurus glanis* L. Aquaculture. 235: 619-632.
- 1414 Linhart, O., Kouril, J., Hamacková, J., 1987. Increase rate of egg fertilization in artificial
propagation of sheatfish (*Silurus glanis* L.) by means of suppressing the movements of
1416 spermatozoa with immobilization solution. Aquaculture. 65: 353-358.
- Liu, F.-G., Lin, T.-S., Huang, D.-U., Perng, M.-L., Liao, I. C., 2000. An automated system for
1418 egg collection, hatching, and transfer of larvae in a freshwater finfish hatchery. Aquaculture.
182: 137-148.
- 1420 Loir, M., Sourdain, P., Mendis-Handagama, S. M., Jegou, B., 1995. Cell-cell interactions in the
testis of teleosts and elasmobranchs. Microscopy Research And Technique. 32: 533-552.
- 1422 Luckenbach, J. A., Iliev, D. B., Goetz, F. W., Swanson, P., 2008. Identification of differentially
expressed ovarian genes during primary and early secondary oocyte growth in coho salmon,
1424 *Oncorhynchus kisutch*. Reproductive Biology and Endocrinology. 6: 2.
- Ludwig, M., Felberbaum, R. E., Diedrich, K., Lunefeld, B., 2002. Ovarian stimulation: from
1426 basic science to clinical application. Reproductive Biomedicine Online. 5: 73-86.
- Lutes, P. B., Doroshov, S. I., Chapman, F., Harrah, J., Fitzgerald, R., Fitzpatrick, M., 1987.
1428 Morpho-physiological predictors of ovulatory success in white sturgeon, *Acipenser*
transmontanus Richardson. Aquaculture. 66: 43-52.
- 1430 Malison, J. A., Procarione, L. S., Barry, T. P., Kapuscinski, A. R., Kayes, T. B., 1994. Endocrine
and gonadal changes during the annual reproductive cycle of the freshwater teleost,
1432 *Stizostedion vitreum*. Fish Physiol. Biochem. 13: 473-484.
- Malison, J. A., Procarione, L. S., Kayes, T. B., Hansen, J. F., Held, J. A., 1998. Induction of out-
1434 of-season spawning in walleye (*Stizostedion vitreum*). Aquaculture. 163: 151-161.

Broodstock management and hormonal manipulations of fish reproduction

- Mañanos, E., Carrillo, M., Sorbera, L. S., Mylonas, C. C., Asturiano, J. F., Bayarri, M. J., Zohar, Y., Zanuy, S., 2002. Luteinizing hormone and sexual steroid plasma levels after treatment of European sea bass with sustained-release delivery systems for gonadotropin-releasing hormone analogue. *J. Fish Biol.* 60: 328-339.
- Mañanos, E., Duncan, N., Mylonas, C. C., 2008. Reproduction and control of ovulation, spermatogenesis and spawning in cultured fish. In: Cabrita, E., Robles, V., Herráez, M.P., (Eds.), *Methods in Reproductive Aquaculture: Marine and Freshwater Species*. CRC Press, Taylor and Francis Group, Boca Raton, pp. 3-80.
- Manning, A. J., Crim, L. W., 1998. Maternal and interannual comparison of the ovulatory periodicity, egg production and egg quality of the batch-spawning yellowtail flounder. *J. Fish Biol.* 53: 954-972.
- Mansour, N., Lahnsteiner, F., Berger, B., 2003. Metabolism of intratesticular spermatozoa of a tropical teleost fish (*Clarias gariepinus*). *Comp. Biochem. Physiol.* B135: 285-296.
- Mansour, N., Lahnsteiner, F., Berger, B., 2004. Characterization of the testicular semen of the African catfish, *Claria gariepinus* (Burchell, 1822), and its short-term storage. *Aqua. Res.* 35: 232-244.
- Marino, G., Panini, E., Longobardi, A., Mandich, A., Finoia, M. G., Zohar, Y., Mylonas, C. C., 2003. Induction of ovulation in captive-reared dusky grouper, *Epinephelus marginatus* (Lowe, 1834) with a sustained-release GnRHa implant. *Aquaculture*. 219: 841-858.
- Martínez-Pastor, F., Cabrita, E., Soares, F., Anel, L., Dinis, M. T., 2008. Multivariate cluster analysis to study motility activation of *Solea senegalensis* spermatozoa: a model for marine teleosts. *Reproduction*. 135: 449-459.
- Masuma, S., 2006. Maturation and spawning of bluefin tuna in captivity. In: Sakamoto, W., Miyashita, S., Nakagawa, Y., (Eds.), *Ecology and Aquaculture of Bluefin Tuna*. Proceedings

of the Joint International Symposium on Bluefin Tuna, 2006. Fisheries Laboratory, Kinki
University, Shirahama, Wakayam, Japan, pp. 15-20.

Mateos, J., Mananos, E., Martinez-Rodriguez, G., Carrillo, M., Querat, B., Zanuy, S., 2003.

Molecular characterization of sea bass gonadotropin subunits (α , FSH β , and LH β) and their
expression during the reproductive cycle. Gen. Comp. Endocrinol. 133: 216-232.

Matsubara, T., Nagae, M., Ohkubo, N., Andoh, T., Sawaguchi, S., Hiramatsu, N., Sullivan, C.
V., Hara, A., 2003. Multiple vitellogenins and their unique roles in marine teleosts. Fish
Physiol. Biochem. 28: 295-299.

Matsubara, T., Ohkubo, N., Andoh, T., Sullivan, C. V., Hara, A., 1999. Two forms of
vitellogenin, yielding two distinct lipovitellins, play different roles during oocyte maturation
and early development of barfin flounder, *Verasper moseri*, a marine teleost that spawns
pelagic eggs. Dev. Biol. 213: 18-32.

Matsuyama, M., Chuda, H., Ikeda, Y., Tanaka, H., Matsuura, S., 1997. Induction of ovarian
maturation and ovulation in the cultured tiger puffer *Takifugu rubripes* by different hormonal
treatments. Suisanzoshoku. 45: 67-73.

