Chapter 4

Reproduction and broodstock management

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Chapter contents

Abstract: The Sparidae is a cosmopolitan family, and sparid fishes can be found and reproduced in both temperate and tropical seas around the world. Most of the members of this family are sequential hermaphrodites—either protogynous, such as the red porgy (*Pagrus pagrus*), or protandrous, such as the gilthead sea bream (*Sparus aurata*)– but gonochoristic species also exist, such as the common dentex (*Dentex dentex*). Fish in this family have a long spawning season, ranging between 60 and 150 days, and spawn daily or in a highly cyclical fashion. Fecundity is very high ranging between 0.4 and 3.2 $\times$ 10^6 eggs kg^{-1} of female body weight. The eggs are pelagic and transparent and have a diameter between 800 and 1,000 μm. Hormonal therapies have been developed to address the reproductive dysfunctions that exist in the early days of establishing captive wild or hatchery-produced broodstocks. At present, most Sparidae that are cultured commercially reproduce spontaneously in captivity and hatchery produced broodstocks have been developed and in some cases selected for traits of commercial importance.

Key words: broodstock management; controlled-release delivery systems; gametogenesis; GnRH; hermaphroditism; spawning induction; spermatogenesis

4.1 Introduction

The Sparidae is a cosmopolitan family, and sparid fishes can be found and reproduced in both temperate and tropical seas around the world (Sheaves 2006). Certain species may be present both in the Northern and

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Southern Hemispheres, such as the white sea bream (*Diplodus sargus*) (Mann & Buxton 1998; Mouine *et al.* 2007), whereas others may be present also in the Eastern and Western Hemispheres, such as the red porgy (*Pagrus pagrus*) (Manooch & Hassler 1978; Vassilopoulou & Papaconstantinou 1992; Pajuelo & Lorenzo 1996; Aristizabal *et al.* 2009). In terms of the study of reproduction in fish, the Sparidae is an important family, and the reproductive biology of some members of this family has been investigated extensively. Most of the Sparidae are sequential hermaphrodites—either protogynous, such as the red porgy, or protandrous, such as the gilthead sea bream (*Sparus aurata*)—but gonochoristic species also exist, such as the common dentex (*Dentex dentex*). A major finding in vertebrate reproductive biology was the discovery of three forms of gonadotropin releasing hormone (GnRH) in the brain, which was done in the gilthead sea bream (Powell *et al.* 1994; Holland *et al.* 1998). Fish in this family have a long spawning season, and spawn daily or in a highly cyclical fashion for a period of many months (3–5), and, therefore, have very high annual fecundity that can reach well above 2 million eggs kg^{-1} body weight per season. The present chapter attempts to summarize the available information on the reproductive biology-–hermaphroditism and reproductive cycles—of the main cultured Sparidae species, providing also some information on broodstock management of these species in captivity and the available methods for the control of reproductive function and spawning for commercial aquaculture purposes. Therefore, not all available information on the reproduction of Sparidae has been included, and some species that do not have any interest for commercial aquaculture have been left out.

4.2 Hermaphroditism and puberty in Sparidae

The phenomena of hermaphroditism and sex reversal in Sparidae caught the attention of reproductive biologists many decades ago (D'Ancona 1941, 1949; Pasqualli 1941; Reinboth 1962). Sparidae express protandrous, protogynous, as well as simultaneous and rudimentary hermaphroditism (Buxton & Garratt 1990). In rudimentary hermaphroditism, the fish do not change sex, but before puberty they go through a phase when the gonad has both an ovarian and a testicular section, both in an immature state. As in other fishes, sequential hermaphroditism has been proposed to be an adaptation, enabling the individual to maximize its reproductive success, in situations where this is size-related: the individual functions as one sex when small and changes to the opposite sex when larger (Sadovy & Shapiro 1987; Buxton & Garratt 1990; Sadovy & Liu 2008).

Together with the black porgy (*Acanthopagrus schlegeli*), the gilthead sea bream has been one of the most well-studied hermaphrodite fish models and is presented here even though it may not be a representative model for other Sparidae considered in this chapter. The earliest studies, analyzing wild gilthead sea bream, demonstrated its protandrous hermaphrodite gonadal cycle with the entire population functioning as males at the age of two years and changing sex at the age of three years. The selection of the gilthead sea bream in the 1960s as one of the main species of interest in the unfolding Mediterranean marine aquaculture industry, and the ensuing need to establish reliable broodstocks and controlled spawning, led to a renewed interest in studying gilthead sea bream reproductive biology. A complete description of the gonadal cycle of captive gilthead sea bream populations demonstrated the complex and unique processes involved in its gametogenesis and sex reversal (Zohar *et al.* 1978, 1984). During the first year of life, gilthead sea bream undergoes juvenile hermaphroditism, with the young bisexual gonad developing toward an ovary and then subsequently toward a testis (Figure 4.1). Ultimately, the entire year class functions as males during their first reproductive maturity as one-year-old fish, a year earlier than in the wild. This ephemeral oogenesis in the gonad's dorsal portion reaches the stage of multiple oogonia densely populating ovigerous lamellae surrounding the gonadal central cavity, thereby forming a dominant dorsal ovary (Figure 4.1a). However, prior to further development, all but a few layers of oogonia lining the gonadal central cavity undergo massive atresia. Simultaneously, the ventral testicular portion of the gonad undergoes intensive spermatogenesis and functions as a mature testis during the first reproductive

season (Figure 4.1b and c). After the end of the first reproductive season in captivity, the testicular part of the bisexual gonad regresses quickly to form a dormant testis containing testicular lobules lined with spermatogonia (Figure 4.1d). Concomitantly, the remaining oogonia in the dorsal part of the gonad enter intensive oogenesis and proliferate into a young ovary consisting of oogonia and primary oocytes (Figure 4.1d). At this stage, the gonads in all one-year-old fish are still bisexual and bipotential, with a dominant ovarian part and a smaller testicular portion (Zohar *et al.* 1978; Zohar *et al.* 1984; Bruslé-Sicard & Fourcault 1997). From this point onward and until the second reproductive season (22–27 months old), two opposing patterns develop. Some fish continue to develop the ovary through oogenesis and function as two-year-old females (Figure 4.2a). As the ovarian part of the gonad develops in these fish, the testicular portion regresses completely and irreversibly. These individuals continue to function as females for the duration of their reproductive life. The percentage of fish changing sex during the second year can vary from 15 to 80% (Zohar *et al.* 1978; Kadmon *et al.* 1985; Bruslé-Sicard & Fourcault 1997), and in the third year, this percentage may increase to 63% (Kadmon *et al.* 1985). In the remaining fish, the dormant testicular part of the gonad undergoes intensive spermatogenesis and the ovarian portion undergoes massive apoptosis (also called atresia) of the oogonia and primary oocytes, very much similar to the process witnessed a year earlier in all fish. These individuals function again as males, two-year-olds at this time (Figure 4.2b). In these functional males, a few layers of oogonia remain intact and line the central cavity of the gonad. At the end of the second spawning season, these males undergo the same process described above for the one-year-old males (Figure 4.1d): the testis regresses, the ovary develops, and the bipotent ambisexual gonad again progresses in one of two opposing directions, with a percentage of the fish becoming 3-year-old females and the remaining developing again into functional males. A similar pattern of gonadal development and sex inversion has been described also in the black porgy (Lee *et al.* 2001, 2002; Du *et al.* 2003).

The red sea bream (*Pagrus major*) also exhibits juvenile hermaphroditism after a short ovarian differentiation period (Kato *et al.* 1999; Lim, 2004). Under culture conditions, formation of the ovarian cavity takes place at 75 days after hatch (dah) and synaptic oocytes are observed around 150 dah in all juveniles. Shortly after the appearance of oocytes, the testicular section in which spermatogonia are localized begins forming around 180 dah. Juvenile hermaphroditism continues to be exhibited until completion of sex differentiation at around 500 dah. Fish from wild populations of the southern hemisphere congener Australasian snapper (synonym: squirefish) *Pagrus* (= *Chrysophrys*) *auratus* show a similar pattern of development, with all 0+ year class fish having undifferentiated gonads, 1+ year class fish having immature ovaries (oogonia and primary oocytes only), and 2+ to 4+ year class fish having ovary, ovotestis, or testis (Francis & Pankhurst 1988). The sex ratio of red sea bream in the wild is approximately 1:1 (Matsuyama *et al.* 1988b), but high female percentages are observed sometimes under culture conditions. Fadrozole, a P450 aromatase inhibitor, and rearing under high water temperature conditions suppress ovarian cavity formation (Lim *et al.* 2003), suggesting the role of estrogen in ovarian cavity formation in the red sea bream. During testicular differentiation, the expression of follicle stimulating hormone (FSH) β subunit mRNA in the pituitary and 11β-hydroxylase mRNA in the gonad increase, while mRNA expression of P450 aromatase in the gonad decreases (Lim *et al*. 2003; Lim 2004). These data indicate that FSH may be implicated in male sex differentiation through androgen synthesis in the gonad. The red sea bream attains puberty at 2–3 years of age in both males and females, wherein they acquire full reproductive capacity (Matsuyama *et al.* 1988b). However, precocious puberty and spawning has been recorded in 1-year-old fish reared under relatively high water temperature conditions (Kato *et al.* 1985).

The common (or red) pandora (*Pagellus erythrinus*) is a protogynous hermaphrodite and fish larger than 210 mm (>4 years old, 700–800 g) in the wild were found to be all males, whereas in fish less than 160 mm (<2 years old) the sex ratio was heavily (80%) skewed toward females (Pajuelo & Lorenzo 1998; Somarakis & Machias 2002; Valdéz *et al.* 2004). In captivity, however, 20–30% of fish larger than 250 mm were found to be females (Klaoudatos *et al.* 2004). Puberty occurs at an age of 1+ to 3+ years, with the size at first maturity of

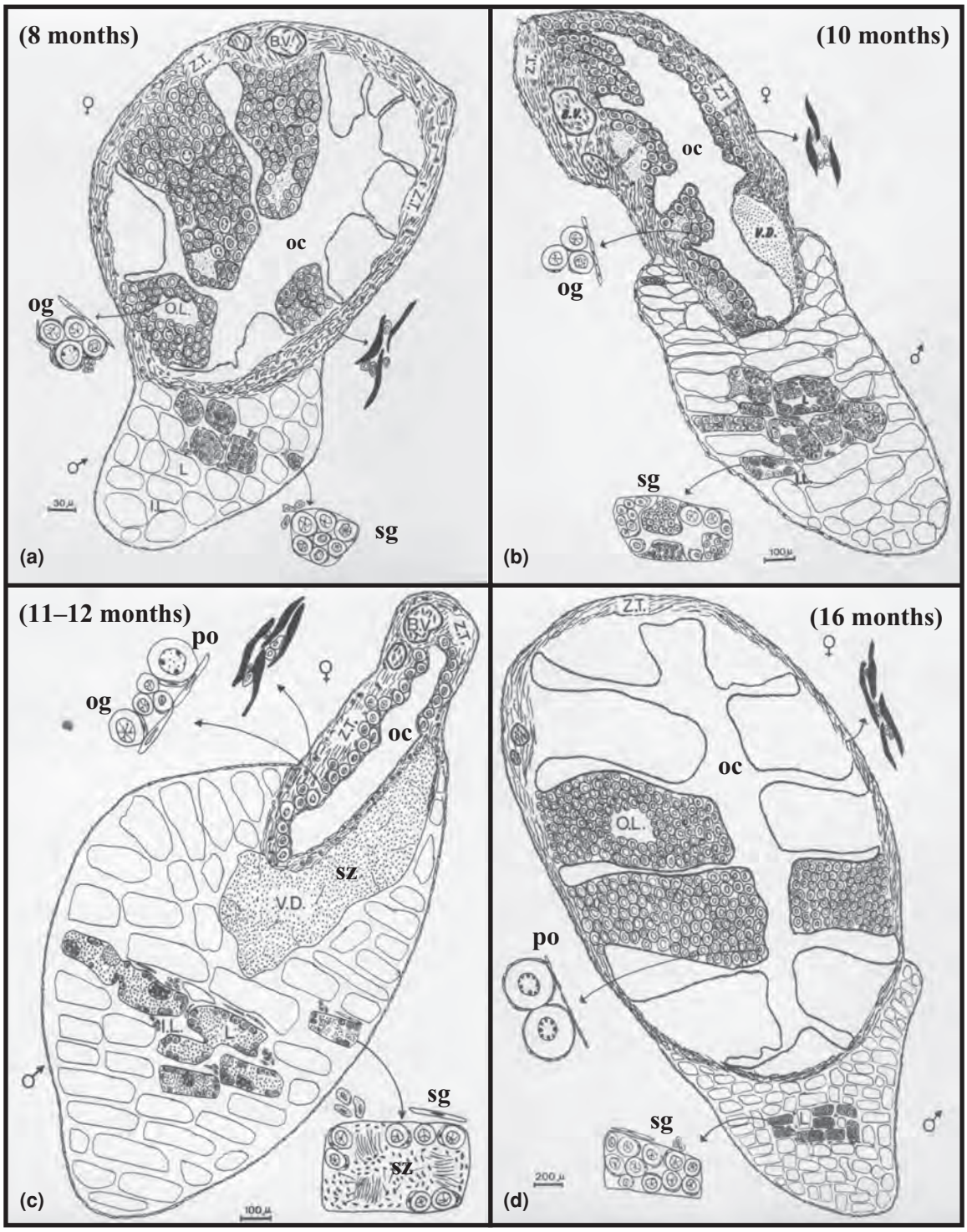

Figure 4.1 (*Continued*)

50% of the population (L_{50}) at 142 and 134 mm for males and females, respectively, in the eastern Mediterranean (Somarakis & Machias 2002). However, in the eastern Atlantic (Portugal) puberty may occur at a larger body size of 197 and 209 mm in males and females, respectively (Santos *et al.* 1995). The common pandora is a diandric species, with a percentage of the males developing directly after sex differentiation, without first maturing as females (Valdéz *et al.* 2004), a characteristic also of the red porgy (Kokokiris *et al.* 1999). In contrast, the congener blackspot sea bream (*Pagellus bogaraveo*) is a protandrous hermaphrodite with a high incidence of gonochoric females in captivity, and with a size at first maturity of 262–282 mm for males and 292–323 mm for females (Krug 1998; Micale *et al.* 2002; Chilari *et al.* 2006). Puberty also occurs at a later age in this species and has been reported to take place after the fourth year of age in both sexes (Micale *et al.* 2002). Similar to the common pandora, sex inversion in captivity does not take place in all individuals of the population, and some females develop directly from the undifferentiated juveniles (Micale *et al.* 2002).

As mentioned above, the red porgy is a protogynous diandric hermaphrodite (Vaughan *et al.* 1992; Pajuelo & Lorenzo 1996; Kokokiris *et al.* 1999; Fostier *et al.* 2000). Sex differentiation in this species begins at an age of 6 months, when all individuals develop a bisexual gonad with well-delineated heterosexual zones, but with the ovarian section dominating the gonad. Thus, in all individuals, the gonad develops first into an ovary, but not all individuals become functional (i.e., vitellogenic) females. A very small percentage of fish become functional females at an age of 3 years, but the majority of individuals mature at an age of 4 years (Fostier *et al.* 2000). Similarly, the first functional males appear at an age of 3 years, with the majority maturing at an age of 4 years. Males may be produced directly from immature females (primary males) after a progressive degeneration of the ovarian section of the gonad, and the development and maturation of the testicular part (Kokokiris *et al.* 1999, 2006; Fostier *et al.* 2000). This sex inversion may not be achieved within one reproductive season, and it may take 2–3 years to be completed. The second pathway of male production involves the degeneration of the ovarian section of functional females (secondary males), which may start with fish at an age of 3 years. Similar to primary males, the sex change from functional females may also require more than one reproductive season to be completed. During this sex inversion, the ovarian section does not undergo full vitellogenesis, whereas the testicular section may contain fully mature spermatozoa (Kokokiris *et al.* 1999). However, these spermatozoa cannot be released, since the vas deferens is not yet completed.

The sharpsnout sea bream (*Diplodus puntazzo*) has been shown to have rudimentary hermaphroditism with some protandry in the wild (Micale *et al.* 1996; Pajuelo *et al.* 2008). In captivity, gonadal differentiation commenced at 5 months of age (total length 60.1 mm, body weight 3.67 g) with the formation of the ovarian cavity (Papadaki *et al.*, in review). By the tenth month of age (length 128 mm, weight 41 g) bisexual gonads

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Figure 4.1 Schematic representation of the evolution of hermaphroditism in cultured gilthead sea bream (*Sparus aurata*) during the first reproductive cycle. The upper side of all figures is the dorsal one. (a) Gonad at 8 months of age having a dominant ovarian section with oogonia (og) on the dorsal side. The smaller section in the ventral side is organized as a testis with primary spermatogonia in the spermatogenic tissue (sg). (b) Gonad at 10 months of age with a decreasing ovarian section and a diminishing number of oogonia (og) on the dorsal side (upper). The ventral testicular section is now larger than the ovarian one and contains spermatogenic tissue at various stages of spermatogenesis. (c) Gonad at 11–12 months of age with a small, degenerated ovarian section (upper) with few oogonia and primary oocytes (po). The testis constitutes the major section (lower), containing spermatogenic tissue at the peak of spermatogenesis (spermiation). A large vas deferens (V.D.) lays between the ovarian and testicular section and is full of spermatozoa (sz). (d) Gonad after the end of the first reproductive period at 16 months of age, showing the enlargement of the ovarian section (upper) which contains dense ovigerous lamellae with primary oocytes. The regressed testicular section contains spermatogenic tissue with primary spermatogonia. B.V., blood vessel; I.L., inter-lobule space; L, Leydig cell; oc = ovarian cavity; O.L., ovigerous lamellae; Z.T., zona trabeculata.

