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Broodstock management and hormonal manipulations of fish reproduction

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

1. Introduction

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

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

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gamete maturation and enable artificial collection in order to implement inter-specific hybridization, chromosome set manipulation or artificial fertilization for genetic selection programmes.

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

2. Gametogenesis and final maturation

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

2.1. Spermatogenesis and spermiation

The gametogenic process in the males is separated into two phases (Fig. 1). Spermatogenesis is the first phase and it includes the proliferation of the spermatogonia, the multiplication of the spermatocytes I with multiple mitotic divisions, the production of spermatocytes II with meiotic division and their differentiation to spermatids (Fig. 2). The process is completed with the production of flagellated spermatozoa, i.e., spermiogenesis (reviewed by Billard, 1986; Schulz and Miura, 2002; Vizziano et al., 2008). The spermatozoa are released in the sperm ducts during the second phase of the male reproductive cycle, i.e., spermiation, which occurs during the spawning season. Sperm is ejaculated spontaneously by the fish and with the exception of catfishes (Mansour et al., 2004; Viveiros et al., 2002), it can also be expressed easily from the testes after application of gentle abdominal pressure (i.e., stripping). Spermiation and ejaculation can be synchronized with female spawning via pheromonal communications (Stacey, 2003).

Spermatogenesis may be continuous in species showing a tubular testis type or discontinuous in species showing a lobular testis type, which is the most frequent among teleostean fish (Billard, 1986; Schulz and Miura, 2002; Vizziano et al., 2008). Also, spermatogenesis and spermiation may be temporally separated and during the spawning season the testes may contain exclusively spermatozoa (Billard, 1986; Malison et al., 1994). In most species, however, there is significant overlap between the two processes, with both spermatogenesis and spermiation taking place during the spawning season (Jackson and Sullivan, 1995; Mylonas et al., 2003a; Rainis et al., 2003), and it has been shown in the gilthead sea bream (*Sparus aurata*) that the spermatogonia and Sertoli cell proliferation activity is not blocked by the presence of spermatozoa (Chaves-Pozo et al., 2005), in contrast to fish with synchronous spermatogenesis.

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

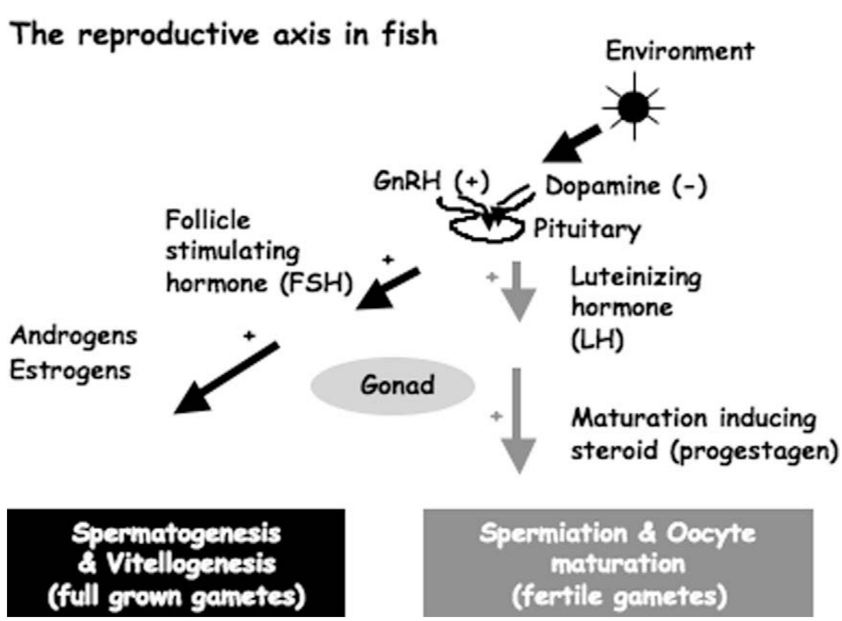

Fig. 1. Schematic representation of the reproductive axis in fish, its major components and phases, and its environmental and endocrine control.