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. Fisheries Sci. 61: 472-477.

Meseda, M. E. G., Samira, S. A., 2006. Spawning induction in the Mediterranean mullet *Mugil
cephalus* and larval developmental stages. African Journal of Biotechnology. 5: 1836-1845.

Mikolajczyk, T., Chyb, J., Sokolowska-Mikolajczyk, M., Enright, W. J., Epler, P., Filipiak, M.,
Breton, B., 2003. Attempts to induce an LH surge and ovulation in common carp (*Cyprinus
carpio* L.) by differential application of a potent GnRH analogue, azagly-nafarelin, under
laboratory, commercial hatchery, and natural conditions. Aquaculture. 223: 141-157.

Broodstock management and hormonal manipulations of fish reproduction

- Mikolajczyk, T., Chyb, J., Szczerbik, P., Sokolowska-Mikolajczyk, M., Epler, P., Enright, W. J.,
 1484 Filipiak, M., Breton, B., 2004. Evaluation of the potency of azagly-nafarelin (GnRH
 analogue), administered in combination with different formulations of pimozide, on LH
 1486 secretion, ovulation and egg quality in common carp (*Cyprinus carpio* L.) under laboratory,
 commercial and natural conditions. *Aquaculture*. 234: 447-460.
- 1488 Milla, S., Jalabert, B., Rime, H., Prunet, P., Bobe, J., 2006. Hydration of rainbow trout oocyte
 during meiotic maturation and in vitro regulation by 17,20beta-dihydroxy-4-pregne-3-one and
 1490 cortisol. *J. Exp. Biol.* 209: 1147-1156.
- Miranda, L. A., Cassará, M. C., Somoza, G. M. I. A. R., 2005. Increase in milt production by
 1492 hormonal treatment in the pejerrey fish *Odontesthes bonariensis* (Valenciennes 1835). *Aqua.*
Res. 36: 1473-1479.
- 1494 Miura, T., Miura, C. I., 2003. Molecular control mechanisms of fish spermatogenesis. *Fish*
Physiol. Biochem. 28: 181-186.
- 1496 Miura, T., Yamauchi, K., Takahashi, H., Nagahama, Y., 1992. The role of hormones in the
 acquisition of sperm motility in salmonid fish. *J. Exp. Zool.* 261: 359-363.
- 1498 Miwa, S., Yan, L., Swanson, P., 1994. Localization of two gonadotropin receptors in the salmon
 gonad by *in vitro* ligand autoradiography. *Biol. Reprod.* 50: 629-642.
- 1500 Molés, G., Gómez, A., Rocha, A., Carrillo, A., Zanuy, S., 2008. Purification and characterization
 of follicle-stimulating hormone from pituitary glands of sea bass (*Dicentrarchus labrax*).
 1502 *Gen. Comp. Endocrinol.* 158: 68-76.
- Molony, B. W., Sheaves, M. J., 2001. Challenges of external insemination in a tropical sparid
 1504 fish, *Acanthopagrus berda*. *Env. Biol. Fish.* 61: 65-71.
- Montserrat, N., González, A., Méndez, E., Piferrer, F., Planas, J. V., 2004. Effects of follicle
 1506 stimulating hormone on estradiol-17 β production and P-450 aromatase (CYP19) activity and

mRNA expression in brown trout vitellogenic ovarian follicles in vitro. Gen. Comp.

Endocrinol. 137: 123-131.

Moon, S. H., Lim, H. K., Kwon, J. Y., Lee, J. K., Chang, Y. J., 2003. Increased plasma 17-

hydroxyprogesterone and milt production in response to gonadotropin-releasing hormone
analogue in captive male starry flounder, *Platichthys stellatus*. Aquaculture. 218: 703-716.

Morehead, D. T., Pankhurst, N. W., Ritar, A. J., 1998. Effect of treatment with LHRH analogue
on oocyte maturation, plasma sex steroid levels and egg production in female striped

trumpeter *Latris lineata* (Latrididae). Aquaculture. 169: 315-331.

Mousa, S. A., Mousa, M. A., 2006. Involvement of corticotropin-releasing factor and

adrenocorticotrophic hormone in the ovarian maturation, seawater acclimation, and induced
spawning of *Liza ramada*. Gen. Comp. Endocrinol. 146: 167-179.

Mugnier, C., Fostier, A., Guezou, S., Gaignon, J. L., Quemener, L., 1998. Effect of some
repetitive factors on turbot stress response. Aquacult. Int. 6: 33-45.

Mugnier, C., Gaignon, J. L., Lebegue, E., Fostier, A., Breton, B., 2000. Induction and
synchronisation of spawning in cultivated turbot (*Scophthalmus maximus* L.) broodstock by
implantation of sustained-release GnRH-a pellet. Aquaculture. 181: 241-255.

Mylonas, C. C., Bridges, C. R., Gordin, H., Belmonte Ríos, A., García, A., De la Gándara, F.,

Fauvel, C., Suquet, M., Medina, A., Papadaki, M., Heinisch, G., De Metrio, G., Corriero, A.,
Vassallo-Agius, R., Guzmán, J. M., Mañanos, E., Zohar, Y., 2007. Preparation and

administration of gonadotropin-releasing hormone agonist (GnRHa) implants for the artificial
control of reproductive maturation in captive-reared Atlantic bluefin tuna (*Thunnus thynnus*

thynnus). Reviews in Fisheries Science. 15: 183-210.

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.