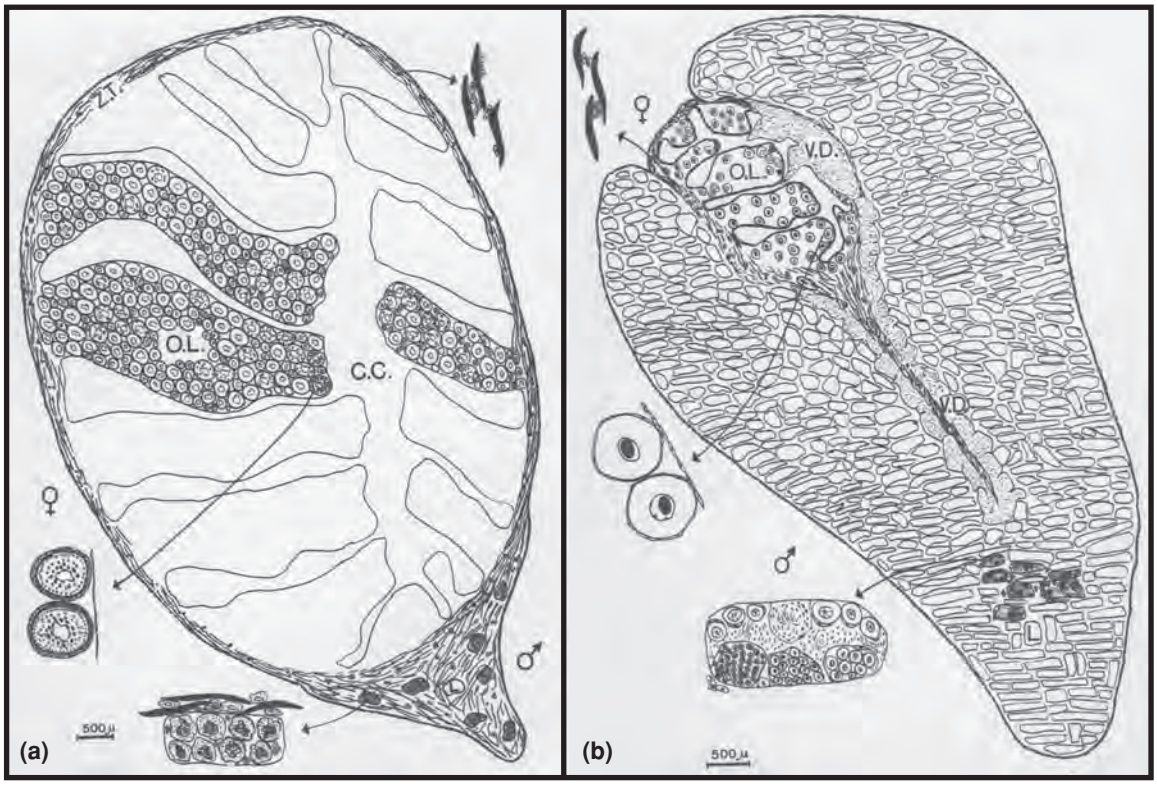


Figure 4.2 Schematic representation of the gonadal histology of gilthead sea bream during the second reproductive season (23–24 months old). The upper side of all figures is the dorsal one. (a) Gonad of a functional female with the ovary (upper) organized in dense ovigerous lamellae surrounding the ovarian cavity (oc), containing primary oocytes (po). A residual fully regressed testicular lobe is present in the ventral region (lower) containing some necrotic spermatogenic tissue (sg). (b) Gonad of a functional male with a dominant and mature testes containing spermatogenic tissue at the peak of spermatogenesis. The vas deferens (V.D.) contains spermatozoa (sz). A residual ovarian section is present in the dorsal region (upper) surrounded by the testes, containing primarily oocytes in various degenerative stages, as well as healthy oogonia. L, Leydig cell; oc, ovarian cavity; O.L., ovigerous lamellae; Z.T., zona trabeculata.

appeared, exhibiting both testicular tissue with the first visible spermatogonia and spermatocytes, and ovarian tissue with an ovarian cavity. Until achieving a length of 210 mm (at the end of the second year of age), gonads were always bisexual, belonging to three different categories: predominant testicular tissue with rudimentary ovarian tissue (Mf), substantial amounts of both tissues (mf) and predominant ovarian tissue with rudimentary testicular tissue (mF). In the beginning of the third year of age (length 227 mm, weight 240 g), the first males (M) and females (F) appeared, the percentage of the latter increased during the third year of age. At the same time, the number of fish with bisexual gonads decreased. Considering that Mf fish develop to males and mF fish to females, the sex ratio (F:M) of the studied populations during the first, second, and third years of life was 1:1. Similarly, in a study of another cultured stock (Micale *et al.* 1996) it was found that during the third year of life all examined fish contained gonads with both ovarian and testicular areas, but the sex ratio of the predominantly male (spermiating) and predominantly female fish (only spermatogonia in the testicular section) was near 1:1. Therefore, unlike the gilthead sea bream described earlier, the sex ratio of a captive sharpsnout

sea bream broodstock may not be changing significantly over the years, at least during the first 4 years of life in this species, although the gonads maintain always both ovarian and testicular sections in varying degrees of development or regression.

Similarly, the white sea bream has been described as a digynic hermaphrodite, which means that functional females can be produced directly from an immature, rudimentary hermaphroditic gonad or from sex inversion of functional males at a later age (Morato *et al.* 2003; Mouine *et al.* 2007), though other studies on cultured populations classified this species as protandrous hermaphrodite (Micale *et al.* 1987; Micale & Perdichizzi 1994). Puberty occurs at the second year of life at a mean total length of 16.7 cm (Micale *et al.* 1987; Morato *et al.* 2003), though late maturing populations at 4 years of age and mean total length of 21 cm have been described recently (Mouine *et al.* 2007).

The common dentex is one of the exceptions to the rule of hermaphroditism in the family of Sparidae, as it is a gonochoristic species (Abellan 2001; Loir *et al.* 2001; Rueda & Martínez 2001). Gonadal differentiation occurs between 5 and 12 months of age, at a size of 180–190 mm and a body weigh of 70 g, similar to other sparids, such as the gilthead sea bream (Zohar *et al.* 1978) and the red porgy (Kokokiris *et al.* 1999). Puberty begins on the second year of life, when all males are spermiating during the reproductive season, whereas 70% of females contain oocytes at the maturation stage (Loir *et al.* 2001). The L_{50} for reproductive maturation in the wild has been reported at 1,960 and 563 g for males and females, respectively, while the first mature males and females had a mean size of 692 and 443 g, respectively (see review by Rueda & Martínez 2001).

4.3 Reproductive cycles in Sparidae

The gametogenic process in fish is separated into two phases. In females, these are the periods of oocyte growth and maturation (Zohar 1989a, 1989b; Tyler & Sumpter 1996; Bobe *et al.* 2008; Lessman 2009). Gametogenesis begins with the primary oocytes (Figure 4.3a), which are arrested at prophase I of meiosis, at which stage they remain until oocyte maturation (OM), just prior to ovulation. The major event characterizing gametogenesis in the female is the sequestration of the yolk-precursor (vitellogenin, VTG) into the growing oocytes, hence the process is more commonly referred to as "vitellogenesis" (Polzonetti-Magni *et al.* 2004; Babin *et al.* 2007; Mommsen & Korsgaard 2008; Mylonas *et al.* 2009). Just prior to vitellogenesis, the primary oocytes go through a hormone-independent primary growth phase, which involves the appearance of pale material in the cytoplasm, and the appearance of the follicular layers of granulosa and theca cells. The secondary growth phase is characterized by the synthesis of VTG in the liver and its sequestration into the growing oocyte (Figure 4.3b), resulting in a tenfold increase in size. At the end of vitellogenesis, the postvitellogenic oocyte is characterized by a large opaque cytoplasm completely filled with yolk granules and lipid globules, a centrally located nucleus (or germinal vesicle, gv), and a thick, clearly striated zona radiata—the future chorion of the egg—surrounded by the granulosa and theca layers (Figure 4.3c). After vitellogenesis, the oocytes undergo OM (Nagahama *et al.* 1994), a process that includes migration of the gv to the area below the micropyle—where a single spermatozoon will enter during fertilization—the coalescence of the lipid globules and later of the yolk granules, the dissolution of the nuclear wall and the resumption of meiosis that is completed after fertilization (Kinsey *et al.* 2007). During OM, an extensive enzymatic degradation of the lipid and yolk components modifies the chemical composition and osmotic pressure of the cytoplasm and causes drastic morphological changes in the oocyte together with a dramatic increase in volume due to water uptake (Cerdá *et al.* 2007). This tremendous hydration is especially relevant in marine fish with pelagic eggs (such as the Sparidae) and causes a rapid two- and threefold increase in oocyte volume. After hydration, the follicular wall ruptures and the oocyte is ovulated into the ovarian cavity and released to the water during spawning.

In terms of ovarian development, female fish may be separated into two classifications (Zohar 1989a, 1989b; Mylonas *et al.* 2009): fish spawning only once during the reproductive season (synchronous and single-batch

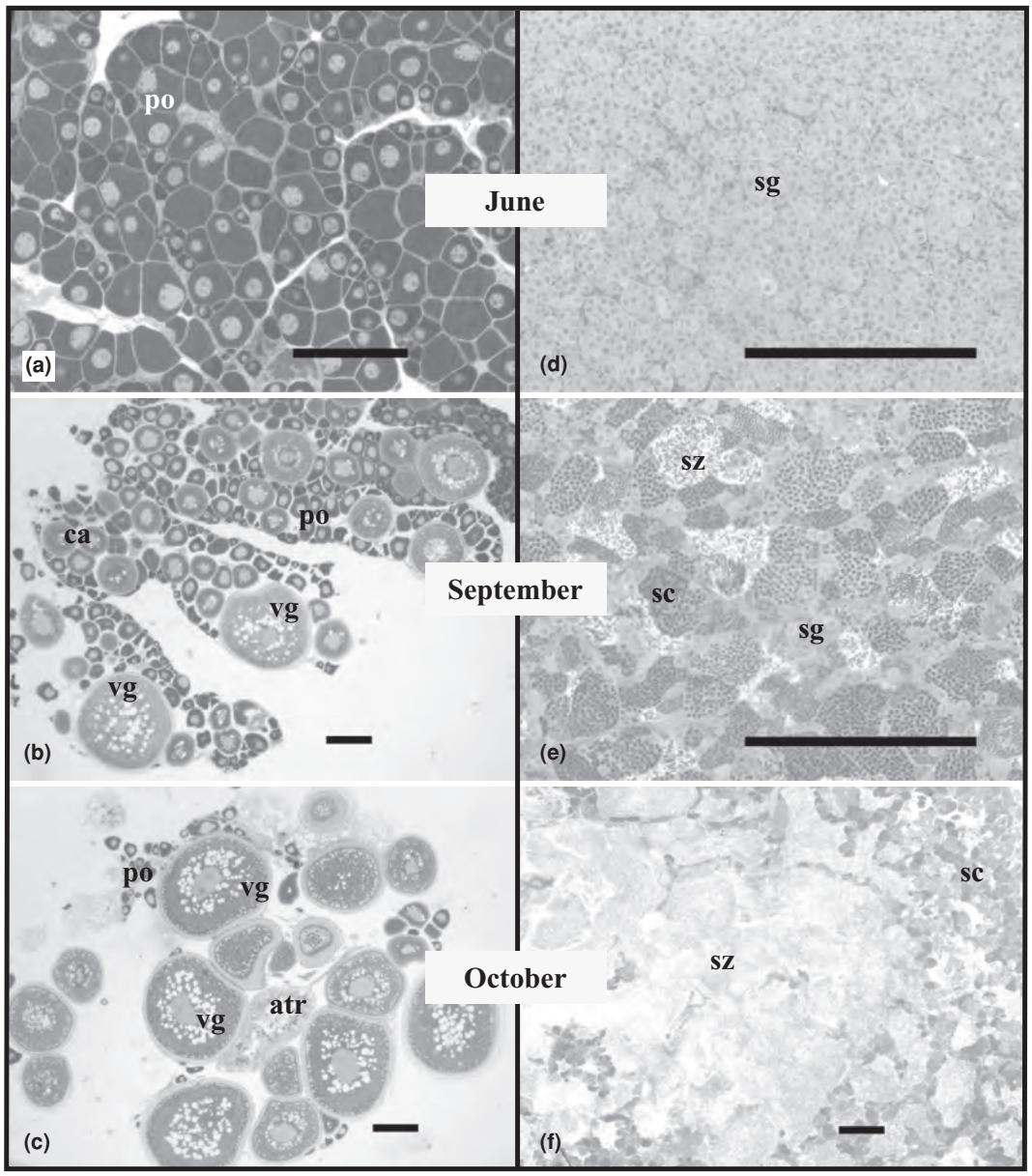


Figure 4.3 Histological description of oogenesis and spermatogenesis in a representative Sparidae, the sharpsnout sea bream (*Diplodus puntazzo*). During the quiescent period in June, the ovary (a) contains only primary oocytes (po) and the testes (d) are occupied exclusively by spermatogonia (sg). A month before the spawning season in September, the ovary (b) contains oocytes at vitellogenesis (vg) and the cortical alveoli stage (ca), along with primary oocytes. At the same time the testes (e) contain spermatocysts (sc) at all stages of development, including some pockets with spermatozoa (sz). During the spawning season in October, the ovary (c) contains mostly vitellogenic oocytes of various sizes (i.e., different stages of vitellogenesis), as well as some vitellogenic oocytes undergoing atresia (atr). The testes (f) are occupied by a large volume of spermatozoa in the central region, whereas the cortical area still contains spermatocysts at various stages of development. Bar at the bottom is 200 μm.

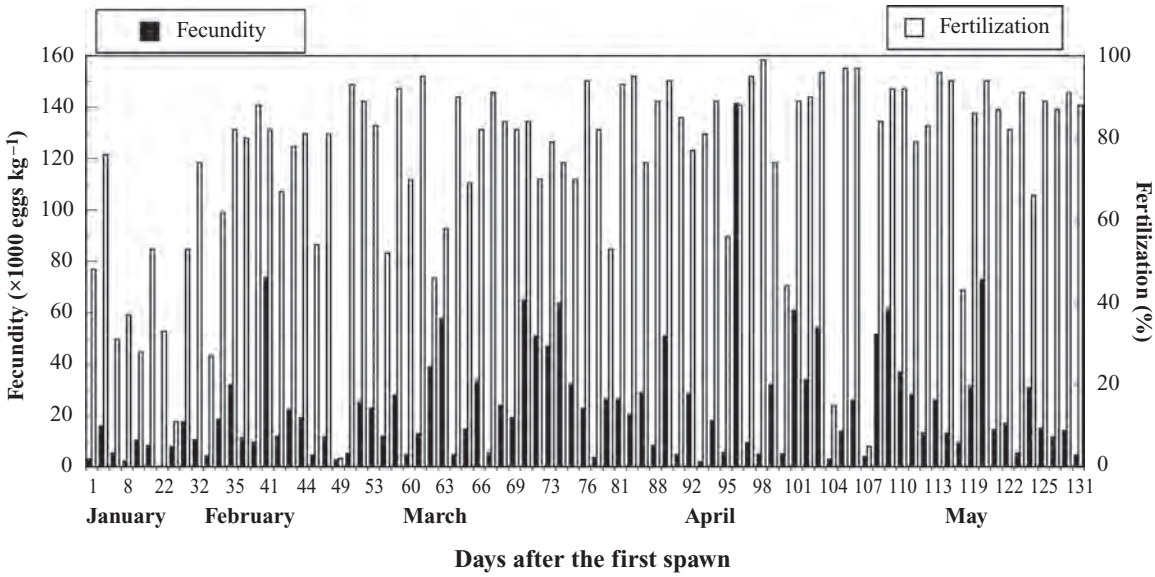


Figure 4.4 Daily egg production and fertilization success of a white sea bream (*Diplodus sargus*) broodstock at the Hellenic Center for Marine Research, Crete, Greece, during the 2005 reproductive season. The female biomass was estimated to be 14 kg.

group-synchronous), which are referred to as single spawners, and those that spawn multiple times (multiple-batch group-synchronous and asynchronous), which are called multiple spawners (Tyler & Sumpter 1996). To our knowledge, the vast majority of Sparidae are multiple spawning fishes, most of them of the asynchronous type of ovarian development (Wallace & Selman 1981). This means that spawning is frequent and regular, for example, daily or every other day for a period of 3–5 months (Figure 4.4) (Zohar & Gordin 1979; Zohar *et al.* 1995a; Mylonas *et al.* 2004; in review 2008). Therefore, the processes of oogenesis and OM are not temporally well-separated as in fishes that spawn once during the reproductive season, and during the spawning season the ovary contains oocytes at all stages of development.

Spermatogenesis is the first phase of the male reproductive cycle, and it includes spermatogonia proliferation (Figure 4.3d), mitotic multiplication of spermatocytes I, meiotic division and production of spermatocytes II, their differentiation to spermatids and the production of flagellated spermatozoa (Figure 4.3e) (reviewed by Billard 1986; Schulz & Miura 2002; Vizziano *et al.* 2008). The second phase, referred to as spermiation, includes spermatozoa release in the sperm ducts (Figure 4.3f). Sperm (also called milt) may be obtained from the fish using abdominal pressure during the period of spermiation (Alavi *et al.* 2008), which often encompasses the spawning season of females by a few months. Spermiation and ejaculation may be synchronized with female spawning through pheromonal communications (Stacey & Sorensen 2008). Spermatogenesis and spermiation may be temporally separated and during the spawning season the testes may contain exclusively spermatozoa (Billard 1986; Malison *et al.* 1994). However, in most species both spermatogenesis and spermiation take place during the spawning season (Jackson & Sullivan 1995; Mylonas *et al.* 2003; Rainis *et al.* 2003). Especially in Sparidae that have a prolonged spawning season, it has been shown that the proliferation activity of both spermatogonia and Sertoli cells is not blocked by the presence of spermatozoa (Chaves-Pozo *et al.* 2005).

Gametogenesis (spermatogenesis and vitellogenesis) and maturation (spermiation and OM) are regulated by a cascade of hormones along the brain-pituitary-gonad (BPG) axis (Yaron *et al.* 2003; Pankhurst 2008; Planas & Swanson 2008). The brain provides stimulatory inputs to the pituitary in the form of the GnRHs (Zohar

1989b; Peter & Yu 1997; Yu *et al.* 1997; Alok & Zohar 2005), which are the primary neuropeptides regulating reproduction, acting as integrators of external information (e.g., environment, temperature, waterfall, and social interactions). Dopamine (DA) in some fishes exerts a negative effect on the functions of GnRH on the pituitary gonadotrophs (Chang *et al.* 2009), but this inhibition is not very important in Sparidae (Zohar 1989b). In response to these positive and negative inputs, the pituitary is secreting the gonadotropins FSH and luteinizing hormone (LH) (Rosenfeld *et al.* 2007; Planas & Swanson 2008). The FSH and LH are released into the bloodstream and stimulate the synthesis by the gonads of the sex steroid hormones (androgens, estrogens, and progestogens), which are the ultimate effectors of gonadal development (Nagahama 1994; Pankhurst 2008).

4.3.1 Endocrine control of oogenesis and oocyte maturation

As indicated earlier, the processes of oogenesis and OM are not well-separated temporally in Sparidae, since they have an asynchronous ovarian development and during the spawning season the ovary contains oocytes at all stages of development. As a result, the definition of the roles of the various reproductive hormones described above, on the process of gametogenesis, and especially on oogenesis, is rather difficult. This is because, in most cases, all hormones may be present at any point in time, each supporting a different process (Meiri *et al.* 2004). As an example of the Sparidae, the following section describes the current knowledge on the reproductive endocrinology of the gilthead sea bream, one of the better-studied fishes in this family.

The gilthead sea bream is a daily spawner with an asynchronous ovary and its spawning season may last for 5 months (Zohar & Gordin 1979; Zohar *et al.* 1995a). Driven by the quest to develop broodstock management and spawning technologies for the rapidly developing aquaculture industry in the Mediterranean region, studies on its reproductive endocrinology started rather early and focused on the regulation of OM, ovulation and spawning. The development of a homologous radioimmunoassay for gilthead sea bream gonadotropin (Zohar *et al.* 1990b) and the later cloning of its LH and FSH subunits (Elizur *et al*. 1996, 2000) paved the way to studying the dynamics of the gonadotropins and other reproductive hormones during gametogenesis. The daily pattern of OM, ovulation, and spawning was shown to be accompanied by diurnal cycles of LH protein levels, FSH and LH transcripts, and levels of 17β-estradiol (E2), but not testosterone (T) and the putative maturation-inducing hormones (MIHs) 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) and 17,20β,21-trihydroxy-4-pregnen-3-one (17,20β,21P) (Zohar *et al.* 1988; Canario *et al.* 1995; Gothilf *et al.* 1997). The characterization of the novel GnRH in the gilthead sea bream (sbGnRH; Powell *et al.* 1994) and the cloning of the three forms of GnRH in this species (Gothilf *et al.* 1995, 1996; Chow *et al.* 1998) enabled the demonstration that the daily rhythms in the hormonal and spawning activities are driven by diurnal cycles of the three GnRHs and their messages (Gothilf *et al.* 1997). Interestingly, when males were removed from a group of daily spawning females, the vitellogenic oocytes quickly entered apoptosis and brain mRNA levels for all three GnRHs, pituitary LH mRNA levels, and circulating levels of LH, E2, T, 17,20βP and 17,20β,21P declined significantly (Meiri *et al.* 2002), indicating the existence of an endocrine response to sociosexual stimuli during the reproductive process.

