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Mylonas C.

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Hormonal induction of spawning with reference to the bluefin tuna

C. Mylonas
Aquaculture Department, Institute of Marine Biology of Crete,
PO Box 2214, Crete 71003, Greece

SUMMARY – Fish reared in captivity often fail to undergo final oocyte maturation, ovulation and spawning. This failure was shown to be due to lack of luteinizing hormone (LH) release from the pituitary. To ameliorate these problems, it is possible to use synthetic agonists of the gonadotropin-releasing hormone (GnRHa), in order to induce the release of LH and initiate the cascate of events leading to maturation and spawning. This approach will be employed to induce spawning of bluefin tuna (*Thunnus thynnus*) broodstock collected from the capture fisheries in the Mediterranean Sea and maintained in sea cages.

Key words: Tuna, induced spawning, GnRHa, implants.

RESUME – *"Induction hormonale de la ponte chez le thon rouge". Les poissons élevés en captivité souvent ne parviennent pas à la maturation finale des ovocytes, l'ovulation et la ponte. Cet échec s'est avéré être dû au manque d'hormone lutéinisante (LH) sécrétée par la glande pituitaire. Pour résoudre ces problèmes, il est possible d'utiliser des agonistes synthétiques de la GnRHa (gonadotropin-releasing hormone), afin d'induire la sécrétion de LH et d'entamer l'enchaînement des évènements qui mèneront à la maturation et à la ponte. Cette approche sera employée pour induire la ponte chez des reproducteurs de thon rouge (Thunnus thynnus) capturés dans les pêcheries de la mer Méditerranée et maintenus en cages marines.*

Mots-clés : *Thon, ponte induite, GnRHa, implants.*

Almost all fishes reared in captivity exhibit some form of reproductive dysfunction (Zohar and Mylonas, 2001). In females, which are the most seriously affected, there is commonly a failure to undergo final oocyte maturation (FOM), ovulation and spawning; while in males there is reduction of milt quantity or quality. Dysfunctions probably result from the combination of captivity-induced stress (Pankhurst and Van Der Kraak, 1997) and the lack of the appropriate "natural" spawning environment. Reproductive dysfunctions often weaken as consecutive generations of broodstock are being produced from cultured parents, as fish are inadvertently selected for characteristics adaptive to the cultured environment. A good example of such a "domestication" effect is the gilthead seabream (*Sparus aurata*). When the culture of this Mediterranean species began in the early 1970s, the only way a significant number of eggs could be obtained was through exogenous hormone manipulations (Gordin and Zohar, 1978), and even then strip-spawning was required. As time progressed and more generations of captive fish were produced, the reliance of the industry on hormonal manipulations was reduced tremendously. Today, gilthead seabream spawn daily for more than three months during the regular spawning season, and hormonal manipulations are used only for non-responsive fish reared under artificial photothermal conditions (Barbaro *et al.*, 1997). Similarly, early in the development of domestic striped bass (*Morone saxatilis*) broodstocks, all females were found to be arrested at the vitellogenesis stage of ovarian development, and instead of undergoing FOM and spawning, their oocytes became atretic and were reabsorbed (Woods and Sullivan, 1993). In more recent years, female striped bass initiating FOM are observed more often, although hormonal treatments are still employed for the induction and synchronization of spawning (Sullivan *et al.*, 1997; Mylonas and Zohar, 2001).

Spontaneous spawning of bluefin tuna in sea cages has been reported, and eggs successfully collected (Iioka *et al.*, 2000). However, spawning is highly depended on environmental conditions at the site and is successful only when broodstocks are maintained in captivity for more than 4 years. We expect that there will be a need for appropriate tools to induce spawning in wild bluefin tuna recently captured and maintained in sea cages for periods of only a few months to a few years. In preliminary experiments carried out with recently captured migrating bluefin tuna in the Mediterranean

Sea (A. Medina, this volume), it was observed that females contained post-vitellogenic oocytes at the time of capture, but no spawning was observed and at the end of the spawning season the ovaries had undergone atresia.

The failure of cultured fish to spawn is located at the level of the pituitary. Although luteinizing hormone (LH) is produced in the gonadotrophs, there is no release into the blood during the spawning season. Therefore, current hormone-based spawning induction protocols employ super-active agonists of the hypothalamic peptide gonadotropin-releasing hormone (GnRHa). Treatment of mature broodstock during the spawning season with GnRHa induces release of LH from the pituitary, which in turn regulates the necessary changes in steroid hormone production required for maturation and spawning (Zohar and Mylonas, 2001). However, simple injections of GnRHa are often partly effective, unless given repetitively over the course of a few hours or days. Multiple GnRHa treatments are cumbersome and labour intensive in all aquaculture operations, but are entirely prohibitive in a potential bluefin tuna broodstock operation, due to the problems encountered in handling such large and constantly swimming species. Solving the problem of multiple treatments, GnRHa-delivery implants have been developed to release the hormone continuously, resulting in elevated plasma GnRHa for periods of days to weeks (Mylonas and Zohar, 2000). Some of the more spectacular results from GnRHa-implants have been obtained from various fishes with multiple-batch group-syncrhonous or, like the bluefin tuna, with asynchronous ovarian development.

A GnRHa-delivery system will be developed for bluefin tuna, in order to induce maturation and spawning of captured fish. The GnRHa-implant will be manufactured using p[Ethylene-Vinyl acetate], modifying existing methods. These implants have been shown to produce continuous release of GnRHa for periods of 1 to 5 weeks (Mylonas and Zohar, 2000). At the onset of the natural spawning season for bluefin tuna in the Mediterranean, fish maintained in sea cages or transferred to land-based facilities will be treated with the GnRHa-implants. In the cages, implantation will done by SCUBA divers using a jab-stick implanter, which will insert the GnRHa-implant into the fish's muscle. Implantation will be initially done without immobilizing or anaesthetizing the fish, until appropriate methods are developed.

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