Broodstock management and hormonal manipulations of fish reproduction

- 1532 Mylonas, C. C., Kyriakou, G., Sigelaki, I., Georgiou, G., Stephanou, D., Divanach, P., 2004a.
Reproductive biology of the shi drum (*Umbrina cirrosa*) in captivity and induction of
spawning using GnRHa. *Isr. J. Aquacult.-Bamidgeh*. 56: 75-92.
- 1534 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 x striped
1536 bass hybrids (sunshine bass), using captive broodstocks. *Aquaculture*. 140: 265-280.
- 1538 Mylonas, C. C., Magnus, Y., Gissis, A., Klebanov, Y., Zohar, Y., 1997a. Reproductive biology
and endocrine regulation of final oocyte maturation of captive white bass. *J. Fish Biol.* 51:
234-250.
- 1540 Mylonas, C. C., Papadaki, M., Divanach, P., 2003a. Seasonal changes in sperm production and
quality in the red porgy *Pagrus pagrus* (L.). *Aqua. Res.* 34: 1161-1170.
- 1542 Mylonas, C. C., Papadaki, M., Pavlidis, M., Divanach, P., 2004b. Evaluation of egg production
and quality in the Mediterranean red porgy (*Pagrus pagrus*) during two consecutive spawning
1544 seasons. *Aquaculture*. 232: 637-649.
- 1546 Mylonas, C. C., Papandroulakis, N., Smboukis, A., Papadaki, M., Divanach, P., 2004c.
Induction of spawning of cultured greater amberjack (*Seriola dumerili*) using GnRHa
implants. *Aquaculture*. 237: 141-154.
- 1548 Mylonas, C. C., Scott, A. P., Vermeirssen, E. L. M., Zohar, Y., 1997b. Changes in plasma
gonadotropin II and sex steroid hormones, and sperm production of striped bass after
1550 treatment with controlled-release gonadotropin-releasing hormone agonist-delivery systems.
Biol. Reprod. 57: 669-675.
- 1552 Mylonas, C. C., Scott, A. P., Zohar, Y., 1997c. Plasma gonadotropin II, sex steroids, and thyroid
hormones in wild striped bass (*Morone saxatilis*) during spermiation and final oocyte
1554 maturation. *Gen. Comp. Endocrinol.* 108: 223-236.

- Mylonas, C. C., Sigelaki, I., Divanach, P., Mañanos, E., Carillo, M., Afonso-Polyviou, A.,
 1556 2003b. Multiple spawning and egg quality of individual European sea bass (*Dicentrarchus labrax*) females after repeated injections of GnRHa. *Aquaculture*. 221: 605-620.
- Mylonas, C. C., Tabata, Y., Langer, R., Zohar, Y., 1995a. Preparation and evaluation of
 1558 polyanhydride microspheres containing gonadotropin-releasing hormone (GnRH), for
 1560 inducing ovulation and spermiation in fish. *J. Control. Release*. 35: 23-34.
- Mylonas, C. C., Woods, L. C., III, Thomas, P., Schulz, R. W., Zohar, Y., 1998a. Hormone
 1562 profiles of captive striped bass (*Morone saxatilis*) during spermiation, and long-term
 enhancement of milt production. *J. World Aquac. Soc.* 29: 379-392.
- Mylonas, C. C., Woods, L. C., III, Thomas, P., Zohar, Y., 1998b. Endocrine profiles of female
 1564 striped bass (*Morone saxatilis*) in captivity, during post-vitellogenesis and induction of final
 1566 oocyte maturation via controlled-release GnRHa-delivery systems. *Gen. Comp. Endocrinol.*
 110: 276-289.
- Mylonas, C. C., Woods, L. C., III, Zohar, Y., 1997d. Cyto-histological examination of post-
 1568 vitellogenesis and final oocyte maturation in captive-reared striped bass. *J. Fish Biol.* 50: 34-
 1570 49.
- Mylonas, C. C., Zohar, Y., 2001a. Endocrine regulation and artificial induction of oocyte
 1572 maturation and spermiation in basses of the genus *Morone*. *Aquaculture*. 202: 205-220.
- Mylonas, C. C., Zohar, Y., 2001b. Use of GnRHa-delivery systems for the control of
 1574 reproduction in fish. *Rev. Fish Biol. Fish.* 10: 463-491.
- Mylonas, C. C., Zohar, Y., 2007. Promoting oocyte maturation, ovulation and spawning in
 1576 farmed fish. In: Babin, P.J., Cerdá, J., Lubzens, E., (Eds.), *The Fish Oocyte: from Basic
 Studies to Biotechnological Applications*. Kluwer Academic Publishers, Dordrecht, The
 1578 Netherlands, pp. 433-470.

Broodstock management and hormonal manipulations of fish reproduction

- 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 Aquac. Soc. 26: 240-251.
- Nagahama, Y., Yoshikuni, M., Yamashita, M., Tanaka, M., 1994. Regulation of oocyte maturation in fish. In: Sherwood, N.M., Hew, C.L., (Eds.), Fish Physiology. Academic Press, San Diego, California, pp. 393-439.
- Negatu, Z., Hsiao, S. M., Wallace, R. A., 1998. Effects of insulin-like growth factor-I on final oocyte maturation and steroid production in *Fundulus heteroclitus*. Fish Physiol. Biochem. 19: 13-21.
- Ohta, H., Kagawa, H., Tanaka, H., Okuzawa, K., Hirose, K., 1996. Changes in fertilization and hatching rates with time after ovulation induced by 17, 20 β -dihydroxy-4-pregnen-3-one in the Japanese eel, *Anguilla japonica*. Aquaculture. 139: 291-301.
- 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.
- Okumura, S., Okamoto, K., Oomori, R., H., S., Nakazono, A., 2003. Improved fertilization rates by using a large volume spawning tank in red spotted grouper (*Epinephelus akaara*). Fish Physiol. Biochem. 28: 515-516.
- Okumura, S., Okamoto, K., Oomori, R., Nakazono, A., 2002. Spawning behavior and artificial fertilization in captive reared red spotted grouper, *Epinephelus akaara*. Aquaculture. 206: 165-173.
- Ottolenghi, F., Silvestri, C., Giordano, P., Lovatelli, A., New, M. B., 2004. Capture-based Aquaculture. The fattening of eels, groupers, tunas and yellowtails. Food and Agriculture Organization of the United Nations, Rome.