As individual female gilthead sea bream spawn daily for periods of up to 5 months (Zohar & Gordin 1979; Zohar *et al.* 1984, 1995a), vitellogenesis in younger clusters of oocytes continues at the same time as the more advanced ones undergo OM. This scenario presumably requires finely tuned and well-coordinated hormonal secretion patterns, especially of sex steroid hormones. Based on the correlation of the circadian pattern of hormonal levels with that of follicular developmental processes, it was suggested that in the female gilthead sea bream, OM and ovulation are induced by a preovulatory surge of sbGnRH secretion that drives surges of LH, 17,20βP, and 17,20β,21P release (Gothilf *et al.* 1997). A daily surge in E2 levels, peaking as levels of 17,20βP are at their minimum, was suggested to be responsible for stimulating vitellogenesis in earlier developing follicles.

Studying the ontogeny of hormonal patterns throughout the entire reproductive cycle in the gilthead sea bream demonstrated the very early establishment of the hypothalamus-pituitary-gonadal axis. Transcripts of the three GnRHs, GnRH receptor, FSHβ, FSH and LH receptor and VASA were expressed and fluctuated

significantly as early as 1–36 days postfertilization, well before the differentiation of the gonads (Wong *et al.* 2004). The functional significance of these early hormonal activities is not yet fully understood. During active gametogenesis, pituitary, and plasma LH, and both FSHβ and LHβ transcripts increased clearly as both males and females reached advanced stages of gametogenesis and entered the spawning season (Holland *et al.* 1998; Meiri *et al.* 2004). This increase in pituitary and plasma LH levels was accompanied by a significant rise in pituitary levels of sbGnRH, but not the other GnRH forms present in the gilthead sea bream (Holland *et al.* 1998). These data, together with the brain distribution of the three GnRH forms in the gilthead sea bream brain (Gothilf *et al.* 1996), indicate strongly that sbGnRH is the hypophysiotrophic GnRH form in gilthead sea bream, and the most relevant to the control of reproduction in this species.

Of special interest is the finding in the gilthead sea bream, for the first time in any vertebrate species, that FSH and LH are expressed also in both the ovaries and testes, at the mRNA and protein levels (Wong & Zohar 2004b). Taken together with previous evidence for the expression of GnRHs and their receptors in the gilthead sea bream gonads (Nabissi 1997), this finding indicates that in this species, and possibly other vertebrates, the gonads contain the equivalent of the entire hypothalamus-pituitary-gonadal axis. It is hypothesized that such redundancy may provide a gonadal mechanism to fine-tune hormonal inputs important to gametogenesis and reproduction.

In another very well-studied Sparidae, the red sea bream, the physiologically important form of GnRH is also sbGnRH, one of the three GnRHs synthesized in its brain (Okuzawa *et al.* 1993, 1994, 1997, 2002, 2003a; Okuzawa & Kobayashi 1999; Senthilkumaran *et al.* 1999; Okuzawa, 2002). The mRNA levels of sbGnRH in the brain (Okuzawa *et al.* 2003b) and its contents in both brain and pituitary (Senthilkumaran *et al.* 1999) increase during the reproductive season with the increase in gonadosomatic index (GSI, the weight of the gonad divided by the body weight). The expression of gonadotropin subunit genes shows unique changes during the reproductive cycle (Gen *et al.* 2000; Gen *et al.* 2003). The FSHß subunit mRNA levels in the female pituitary are maintained at low levels during sexual maturation, whereas LHß mRNA levels are maintained at high levels from the start of gametogenesis up to the spawning season (Gen *et al.* 2000). Moreover, serum LH levels increase with gonadal development and in the mature fish after induction by GnRH injection (Tanaka *et al.* 1993). These patterns of change observed in the red sea bream are different from those described in salmonids (Planas & Swanson 2008) and suggest that LH has taken some of the actions of FSH in female red sea bream reproduction. This hypothesis is supported by *in vitro* experiments showing that LH, but not FSH, stimulates E2 production in vitellogenic follicles (Tanaka *et al.* 1995) through increased levels of P450 aromatase mRNA (Gen *et al.* 2001), as well as stimulating OM (Kagawa *et al.* 1998). Serum E2 and T levels increase during vitellogenesis and remain high during the spawning season (Okuzawa *et al.* 2003b). Studies on the steroidogenic pathway for E2 biosynthesis in the ovarian follicles showed that estrone, not T, is the major precursor of E2 in the red sea bream (Ohta *et al.* 2002b). During vitellogenesis, three different vitellogenin (VTG) genes were expressed and the synthesis of their product yolk proteins was first demonstrated in the red sea bream (Sawaguchi *et al.* 2006). After completion of vitellogenesis, the ovarian follicle produce the MIH and the oocytes acquire the ability to respond to it (i.e., oocyte maturational competence) (Kagawa *et al.* 1994b; Patiño *et al.* 2001). Plasma LH from the pituitary and insulin-like growth factor I (IGF-I) being synthesized in the ovarian follicles (Kagawa *et al.* 1994a; Patino & Kagawa 1999) are involved in the induction of maturational competence (Kagawa *et al.* 1995, 1998, 1999). As in the gilthead sea bream, the MIH of the red sea bream is thought to be either 17,20βP or 17,20β,21P (Adachi *et al.* 1988; Matsuyama *et al.* 1988a; Ohta *et al.* 2002a) and its levels during the spawning season are high and show diurnal changes in association with the diurnal rhythm of OM (Kagawa *et al.* 1991). High water temperature affects negatively all hormone levels in the brain-pituitary-gonad axis, and this becomes the terminal factor of the spawning season of this species (Okuzawa *et al.* 2003a).

Plasma steroids in female Australasian snapper follow a similar pattern to that described for red sea bream. Plasma levels of both T and E2 rise through recrudescence to peak in fish undergoing daily cycles of OM and ovulation. In contrast, substantial changes in plasma levels of 17,20βP were not evident during the spawning

season (Carragher & Pankhurst 1993). More closely spaced (4 hourly) sampling of wild fish during the peak spawning period showed that there were some daily changes in plasma levels of T and E2, but more marked increases in ovarian levels of T and E2 were observed toward the end of the day in association with the recruitment of the next vitellogenic clutch. Plasma 17,20βP peaked in the early part of the day in association with OM of the leading oocyte clutch (Hobby & Pankhurst 1997). In terms of the MIHs, in Australasian snapper 17,20βP was more effective than 17,20β,21P at inducing OM *in vitro*, and the response was further augmented by cotreatment with human chorionic gonadotropin (hCG) (Ventling & Pankhurst 1995). This is consistent with the maturational competence promoting effects of LH described for red sea bream (Kagawa *et al.* 1998).

4.3.2 Endocrine control of spermatogenesis and spermiation

The endocrine regulation of spermatogenesis and spermiation is not as well studied in Sparidae. In red sea bream, some fragmentary information exists on the morphological appearance of the testis. Just after the spawning season, spermatogonia are the only germ cells present in the testes in June and males have a GSI of <0.3% (Gen *et al.* 2000). In November, small numbers of spermatocytes and spermatids begin to appear (Matsuyama *et al.* 1993) and some spermatozoa are first found in December (Yamaguchi *et al.* 2005). Testes sampled in February show more advanced stages of gonadal development when GSI is >1% and germ cells at all stages of maturation are observed. The GSI continues to increase in March and reaches a maximum of >7% in the spawning season in April, at which time the testes are occupied predominantly by spermatozoa (Gen *et al.* 2000). As compared to the changes in the female red sea bream, FSHβ subunit mRNA levels in males increase with testicular development, while the LHβ subunit mRNA levels are maintained at high levels through the reproductive cycle, from the beginning of gametogenesis to the spawning season, and decrease rapidly to low levels after the spawning season (Gen *et al.* 2000, 2003). Moreover, both FSH and LH were equipotent in stimulating *in vitro* production of 11-ketotestosterone (KT) by testis slices (Kagawa *et al.* 1998). These findings suggest that both gonadotropins, but especially LH, are physiologically important for regulating all stages of spermatogenesis in the red sea bream, contrary to what is observed in salmonids, where only FSH is thought to be important in early spermatogenesis (Planas & Swanson 2008). The GnRH may downregulate the expression of FSHβ mRNA and upregulate LHβ mRNA through the production of KT in the testis of the immature male red sea bream (Yamaguchi *et al.* 2003, 2005, 2006).

Reproductive cycles have been described for male Australasian snapper with males showing a rapid increase in GSI in austral spring in concert with females, in association with a shift from dominance of the testis by spermatocytes to spermatozoa (Scott & Pankhurst 1992). This was associated with seasonal increases in plasma levels of T and KT, and, in contrast to females, with increase in 17,20βP. Males also showed a daily cycle of sex steroid production with elevated plasma T and KT levels before noon, but no detectable diel changes in 17,20βP (Carragher & Pankhurst 1993). Finally, in the gilthead sea bream both FSH and LH were expressed during the period of spermatogenesis, but the season profiles were clearly different from the females (Meiri *et al.* 2004). Expression of FSHβ was always higher in males compared to females, whereas expression of LHβ was lower in males at the onset of the reproductive season and higher at the end of the reproductive season, when the testes begin to be absorbed (Meiri *et al.* 2004).

In the red porgy, only T and KT correlated with spermatogenesis and spermiation, with maximal levels observed when fish were in full spermiation (Kokokiris *et al.* 2000). Plasma KT levels were slightly lower than of T throughout the reproductive cycle. Plasma 17,20βP did not fluctuate during the reproductive cycle and remained below 1 ng mL^{-1}.

4.3.3 Annual spawning season

As mentioned earlier, most Sparidae have an asynchronous ovarian development and spawn with a daily cyclicity for periods up to 5 months, usually in the winter–spring (Table 4.1). According to a recent meta-analysis article

Table 4.1 Spawning characteristics of various cultured Sparidae

Common name	Scientific name	Spawning season (month to month)	Spawning period (days)	Total fecundity mean annual ($\times 10^6$ eggs kg^{-1})	Fertilization mean annual (%)	References
Black porgy	*Acanthopagrus schlegelii*	Feb–Apr	30	0.18–0.48	77	(Leu 1997; Gonzalez *et al.* 2008)
Yellowfin porgy	*Acanthopagrus latus*	Nov–Dec	n/a[a]	0.43–1.01	72–84	(Leu & Chou 1996)
Sobaity	*Acanthopagrus cuviery*	Feb–Mar	40	0.54[b]	49	(Hussain *et al.* 1981)
Common dentex	*Dentex dentex*	Apr–June Mar–June	90 71–99	0.76–1.50 0.60–0.72[c]	50–70 34–80[c]	(Abellan 2001) (Loir *et al.* 2001)
Sharpsnout sea bream	*Diplodus puntazzo*	Oct–Dec	120	4.9	70–80	(in review 2008)
White sea bream	*Diplodus sargus*	Jan–May May–Nov[d] Mar–Jun	140 122	n/a n/a	75 n/a	(C.C. Mylonas, unpublished data) (Mann & Buxton 1998) (Morato *et al.* 2003)
Blackspot sea bream	*Pagellus bogaraveo*	Feb–May	110	0.69	30–60	(Peleteiro *et al.* 2001)
Common pandora	*Pagellus erythrinus*	Apr–Aug	100–130	2.50	85–90	(C.C. Mylonas, unpublished data)
		June–July	65	3.21	97	(Güner *et al.* 2004)
Red sea bream	*Pagrus major*	Apr–June	60–90	2.16	60–90	(Foscarini 1988; Matsuura *et al.* 1988; Watanabe & Kiron 1995)
Red porgy	*Pagrus pagrus*	Mar–May Feb–June Oct–Feb[d]	90 114 120	0.44 1.08 0.33–0.96	36–68 86 53	(Mylonas *et al.* 2004) (Mihelakakis *et al.* 2001) (Aristizabal *et al.* 2009)
Gilthead sea bream	*Sparus aurata*	Dec–Apr	100–150	2.00	80–85	(Zohar *et al.* 1995a; Barbaro *et al.* 1997; C.C. Mylonas, unpublished data)

[a]Not available.
[b]Estimated from the reported standard length of the fish (31 cm).
[c]This value is the product of the reported fertilization and viability percentage in the manuscript, the latter not being explained by the authors.
[d]In the Southern Hemisphere, the spawning season is at similar temperatures as in the Northern Hemisphere.

(Sheaves 2006), sparid fishes of lower latitudes (e.g., ≤30°N or S) spawn close to the month of the lowest annual temperature (both before and after), whereas at higher latitudes spawning time is more variable in regard to the ambient temperature. It appears that in species that live in areas with mean annual sea surface temperature (SST) >21°C, the spawning season is within 2 months of the minimum annual temperature, whereas in areas with mean annual SST ≤20°C spawning may take place at any time of the year (Sheaves 2006). This observation holds true for species in both the Northern and Southern Hemispheres. A similar, though less powerful, trend was found for the relation between spawning time and the time of minimum photoperiod, though in this case the deviation was almost always positive, that is, the spawning season was after the month of minimum photoperiod. In some species, such as the Australasian snapper, it has been shown that an interannual variation in spawning time existed and was closely related to ambient water temperatures, in that spawning always started at 15°C and finished at 20°C (Scott & Pankhurst 1992).

In the gilthead sea bream, gametogenesis begins in November and the spawning season starts soon after the shortest day of the year and lasts for 3–5 months (Zohar & Gordin 1979; Zohar *et al.* 1995a). Spawning time is consistent usually within a broodstock, and it occurs in the late afternoon (~1600 hours) (Meseguer *et al.* 2008), although populations spawning early in the morning (~0600 hours) also exist (C.C. Mylonas, unpublished data). Furthermore, it is also possible that a single broodstock tank may contain both an afternoon and a morning spawning population. Studies using microsatellite genetic analysis have demonstrated recently that in a given broodstock in captivity, only a small percentage (27–33%) of the fish contributed to the progeny of each day's spawn, with male broodstock having a smaller parental representation than females (Brown *et al.* 2005). Similarly, in another study, out of a total of 66 individuals in a broodstock only 17 females and 11 males participated in a single day's spawning (Navarro *et al.* 2009). The above data indicate that although the gilthead sea bream has an asynchronous ovarian development and has been reported to spawn daily, not all individuals may be undergoing daily spawning during the spawning season. More research is necessary to describe not only the spawning season duration of a captive broodstock, but of individual females as well.

The spawning season of the red sea bream in nature, as well as under culture conditions, lasts for 70–90 days from April to early June, at water temperatures between 12 and 20°C (Foscarini 1988; Watanabe & Kiron 1995). In warmer waters, however, the season starts earlier with relatively high water temperatures (15–22°C) (Kato *et al.* 1985). Vitellogenesis starts in February and mean GSI in females increases gradually attaining maximum values during the spawning season (Gen *et al.* 2003; Okuzawa *et al.* 2003b). The red sea bream has an asynchronous ovary containing oocytes at various developmental stages during the spawning season and shows a diurnal ovarian maturation rhythm, in which the most advanced oocytes in the ovary complete gv breakdown at 0700 hours, attain maturation at 1000 hours, and begin ovulation at 1300 hours. Spawning occurs between 1800 and 1900 hours (Matsuyama *et al.* 1988a). Under natural photothermal conditions, red sea bream spawns approximately 1,900,000 eggs (4-year-old fish, 880 g in body weight) during the spawning season (Matsuura *et al.* 1988).

The Australia snapper mirrors the above pattern of spawning with an extended spawning period of 3–5 months in austral spring and early summer. Gonadal recrudescence occurs consistently in October but the actual onset of spawning varies annually by as many as 3 weeks and is associated with the attainment of water temperatures of 15–16°C. The spawning season is truncated by the development of SST of over 20°C (Scott & Pankhurst 1992). Within this, there is daily spawning by most of the population with the leading clutch of ovarian follicles undergoing OM during the morning, and ovulation occurring in early afternoon (Scott *et al.* 1993). Spawning in the wild has not been observed, but in captive stocks it occurs in early evening (see Section 4, this chapter).

The reproductive cycle of the red porgy in the Mediterranean region begins in the winter (December) with the first significant increase in GSI, and the appearance of oocytes accumulating VTG in the ovary and spermatozoa in the testes (Kokokiris *et al.* 2001). The spawning season is between March and May at ambient water temperatures of 15–19°C. In the Southern Hemisphere, the spawning season extends between October and February, at water temperatures of 15–18°C (Aristizabal *et al.* 2009). The mean maximum GSI during the spawning season was

~3% for females and 2.4% for males in captivity (Kokokiris *et al.* 2001), whereas in a wild stock in the Canary Islands, Spain, mean maximum values were 2.5 and 0.8% for females and males, respectively (Pajuelo & Lorenzo 1996). In another report, the spawning season of some stocks in captivity appears to occur earlier and last longer (January–June) at water temperatures of 12.2–18.5°C, though peak spawning was also between April and May (Mihelakakis *et al.* 2001). Egg production was daily, but it could not be ascertained how many of the 49 females spawned every day. Maximum daily fecundity was 20,000 eggs kg^{-1}, assuming that all females spawned. Wild red porgy from the eastern coast of North America spawn in the months of March to April, at water temperatures of 16–22°C and depths between 21 and 100 m (Manooch 1976). The red porgy is among the least fecund species of the Sparidae, producing only 0.44×10^6 eggs kg^{-1} female body weight per season in captivity (Mylonas *et al.* 2004), while in the wild it was estimated using histological methods that the fish may spawn 0.27×10^6 eggs kg^{-1} (Manooch 1976). Another study from a captive stock in Greece reported fecundities of 1.08×10^6 eggs kg^{-1} (Mihelakakis *et al.* 2001).

The common dentex has also an asynchronous ovarian development with continuous vitellogenesis and may spawn between 4 and 18 different clutches of eggs between April and June, with an estimated annual spawning interval of 1.4–2.0 days (Loir *et al.* 2001). However, the latter data were obtained from tank spawning of a group of fish, not individual females, and other researchers suggested that the common dentex spawns daily (Abellan 2001). In fact, in the study of Loir *et al.* (2001) during the peak of the spawning season eggs were collected almost daily, pointing to the possibility that all females were participating in daily spawning, at least for some part of the spawning season. In Crete (southern Greece) vitellogenesis and spermatogenesis begin in February, and by the end of March all males are spermiating and some females undergo OM (Loir *et al.* 2001). Maximum GSI can be 6 and 5% for males and females, respectively, and spawning takes place at a temperature range of 16–22°C. Although the GSI is relatively high compared to other sparids, annual fecundity in common dentex is relatively small at $0.70–1.5 \times 10^6$ eggs kg^{-1} (Table 4.1).

The white sea bream reproduces readily in captivity for a period of 3–4 months (Figure 4.4) between January and June in the Northern Hemisphere at water temperatures of 13–18°C (Micale *et al.* 1987; Micale & Perdichizzi 1994; Morato *et al.* 2003; Mouine *et al.* 2007) and between August and March in the Southern Hemisphere at water temperatures of 17–20°C (Mann & Buxton 1998). Published data on the annual fecundity of this species in captivity do not exist to our knowledge, but data from commercial hatcheries indicate that this species may have an annual production ranging from 600,000 eggs kg^{-1} (P. Pavlidou, SELONDA S. A., Greece, personal communication) to almost 2 million eggs kg^{-1} (Figure 4.4). Fertilization success is usually between 40 and 96%, with annual averages between 70 and 75%.

The sharpsnout sea bream is the only Sparidae species cultured in the Mediterranean that spawns in the fall (Georgiou & Stephanou 1995; Papadaki *et al.*, 2008). Spawning starts in September and finishes in December at water temperatures between 21 and 19°C. This is the Sparidae member with the highest reported annual fecundity, which can reach a mean of 4.9 million eggs kg^{-1} (Papadaki *et al.*, 2008). Reproduction of the sharpsnout sea bream in captivity has been and still is problematic (Faranda *et al.* 1985; Georgiou & Stephanou 1995; Bodington 2000). Fish do not reproduce readily in captivity and even after successful hormonal induction of maturation, ovulation is not always followed with spawning, resulting in the latter release of overripe eggs or the death of the female due to "bloating" of the ovarian cavity from the daily ovulation and hydration of eggs (Faranda *et al.* 1985). Originally it was considered that this fish has very strict temperature requirements for spawning (Georgiou & Stephanou 1995; Bodington 2000), but this was later shown not to be the case (Papadaki *et al.*, 2008). What seems to be certain, however, is that hatchery-produced broodstocks (F1 generation) do not spawn in captivity, even though males are in spermiating condition and females undergo vitellogenesis, OM and ovulation readily (C.C. Mylonas, unpublished data).