- Palstra, A. P., Cohen, E. G. H., Niemantsverdriet, P. R. W., van Ginneken, V. J. T., van den
1604 Thillart, G. E. E. J. M., 2005. Artificial maturation and reproduction of European silver eel:
development of oocytes during final maturation. *Aquaculture*. 249: 533-547.
- 1606 Pankhurst, N. W., Fitzgibbon, Q. P., 2006. Characteristics of spawning behaviour in cultured
greenback flounder, *Rhombosa tapirina*. *Aquaculture*. 253: 279-289.
- 1608 Pankhurst, N. W., Purser, G. J., Van Der Kraak, G., Thomas, P. M., Forteach, G. N. R., 1996.
Effect of holding temperature on ovulation, egg fertility, plasma levels of reproductive
1610 hormones and in vitro ovarian steroidogenesis in the rainbow trout *Oncorhynchus mykiss*.
Aquaculture. 146: 277-290.
- 1612 Pankhurst, N. W., Thomas, P. M., 1998. Maintenance at elevated temperature delays the
steroidogenic and ovulatory responsiveness of rainbow trout *Oncorhynchus mykiss* to
1614 luteinizing hormone releasing hormone analogue. *Aquaculture*. 166: 163-177.
- Papadaki, M., Papadopoulou, M., Sigelaki, I., Mylonas, C. C., 2008. Egg and sperm production
1616 and quality of sharpsnout sea bream (*Diplodus puntazzo*) in captivity. *Aquaculture*. 276: 187-
197.
- 1618 Papanikos, N., Phelps, R. P., Williams, K., Ferry, A., Maus, D., 2003. Egg and larval quality of
natural and induced spawns of red snapper, *Lutjanus campechanus* *Fish Physiol. Biochem.*
1620 28: 487-488.
- Park, I. S., Choi, G. C., Nam, Y. K., Kim, D. S., 2002. The effect of exogenous hormone
1622 treatment on spermiation in *Rhynchocypris oxycephalus* (Sauvage and Dabry). *J. World*
Aquac. Soc. 33: 494-500.
- 1624 Paspatis, M., Markakis, G., Koumoundouros, G., Kentouri, M., 1999. Preliminary results on
rearing of *Sparus Aurata* × *Pagrus Pagrus* hybrids. Performance comparison with the
1626 parental species. *Aquacult. Int.* 7: 29-44.

Broodstock management and hormonal manipulations of fish reproduction

- Patiño, R., Sullivan, C. V., 2002. Ovarian follicle growth, maturation, and ovulation in teleost
1628 fish. *Fish Physiol. Biochem.* 26: 57-70.
- Patiño, R., Yoshizaki, G., Thomas, P., Kagawa, H., 2001. Gonadotropic control of ovarian
1630 follicle maturation: the two-stage concept and its mechanisms. *Comp. Biochem. Physiol.*
B129: 427-439.
- Pavlidis, M., Greenwood, L., Scott, A. P., 2004. The role of sex ratio on spawning performance
1632 and on the free and conjugated sex steroids released into the water by common dentex
(*Dentex dentex*) broodstock. *Gen. Comp. Endocrinol.* 138: 255-262.
- Peter, R. E., Yu, K. L., 1997. Neuroendocrine regulation of ovulation in fishes: basic and applied
1636 aspects. *Rev. Fish Biol. Fish.* 7: 173-197.
- Petersson, J., Järvi, T., 2001. 'False orgasm' in female brown trout: trick or treat? *Anim. Behav.*
1638 61: 497-501.
- Piper, R. G., McElwain, I. B., Orme, L. E., McCraren, J. P., Fowler, L. G., Leonard, J. R., 1982.
1640 *Fish Hatchery Management*. Washington, D.C.
- Polzonetti-Magni, A., Mosconi, G., Soverchia, L., Kikuyama, S., Carnevali, O., 2004.
1642 *Multihormonal control of vitellogenesis in lower vertebrates*. *Int. Rev. Cytol.* 239: 1-45.
- Poortenaar, C. W., Pankhurst, N. W., 2000. Effect of luteinising hormone-releasing hormone
1644 analogue and human chorionic gonadotropin on ovulation, plasma and ovarian levels of
gonadal steroids in greenback flounder *Rhombosolea tapirina*. *J. World Aquac. Soc.* 31: 175-
1646 185.
- Pudney, J., 1995. Spermatogenesis in nonmammalian vertebrates. *Microscopy Research And*
1648 *Technique*. 32: 459-497.

- 1650 Rainis, S., Mylonas, C. C., Kyriakou, Y., Divanach, P., 2003. Enhancement of spermiation in
European sea bass (*Dicentrarchus labrax*) at the end of the reproductive season using GnRH
implants. *Aquaculture*. 219: 873-890.
- 1652 Rocha, A., Zanuy, S., Carrillo, M., Gómez, A., 2008. Seasonal changes in gonadal expression of
gonadotropin receptors, steroidogenic acute regulatory protein and steroidogenic enzymes in
1654 the European sea bass. *J. Endocrinol.* 000: 000-000.
- Rodríguez, R., Celada, J. D., Saez-Royuela, M., Carral, J. M., Aquilera, A., Melendre, P. M.,
1656 2004. Artificial reproduction in 1-year-old tench (*Tinca tinca* L.). *J. Appl. Ichthyol.* 20: 542-
544.
- 1658 Rosenfeld, H., Meiri, I., Elizur, A., 2007. Gonadotropin regulation of oocyte development. In:
Babin, P.J., Cerdá, J., Lubzens, E., (Eds.), *The Fish Oocyte: from Basic Studies to*
1660 *Biotechnological Applications*. Kluwer Academic Publishers, Dordrecht, The Netherlands,
pp. 171-198.
- 1662 Rudolfson, G., Figenschou, L., Folstad, I., Kleven, O., 2008. Sperm velocity influences paternity
in the Atlantic cod (*Gadus morhua* L.). *Aqua. Res.* 39: 212-216.
- 1664 Rurangwa, E., Kime, D. E., Ollevier, F., Nash, J. P., 2004. The measurement of sperm motility
and factors affecting sperm quality in cultured fish. *Aquaculture*. 234: 1-28.
- 1666 Rzemieniecki, A., Domagala, J., Glogowski, J., Ciereszko, A., Trzebiatowski, R., Kouril, J.,
Hamackova, J., Babiak, I., 2004. Induced spermiation in 3-year-old sterlet, *Acipenser*
1668 *ruthenus* L. *Aqua. Res.* 35: 144-151.
- Saito, K., O'Donnell, L., McLachlan, R. I., Robertson, D. M., 2000. Spermiation failure is a
1670 major contributor to early spermatogenic suppression caused by hormone withdrawal in adult
rats. *Endocrinol.* 141: 2779-2785.

Broodstock management and hormonal manipulations of fish reproduction

- 1672 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
1674 abnormal alevins during the process of over-ripening. Bull. Jap. Soc. Sci. Fish. 41: 855-860.
- Sato, N., Kawazoe, I., Shiina, Y., Furukawa, K., Suzuki, Y., Aida, K., 1995. A novel method of
1676 hormone administration for inducing gonadal maturation in fish. Aquaculture. 135: 51-58.
- Sawada, Y., Okada, T., Miyashita, S., Murata, O., Kumai, H., 2005. Completion of the Pacific
1678 bluefin tuna *Thunnus orientalis* (Temmich et Schlegel) life cycle. Aqua. Res. 36: 413-421.
- Sawaguchi, S., Kagawa, H., Ohkubo, N., Hiramatsu, K., Sullivan, C. V., Matsubara, M., 2006.
1680 Molecular characterization of three forms of vitellogenin and their yolk protein products
during oocyte growth and maturation in red seabream (*Pagrus major*), a marine teleost
1682 spawning pelagic eggs. Mol. Repro. Dev. 73: 719-736.
- Schally, A. V., 1978. Aspects of hypothalamic regulation of the pituitary gland. Science. 202:
1684 18-28.
- Schiavone, R., Zilli, L., Vilella, S., Fauvel, C., 2006. Human chorionic gonadotropin induces
1686 spermatogenesis and spermiation in 1-year-old European sea bass (*Dicentrarchus labrax*):
assessment of sperm quality. Aquaculture. 255: 522-531.
- Schulz, D. R., Perez, N., Tan, C.-K., Mendez, A. J., Capo, T. R., Snodgrass, D., Prince, E. D.,
1688 Serafy, J. E., 2005a. Concurrent levels of 11-ketotestosterone in fish surface mucus, muscle
1690 tissue and blood. J. Appl. Ichthyol. 21: 394-398.
- Schulz, R. W., Miura, T., 2002. Spermatogenesis and its endocrine regulation. Fish Physiol.
1692 Biochem. 26: 43-56.
- Schulz, R. W., Sandra Menting, S., Bogerd, J., França, L. R., Vilela, D. A. R., Godinho, H. P.,
1694 2005b. Sertoli cell proliferation in the adult testis—Evidence from two fish species belonging
to different orders. Biol. Reprod. 73: 891-898.

- 1696 Scott, A. P., Baynes, S. M., 1980. A review of the biology, handling and storage of salmonid
spermatozoa. J. Fish Biol. 17: 707-739.
- 1698 Shiraishi, T., Ohta, K., Yamaguchi, A., Yoda, M., Chuda, H., Matsuyama, M., 2005.
Reproductive parameters of the chub mackerel *Scomber japonicus* estimated from human
1700 chorionic gonadotropin-induced final oocyte maturation and ovulation in captivity. Fisheries
Sci. 71: 531-542.
- 1702 Silverstein, J. T., Bosworth, B. G., Wolters, W. R., 1999. Evaluation of dual injection of LHRHa
and the dopamine receptor antagonist pimozide in cage spawning of channel catfish *Ictalurus*
1704 *punctatus*. J. World Aquac. Soc. 30: 263-268.
- Slater, C., Schreck, C. B., Swanson, P., 1994. Plasma profiles of the sex steroids and
1706 gonadotropins in maturing female spring chinook salmon (*Oncorhynchus tshawytscha*).
Comp. Biochem. Physiol. 109A: 167-175.
- 1708 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
1710 water. Aquaculture. 130: 279-285.
- Solar, I. I., Smith, J., Dye, H. M., MacKinlay, D. D., Zohar, Y., Donaldson, E. M., 1995.
1712 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
1714 of Fish. Fish Symposium 95, Austin, Texas, pp. 144.
- Sorbera, L. A., Asturiano, J. F., Carrillo, M., Cerda, J., Kime, D. E., Zanuy, S., 1999. *In vitro*
1716 maturation in the sea bass: effects of hCG, pituitary extract and steroids. J. Fish Biol. 55: 9-
25.

Broodstock management and hormonal manipulations of fish reproduction

- 1718 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.
1720 276: 361-368.
- Sower, S., Shreck, C., 1982. Steroid and thyroid hormones during sexual maturation of coho
1722 salmon (*Oncorhynchus kisutch*) in seawater or fresh water. Gen. Comp. Endocrinol. 47:42-
53.
- 1724 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 eyeing,
1726 hatch and swim-up in the rainbow trout (*Salmo gairdneri*). Aquaculture. 43: 313-322.
- Stacey, N., 2003. Hormones, pheromones and reproductive behavior. Fish Physiol. Biochem. 28:
1728 229-235.
- Steven, C., Studies on the GnRH-GtH system of female striped bass (*Morone saxatilis*): effects
1730 of GnRH agonist therapy and comparison of reproductive endocrine parameters between wild
and captive fish. Marine Estuarine and Environmental Sciences. Univeristy of Maryland,
1732 College Park, 2000.
- Steven, C., Gothilf, Y., Holland, M. C. H., Stubblefield, J., Mylonas, C. C., Zohar, Y., 2000.
1734 Differential expression of the three GnRH genes in wild and captive striped bass, *Morone*
saxatilis, in response to natural and hormonally induced maturation. In: Norberg, B., Kjesbu,
1736 O.S., Taranger, G.L., Andersson, E., Stefansson, S.O., (Eds.), Reproductive Physiology of
Fish. University of Bergen, Bergen, pp. 66.
- 1738 Stoltz, J. A., Neff, B. D., 2006. Sperm competition in a fish with external fertilization: the
contribution of sperm number, speed and length. J. Evol. Biol. 19: 1873-1881.