The spawning season for the common pandora is between April and August at temperatures of 19–24°C (Pajuelo & Lorenzo 1998; Somarakis & Machias 2002; Güner *et al.* 2004; Valdéz *et al.* 2004). Females spawn daily with a high fecundity in captivity of 3.2×10^6 eggs kg^{-1} body weight (Table 4.1). The mean GSI of this

species during the spawning season was 3.9% for females and 2.0% for males, which ranks in the middle of the scale for other Mediterranean sparids (Valdéz *et al.* 2004). An egg size of 700–800 mm in diameter (Güner *et al.* 2004; Klaoudatos *et al.* 2004), however, is smaller in common pandora than in other sparids (Valdéz *et al.* 2004)—which are more in the 850–1000 μm range (Rueda & Martínez 2001)—perhaps explaining the higher fecundity of the species.

Another *Pagellus* spp. with interest for the aquaculture industry in the Mediterranean Sea and Atlantic coast of southern Europe is the blackspot sea bream. Contrary to its congener the common pandora, this species has been reported to spawn at widely different times of the year, depending on geographic latitude and longitude (see Peleteiro *et al.* 2001). Some studies report a winter spawning season between November and May (Orsi Relini & Fida 1992), whereas others a March–April spawning season (Chilari *et al.* 2006). In captivity, the spawning season for this species has been described to be March–April in Sicily, Italy (Micale *et al.* 2002), and February–May in the north-western Atlantic coast of Spain (Peleteiro *et al.* 2001). In captivity, females have been reported to spawn every other day, with a fecundity of 18,000 eggs kg^{-1} body weight (Peleteiro *et al.* 2001).

4.4 Reproductive behavior and spawning in Sparidae

Available data indicate that under natural conditions, sparids are mostly pelagic spawners, with spawning occurring in group-spawning aggregations in mid-water, away from reef areas. However, there is very little observational data on spawning behavior in the wild and the majority of descriptions come from observations made in large public aquaria or aquaculture systems. Natural spawning can occur in volumes as low as 1 m^3 (e.g., Haddy & Pankhurst 2000a), but generally larger volumes are required to initiate spawning in large pelagic spawners, including some sparids (Pankhurst 1998). Behavior in these larger volume situations is also more likely to replicate the behavior occurring in the natural environment.

Observations of spawning in captive or cultured populations of various Sparidae show a high degree of commonality. Spawning of Australasian snapper was described first by Smith (1986) from observations made on acclimated wild fish in a public aquarium. In a typical event, spawning males characterized by a marked gray color on the head, dorsal surface and abdomen, pursued a single female around the tank margin until she dropped to the tank floor and then made a rapid "spawning rush" to the surface followed closely by the attendant males. Egg and sperm release occurred at the apex of the rush, at or near the water surface. Later observations of the same species in a larger public aquarium (Kelly Tarlton's Aquarium, Auckland, New Zealand) (N.W. Pankhurst, unpublished observations) showed the same basic pattern, but with females first initiating a marked increase in swimming speed, often with tight circling near the tank floor, again with 3–6 attendant males following closely. After 30–60 seconds of the circling behavior there was the spawning rush and eggs and sperm were released at the surface as described by Smith (1986). Spawning rushes involving more than one female were not seen in either set of observations; however, Smith (1986) did report one spawning event involving only a single male and female, with the male repeatedly nudging the vent and caudal region of the female during the pursuit stage of spawning behavior. The same vent-nudging behavior has also been seen among larger groups of pursuing males, but was not a common feature of all spawning rushes in later observations (N.W. Pankhurst, unpublished observations). The significance of the nudging behavior is not clear. It may be part of the behavioral priming required to initiate a spawning rush by the female, but as it does not appear to be requisite for spawning to occur, it appears more likely that it involves olfactory testing of the female by the male. In many teleost species, prostaglandin F_{2a} and its metabolites produced during the periovulatory period and released in urine and ovarian fluid serve as pheromonal triggers for courtship and spawning behavior by males (reviewed in Stacey & Sorensen 2008). It is currently not known whether prostaglandins and their metabolites have a similar function in Sparidae.

Similar behavioral suites during spawning were described for other Sparidae. In cultured populations of the closely related red sea bream, males were also reported to adopt the dark/gray spawning coloration prior to

spawning (Foscarini 1988). Spawning of the santer sea bream (*Cheimerius nufar*) acclimated from the wild was reported from a large public aquarium (Garratt 1991). As with the Australasian snapper, male santer sea bream showed a color change from the normal silvery-pink to a dull gray accentuated by white patches on the flank and head. Males became territorial and patrolled in tight circles until approached by a female, with receptive females joining the circling behavior. This was followed by ascent to the surface with vent nudging by the male, and egg and sperm release near the water surface. Spawning pairs were sometimes joined by additional males during the spawning rush, with subsequent sperm release from multiple males. In a subtle variation on the Australasian snapper behavior pattern, santer sea bream females assumed a rigid posture as they neared the surface, with the male nudging the female toward the surface immediately before egg release (Garratt 1991). A similar behavioral pattern was described for captive Roman sea bream (*Chrysoblephus latticeps*) with the addition of a "mouth bumping" component during the approach and circling stages (Buxton 1990).

Goldlined sea bream (synonym: silver bream) (*Rhabdosargus sarba*) also spawn naturally in captivity with one female being followed closely by 3–4 males and spawning occurring as before at the surface after a spawning rush (Leu 1994). Similarly, males of cultured black porgy compete for space near ovulated females and engage in pursuit behavior with nudging of the females' heads and abdomens prior to egg release and fertilization (Hu *et al.* 1981). The Australian congener black bream (*A. butcheri*) also spawns readily in captivity where there does not appear to be a requirement for ascent through the water column for spawning to occur. Observation of a spawning stock held in large volume systems of ~1.5 m in depth at Fremantle TAFE (Fremantle, Australia) showed a variation on the spawning behavior described above. As before, attendant males pursued a receptive female culminating in a high speed spawning rush with egg and sperm release. However, spawning occurred both at midlevels following lateral pursuit, and at the tank surface (N.W. Pankhurst, unpublished observations) indicating that a rush to the surface was not requisite for egg release. Later experiments showed that suitably acclimated wild fish spawn in tank volumes as low as 1 m^3 (Haddy & Pankhurst 2000b). Black bream is an estuarine species and spawns in the middle to upper reaches of estuaries, often in water of depth of only a few meters (Haddy & Pankhurst 1998). Its capacity to spawn in low-volume systems in captivity may relate to the often shallow conditions of the natural spawning habitat.

The timing of spawning appears to be highly consistent within species, with daily spawning occurring in most species, and the majority of them spawning at dusk or in the early evening, including the Australasian snapper (Smith 1986; Matsuyama *et al.* 1988a; Kagawa *et al.* 1991; Scott *et al.* 1993), gilthead sea bream (Zohar & Gordin 1979; Zohar *et al.* 1988), black porgy (Hu *et al.* 1981; Haddy & Pankhurst 1998), goldlined sea bream (Leu 1994) and Roman sea bream (Buxton 1990). An exception is santer sea bream, where spawning occurs at sunrise (Garratt 1991). Spawning at dawn or dusk is usually proposed to be a mechanism for minimizing egg predation losses from diurnal planktivorous predators (Johannes 1978) and is a common feature of species that form aggregations culminating in pelagic spawning. With the proviso that spawning behavior in the wild is assumed to mirror that seen in captivity, sparid spawning behaviors are typical of those shown by the wide range of families where spawning aggregations are formed, and spawning generally occurs at the apex of a spawning rush (Domeier & Colin 1997). These authors also note that spawning rushes may involve multiple fish, or short-term pair formation within the aggregation, and both situations also appear to occur in sparid spawning behavior.

The question, however, does remain as to the degree to which behavior in captivity reflects that shown in the wild, and this is generally not known. In the case of the Australasian snapper, fisheries capture data from a natural population do suggest that the general pattern of events is similar in the wild. Extensive trawl surveys from the Hauraki Gulf in northern New Zealand conducted over two successive spawning seasons showed that there was daily spawning involving most of the adult population, ovulation peaked in the early afternoon and assessment of postovulatory egg viability showed that the maximum fertilization window was 14:00–22:00 hours, but with maximum viability extending from ovulation to only 18:00–19:00 hours (Scott *et al.* 1993). From this information, it was concluded that as in captivity, the most likely timing of spawning was early evening.

Diver observation surveys conducted on near-shore reefs in the adjacent Cape Rodney-Okakari Point Marine Reserve where snapper are very common and highly diver-approachable by day, showed disappearance of fish from reef areas in late afternoon. Midwater dives at dusk did not reveal the presence of any fish and spawning behavior in the wild remains to be observed (N.W. Pankhurst, unpublished observations). From this, it was concluded that fish were moving off-reef to spawn on a daily basis. Early morning dives showed the presence of snapper back on the diurnal range reef areas, with movement back into the diurnal range presumably occurring during the night. More complete description of spawning behavior in natural populations of Sparidae remains a gap in our knowledge, as does information on the hormonal and possible pheromonal mechanisms involved in the regulation of spawning behavior.

4.5 Broodstock management in Sparidae

The development of commercial-scale aquaculture requires control of reproductive function and development of cultured broodstocks that would, ideally, reproduce spontaneously in captivity and produce eggs of high fecundity and quality for the production of fingerlings for ongrowing operations. Cultured broodstocks also allow the establishment of selective breeding programs, which take advantage of the heritability of certain traits (such as growth, shape, disease resistance, etc.) for the optimization of the production cycle of a given fish species. However, being a very recent activity for most of the world, aquaculture of many species still depends on the acquisition—even occasional one—of mature fish from the wild for the establishment of cultured broodstocks, as domesticated stocks hardly exist, with the exception of Chinese carps and trout. Therefore, acclimation of wild fish to captivity is an important consideration for their successful reproduction and the production of viable gametes in the beginning of the establishment of an aquaculture industry for any species.

Furthermore, and contrary to what one would expect, it appears that some Sparidae when reared entirely in captivity (i.e., from egg) do not spawn spontaneously, even though they may undergo complete gametogenesis and maturation, including spermiation and ovulation. It is not known why this occurs, but most probably the dysfunction relates to breeding behavior, as both males and females produce viable gametes, but do not seem to release them at all in the case of the sharpsnout sea bream or not at the right time for successful fertilization in the case of common dentex. This phenomenon has been described also for the Senegalese sole (*Solea senegalensis*) (Guzmán *et al.* 2009). Therefore, it is necessary to obtain fish continually from the wild in order to establish spawning populations in captivity. Common dentex collected from the wild adapt easily to captive conditions and can produce good quality spawns in tanks of 5–100 m^3, stocking densities of 1–15 kg m^{-3} and sex ratio of around 1:1 (Abellan 2001; Loir *et al.* 2001). On the contrary, wild sharpsnout sea bream is more difficult to reproduce in captivity and the complete failure of cultured F1 broodstock to reproduce has hampered the industry significantly (Faranda *et al.* 1985; Georgiou & Stephanou 1995; Bodington 2000).

4.5.1 Acclimation of wild stocks

The well-documented effects of stress on reproduction in most teleosts (reviewed in Pankhurst & Van Der Kraak 1997) apply equally to Sparidae and are most keenly expressed in stocks early in the domestication process. Wild fish show a marked increase in plasma cortisol levels soon after capture with the severity of the response being a function of the capture technique, and subsequent recovery from capture stress can be slow (Pankhurst & Sharples 1992). In Australasian snapper, even short episodes of stress resulted in depression of plasma T and E2 levels whereas plasma 17,20βP levels were elevated in fish stressed for 1–6 hours (Carragher & Pankhurst 1991). This effect was maintained, if stress episodes were repeated, and resulted in the proportion of fish ovulating declining to zero over a period of 4–5 days in association with a decline in plasma E2 to undetectable levels, and significant depressions in plasma T. Black bream showed similar postcapture increases

in plasma cortisol and concomitant reductions in plasma T and E2 within 1 hour in females, and T and KT (within 30 minutes and 6 hours, respectively) in males. In contrast, plasma 17,20βP levels were elevated, following stress in both sexes (Haddy & Pankhurst 1999). The longer-term implication of suppression of E2 secretion, in particular, is the interruption of cycles of oocyte recruitment and growth and increased incidence of ovarian atresia (Clearwater & Pankhurst 1997; Cleary *et al.* 2000).

Because capture of broodstock from wild populations inevitably involves the imposition of stress, and also the fact that in many species this will result in ovarian and testicular regression, the acclimation process does initially pose some challenges. Subsequent hormonal manipulation of stress-compromised fish may offer remediation (see Section 6, this chapter) but the timing of any intervention is important. Treatment of wild female black bream with GnRH agonists (GnRHa) either at capture or 24 hours postcapture resulted in a marked difference in response depending on the time after capture when fish were treated (Haddy & Pankhurst 2000b). Treatment at capture resulted in higher proportions of fish ovulating, the occurrence of serial ovulations, and a higher mean number of ovulations per fish. Injection at the time of capture also maintained plasma E2 at capture levels, whereas treatment 24 hours later failed to maintain plasma E2. This suggests that serial ovulations were dependent on the continued E2-regulated recruitment of fresh cohorts of vitellogenic follicles. A similar protocol applied to Australasian snapper resulted in reduced plasma T and E2 levels in fish treated with GnRHa or hCG 24 hours after capture, compared with those treated at the time of capture. In contrast to black bream, there was no effect of time of injection on the ovulatory response (Cleary *et al.* 2002). The volume and quality of eggs produced from these ovulations was, however, lower with treatment at 24 hours postcapture.

The available data suggest that in species where there is high stress sensitivity, and where there is any dependence on wild-sourced broodstock, hormone treatment of fish at the time of capture, rather than attempts at short-term acclimation may be the most productive approach. It also appears that the response to stress and its effect on reproductive events changes quite quickly with domestication. Comparison of inhibitory effects of stress was made in Australasian snapper among (a) fish freshly captured from the wild, (b) wild fish that had been acclimated for 4–5 years, and (c) 2- and 3-year-old hatchery-produced cultured fish, which were in turn progeny of wild broodstock. All groups showed an increase in cortisol in response to confinement, but the increase was smallest in cultured fish (Cleary *et al.* 2000). All groups also showed suppression of plasma E2, but by 1 week after stress E2 levels were increasing again in 3-year-old cultured fish, whereas they remained low or undetectable in other groups. In concert with this, there was a low proportion of atretic follicles in 3-year-old cultured fish 1 week after stress, whereas both groups of wild fish had high and increasing proportions of atretic follicles (Cleary *et al.* 2000). This may indicate that domestication results in fairly rapid forced selection for stress tolerance. However, the same study also showed that all stocks of fish remained highly sensitive to serial disturbance associated with removal of other fish from a large stock tank, and that this was correlated with a fall in plasma E2 levels (Cleary *et al.* 2000). This means that the requirement for attention to stress management in reproductive husbandry is likely to be ongoing.

4.5.2 Management of cultured stocks

Feeding is very important during the reproductive season of sparids (Tandler *et al.* 1995; Watanabe & Kiron 1995; Zohar *et al.* 1995a; Izquierdo *et al.* 2001; Watanabe & Vassallo-Agius 2003), especially during the spawning period, as their asynchronous ovaries require nutrients for the intensive process of vitellogenesis, which continues throughout the spawning season, parallel to the daily cycles of OM, ovulation and spawning. It was shown in the gilthead sea bream that a change in the feeding of a spawning stock to a nutrient deficient diet resulted in rapid and dramatic reduction in the quality of the eggs produced, with fertilization success decreasing to 0% within a period of <10 days (Zohar *et al.* 1995a). The nutritional requirements of reproductive fish in commercial facilities are usually met with a combination of commercial broodstock diets, and raw fish or squid. Studies in the gilthead sea bream and red sea bream have shown that the n-3 highly unsaturated fatty acids (HUFA) are

essential nutrients required for optimal reproductive performance (Watanabe & Kiron 1995; Zohar *et al.* 1995a; Fernández-Palacios *et al.* 1997). Feeding of a broodstock diet (which contains high lipid/protein, krill and/or squid meals, and vitamin supplements) may be initiated 1–2 months prior to the spawning season and continue until at least a month after spawning ceases. In the quiescent period, a less expensive diet may be offered (e.g., ongrowing diet), which contains slightly lower levels of HUFA, proteins, and vitamins than the broodstock diet.

Egg quality—that is, the ability of an egg to produce a viable larva (Brooks *et al.* 1997)—in cultured fishes is considered an important parameter affecting the operations of commercial hatcheries (Bromage 1995). Some recent studies attempted to correlate morphological, biochemical, and molecular aspects of fertilized eggs, with embryo development, hatching, and larval survival (Carnevali *et al.* 2001; Lahnsteiner & Patarnello 2003, 2004, 2005). This was done in order to develop quick methods for determining the potential of a batch of eggs for providing larvae of good quality, before a large investment in time and money has been done during larval rearing. Buoyancy of spawned eggs—regardless of fertilization success—is one parameter used in marine fish hatcheries to evaluate the potential of a batch of eggs to produce viable embryos and hatched larvae. This relates to the fact that proper hydration of the egg during OM and immediately after spawning is essential for its further development and survival (Cerdá *et al.* 2007). In the gilthead sea bream, it was found that nonfloating eggs were defective in their vitelline envelope components, which affected the hydration but not the fertilization process (Carnevali *et al.* 2001). In addition, the concentration of cathepsin D and L—both proteolytic enzymes involved in the processing of yolk proteins during OM and the associated increase in the osmotic pressure necessary for egg hydration—differed between floating and nonfloating eggs, pointing to the processing of the yolk proteins as one of the reasons for the reduced viability of nonfloating eggs. Furthermore, it was shown that these nonfloating eggs were already undergoing apoptosis (Carnevali *et al.* 2003). In the gilthead sea bream and sharpsnout sea bream, a multiple regression equation correlating values of various biochemical parameters—such as amino acid and monosaccharide concentrations and the levels of acid phosphatase, adenylate kinase and others—to a predicted prehatch embryo survival was developed and was shown to be a reliable method for predicting the quality of the eggs (Lahnsteiner & Patarnello 2003, 2004). However, such method would be very difficult to implement in a commercial setting. On the other hand, larval survival of the above species was also shown to be correlated with the shape of the lipid droplet of fertilized eggs (Lahnsteiner & Patarnello 2005), and this is a parameter that can be monitored easily in a commercial hatchery. Still a lot more work needs to be done, both in Sparidae and in other cultured fishes, before the determinants of egg quality are known and reliable indicators are developed, which can be employed easily and rapidly in the field (Brooks *et al.* 1997; Bobe & Labbé 2009).

Tank size is not usually a problem with Sparidae, since their relatively small size allows their rearing in small broodstock tanks (even 2 m^3, if needed). However, in the blackspot sea bream, it has been reported that spontaneous spawning can be better achieved in large tanks ranging in size between 10 and 120 m^3, and at stocking densities of 1.7–3.0 kg m^{-3} (see Peleteiro *et al.* 2001). In most commercial hatcheries of Sparidae species, spawning tanks are in the range of 10–40 m^3 in volume, circular or square in the case they are constructed of concrete.