- 1740 Sullivan, C. V., Bernard, M. G., Hara, A., Dickhoff, W. W., 1989. Thyroid hormones in trout
reproduction: enhancement of GnRH α and partially purified salmon GtH-induced ovarian
1742 maturation *in vivo* and *in vitro*. J. Exp. Zool. 250: 188-195.
- Suquet, M., Billard, R., Cosson, J., Dorange, G., Chauvaud, L., Mugnier, C., Fauvel, C., 1994.
1744 Sperm features in turbot (*Scophthalmus maximus*): a comparison with other freshwater and
marine fish species. Aquat. Living Resour. 7: 283-294.
- 1746 Suquet, M., Billard, R., Cosson, J., Normant, Y., Fauvel, C., 1995. Artificial insemination in
turbot (*Scophthalmus maximus*): determination of the optimal sperm to egg ratio and time of
1748 gamete contact. Aquaculture. 133: 83-90.
- Suwa, K., Yamashita, M., 2007. Regulatory mechanisms of oocyte maturation and ovulation. In:
1750 Babin, P.J., Cerdá, J., Lubzens, E., (Eds.), The Fish Oocyte: from Basic Studies to
Biotechnological Applications. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- 1752 Szabó, T., 2001. Hormonally induced ovulation of northern pike via sustained-release vehicles.
North American Journal of Aquaculture. 63: 137-143.
- 1754 Takashima, F., Weil, C., Billard, R., Crim, L. W., Fostier, A., 1984. Stimulation of spermiation
by LHRH analogue in carp. Bull. Jap. Soc. Sci. Fish. 50: 1323-1329.
- 1756 Takushima, M., Nozaki, R., Kadomura, K., Yasumoto, S., Soyano, K., 2003. Induced ovulation
using LHRH α and artificial fertilization in devil stinger, *Inimicus japonicus*. Fish Physiol.
1758 Biochem. 28: 521-522.
- Thomas, P., Arnold, C. R., Holt, G. J., 1995. Red drum and other sciaenids. In: Bromage, N.R.,
1760 Roberts, R.J., (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell
Science, Oxford, pp. 118-137.

Broodstock management and hormonal manipulations of fish reproduction

- 1762 Tsadik, G. G., Bart, A. N., 2007. Effects of feeding, stocking density and water-flow rate on
fecundity, spawning frequency and egg quality of Nile tilapia, *Oreochromis niloticus* (L.).
1764 *Aquaculture*. 272: 380-388.
- Tucker, J. W., 1994. Spawning of captive serranid fishes: a review. *J. World Aquac. Soc.* 25:
1766 345-359.
- Tveiten, H., Solevac, S. E., Johnsen, H. K., 2001. Holding temperature during the breeding
1768 season influences final maturation and egg quality in common wolffish. *J. Fish Biol.* 58: 374-
385.
- 1770 Tyler, J. R., Sumpter, J. P., 1996. Oocyte growth and development in teleosts. *Rev. Fish Biol.*
Fish. 6: 287-318.
- 1772 Uchida, K., Kaneko, T., Yamaguchi, A., Ogasawara, Y., Hirano, T., 1997. Reduced
hypoosmoregulatory ability and alteration in gill chloride distribution in mature chum salmon
1774 (*Oncorhynchus keta*) migrating upstream for spawning. *Mar. Biol.* 129: 247-253.
- Ulloa-Aguirre, A., Timossi, C., 2000. Biochemical and functional aspects of gonadotrophin-
1776 releasing hormone and gonadotrophins. *Reproductive Biomedicine Online*. 1: 48-62.
- Unuma, T., Kondo, S., Tanaka, H., Kagawa, H., Nomura, K., Ohta, H., 2005. Relationship
1778 between egg specific gravity and egg quality in the Japanese eel, *Anguilla japonica*.
Aquaculture. 246: 493-500.
- 1780 Van Der Kraak, G., Dye, H. M., Donaldson, E. M., Hunter, G. A., 1985. Plasma gonadotropin,
17β-estradiol, and 17α,20β-dihydroxy-4-pregnen-3-one levels during luteinizing hormone-
1782 releasing hormone analogue and gonadotropin induced ovulation in coho salmon
(*Oncorhynchus kisutch*). *Can. J. Zool.* 63: 824-833.

- 1784 Van Der Kraak, G., Pankhurst, N. W., 1996. Temperature effects on the reproductive
performance of fish. In: Wood, C.M., McDonald, D.G., (Eds.), Global Warming: Implications
1786 for Freshwater and Marine Fish. Cambridge University Press, Cambridge, pp. 159-176.
- van Ginneken, V. J. T., Maes, G. E., 2005. The European eel (*Anguilla anguilla*, Linnaeus), its
1788 lifecycle, evolution and reproduction: a literature review. Rev. Fish Biol. Fish. 15: 367-398.
- Vermeirssen, E. L. M., Mazorra de Quero, C., Shields, R. J., Norberg, B., Kime, D. E., Scott, A.
1790 P., 2003. Fertility and motility of sperm from Atlantic halibut (*Hippoglossus hippoglossus*) in
relation to dose and timing of gonadotropin-releasing hormone agonist implant. Aquaculture.
1792 230: 547-567.
- Vermeirssen, E. L. M., Scott, A. P., Mylonas, C. C., Zohar, Y., 1998. Gonadotrophin-releasing
1794 hormone agonist stimulates milt fluidity and plasma concentrations of 17,20 β -dihydroxylated
and 5 β -reduced, 3 α -hydroxylated C21 steroids in male plaice (*Pleuronectes platessa*). Gen.
1796 Comp. Endocrinol. 112: 163-177.
- Vermeirssen, E. L. M., Shields, R. J., Mazorra de Quero, C., Scott, A. P., 2000. Gonadotrophin-
1798 releasing hormone agonist raises plasma concentrations of progestogens and enhances milt
fluidity in male Atlantic halibut (*Hippoglossus hippoglossus*). Fish Physiol. Biochem. 22: 77-
1800 87.
- Viveiros, A. T. M., Eding, E. H., Komen, J., 2001. Effects of 17 α -methyltestosterone on seminal
1802 vesicle development and semen release response in the African catfish, *Clarias gariepinus*.
Reproduction. 122: 817-827.
- 1804 Viveiros, A. T. M., Fessehaye, Y., ter Veld, M., Schulz, R. W., Komen, J., 2002. Hand-stripping
of semen and semen quality after maturational hormone treatments, in African catfish *Clarias*
1806 *gariepinus*. Aquaculture. 213: 373-386.