In commercial hatchery production, one of the issues that need to be addressed is the seasonal egg production in fish from the temperate zone. Although members of the Sparidae family have a rather long spawning season—compared to other fishes, there is still a need to extend the reproductive season in order to achieve a steady supply of fingerlings for growout operations. This seasonality can be managed using photothermal manipulations, to shift the spawning season of broodstocks in a way that egg production can be extended to almost all year round (Munro *et al.* 1990; Bromage & Roberts 1995; Bromage *et al.* 2001). This approach has been studied and implemented first in the gilthead sea bream (Zohar *et al.* 1995a), which is the species cultured most in the Mediterranean region. Three or four different populations of broodfish may be exposed to phase-shifted, year-long photothermal manipulations, simulating natural conditions. The environmental conditions are phase-shifted by 3, 6, or 9 months relative to the natural conditions. Since each group spawns daily for 4–5 months, using this approach can ensure year-round egg production (Zohar *et al.* 1995b). Similarly, in common

dentex, 3-month phase shifted photoperiods-induced maturation and spawning 4 months in advance and 2 months in delay for the advance and delayed regime, respectively (Pavlidis *et al.* 2001). The quality of the spawns in terms of spawns per females, fecundity, and fertilization success in photoperiod-shifted broodstocks was higher than or equal to nonmanipulated controls, demonstrating that egg production in common dentex can be achieved for a period of 9 months of a year. It is often observed that photoperiod-manipulated fish spawn more reliably and give better quality eggs than fish allowed to spawn under natural photothermal regimes. This improved reproductive success may be the result of better isolation and management of the environmental conditions of the fish in the photoperiod-controlled tanks, as these are monitored and controlled more carefully from hatchery personnel.

Although the photoperiod control protocols are usually combined with the corresponding thermal manipulations, recent work suggests that the reproductive function of gilthead sea bream is not very sensitive to ambient temperature, and fish may spawn well under semiconstant water temperatures ranging between 19 and 21°C, if exposed to the necessary photoperiod (M. Pavlidis, unpublished data). On the other hand, spawning has been reported to be blocked in sharpsnout sea bream exposed to even ±1°C different from what is required for natural spawning (Georgiou & Stephanou 1995; Bodington 2000). So, in order to simplify and reduce the cost of the environmental manipulations employed in commercial operations for the control of reproductive function, it is necessary to investigate separately in each species their sensitivity to different conditions, and manipulate only the ones absolutely necessary and to the degree required for the production of good quality eggs.

Since most Sparidae are hermaphroditic (either protandrous or protogynous), the sex ratio of a broodstock in culture may change every year, with the danger of the stock becoming monosex (Zohar *et al.* 1995a). Therefore, this is of concern to the hatchery manager and needs to be monitored and managed (see next section). For example, in the gilthead sea bream, up to 80% of the population may become females after the second reproductive season, and this percentage may continue to increase in the following years, as more males become irreversibly females. Although it is possible that highly skewed populations (up to 90% females) may still produce eggs of good quality and extremely high fertilization success approaching 100% (C.C. Mylonas, personal observations), the genetic variability of the population will be affected significantly, as all produced fish will be the offspring of only a small number of males. It is customary, therefore, for commercial hatcheries to remove older and larger individuals (i.e., females in the case of gilthead sea bream) from the broodstock populations and add younger and smaller individuals (i.e., males) just prior to the reproductive season every few years. In contrast, such manipulation does not seem to be as critical in the sharpsnout sea bream or the red porgy, since the sex ratio of the functional males and females does not change during the first few years. This occurs because there is a "pool" of fish of the same age that are either immature or undergoing sex inversion, each supplying the pool of functional males and females with more individuals (Kokokiris *et al.* 1999).

4.6 Hormonal manipulation of reproduction in Sparidae

4.6.1 Hermaphroditism

Developing strategies to manipulate the sex reversal process is important for broodstock management in Sparidae aquaculture. A gradual shift in the sex ratio of the population over the lifetime of a stock may have significant influences on the fecundity (due to reduction in the number of females), fertilization success (due to reduction in the number of males), and parental contribution to the progeny (due to reduction in the number of individuals of either sex), and hence genetic variability. This need of commercial hatcheries, as well as basic interest in understanding the regulation of sex reversal in Sparidae, led to studies on social, pheromonal, and hormonal control of sex reversal. While a significant volume of data has been generated on the endocrine regulation of sex reversal in Sparidae, little progress has been made on social control, which is known to play a significant

role in sex inversion in another typical hermaphrodite family, the Serranidae (see Liu & Sadovy 2004). Such information may be helpful in managing sex ratios of broodstocks in aquaculture, without the need for exogenous hormonal therapies (see below), the latter being costly, cumbersome for the hatchery personnel and not accepted well by the consumers. One study provided evidence for social control in the gilthead sea bream, showing that the presence of older (and larger) females reduced significantly the number of younger males reversing sex (Happe & Zohar 1988), which was hypothesized, but not demonstrated to be mediated by pheromones (For review, see also Zohar *et al.* 1995a). A similar social effect was demonstrated in the protogynous black sea bass (*Centropristis striata*), where the presence of males in the population prevented sex inversion of the females (Benton & Berlinsky 2006). Though still not clear how this process works, this procedure has been proposed as an approach to regulating the sex ratio in gilthead sea bream commercial hatcheries (Zohar *et al.* 1995a).

As in other fishes—both hermaphrodites and gonochores—sex steroid hormones have been used to prevent or induce sex change (Pandian & Sheela 1995; Piferrer 2001; Devlin & Nagahama 2002). The general principle is that androgens induce sex inversion from female to male, whereas estrogens induce sex inversion from male to female. In addition, androgens may be used to prevent natural sex inversion toward females and estrogens may be used to prevent natural sex inversion toward males. The following paragraphs provide some representative examples of controlling sex inversion in Sparidae.

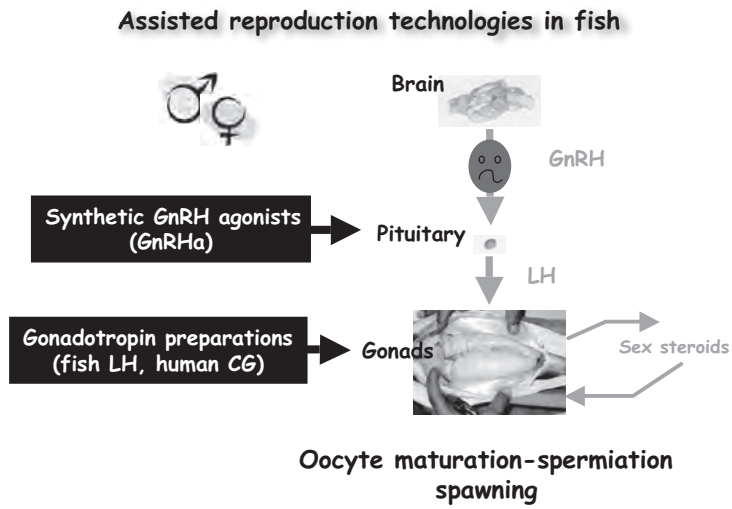
In the protandrous gilthead sea bream, feeding young males with E2 (1 mg kg^{-1} body weight mixed in the diet for 60 days) resulted in 90% of the males reversing to females (Happe & Zohar 1988). Estrogens inhibited testicular growth and male germ development beyond the spermatogonia stage (Condeca & Canario 1999). Wong *et al.* (2006) demonstrated that ovarian aromatase—the enzyme that converts C-18 androgens to estrogens—and E2 plays a significant role in the sex reversal process in the gilthead sea bream, as demonstrated earlier in another protandrous sparid, the black porgy (Lee *et al.* 2001). In the latter fish, oral administration of E2 stimulated gonadal aromatase activity, increased plasma LH levels and resulted in sex change from male to female in two-year-old fish (Chang & Lin 1998). However, sustained treatment with a T implant did not prevent the natural sex inversion into females (Lee *et al.* 2001), presumably because it could be aromatized to E2, while the administration of an aromatase inhibitor blocked completely the natural sex change of fish, and resulted in all fish maturing as functional males during the reproductive season (Du *et al.* 2003).

Less information is available on the use of androgens to induce or prevent sex inversion in Sparidae. For example, oral administration of 17α-methyltestosterone (0.01–1.0 mg kg^{-1} body weight per day) for 16 weeks to 281-day-old meiotic gynogenetic diploids resulted in 100% functional male red sea bream (Kato *et al.* 2003). However, male red sea bream is sometimes found in heterozygous and homozygous clones produced by chromosome manipulation (Kato *et al.* 2002), suggesting that the sex of the red sea bream is determined by both genetic and environmental factors (Kato *et al.* 2003).

Attempts have also been made to control sex inversion through the use of GnRHa or hCG treatments in some Sparidae (Chang *et al.* 1991, 1995b). For example, in the black porgy, GnRHa and hCG increased plasma circulating levels of T in bisexual individuals and functional males, while both T and E2 were elevated in already sex-inverted females (Chang *et al.* 1991). However, no sex inversion was observed. In another study, microncapsulated GnRHa accelerated maturation of the fish, but did not increase the percentage of spermiating males compared to controls (Chang *et al.* 1995b). Some other recent studies attempted to correlate endocrine changes with sex reversal in gilthead sea bream, in an effort to elucidate the hormonal mechanisms regulating hermaphroditism, and perhaps develop a method for controlling sex reversal of this species in aquaculture. The FSHβ transcript levels in the pituitary were shown to be higher in males than in females during the spawning season, while LH transcript levels were higher in the females (Meiri *et al.* 2004). During the sex reversal period, FSH receptor transcripts were shown to be higher than LH receptor transcripts in the testicular portion of the ambisexual gonads, while LH receptor mRNA levels were higher than FSH receptor mRNA levels in the ovarian portion of the gonad (Wong & Zohar 2004a). Considered together, these data suggest that FSH and its receptor are involved in testicular and male development while LH and its receptor may drive sex reversal toward female development.

4.6.2 *Induction of oocyte maturation, ovulation, and spawning*

Hormonal therapies for the induction of maturation and spawning have been often necessary in the early days of maintaining broodstocks in captivity, in both Sparidae and other aquacultured species. This is because it takes some time to gather the necessary knowledge on the optimal environmental conditions required for successful reproductive function in captivity, and until that time captive broodstocks may fail to reproduce efficiently. In captivity, the reproductive dysfunctions observed may range from the total absence of reproductive development observed in freshwater eel (*Anguilla anguilla*) (Kagawa *et al.* 2005; Palstra *et al.* 2005; van Ginneken & Maes 2005), to the absence of only gamete release (i.e., spawning) observed in cultured salmonids (Bromage *et al.* 1992) and described earlier for F1 generation broodstocks of common dentex and sharpsnout sea bream. In Sparidae, as in other fishes, the most common dysfunctions include the production of lower quantity of sperm during the spermiation period and the failure to undergo OM at the completion of vitellogenesis (Mylonas & Zohar 2001b; Zohar & Mylonas 2001; Mylonas & Zohar 2007; Mañanos *et al.* 2008). Therefore, hormonal therapies targeted the induction of LH release through treatment with GnRHa or gonadotropin preparations, such as hCG, carp pituitary extracts (CPE) or purified gonadotropins of piscine origin (Figure 4.5). The following paragraphs provide some representative examples of hormonal therapies employed in Sparidae for the induction of OM, ovulation, and, hopefully, spawning.



Therapy	Advantages	Disadvantages
GnRHa	Almost no species specificity Synthetic, pure, no diseases Widely available	Highly degradable Requires active pituitary May also require DA antagonist
Gonadotropin preparations	Fast response Active pituitary not necessary Crude pituitaries inexpensive	High species specificity Immunogenic Fish LH very expensive Disease transfer (CPE)

Figure 4.5 Schematic representation of the reproductive axis of fish and the level of exogenous interventions for the induction of oocyte maturation, spermiation and spawning. Also shown are the advantages and disadvantages of the different levels of hormonal therapy.

When gilthead sea bream aquaculture in the Mediterranean began to gain momentum in the 1970s, most broodstocks available at hatcheries were collected from the wild (Zohar *et al.* 1995a). These initial populations, and even the first generations of broodstocks produced in captivity, only rarely spawned spontaneously in confinement (Barnabe & Rene 1973; Alessio *et al.* 1975; Gordin & Zohar 1978). While spermatogenesis was completed in the males, oocytes in the females developed only to the final stages of vitellogenesis and then rapidly underwent atresia (Zohar *et al.* 1978, 1984). Thus, OM, ovulation, and spawning did not occur. It became clear that in order to produce reliably gilthead sea bream eggs and juveniles, the spawning "bottleneck" had to be resolved and captive-held gilthead sea bream females had to be induced to undergo OM, ovulation, and spawning. As the gilthead sea bream female can ovulate and spawn daily for long periods, a spawning induction technology must achieve this pattern and level of fecundity. Based on earlier studies in other fish species, an hCG-based spawning induction technology was also examined for the gilthead sea bream (reviewed by Zohar *et al.* 1995a; Zohar & Mylonas 2001). However, hCG did not lead to the desired repetitive spawning, and females treated with hCG in consecutive years did not respond to the hormone. This lack of subsequent response was hypothesized to reflect an immune resistance developed by the fish to the large hCG protein, which was confirmed in later studies (Zohar & Mylonas, 2001).

Consequently, and because the pituitary of gilthead sea bream was shown to be loaded with the fish's own LH (Zohar *et al.* 1988, 1995b), further efforts to induce spawning in gilthead sea bream (and other Sparidae) focused on the use of GnRH and GnRHa. Initially, native mammalian and fish GnRHs were injected into females, but were not efficient in inducing OM, ovulation, or spawning. This was due to the very short-term (6–8 hours) surge of LH secretion from the pituitary induced by the native GnRHs (Zohar *et al.* 1988, 1989, 1995b), which reflected the fact that the native decapeptides are very quickly degraded and inactivated by specific enzymes located in the fish pituitary, kidney, and liver (Goren *et al.* 1990; Zohar *et al.* 1990a). Based on the characterization of these proteases and their sites of action, GnRHas were designed and synthesized with modifications at the sites within the native decapeptides that were specifically cleaved by the enzymes. These agonists were not recognized by the proteases and were shown to be highly resistant to enzyme degradation (Goren *et al.* 1990; Zohar *et al.* 1990a), and thus to be cleared more slowly from the fish circulation after their injection (Gothilf & Zohar 1991). These highly resistant GnRHas were further screened and a few superactive ones were selected based on their increased binding affinity to the GnRH receptors in the gilthead sea bream pituitary (Zohar *et al.* 1989; Pagelson & Zohar 1992; Alok & Zohar 2005) and enhanced potency to induce LH release from *in vitro* cultured pituitary cells (Zohar *et al.* 1989). However, when tested *in vivo*, even the most resistant GnRHas with the best GnRH receptor affinity were still cleared from the fish circulation relatively quickly, albeit more slowly than the native GnRHs (Gothilf & Zohar 1991), induced a relatively short-term (48–72 hours) surge of LH secretion and did not result in daily spawning for long periods of time (Zohar *et al.* 1989; Zohar *et al.* 1995b).

Incorporating the selected highly resistant and superactive GnRHas into polymer-based, controlled release delivery systems (implants or microspheres) led to the sustained release and presence of the GnRHa in the fish circulation and, therefore, to the induction of a prolonged secretion of LH for periods ranging from 8 to 90 days, depending on the polymer used and the composition of the delivery system (Mylonas & Zohar 2001b). These GnRHa delivery systems induced daily cycles of OM, ovulation, and spawning lasting up to three months, not different in pattern, fecundity or egg quality from those observed in spontaneously spawning fish, and have since been used for the induction of maturation and spawning in a wide variety of fishes (Zohar 1989a, 1989b; Mylonas *et al*. 1995, 2009; Zohar *et al.* 1995a; Crim & Bettles 1997; Mylonas & Zohar 2001a, 2001b, 2007; Zohar & Mylonas 2001; Mañanos *et al.* 2008).

In the case of the red sea bream, implantation of another GnRHa delivery system (a cholesterol pellet) was effective in inducing OM in the spawning season, but also sexual maturation and spawning during the nonspawning season from November to December. Spawning was induced 17 days after the implantation in completely immature fish, which had ovaries containing oocytes at the perinucleolus stage (Matsuyama *et al.*

1992). Similar results were obtained in females implanted also with a nonbiodegradable copolymer delivery system containing GnRHa, polymerized by ultraviolet rays (Matsuyama *et al.* 1993). Also, interesting results have been reported in 16-month-old prepubertal red sea bream (Kumakura *et al.* 2003), where implantation of a cholesterol pellet containing GnRHa induced precocious puberty; vitellogenesis and ovulation were observed at 10 and 20 days after implantation, respectively (Kumakura *et al.* 2003). However, precocious puberty could not be induced in pubertal 12-month-old fish (Kumakura *et al.* 2004). Molecular analysis indicated that the pituitary gonadotrophs of these fish were already receptive to the GnRH stimulus and had the ability to synthesize and release LH as in the case of adult fish, although serum concentrations of T and E2 were maintained at relatively low levels (Kumakura *et al.* 2004). These results indicate that the brain-pituitary-gonad axis in the red sea bream is developed by 16 months of age, and GnRHa (or the commencement of *de novo* sbGnRH secretion) can induce precocious puberty.

As mentioned previously, cholesterol (Matsuyama *et al.* 1992) and copolymer GnRHa delivery systems (Matsuyama *et al.* 1993) induced vitellogenesis in female red sea bream. Spawning was also induced by these exogenous hormonal treatments, 9–17 days after implantation (Matsuyama *et al.* 1993). Percentages of buoyant (viable) eggs and hatching rates were relatively low (approximately 50 and 20%, respectively). The precise reasons why egg quality was low in GnRHa-implanted female fish are not known. GnRHa implantation may disturb the timing of spawning in other Sparidae (Barbaro *et al.* 1997), which in red sea bream takes place very soon after ovulation under natural environmental conditions (Matsuyama *et al.* 1988a). A prolongation of the period of time between ovulation and spawning (i.e., egg release) may cause overripening in a matter of minutes or hours depending on species and water temperature (Hirose *et al.* 1979; Mollah & Tan 1983; Bromage 1995; Mylonas *et al.* 1996; Lahnsteiner 2000; Shiraishi *et al.* 2005).

Sexually mature wild Australasian snapper responded to treatment with hCG (1000 IU kg^{-1}) with OM and ovulation indicating that LH agonists can also synchronize ovulation in adult fish. Injections of hCG were followed by increases in plasma 17,20βP levels within 6 hours, and increased T and E2 in 6–24 hours suggesting that steroidogenesis by at least two follicle clutches (maturing follicles and the subsequent vitellogenic clutch) gave rise to the plasma profiles observed (Pankhurst & Carragher 1992). Common pandora have also been induced to undergo OM and spawning using hCG treatments (250–500 IU kg^{-1}), but the produced spawns were of low fecundity and fertilization success, compared to spontaneously spawning fish of the same stock (Klaoudatos *et al.* 2004). However, these broodstock were transferred from their original facility just prior to the spawning induction experiment, and were placed in higher stocking densities (3.2–3.4 kg m^{-3}) compared to the broodstock that remained in the original holding facility (1.2–1.3 kg m^{-3}) and was allowed to spawn spontaneously.

Wild common dentex do spawn spontaneously in captivity, but hormonal induction methods have been investigated in order to synchronize spawning and enhance the yield of fertile gametes (Pavlidis 2000). Use of a GnRHa delivery system resulted in induction of OM and ovulation, producing a 10× increase in fecundity (Pavlidis 2000). On the other hand, hormonal therapies were not effective in inducing spawning in F1 cultured broodstocks—which fail to spawn in captivity, as mentioned above—even though OM was achieved and spermiation was enhanced (C.C. Mylonas, unpublished data). Sharpsnout sea bream have also been very difficult to reproduce in captivity, forcing the investigation of hormonal therapies for the induction of OM, ovulation and spawning. However, presumably due to problems observed with breeding behavior, hormonal inductions of maturation often achieve only ovulation, but not spawning, resulting in the death of the fish due to bloating of the ovarian cavity (Faranda *et al.* 1985). As a result, studies using hormonal therapies to induce spawning in this fish are very limited. However, once a wild broodstock is acclimatized well to hatchery conditions, it is possible to reproduce reliably year after year (Papadaki *et al.*, 2008), producing the highest fecundity reported in cultured Sparidae (Table 4.1).

Red porgy also presents problems in reproducing in captivity, with spawning being unreliable from year to year and broodstock to broodstock (Mylonas *et al.* 2003, 2004). Therapies with GnRHa have been used successfully

to enhance spawning performance, with controlled-release delivery systems loaded with GnRHa resulting in a more prolonged stimulation and higher fecundity compared to a single injection (Zohar & Mylonas 2001b). Finally, the yellowfin porgy (*Acanthopagrus latus*) has been also induced to spawn using hCG or combinations of GnRHa and DA antagonists (Leu & Chou 1996).

4.6.3 Induction of spermiation

As mentioned above, the most commonly observed problem in captive male broodstocks is not a failure in spermatogenesis or spermiation, but rather a reduction in the volume and sometimes quality of the produced sperm (Mañanos *et al.* 2008; Mylonas *et al.* 2009). As a result, exogenous hormonal therapies have also been investigated for the enhancement of spermiation in cultured Sparidae. In the male red sea bream, spermatogenesis and spermiation was induced by the implantation of a cholesterol (Matsuyama *et al.* 1992) or copolymer delivery system (Matsuyama *et al.* 1993) containing GnRHa, similar to the ones used in the females. Such GnRHa treatment increased significantly LHβ and alpha glycoprotein subunit (aGSU) mRNA levels concomitant with the increase in serum KT levels, while the FSHβ mRNA levels were downregulated by the same treatment, suggesting that GnRHa stimulates spermatogenesis and spermiation through the production of LH in the pituitary and KT in the testis (Yamaguchi *et al.* 2005).

Increases in milt volume could be also induced by injection with GnRHa, hCG, or sex steroids in Australasian snapper (Pankhurst 1994). Injection of GnRHa at 100 μg kg^{-1} resulted in increased milt volume and was accompanied by increases in plasma levels of T and KT. Treatment with hCG had a similar effect on milt volume, but resulted only in increased plasma T levels. Injections of 17,20βP or 17-hydroxy progesterone (17P) both stimulated increases in milt volume, whereas 17,20αP, T and KT were ineffective. Treatment with 17P also resulted in substantial increases in plasma 17,20βP levels suggesting that failure to detect increases in 17,20βP following treatment with GnRHa or hCG resulted from rapid metabolism and clearance, and that 17,20βP was the prime candidate for testicular regulation of milt hydration (Pankhurst 1994). In contrast, use of GnRHa delivery systems in common dentex did not result in a marked increase in sperm production, although an elevation of KT was measured after the treatment (Pavlidis 2000).

Sex steroid hormones have also been used to enhance spermiation in Sparidae. In the protandrous black porgy, T treatment induced significant increases in the percentage of spermiating males and prolonged the spermiation period (Lau *et al.* 1997). In addition, it increased the volume of expressible milt, without reducing spermatozoa concentration. This enhancement of spermiation was associated with significant increases in plasma KT, leading the authors to conclude that the administered T was acting through its conversion to KT (Lau *et al.* 1997). However, contradictory results were obtained with exogenous administration of KT in another study, which failed to enhance spermiation (Yueh & Chang 1997). In the latter study, 17,20βP and 17,20β,21P—the MIH in this and other Sparidae—were more effective in enhancing spermiation. Furthermore, it was also shown that low levels of exogenous E2 also stimulated spermatogenesis (instead of sex inversion from male to female) and increased milt production (Chang *et al.* 1995a).

Increasing knowledge acquired over the last decades of the biology of cultured Sparidae and their optimal environmental requirements for reproductive maturation has limited the use of hormonal therapies for the induction of maturation and spawning, and the production of viable eggs in commercial operations. Nevertheless, hormonal therapies will continue to have a role in the control of reproduction in cultured Sparidae, in the case of (a) new species adopted by the aquaculture industry, (b) species that have inherent problems in reproducing in captivity, and (c) situations where synchronization of spawning is necessary in order to improve production schedules or implement selective breeding programs. As a result, such therapies are continuing to be investigated in Sparidae, as well as in other fishes.

References

Abellan, E. (2001) Culture of common dentex (*Dentex dentex* L.): present knowledge, problems and perspectives. *Cahiers Options Méditerranéenes*, **47**, 157–168.

Adachi, S., Ouchi, K., Hirose, K., *et al.* (1988) Induction of oocyte maturation *in vitro* by steroid hormones in the red sea bream *Pagrus major*. *Nippon Suisan Gakkaishi*, **54**, 1665.

Alavi, H.S.M., Cosson, J., Coward, K., *et al.* (2008) *Fish Spermatology*. Alpha Science International Ltd., Oxford, UK.

Alessio, G., Gandolfi, G., & Schreiber, B. (1975) Techniche e methodiche generali di riproduzione artificiale dell'orata, *Sparus aurata* (L.) (Osteichthys, Sparidae). *Inv Pesq*, **39**, 417–428.

Alok, D. & Zohar, Y. (2005) Genes for fish GnRHs and their receptor: relevance to aquaculture biotechnology. In: *Fish Genetics and Aquaculture Biotechnology* (eds. T.J. Pandian, C.A. Strüssman, & M.P. Marian), pp. 81–101. Science Publishers, Enfield (NH), USA.

Aristizabal, E., Suárez, J., Vega, A., *et al.* (2009) Egg and larval quality assessment in the Argentinean red porgy (*Pagrus pagrus*). *Aquaculture*, **287**, 329–334.

Babin, P.J., Carnevali, O., Lubzens, E., *et al.* (2007) Molecular aspects of oocyte vitellogenesis in fish. In: *The Fish Oocyte: From Basic Studies to Biotechnological Applications* (eds. P.J. Babin, J. Cerdá, & E. Lubzens), pp. 39–76. Springer, The Netherlands.

Barbaro, A., Francescon, A., Bozzato, G., *et al.* (1997) Induction of spawning in gilthead seabream, *Sparus aurata* L., by long-acting GnRH agonist and its effects on egg quality and daily timing of spawning. *Aquaculture*, **154**, 349–359.

Barnabe, G. & Rene, F. (1973) Reproduction controlee et production d'alevins chez la daurade *Sparus aurata* (Linne 1758). *C.R. Acad. Sci. Paris, Ser. D*, **276**, 1621–1624.

Benton, C.B. & Berlinsky, D.L. (2006) Induced sex change in black sea bass. *Journal of Fish Biology*, **69**, 1491–1503.

Billard, R. (1986) Spermatogenesis and spermatology of some teleost fish species. *Reproduction Nutrition Development*, **26**, 877–920.

Bobe, J. & Labbé, C. (2009) Egg and sperm quality in fish. *General and Comparative Endocrinology*, **165**, 535–548.

Bobe, J., Jalabert, B. & Fostier, A. (2008) Oogenesis: post-vitellogenic events leading to a fertilizable oocyte. In: *Fish Reproduction* (eds. B.G. Rocha, M.J. Arukwe, & A. Kapoor), pp. 1–36. Science Publishers, Enfield.

Bodington, P. (2000) Enterprise experiences in the culture of new sparids. *Cahiers Options Méditerranéennes*, **47**, 135–139.

Bromage, N.R. (1995) Broodstock management and seed quality—general considerations. In: *Broodstock Management and Egg and Larval Quality* (eds. N.R. Bromage & R.J. Roberts), pp. 1–24. Blackwell Publishing Ltd., Oxford, UK.

Bromage, N.R. & Roberts, R.J. (1995) *Broodstock Management and Egg and Larval Quality*. Blackwell Publishing Ltd., Oxford, UK.

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

Bromage, N., Porter, M. & Randall, C. (2001) The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. *Aquaculture*, **197**, 63–98.

Brooks, S., Tyler, C.R. & Sumpter, J.P. (1997) Egg quality in fish: what makes a good egg? *Reviews in Fish Biology and Fisheries*, **7**, 387–416.

Brown, R.C., Woolliams, J.A. & Mcandrew, B.J. (2005) Factors influencing efffective population size in commercial populations of gilthead seabream, (*Sparus aurata*). *Aquaculture*, **247**, 219–225.

Bruslé-Sicard, S. & Fourcault, B. (1997) Recognition of sex-inverting protandric *Sparus aurata*: ultrastructural aspects. *Journal of Fish Biology*, **50**, 1094–1103.

Buxton, C.D. (1990) The reproductive biology of *Chrysoblephus latticeps* and *C. cristiceps* (Teleostei: Sparidae). *Journal of Zoology (London)*, **220**, 497–511.

Buxton, C.D. & Garratt, P.A. (1990) Alternative reproductive styles in seabreams (Pisces: Sparidae). *Environmental Biology of Fishes*, **28**, 113–124.

Canario, A.V.M., Couto, E., Vilia, P., *et al.* (1995) Sex steroids during the ovulatory cycle of gilthead seabream (*Sparus aurata*). In: *Reproductive Physiology of Fish, 1995* (eds. F.W. Goetz & P. Thomas), pp. 290–292. Fish Symposium 95, Austin, Texas.

Carnevali, O., Mosconi, G., Cardinali, M., *et al.* (2001) Molecular components related to egg viability in the gilthead sea bream, *Sparus aurata*. *Molecular Reproduction and Development*, **58**, 330–335.

Carnevali, O., Polzonetti, V., Cardinali, M., *et al.* (2003) Apoptosis in sea bream *Sparus aurata* eggs. *Molecular Reproduction and Development*, **66**, 291–296.

Carragher, J.F. & Pankhurst, N.W. (1991) Stress and reproduction in a commercially important marine fish, *Pagrus auratus* (Sparidae). In: *Reproductive Physiology of Fish 1991* (eds. A.P. Scott, J.P. Sumpter, D.E. Kime, & M.S. Rolfe), pp. 253–255. FishSymp 91, Sheffield.

Carragher, J.F. & Pankhurst, N.W. (1993) Plasma levels of sex steroids during sexual maturation of snapper, *Pagrus auratus* (Sparidae), caught from the wild. *Aquaculture*, **109**, 375–388.

Cerdá, J., Fabra, M. & Raldúa, D. (2007) Physiological and molecular basis of fish oocyte hydration. In: *The Fish Oocyte: from Basic Studies to Biotechnological Applications* (eds. P.J. Babin, J. Cerdá, & E. Lubzens), pp. 349–396. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Chang, C.F. & Lin, B.Y. (1998) Estradiol-17ß stimulates aromatase activity and reversible sex change in protandrous black porgy, *Acanthopagrus schlegeli*. *The Journal of Experimental Zoology*, **280**, 165–173.

Chang, C.F., Yueh, W.S. & Lee, M.F. (1991) Effects of LHRH-A and HCG on the steroid profiles of bisexual and mature male and female protandrous black porgy, *Acanthopagrus schlegeli*. *Aquaculture*, **92**, 83–92.

Chang, C.F., Lau, E.N. & Lin, B.Y. (1995a) Stimulation of spermatogenesis or of sex reversal according to the dose of exogenous estradiol-17ß in juvenile males of protandrous black porgy, *Acanthopagrus schlegeli*. *General and Comparative Endocrinology*, **100**, 355–367.

Chang, C.F., Yueh, W.S., Lee, M.F., *et al.* (1995b) A microencapsulated analog of LH-RH accelerates maturation but without stimulating sex reversal in the protandrous black porgy, *Acanthopagrus schlegeli*. *Reproduction Nutrition Development*, **35**, 339–349.

Chang, J.P., Johnson, J.D., Sawinsky, G.R., *et al.* (2009) Signal transduction in multifactorial neuroendocrine control of gonadotropin secretion and synthesis in teleosts-studies on the goldfish model. *General and Comparative Endocrinology*, **161**, 42–52.

Chaves-Pozo, E., Mulero, V., Meseguer, J., *et al.* (2005) An overview of cell renewal in the testis throughout the reproductive cycle of a seasonal breeding teleost, the glthead seabream (*Sparus aurata* L.). *Biology of Reproduction*, **72**, 593–601.

Chilari, A., Petrakis, G. & Tsamis, E. (2006) Aspects of the biology of blackspot seabream (*Pagellus bogaraveo*) in the Ionian Sea, Greece. *Fisheries Research*, **77**, 84–91.

Chow, M.M., Kight, K.E., Gothilf, Y., *et al.* (1998) Multiple GnRHs present in a teleost species are encoded by separate genes: Analysis of the sbGnRH and cGnRH-II genes from the striped bass, *Morone saxatilis*. Journal of Molecular Endocrinology, **21**, 277–289.

Clearwater, J.C. & Pankhurst, N.W. (1997) The response to capture and confinement stress of plasma cortisol, plasma sex steroids and vitellogenic oocytes in the marine teleost, red gurnard (*Chelidonichthys kumu*) (Triglidae). *Journal of Fish Biology*, **50**, 429–441.

Cleary, J.J., Battaglene, S.C. & Pankhurst, N.W. (2002) Capture and handling stress affects the endocrine and ovulatory response to exogenous hormone treatment in snapper, *Pagrus auratus* (Bloch & Schneider). *Aquaculture Research*, **33**, 1–10.

Cleary, J.J., Pankhurst, N.W. & Battaglene, S.C. (2000) The effect of capture and handling stress on plasma steroid levels and gonadal condition in wild and farmed snapper, *Pagrus auratus* (Sparidae). *Journal of the World Aquaculture Society*, **31**, 558–569.

Condeca, J.B. & Canario, A.V.M. (1999) The effect of estrogen on the gonads and on *in vitro* conversion of androstenedione to testosterone, 11-ketotestosterone, and estradiol-17ß in *Sparus aurata* (Teleostei, Sparidae). *General and Comparative Endocrinology*, **116**, 59–72.

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

D'Ancona, U. (1941) Ulteriori osservazioni e considerationi sull'ermafroditismo ed il differenziamento sessuale dell'orata (*Sparus aurata*). *Pubbl Staz Zoool Napoli*, **18**, 313–336.

D'Ancona, U. (1949) Il differenziamento della gonado e l'inversione sessuale degli sparidi. *Archives of Oceanography and Limnology*, **6**, 97–163.

Devlin, R.H. & Nagahama, Y. (2002) Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture*, **208**, 191–364.

Domeier, M.L. & Colin, P.L. (1997) Tropical reef fish spawning aggregations defined and reviewed. *Bulletin of Marine Science*, **60**, 698–726.

Du, J., Lin, B., Lee, Y., *et al.* (2003) The possible mechanism of sex change in the protandrous black porgy, *Acanthopagrus schlegeli*. *Journal of the Fisheries Society Taiwan*, **30**, 101–109.

Elizur, A., Zmora, N., Meiri, I., *et al.* (2000) Gonadotropins—from genes to recombinant proteins. In: *Reproductive Physiology of Fish* (eds. B. Norberg, O.S. Kjesbu, G.L. Taranger, E. Andersson, & S.O. Stefansson), pp. 462–465. University of Bergen, Bergen, Norway.

Elizur, A., Zmora, N., Rosenfeld, H., *et al.* (1996) GtH-I and GtH-II from the gilthead seabream, *Sparus aurata*, isolation and molecular characterization of beta subunit cDNAs. *General and Comparative Endocrinology*, **102**, 39–46.

Faranda, F., Cavaliere, A., Lo Paro, G., *et al.* (1985) Preliminary studies on reproduction of *Puntazzo puntazzo* (Gmelin 1789) (Pisces, Sparidae) under controlled conditions. *Aquaculture*, **49**, 111–123.

Fernández-Palacios, H., Izquierdo, M., Robaina, L., *et al.* (1997) The effect of dietary protein and lipid from squid and fish meals on egg quality of broodstock for gilthead seabream (*Sparus aurata*). *Aquaculture*, **148**, 233–246.

Foscarini, R. (1988) A review: intensive farming procedure for red sea bream (*Pagrus major*) in Japan. *Aquaculture*, **72**, 191–246.

Fostier, A., Kokokiris, L., Le Menn, F., *et al.* (2000) Recent advances in reproductional aspects of *Pagrus pagrus*. *Cahiers Options Méditerranéennes*, **47**, 181–192.

Francis, M.P. & Pankhurst, N.W. (1988) Juvenile sex inversion in the New Zealand snapper *Chrysophrys auratus* (Bloch and Schneider, 1801) (Sparidae). *Australian Journal of Marine and Freshwater Research*, **39**, 625–631.

Garratt, P.A. (1991) Spawning behaviour of *Cheimerius nufar* in captivity. *Environemtal Biology of Fishes*, **31**, 345–353.

Gen, K., Okuzawa, K., Senthilkumaran, B., *et al.* (2000) Unique expression of gonadotropin-I and -II subunit genes in male and female red sea bream (*Pagrus major*) during sexual maturation. *Biology of Reproduction*, **63**, 308–319.

Gen, K., Okuzawa, K., Kumakura, N., *et al.* (2001) Correlation between messenger RNA expression of cytochrome P450 aromatase and its enzyme activity during oocyte development in the red seabream (*Pagrus pagrus*). *Biology of Reproduction*, **65**, 1186–1194.

Gen, K., Yamaguchi, S., Okuzawa, K., *et al.* (2003) Physiological roles of FSH and LH in red seabream, *Pagrus major*. *Fish Physiology and Biochemistry*, **28**, 77–80.

Georgiou, G. & Stephanou, D. (1995) Contribution to the study of maturation and spawning problems of the sharpsnout seabream (*Puntazzo puntazzo*). *Cahiers Options Méditerranéennes*, **16**, 47–50.

Gonzalez, E.B., Umino, T. & Nagasawa, K. (2008) Stock enhancement programme for black sea bream, *Acanthopagrus schlegelii* (Bleeker), in HIrosima Bay, Japan: a review. *Aquaculture Research*, **39**, 1307–1315.

Gordin, H. & Zohar, Y. (1978) Induced spawning of *Sparus aurata* (L.) by means of hormone treatments. *Annales de Biologie Animale Biochimie Biophysique*, **18**, 985–990.

Goren, A., Zohar, Y., Fridkin, M., *et al.* (1990) Degradation of gonadotropin-releasing hormones in the gilthead seabream, *Sparus aurata* I. Cleavage of native salmon GnRH and mammalian LHRH in the pituitary. *General and Comparative Endocrinology*, **79**, 291–305.

Gothilf, Y. & Zohar, Y. (1991) Clearance of different forms of GnRH from the circulation of the gilthead seabream, *Sparus aurata*, in relation to their degradation and bioactivities. In: *Reproductive Physiology of Fish* (eds. A.P. Scott, J.P. Sumpter, D.E. Kime, & M.S. Rolfe), pp. 35–37. Fish Symposium 91, Sheffield.

Gothilf, Y., Chow, M., Elizur, A., *et al.* (1995) Molecular cloning and characterization of a novel gonadotropin-releasing hormone from gilthead seabream (*Sparus aurata*). *Marine Molecular Biology and Biotechnology*, **4**, 27–35.

Gothilf, Y., Muñoz-Cueto, J.A., Sagrillo, C.A., *et al.* (1996) Three forms of gonadotropin-releasing hormone in a perciform fish (*Sparus aurata*): complementary deoxyribonucleic acid characterization and brain localization. *Biology of Reproduction*, **55**, 636–645.

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

Güner, Y., Özden, O., Altunok, M., *et al.* (2004) Spawning and larvae production of common pandora, *Pagellus erythrinus* L. *Israeli Journal of Aquaculture-Bamidgeh*, **56**, 209–217.

Guzmán, J.M., Ramos, J., Mylonas, C.C., *et al.* (2009) Spawning performance and plasma levels of GnRHa and sex steroids in cultured female Senegalese sole (*Solea senegalensis*) treated with different GnRHa-delivery systems. *Aquaculture*, **291**, 200–209.

Haddy, J.A. & Pankhurst, N.W. (1998) Annual change in reproductive condition and plasma concentrations of sex steroids in black bream, *Acanthopagrus butcheri* (Munro) (Sparidae). *Marine and Freshwater Research*, **49**, 389–397.