Broodstock management and hormonal manipulations of fish reproduction

- Vizziano, D., Fostier, A., Loir, M., Le Gac, F., 2008. Testis development, its hormonal
1808 regulation and spermiation induction in teleost fish. In: Alavi, S.M.H., Cosson, J., Coward,
K., Rafiee, G., (Eds.), Fish Spermatology. Alpha Science Intl, Oxford (UK), pp. 103-139.
- Von Ihering, R., 1937. A method for inducing spawning in fish. Prog. Fish-Cult. 34: 15-16.
- 1810 Watanabe, A., Onitake, K., 2008. The regulation of spermatogenesis in fish: recent cellular and
1812 molecular approaches. In: Alavi, S.M.H., Cosson, J., Coward, K., Rafiee, G., (Eds.), Fish
Spermatology. Alpha Science Intl, Oxford (UK), pp. 141-160.
- 1814 Watanabe, T., Kiron, V., 1995. Red sea bream (*Pagrus major*). In: Bromage, N.R., Roberts, R.J.,
(Eds.), Broodstock Management and Egg and Larval Quality. Blackwell Science, Oxford, pp.
1816 398-413.
- Watanabe, W. O., Carroll, P. M., Daniels, H. V., 2001. Sustained, natural spawning of southern
1818 flounder *Paralichthys lethostigma* under an extended photothermal regime. J. World Aquac.
Soc. 32: 153-166.
- 1820 Watanabe, W. O., Ellis, E. P., Ellis, S. C., Feeley, M. W., 1998. Progress in controlled
maturation and spawning of summer flounder (*Paralichthys dentatus*) broodstock. J. World
1822 Aquac. Soc. 29: 393-404.
- Watanabe, W. O., Smith, T. I. J., Berlinsky, D. L., Woolridge, C. A., Stuart, K. R., Copeland, K.
1824 A., Denson, M. R., 2003. Volitional spawning of black sea bass *Centropristis striata* induced
with pelleted luteinizing hormone releasing hormone-analogue. J. World Aquac. Soc. 34:
1826 319-331.
- Webb, M. A. H., Van Eenennaam, J. P., Doroshov, S. I., Moberg, G. P., 1999. Preliminary
1828 observations on the effects of holding temperature on reproductive performance of female
white sturgeon, *Acipenser transmontanus* Richardson. Aquaculture. 176: 315-329.

- 1830 Weber, G. M., Borski, R. J., Powell, J. F. F., Sherwood, N. M., Grau, E. G., 1995. *In vivo* and *in*
1832 *vitro* effects of gonadotropin-releasing hormone on prolactin in the tilapia *Oreochromis*
mossambicus. American Zoologist (Abstract). 34:121A.
- Weil, C., Crim, L. W., 1983. Administration of LHRH analogues in various ways: effect on the
1834 advancement of spermiation in prespawning landlocked salmon, *Salmo salar*. Aquaculture.
35: 103-115.
- 1836 Wen, H. S., Lin, H. R., 2004. Effects of exogenous neurohormone, gonadotropin (GtH) and
dopaminergic drugs on the serum GtH content and ovulatory responsiveness of wild catfish,
1838 *Silurus asotus* (Linnaeus, 1758). Aqua. Res. 35: 204-212.
- Wendling, N. C., Bencic, D. C., Nagler, J. J., Cloud, J. G., Ingermann, R. L., 2000. Adenosine
1840 triphosphate levels of chinook salmon (*Oncorhynchus tshawytscha*) eggs following *in vitro*
maintenance and activation/fertilization. Fish Physiol. Biochem. 22: 217-223.
- 1842 Wexler, J. B., Scholey, V. P., Olson, R. J., Margulies, D., Nakazawa, A., Suter, J. M., 2003.
Tank culture of yellowfin tuna, *Thunnus albacares*: developing a spawning population for
1844 research purposes. Aquaculture. 220: 327-353.
- Williot, P., Brun, R., Rouault, T., Pelard, M., Mercier, D., Ludwig, A., 2005. Artificial spawning
1846 in cultured sterlet sturgeon, *Acipenser ruthenus* L., with special emphasis on hermaphrodites.
Aquaculture. 246: 263-273.
- 1848 Williot, P., Brun, R., Rouault, T., Rooryck, O., 1991. Management of female breeders of the
Siberian sturgeon, *Acipenser baeri* Brandt: first results. In: Williot, P., (Ed.), *Acipenser*.
1850 Cemagref Publications, France, pp. 365-379.
- Williot, P., Gulyas, T., Ceapa, C., 2002. An analogue of GnRH is effective for induction of
1852 ovulation and spermiation in farmed Siberian sturgeon *Acipenser baerii* Brandt. Aqua. Res.
33: 735-737.