Haddy, J.A. & Pankhurst, N.W. (1999) Stress-induced changes in concentrations of plasma sex steroids in black bream. *Journal of Fish Biology*, **55**, 1304–1316.

Haddy, J.A. & Pankhurst, N.W. (2000a) The effects of salinity on reproductive development, plasma steroid levels, fertilisation and egg survival in black bream *Acanthopagrus butcheri*. *Aquaculture*, **188**, 115–131.

Haddy, J.A. & Pankhurst, N.W. (2000b) The efficacy of exogenous hormones in stimulating changes in plasma steroids and ovulation in wild black bream *Acanthopagrus butcheri* is improved by treatment at capture. *Aquaculture*, **191**, 351–366.

Happe, A. & Zohar, Y. (1988) Self-fertilization in the protandrous hermaphrodite *Sparus aurata*: development of the technology. In: *Reproduction in Fish: Basic and Applied Aspects in Endocrinology and Genetics* (eds. Y. Zohar & B. Breton), pp. 177–180. INRA Press, Paris.

Hirose, K., Machida, Y. & Donaldson, E.M. (1979) Induced ovulation of Japanese flounder (*Limanda yokohamae*) with human chorionic gonadotropin and salmon gonadotropin, with special reference to changes in quality of eggs retained in the ovarian cavity after ovulation. *Bulletin of the Japanese Society of Scientific Fisheries*, **45**, 31–36.

Hobby, A.C. & Pankhurst, N.W. (1997) The relationship between plasma and ovarian levels of gonadal steroids in the repeat spawning marine fishes *Pagrus auratus* (Sparidae) and *Chromis dispilus* (Pomacentridae). *Fish Physiology and Biochemistry*, **16**, 65–75.

Holland, M.C.H., Gothilf, Y., Meiri, I., *et al.* (1998) Levels of the native forms of GnRH in the pituitary of the gilthead seabream, *Sparus aurata*, at several characteristic stages of the gonadal cycle. *General and Comparative Endocrinology*, **112**, 394–405.

Hu, S-H., Yen, J-L., Lin, K-J. *et al.* (1981) Sexual conversion and natural spawning of 2 year old cultured black porgy *Acanthopagrus schlegeli*. *Bulletin of Taiwan Fisheries Research Institute*, **33**, 715–722.

Hussain, N., Akatsu, S. & El-Zahr, C. (1981) Spawning, egg and early larval development, and growth of *Acanthopagrus cuvieri* (Sparidae). *Aquaculture*, **22**, 125–136.

Izquierdo, M.S., Fernández-Palacios, H. & Tacon, A.G.J. (2001) Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture*, **197**, 25–42.

Jackson, L.F. & Sullivan, C.V. (1995) Reproduction of white perch: the annual gametogenic cycle. *Transactions of the American Fisheries Society*, **124**, 563–577.

Johannes, R.E. (1978) Reproducive startegies of costal marine fishes in the tropics. *Environmental Biology of Fishes*, **3**, 65–84.

Kadmon, G., Yaron, Z. & Gordin, H. (1985) Sequence of gonadal events and oestradiol levels in *Sparus aurata* (L.) under two photoperiod regimes. *Journal of Fish Biology*, **26**, 609–620.

Kagawa, H., Tanaka, H., Okuzawa, K., *et al.* (1991) Diurnal changes in plasma 17a, 20b-dihydroxy-4-pregnen-3-one levels during spawning season in the red seabream *Pagrus major*. *Bulletin of the Japanese Society of Scientific Fisheries*, **57**, 769.

Kagawa, H., Kobayashi, M., Hasegawa, Y., *et al.* (1994a) Insulin and insulin-like growth factors I and II induce final oocyte maturation of oocytes of red seabream, *Pagrus major*, *in vitro*. *General and Comparative Endocrinology*, **95**, 293–300.

Kagawa, H., Tanaka, H., Okuzawa, K., *et al.* (1994b) Development of maturational competence of oocytes of red seabream, *Pagrus major*, after human chorionic gonadotropin treatment *in vitro* requires RNA and protein synthesis. *General and Comparative Endocrinology*, **94**, 199–206.

Kagawa, H., Moriyama, S. & Kawauchi, H. (1995) Immunocytochemical localization of IGF-I in the ovary of the red seabream, *Pagrus major*. *General and Comparative Endocrinology*, **99**, 307–315.

Kagawa, H., Tanaka, H., Okuzawa, K., *et al.* (1998) GTH II but not GTH I induces final oocyte maturation and the development of maturational competence of oocytes of red seabream *in vitro*. *General and Comparative Endocrinology*, **112**, 80–88.

Kagawa, H., Moriyama, S. & Okuzawa, K. (1999) *In vitro* production of IGF-I by ovarian follicles of the red seabream, *Pagrus major*. In: *Recent Progress in Molecular and Comparative Endocrinology* (eds. H.B. Kwon, J.M.P. Joss, & S. Ishii), pp. 144–148. Hormone Research Center, Chonnam National University, Kwangju, Korea.

Kagawa, H., Tanaka, H., Ohta, H., *et al.* (2005) The first success of glass eel production in the world: basic biology on fish reproduction advances new applied technology in aquaculture. *Fish Physiology and Biochemistry*, **31**, 193–199.

Kato, K., Aoki, Y., Nishimura, K., *et al.* (1985) Spawn taking on one-year-old red seabream *Pagrus major* reared in net cage. *The Aquiculture*, **33**, 143–148.

Kato, K., Hayashi, R., Ishitani, Y., *et al.* (1999) Gonadal sex differentiation of red sea bream of a selected strain. *Suisanzoshoku*, **47**(1), 29–34.

Kato, K., Hayashi, R., Yuasa, D., *et al.* (2002) Production of cloned red sea bream, *Pagrus major*, by chromosome manipulation. *Aquaculture*, **207**, 19–27.

Kato, K., Miyashita, S., Murata, O., *et al.* (2003) Gonadal sex differentiation and sex control in red sea bream, *Pagrus major*. *Fish Physiology and Biochemistry*, **28**, 155–156.

Kinsey, W.H., Sharma, D. & Kinsey, S.C. (2007) Fertilization and egg activation in fishes. In: *The fish Oocyte: From Basic Studies to Biotechnological Applications* (eds. P.J. Babin, J. Cerdá, & E. Lubzens), pp. 397–410. Springer, The Netherlands.

Klaoudatos, S.D., Iakovopoulos, G. & Klaoudatos, D.S. (2004) *Pagellus erythrinus* (common pandora): a promising candidate species for enlarging the diversity of aquaculture production. *Aquaculture International*, **12**, 299–320.

Kokokiris, L., Bruslé, S., Kentouri, M., *et al.* (1999) Sexual maturity and hermaphroditism of the red porgy *Pagrus pagrus* (Teleostei: Sparidae). *Marine Biology*, **134**, 621–629.

Kokokiris, L., Mourot, B., Le Menn, F., *et al.* (2000) Endocrine changes during the annual reproductive cycle of the red porgy, *Pagrus pagrus* (Teleostei, Sparidae). *Fish Physiology and Biochemistry*, **23**, 1–11.

Kokokiris, L., Le Menn, F., Kentouri, M., *et al.* (2001) Seasonal cycle of gonadal development and serum levels of vitellogenin of the red porgy, *Pagrus pagrus* (Teleostei, Sparidae). *Marine Biology*, **139**, 549–559.

Kokokiris, L., Fostier, A., Athanassopoulou, F., *et al.* (2006) Gonadal changes and blood sex steroid levels during natural sex inversion in the protogynous Mediterranean red porgy, *Pagrus pagrus* (Teleostei: Sparidae). *General and Comparative Endocrinology*, **149**, 42–48.

Krug, H.M. (1998) Variation in the reproduction cycle of the blackspot seabream, *Pagellus bogaraveo* (Brünnich, 1768) in the Azores. *Arquipelago Life and Marine Sciences*, **16A**, 37–47.

Kumakura, N., Okuzawa, K., Gen, K., *et al.* (2003) Effects of gonadotropin-releasing hormone agonist and dopamine antagonist on hypothalamus-pituitary-gonadal axis of pre-pubertal female red seabream (*Pagrus major*). *General and Comparative Endocrinology*, **131**, 264–273.

Kumakura, N., Okuzawa, K., Gen, K., *et al.* (2004) Effects of gonadotropin-releasing hormone on pituitary-gonadal axis of one-year old pre-pubertal red seabream. *General and Comparative Endocrinology*, **138**, 105–112.

Lahnsteiner, F. (2000) Morphological, physiological and biochemical parameters characterizing the over-ripening of rainbow trout eggs. *Fish Physiology and Biochemistry*, **23**, 107–118.

Lahnsteiner, F. & Patarnello, P. (2003) Investigations on the metabolism of viable and nonviable gilthead sea bream (*Sparus aurata*) eggs. *Aquaculture*, **223**, 159–174.

Lahnsteiner, F. & Patarnello, P. (2004) Biochemical egg quality determination in the gilthead seabream, *Sparus aurata*: reproducibility of the method and its application for sharpsnout seabream, *Puntazzo puntazzo*. *Aquaculture*, **237**, 433–442.

Lahnsteiner, F. & Patarnello, P. (2005) The shape of the lipid vesicle is a potential marker for egg quality determination in the gilthead seabream, *Sparus aurata*, and in the sharpsnout seabream, *Diplodus puntazzo.* Aquaculture, **246**, 423–435.

Lau, E.L., Lin, B.Y., Lee, F.Y., *et al.* (1997) Stimulation of testicular function by exogenous testosterone in male protandrous black porgy, *Acanthopagrus schlegeli*. *Journal of Fish Biology*, **51**, 327–333.

Lee, Y.-H., Du, J.-L., Yueh, W.-S., *et al.* (2001) Sex change in the protandrous black porgy, *Acanthopagrus schlegeli*: a review in gonadal development, estradiol, estrogen receptor, aromatase activity and gonadotropin. *Journal of Experimental Zoology*, **290**, 715–726.

Lee, Y.-H., Yueh, W.-S., Du, J.-L., *et al.* (2002) Aromatase inhibitors block natural sex change and induce male function in the protandrous black porgy, *Acanthopagrus schlegeli* Bleeker: possible mechanisms of natural sex change. *Biology of Reproduction*, **66**, 1749–1754.

Lessman, C.A. (2009) Oocyte maturation: converting the zebrafish oocyte to the fertilizable egg. *General and Comparative Endocrinology*, **161**, 53–57.

Leu, M-Y. (1994) Natural spawning and larval rearing of silver bream, *Rhabdosargus sarba* (Forsskål), in captivity. *Aquaculture*, **120**, 115–122.

Leu, M.Y. (1997) Natural spawning and mass larviculture of black porgy *Acanthopagrus schlegeli* in captivity in Taiwan. *Journal of the World Aquaculture Society*, **28**, 180–187.

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

Lim, B.S. (2004) Endocrine mechanisms of sex differentiation in the red sea bream, *Pagrus major*. Ph.D. Thesis, Tokyo University of Marine Science and Technology.

Lim, B.S., Kagawa, H., Gen, K., *et al.* (2003) Effects of water temperature on the gonadal development and expression of steroidogenic enzymes in the gonad of juvenile red seabream, *Pagrus major*. *Fish Physiology and Biochemistry*, **28**, 161–162.

Liu, M. & Sadovy, Y. (2004) The influence of social factors on adult sex change and juvenile sexual differentiation in a diandric, protogynous epinepheline, *Cephalopholis boenak* (Pisces, Serranidae). *Journal of the Zoological Society of London*, **264**, 239–248.

Loir, M., Le Gac, F., Somarakis, S., *et al.* (2001) Sexuality and gonadal cycle of the common dentex (*Dentex dentex*) in intensive culture. *Aquaculture*, **194**, 363–383.

Malison, J.A., Procarione, L.S., Barry, T.P., *et al.* (1994) Endocrine and gonadal changes during the annual reproductive cycle of the freshwater teleost, *Stizostedion vitreum*. *Fish Physiology and Biochemistry*, **13**, 473–484.

Mañanos, E., Duncan, N. & Mylonas, C.C. (2008) Reproduction and control of ovulation, spermiation and spawning in cultured fish. In: *Methods in Reproductive Aquaculture: Marine and Freshwater Species* (eds. E. Cabrita, V. Robles, & M.P. Herráez), pp. 3–80. CRC Press, Taylor and Francis Group, Boca Raton.

Mann, B.Q. & Buxton, C.D. (1998) The reproductive biology of *Diplodus sargus capensis* and *D. cervinus hottentotus* (Sparidae) off the south-east Cape coast, South Africa. *Cybium*, **22**, 31–47.

Manooch, C.S., III. (1976) Reproductive cycle, fecundity, and sex ratios of the red porgy, *Pagrus pagrus* (Pisces: Sparidae) in North Carolina. *Fishery Bulletin*, **74**, 775–781.

Manooch, C.S., III & Hassler, W.M. (1978) Synopsis of biological data on the red porgy, *Pagrus pagrus* (Linnaeus). *FAO Fisheries Synopsis*, **116**, 1–19.

Matsuura, S., Furuichi, M., Maruyama, K., *et al.* (1988) Daily spawning and quality of eggs in one female red sea bream. *Suisanzoshoku*, **36**, 33–39.

Matsuyama, M., Adachi, S., Nagahama, Y., *et al.* (1988a) Diurnal rhythm of oocyte development and plasma steroid hormone levels in the female red sea bream during the spawning season. *Aquaculture*, **73**, 357–372.

Matsuyama, M., Lara, R.T. & Matsuura, S. (1988b) Juvenile bisexuality in the red sea bream, *Pagrus major*. *Environmental Biology of Fishes*, **21**, 27–36.

Matsuyama, M., Kagawa, H., Takeuchi, H., *et al.* (1992) Effects of LHRH—a cholesterol pellet on gonadal development, maturation and spawning in *Pagrus major* during winter season. *Suisanzoshoku*, **40**, 159–165.

Matsuyama, M., Hamada, M., Ashitani, T., *et al.* (1993) Development of LHRH-a copolymer pellet polymerized by ultraviolet and its application for maturation in red sea bream *Pagrus major* during non-spawning season. *Nippon Suisan Gakkaishi*, **59**, 1361–1369.

Meiri, I., Gothilf, Y., Zohar, Y., *et al.* (2002) Physiological changes in spawning gilthead seabream, *Sparus aurata*, succeeding in removing the males. *Journal of Experimental Zoology*, **292**, 555–564.

Meiri, I., Knibb, W.R., Zohar, Y., *et al.* (2004) Temporal profile of b follicle-stimulating hormone, b luteinizing hormone, and growth hormone gene expression in the protandrous hermaphrodite, gilthead seabream, *Sparus aurata*. *General and Comparative Endocrinology*, **137**, 288–299.

Meseguer, C., Ramos, J., Bayarri, M.J., *et al.* (2008) Light synchronization of the daily spawning rhythms of gilthead sea brea (*Sparus aurata* L) kept under different photoperiod and after shifting the LD cycle. *Chronobiology International*, **25**, 666–679.

Micale, V. & Perdichizzi, F. (1994) Further studies on the sexuality of the hermaphroditic teleost *Diplodus sargus*, with particular reference to protandrous sex inversion. *Journal of Fish Biology*, **45**, 661–670.

Micale, V., Maricchiolo, G. & Genovese, L. (2002) The reproductive biology of blackspot sea bream *Pagellus bogaraveo* in captivity. I. gonadal development, maturation and hermaphroditism. *Journal of Applied Ichthyology*, **18**, 172–176.

Micale, V., Perdichizzi, F. & Basciano, G. (1996) Aspects of the reproductive biology of the sharpsnout seabream *Diplodus puntazzo* (Cetti, 1777). I. gametogenesis and gonadal cycle in captivity during the third year of life. *Aquaculture*, **140**, 281–291.

Micale, V., Perdichizzi, F. & Santangelo, G. (1987) The gonadal cycle of captive white bream, *Diplodus sargus* (L.). *Journal of Fish Biology*, **31**, 435–440.

Mihelakakis, A., Yoshimatsu, T. & Tsolkas, C. (2001) Spawning in captivity and early life history of cultured red porgy, *Pagrus pagrus*. *Aquaculture*, **199**, 333–352.

Mollah, M.F.A. & Tan, E.S.P. (1983) Viability of catfish (*Clarias macrocephalus*) eggs fertilized at varying post-ovulation times. *Journal of Fish Biology*, **22**, 563–566.

Mommsen, T.P. & Korsgaard, B. (2008) Vitellogenesis. In: *Fish Reproduction* (eds. M.J. Rocha, A. Arukwe, & B.G. Kapoor), pp. 113–169. Science Publishers, Enfield, NH, USA.

Morato, T., Afonso, P., Lourinho, P., *et al.* (2003) Reproductive biology and recruitment of the white sea bream in the Azores. *Journal of Fish Biology*, **63**, 59–72.

Mouine, N., Francour, P., Ktari, M., *et al.* (2007) The reproductive biology of *Diplodus sargus sargus* in the Gulf of Tunis (central Meditteranean). *Scientia Marina*, **71**, 461–469.

Munro, A.D., Scott, A.P. & Lam, T.J. (1990) *Reproductive Seasonality in Teleosts: Environmental Influences*. CRC Press, Inc., Boca Raton, Florida.

Mylonas, C.C. & Zohar, Y. (2001a) Endocrine regulation and artificial induction of oocyte 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 reproduction in fish. *Reviews in Fish Biology and Fisheries*, **10**, 463–491.

Mylonas, C.C. & Zohar, Y. (2007) Promoting oocyte maturation, ovulation and spawning in farmed fish. In: *The Fish Oocyte: From Basic Studies to Biotechnological Applications* (eds. P.J. Babin, J. Cerdá, & E. Lubzens), pp. 433–470. Kluwer Academic Publishers, Dordrecht, The Netherlands.

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

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

Mylonas, C.C., Papadaki, M. & Divanach, P. (2003) Seasonal changes in sperm production and quality in the red porgy *Pagrus pagrus* (L.). *Aquaculture Research*, **34**, 1161–1170.

Mylonas, C.C., Papadaki, M., Pavlidis, M., *et al.* (2004) Evaluation of egg production and quality in the Mediterranean red porgy (*Pagrus pagrus*) during two consecutive spawning seasons. *Aquaculture*, **232**, 637–649.

Mylonas, C.C., Fostier, A. & Zanuy, S. (2009) Broodstock management and hormonal manipulations of reproduction. *General and Comparative Endocrinology*, **165**, 516–534.

Nabissi, M., Pati, D., Polzonetti-Magni, A., *et al.* (1997) Presence and activity of compounds with GnRH-like activity in the ovary of seabream *Sparus aurata*. *American Journal of Physiology*, **272**, R111–R117.

Nagahama, Y. (1994) Endocrine regulation of gametogenesis in fish. *International Journal of Developmental Biology*, **38**, 217–229.

Nagahama, Y., Yoshikuni, M., Yamashita, M., *et al.* (1994) Regulation of oocyte maturation in fish. In: *Fish Physiology* (eds. N.M. Sherwood & C.L. Hew), pp. 393–439. Academic Press, San Diego, California.

Navarro, A., Zamorano, M.J., Hildebrandt, S., *et al.* (2009) Estimates of heritabilities and genetic correlations for growth and carcass traits in gilthead seabream (*Sparus auratus* L.), under industrial conditions. *Aquaculture*, **289**, 225–230.

Ohta, H., Yamaguchi, S., Yamaguchi, A., *et al.* (2002b) Biosynthesis of estradiol-17ß in the ovarian follicles of the red seabream *Pagrus major* during vitellogenesis. *Fisheries Science*, **68**, 680–687.