Broodstock management and hormonal manipulations of fish reproduction

- Williot, P., Sabeau, L., Gessner, J., Arlati, G., Bronzi, P., Gulyas, T., Berni, P., 2001. Sturgeon farming in Western Europe: recent developments and perspectives. *Aquat. Living Resour.* 14: 367-374.
- Woolsey, J., Ingermann, R. L., 2003. Acquisition of the potential for sperm motility in steelhead (*Oncorhynchus mykiss*): effect of pH on dynein ATPase. *Fish Physiol. Biochem.* 29: 47-56.
- Yang, Z., Chen, Y.-F., 2004. Induced ovulation in obscure puffer *Takifugu obscurus* by injections of LHRH-a. *Aquacult. Int.* 12: 215-223.
- Yaron, Z., 1995. Endocrine control of gametogenesis and spawning induction in the carp. *Aquaculture.* 129: 49-73.
- Yu, K. L., Lin, X. W., da Cunha Bastos, J., Peter, R. E., 1997. Neural regulation of GnRH in teleost fishes. In: Parhar, I.S., Sakuma, Y., (Eds.), *GnRH Neurons: Gene to Behavior*. Brain Shuppan, Tokyo, pp. 277-312.
- Zakes, Z., Szczepkowski, M., 2004. Induction of out-of-season spawning of pikeperch, *Sander lucioperca* (L.). *Aquacult. Int.* 12: 11-18.
- Zhuang, P., Kynard, B., Zhang, L., Zhang, T., Zhang, Z., Li, D., 2002. Overview of biology and aquaculture of Amur sturgeon (*Acipenser schrenckii*) in China. *J. Appl. Ichthyol.* 18: 659-664.
- 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., 1989. 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.

- 1878 Zohar, Y., 1996. New approaches for the manipulation of ovulation and spawning in farmed
fish. Bull. Natl. Res. Inst. Aquacult., Suppl. 2: 43-48.
- 1880 Zohar, Y., Harel, M., Hassin, S., Tandler, A., 1995. Gilthead sea bream (*Sparus aurata*). In:
Bromage, N.R., Roberts, R.J., (Eds.), Broodstock Management and Egg and Larval Quality.
Blackwell Science, Oxford, pp. 94-117.
- 1882 Zohar, Y., Mylonas, C. C., 2001a. Endocrine manipulations of spawning in cultured fish: from
hormones to genes. Aquaculture. 197: 99-136.
- 1884 Zohar, Y., Mylonas, C. C., 2001b. Endocrine manipulations of spawning in cultured fish: from
hormones to genes. In: Donaldson, E.M., Lee, C.S., (Eds.), Reproductive Biotechnology in
1886 Finfish Aquaculture. Elsevier, Amsterdam, pp. 99-136.

1888

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11. FIGURE LEGENDS

Figure 1. Schematic representation of the reproductive axis in fish, its major components and phases, and its environmental and endocrine control.

Figure 2. 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 spermatocysts with spermatocytes I and II (sc) and spermatids (st), including some spermatozoa (sz). (C) In December when spermiation 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. The bar at the bottom of each photograph is 200 μ m.

Figure 3. Microphotographs of histological sections from ovaries of (A) striped bass, a synchronous fish having all oocytes at the same stage of maturation (lipid droplet coalescence, germinal vesicle migration), and (B) Atlantic bluefin tuna, an asynchronous fish having oocytes at different stages of vitellogenesis (cortical alveoli, early and late vitellogenesis). gv = germinal vesicle, ld = lipid droplets, y = yolk vesicles, Ca = cortical alveoli stage, Evg = early vitellogenesis stage, Lvg = late vitellogenesis stage. The bar at the bottom of each photograph is 200 μ m.

Figure 4. The process of oocyte maturation (OM) in European sea bass. (A) Time course of *in vitro* OM (%) induced by graded doses of the two maturation inducing steroids (17,20 β P and 20 β S) and hCG (which has LH-like activity). (B) Mean ($\pm$ SEM) plasma levels of 17,20 β P and

20BS in relation to the oocyte stage of development of the most advanced oocytes. The data was compiled from individual females (n in parentheses) in several samplings, independent of the time of sampling. PREVTG = pre-vitellogenesis, eVtG = early vitellogenesis, aVTG = advanced vitellogenesis, POSTVTG = post-vitellogenesis, eMAT = early OM, fMAT = final OM, OVUL = ovulation, ATRE = atresia. Different letters superscripts indicate significant differences (ANOVA, DNMR, $P < 0.05$). (Modified from Asturiano et al., 2000; Sorbera et al., 1999).

Figure 5. Photographs of milt collection from a wreckfish (*Polyprion americanus*) after abdominal pressure (A) and acquisition of an ovarian biopsy using a catheter (B).

Figure 6. Schematic representation of the dysfunction in the reproductive axis of cultured fishes, and the exogenous hormonal interventions for the induction of oocyte maturation and spermiation.

Figure 7. Mean (SEM) volume of expressible milt over a 44-day period, from European sea bass (n = 8) administered different hormonal treatments of GnRH α , including GnRH α fast-release systems (injection, I and EVAc implants), GnRH α slow-release systems (microspheres, MC and EVSL implants) and untreated controls. Asterisks indicate significant ($P \leq 0.05$) differences from the control group, for each sampling point. (Modified from Sorbera et al., 1996).

Figure 8. Batch fecundity (x1000 eggs female) and fertilization success (Viability, %) of an individual greater amberjack (weight 23.5 Kg) after treatment with GnRH α -loaded implants (50

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1938 $\mu\text{g Kg}^{-1}$, arrows) at two times during the reproductive season. The female was maintained with
two males of similar size (23.5 and 36.0 Kg), in a 40-m³ square concrete tank, supplied with
1940 surface water (22-24°C).

The reproductive axis in fish

Mylonas, Foster and Zanuy
Fig. 2

Mylonas, Foster and Zanuy
Fig. 3

Mylonas, Foster and Zanuy
Fig. 4

Mylonas, Foster and Zanuy
Fig. 5

Hormonal induction of oocyte maturation & spermiation

Mylonas, Foster and Zanuy
Fig. 8