Ohta, K., Yamaguchi, S., Yamaguchi, A., *et al.* (2002a) Biosynthesis of steroids in ovarian follicles of red seabream, *Pagrus major* (Sparidae, Teleostei) during final oocyte maturation and the relative effectiveness of steroid metabolites for germinal vesicle breakdown *in vivo*. *Comparative Biochemistry and Physiology*, **B133**, 45–54.

Okuzawa, K. (2002) Puberty in teleosts. *Fish Physiology and Biochemistry*, **26**, 31–41.

Okuzawa, K. & Kobayashi, M. (1999) Gonadotropin-releasing hormone neuronal system in the teleostean brain and functional significance. In: *Neural Regulation in the Vertebrate Endocrine System* (eds. P.D. Rao & R.E. Peter), pp. 85–100. Kluwer Academic/Plenum Publisher, New York, NY.

Okuzawa, K., Amno, M., Aida, K., *et al.* (1993) Chromatographic and immunological identification of gonadotropin-releasing hormone in five marine teleosts. *Fish Physiology and Biochemistry*, **12**, 337–345.

Okuzawa, K., Araki, K., Tanaka, H., *et al.* (1994) Molecular cloning of a cDNA encoding the prepro-salmon gonadotropin-releasing hormone of the red seabream. *General and Comparative Endocrinology*, **96**, 234–242.

Okuzawa, K., Granneman, J., Bogerd, J. *et al.* (1997) Distinct expression of GnRH genes in the red seabream brain. *Fish Physiology and Biochemistry*, **17**, 71–79.

Okuzawa, K., Kumakura, N., Mori, A., *et al.* (2002) Regulation of GnRH and its receptor in a teleost red seabream. In: *Progress in Brain Research* (ed. I.S. Parphar), pp. 95–110. Elsevier Science, Tokyo.

Okuzawa, K., Gen, K., Bruysters, M., *et al.* (2003a) Seasonal variation of the three native gonadotropin-releasing hormone messenger ribonucleic acids levels in the brain of female red seabream. *General and Comparative Endocrinology*, **130**, 324–332.

Okuzawa, K., Kumakura, N., Gen, K., *et al.* (2003b) Effects of high water temperature on brain-pituitary-gonad axis of the red seabream during its spawning season. *Fish Physiology and Biochemistry*, **28**, 439–440.

Orsi Relini, L. & Fida, B. (1992) Note di biologia di *Pagellus bogaraveo* in Mar Ligure. *Oebalia*, **17**(Supplement), 81–86.

Pagelson, G. & Zohar, Y. (1992) Characterization of gonadotropin-releasing hormone binding to pituitary receptors in the gilthead seabream (*Sparus aurata*). *Biology of Reproduction*, **47**, 1004–1008.

Pajuelo, G.C. & Lorenzo, J.M. (1996) Life history of the red porgy *Pagrus pagrus* (Teleostei: Sparidae) of the Canary Islands, central east Atlantic. *Fisheries Research*, **28**, 163–177.

Pajuelo, J.G. & Lorenzo, J.M. (1998) Population biology of the common pandora *Pagellus erythrinus* (Pisces: Sparidae) off the Canary Islands. *Fisheries Research*, **36**, 75–86.

Pajuelo, J.G., Lorenzo, J.M. & Domínguez-Seoane, R. (2008) Gonadal development and spawning cycle in the digynic hermaphrodite sharpsnout seabream *Diplodus puntazzo* (Sparidae) off the Canary Islands, northwest of Africa. *Journal of Applied Ichthyology*, **24**, 68–76.

Palstra, A.P., Cohen, E.G.H., Niemantsverdriet, P.R.W., *et al.* (2005) Artificial maturation and reproduction of European silver eel: development of oocytes during final maturation. *Aquaculture*, **249**, 533–547.

Pandian, T.J. & Sheela, S.G. (1995) Hormonal induction of sex reversal in fish. *Aquaculture*, **138**, 1–22.

Pankhurst, N.W. (1994) Effects of gonadotropin releasing hormone analogue, human chorionic gonadotropin and gonadal steroids on milt volume in the New Zealand snapper, *Pagrus auratus* (Sparidae). *Aquaculture*, **125**, 185–197.

Pankhurst, N.W. (1998) Reproduction. In: *Biology of Farmed Fish* (eds. K. Black & A.D. Pickering), pp. 1–26. Sheffield Academic Press, Sheffield.

Pankhurst, N.W. (2008) Gonadal steroids: functions and patterns of change. In: *Fish Reproduction* (eds. M.J. Rocha, A. Arukwe, & B.G. Kapoor), pp. 67–111. Science Publishers, Enfield, NH, USA.

Pankhurst, N.W. & Carragher, J.F. (1992) Oocyte maturation and changes in plasma steroid levels in snapper *Pagrus* (= *Chrysophrys*) *auratus* (Sparidae) following treatment with human chorionic gonadotropin. *Aquaculture*, **101**, 337–347.

Pankhurst, N.W. & Sharples, D.F. (1992) Effects of capture and confinement on plasma cortisol levels in the snapper *Pagrus auratus*. *Australian Journal of Marine and Freshwater Research*, **43**, 345–356.

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

Papadaki, M., Fakriadis, G. & Mylonas, C.C. (in review) Sex differentiation and hermaphroditism of sharpsnout seabream (*Diplodus puntazzo*) in captivity. *Aquaculture*, **000**, 000–000.

Papadaki, M., Papadopoulou, M., Sigelaki, I., *et al.* (2008) Egg and sperm production and quality of sharpsnout sea bream (*Diplodus puntazzo*) in captivity. *Aquaculture*, **276**, 187–197.

Pasqualli, A. (1941) Contribuo allo studio dell'ermafroditismo e del differenziamento della gonado nell'orata (*Sparus auratus*). *Pubbl Staz Zool Napoli*, **18**, 282–312.

Patino, R. & Kagawa, H. (1999) Regulation of gap junctions and oocyte maturational competence by gonadotropin and insulin-like growth factor-I in ovarian follicles of red seabream. *General and Comparative Endocrinology*, **115**, 454–462.

Patiño, R., Yoshizaki, G., Thomas, P., *et al.* (2001) Gonadotropic control of ovarian follicle maturation: the two-stage concept and its mechanisms. *Comparative Biochemistry and Physiology*, **B129**, 427–439.

Pavlidis, M. (2000) Recent advances in reproductional aspects of *Dentex dentex*. *Cahiers Options Méditerranéennes*, **47**, 169–175.

Pavlidis, M., Keravec, L., Greenwood, L., *et al.* (2001) Reproductive performance of common dentex, *Dentex dentex*, broodstock held under different photoperiod and constant temperature conditions. *Fish Physiology and Biochemistry*, **25**, 171–180.

Peleteiro, J.B., Olmedo, M. & Alvarez-Blázquez, B. (2001) Culture of *Pagellus bogaraveo*: present knowledge, problems and perspectives. *Cahiers Options Méditerranéenes*, **47**, 141–151.

Peter, R.E. & Yu, K.L. (1997) Neuroendocrine regulation of ovulation in fishes: basic and applied aspects. *Reviews in Fish Biology and Fisheries*, **7**, 173–197.

Piferrer, F. (2001) Endocrine sex control strategies for the feminization of teleost fish. *Aquaculture*, **197**, 229–281.

Planas, J.V. & Swanson, P. (2008) Physiological function of gonadotropins in fish. In: *Fish Reproduction* (eds. M.J. Rocha, A. Arukwe, & B.G. Kapoor), pp. 37–66. Science Publishers, Enfield, NH, USA.

Polzonetti-Magni, A., Mosconi, G., Soverchia, L., *et al.* (2004) Multihormonal control of vitellogenesis in lower vertebrates. *International Review of Cytology*, **239**, 1–45.

Powell, J.F.F., Zohar, Y., Elizur, A., *et al.* (1994) Three forms of gonadotropin-releasing hormone characterized from brain of one species. *Proceedings of the National Academy of Sciences*, **91**, 12081–12085.

Rainis, S., Mylonas, C.C., Kyriakou, Y., *et al.* (2003) Enhancement of spermiation in European sea bass (*Dicentrarchus labrax*) at the end of the reproductive season using GnRHa implants. *Aquaculture*, **219**, 873–890.

Reinboth, R. (1962) Morphologische und funktionelle Zweigeschlechtlichkeit bei marinen Teleostiern (Serranidae, Sparidae, Centracanthidae, Labridae). *Zool. Jb. (Physiol.)*, **69**, 405–480.

Rosenfeld, H., Meiri, I. & Elizur, A. (2007) Gonadotropin regulation of oocyte development. In: *The Fish Oocyte: from Basic Studies to Biotechnological Applications* (eds. P.J. Babin, J. Cerdá, & E. Lubzens), pp. 171–198. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Rueda, F.M. & Martínez, F.J. (2001) A review on the biology and potential aquaculture of *Dentex dentex*. *Reviews in Fish Biology and Fisheries*, **11**, 57–70.

Sadovy, Y. & Liu, M. (2008) Functional hermaphroditism in teleosts. *Fish and Fisheries*, **9**, 1–43.

Sadovy, Y. & Shapiro, D. (1987) Criteria for the diagnosis of hermaphroditism in fishes. *Copeia*, **1**, 136–156.

Santos, M.N., Monteiro, C.C. & Erzini, K. (1995) Aspects of the biology and gillnet selectivity of the axillary seabream (*Pagellus acarne*, Risso) and common pandora (*Pagellus erythrinu*s, Linnaeus) from the Algarve (south Portugal). *Fisheries Research*, **23**, 223–236.

Sawaguchi, S., Kagawa, H., Ohkubo, N., *et al.* (2006) 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 spawning pelagic eggs. *Molecular Reproduction and Development*, **73**, 719–736.

Schulz, R.W. & Miura, T. (2002) Spermatogenesis and its endocrine regulation. *Fish Physiology and Biochemistry*, **26**, 43–56.

Scott, S.G. & Pankhurst, N.W. (1992) Interannual variation in the reproductive cycle of the New Zealand snapper *Pagrus pagrus* (Bloch & Schneider)(Sparidae). *Journal of Fish Biology*, **41**, 685–696.

Scott, S.G., Zeldis, J.R. & Pankhurst, N.W. (1993) Evidence of daily spawning in natural populations of the New Zealand snapper *Pagrus auratus* (Sparidae). *Environmental Biology of Fishes*, **36**, 149–156.

Senthilkumaran, B., Okuzawa, K., Gen, K., *et al.* (1999) Distribution and seasonal variations in levels of three native GnRHs in the brain and pituitary of perciform fish. *Journal of Neuroendocrinology*, **11**, 181–186.

Sheaves, M. (2006) Is the timing of spawning in sparid fishes a response to see temperature regimes? *Coral Reefs*, **25**, 655–669.

Shiraishi, T., Ohta, K., Yamaguchi, A., *et al.* (2005) Reproductive parameters of the chub mackerel *Scomber japonicus* estimated from human chorionic gonadotropin-induced final oocyte maturation and ovulation in captivity. *Fisheries Science*, **71**, 531–542.

Smith, P.J. (1986) Spawning behaviour of snapper *Chrysophrys auratus*, in captivity (Note). *New Zealand Journal of Marine and Freshwater Research*, **20**, 513–515.

Somarakis, S. & Machias, A. (2002) Age, growth and bathymetric distribution of red pandora (*Pagellus erythrinus*) on the Cretan shelf (eastern Mediterranean). *Journal of the Marine Biological Association (United Kingdom)*, **82**, 149–160.

Stacey, N. & Sorensen, P.W. (2008) Hormonally derived sex pheromones in fish. In: *Fish Reproduction* (eds. M.J. Rocha, A. Arukwe, & B.G. Kapoor), pp. 201–243. Science Publishers, Enfield, NH, USA.

Tanaka, H., Kagawa, H., Okuzawa, H., *et al.* (1993) Purification of gonadotropins (PmGTH I and II) from red seabream (*Pagrus major*) and development of a homologous radioimmunoassay for PmGTH II. *Fish Physiology and Biochemistry*, **10**, 409–418.

Tanaka, H., Kagawa, H., & Hirose, K. (1995) Steroidogenic activities of two distinct gonadotropins in red seabream, *Pagrus major*. In: Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish (eds F.W. Goetz & P. Thomas). University of Texas at Austin, pp. 10–12.

Tandler, A., Harel, M., Koven, W.M., *et al.* (1995) Broodstock and larvae nutrition in gilthead seabream *Sparus aurata*—new findings on its mode of involvement in improving growth, survival and swimbladder inflation. *The Israeli Journal of Aquaculture-Bamidgeh*, **47**, 95–111.

Tyler, J.R. & Sumpter, J.P. (1996) Oocyte growth and development in teleosts. *Reviews in Fish Biology and Fisheries*, **6**, 287–318.

Valdéz, P., García Alcázar, A., Abdel, I., *et al.* (2004) Seasonal changes on gonadosomatic index and maturaiton stages in common pandora *Pagellus erythrinus* (L.). *Aquaculture International*, **12**, 333–343.

Van Ginneken, V.J.T. & Maes, G.E. (2005) The European eel (*Anguilla anguilla*, Linnaeus), its lifecycle, evolution and reproduction: a literature review. *Reviews in Fish Biology and Fisheries*, **15**, 367–398.

Vassilopoulou, V. & Papaconstantinou, C. (1992) Age, growth and mortality of the red porgy, *Pagrus pagrus*, in the Eastern Mediterranean Sea (Dodecanese, Greece). *Vie Milieu*, **42**, 51–55.

Vaughan, D.S., Huntsman, G.R., Manooch, C.S., *et al.* (1992) Population characteristics of the red porgy, *Pagrus pagrus*, stock off the Carolinas. *Bulletin of Marine Science*, **50**, 1–20.

Ventling, A.R. & Pankhurst, N.W. (1995) Effects of gonadal steroids and human chorionic gonadotrophin on final oocyte maturation *in vitro* in the New Zealand snapper *Pagrus auratus* (Sparidae). *Marine and Freshwater Research*, **46**, 467–473.

Vizziano, D., Fostier, A., Loir, M., *et al.* (2008) Testis development, its hormonal regulation and spermiation induction in teleost fish. In: *Fish Spermatology* (eds. S.M.H. Alavi, J. Cosson, K. Coward, & G. Rafiee), pp. 103–139. Alpha Science Intl, Oxford (UK).

Wallace, R.A. & Selman, K. (1981) Cellular and dynamic aspects of oocyte growth in teleosts. *American Zoologist*, **21**, 325–343.

Watanabe, T. & Kiron, V. (1995) Red sea bream (*Pagrus major*). In: *Broodstock Management and Egg and Larval Quality* (eds. N.R. Bromage & R.J. Roberts), pp. 398–413. Blackwell Publishing Ltd., Oxford, UK.

Watanabe, T. & Vassallo-Agius, R. (2003) Broodstock nutrition research on marine finfish in Japan. *Aquaculture*, **227**, 35–61.

Wong, T.T. & Zohar, Y. (2004a) The involvement of gonadotropin receptors in sex reversal: expression, distribution and regulation of gonadal FSH and LH receptors in the gilthead seabream. *Fish Physiology and Biochemistry*, **28**, 179–180.

Wong, T.T. & Zohar, Y. (2004b) Novel expression of gonadotropin subunit genes in oocytes of the gilthead seabream (*Sparus aurata*). *Endocrinology*, **145**, 5210–5220.

Wong, T.T., Gothilf, Y., Zmora, N., *et al.* (2004) Developmental expression of three forms of gonadotropin-releasing hormone and ontogeny of the hypothalamic-pituitary-gonadal axis in gilthead seabream (*Sparus aurata*). *Biology of Reproduction*, **71**, 1026–1035.

Wong, T.T., Ijiri, S. & Zohar, Y. (2006) Molecular biology of ovarian aromatase in sex reversal: complementary DNA and 5′-flanking region isolation and differential expression of ovarian aromatase in the gilthead seabream (*Sparus aurata*). *Biology of Reproduction*, **74**, 857–864.

Yamaguchi, S., Gen, K., Okuzawa, K., *et al.* (2003) Regulation of gonadotropin subunit genes expression by 11-ketotestoterone during early spermatogenesis in male red seabream, *Pagrus major*. *Fish Physiology and Biochemistry*, **28**, 111–112.

Yamaguchi, S., Gen, K., Okuzawa, K., *et al.* (2005) Effects of 11-ketotestosterone and gonadotropin-releasing hormone on follicle-stimulating hormone and luteinizing hormone gene expression in castrated and sham-operated male red seabream *Pagrus major*. *Fisheries Science*, **71**, 1049–1058.

Yamaguchi, S., Gen, K., Okuzawa, K., *et al.* (2006) Influence of estradiol-17b, testosterone, and 11-ketotestosterone on testicular development, serum steroid hormone, and gonadotropin secretion in male red sea bream *Pagrus major*. *Fisheries Science*, **72**, 835–845.

Yaron, Z., Gur, G., Melamed, P., *et al.* (2003) Regulation of fish gonadotropins. *International Review of Cytology*, **225**, 131–185.

Yu, K.L., Lin, X.W., Da Cunha Bastos, J., *et al.* (1997) Neural regulation of GnRH in teleost fishes. In: *GnRH Neurons: Gene to Behavior* (eds. I.S. Parhar & Y. Sakuma), pp. 277–312. Brain Shuppan, Tokyo.

Yueh, W.S. & Chang, C.F. (1997) 17α, 20ß,21-trihydroxy-4-pregnen-3-one and 17α, 20ß-dihydroxy-4-pregnen-3-one stimulated spermiation in protandrous black progy, *Acanthopagrus schlegeli*. *Fish Physiology and Biochemistry*, **17**, 187–193.

Zohar, Y. (1989a) Endocrinology and fish farming: Aspects in reproduction, growth and smoltification. *Fish Physiology and Biochemistry*, **7**, 395–405.

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

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

Zohar, Y. & Mylonas, C.C. (2001) Endocrine manipulations of spawning in cultured fish: from hormones to genes. *Aquaculture*, **197**, 99–136.

Zohar, Y., Abraham, M. & Gordin, H. (1978) The gonadal cycle of the captivity-reared hermaphroditic teleost *Sparus aurata* (L.) during the first two years of life. *Annales de Biologie Animale, Biochimie, Biophysique*, **18**, 877–882.

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

Zohar, Y., Pagelson, G. & Tosky, M. (1988) Daily changes in reproductive hormone levels in the female gilthead seabream *Sparus aurata* at the spawning period. In: *Reproduction in Fish: Basic and Applied Aspects in Endocrinology and Genetics* (eds. Y. Zohar & B. Breton), pp. 119–125. INRA Press, Paris.

Zohar, Y., Goren, A., Tosky, M., *et al.* (1989) The bioactivity of gonadotropin-releasing hormones and its regulation in the gilthead seabream, *Sparus aurata: in vivo* and *in vitro* studies. *Fish Physiology and Biochemistry*, **7**, 59–67.

Zohar, Y., Goren, A., Fridkin, M., *et al.* (1990a) Degradation of gonadotropin-releasing hormones in the gilthead seabream *Sparus aurata* II. Cleavage of native salmon GnRH, mammalian LHRH and their analogs in the pituitary, kidney and liver. *General and Comparative Endocrinology*, **79**, 306–319.

Zohar, Y., Breton, B., Sambroni, E., *et al.* (1990b) Development of a homologous radioimmunoassay for a gonadotropin of the gilthead seabream, *Sparus aurata*. *Aquaculture*, **88**, 189–204.

Zohar, Y., Harel, M., Hassin, S., *et al.* (1995a) Gilthead sea bream (*Sparus aurata*). In: *Broodstock Management and Egg and Larval Quality* (eds. N.R. Bromage & R.J. Roberts), pp. 94–117. Blackwell Publishing Ltd., Oxford, UK.

Zohar, Y., Elizur, A., Sherwood, N.M., *et al.* (1995b) Gonadotropin-releasing activities of the three native forms of gonadotropin-releasing hormone present in the brain of gilthead seabream, *Sparus aurata*. *General and Comparative Endocrinology*, **97**, 289–299.