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Recent advances in Mediterranean aquaculture finfish species diversification

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## Preliminary data on the reproductive biology and hatchery production of the shi drum (*Umbrina cirrosa*) in Cyprus

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**SUMMARY** – The shi drum (*Umbrina cirrosa*) is a well-known marine species along the Mediterranean coast, where it commands a high market price. Evaluation of the culture potential of this species was initiated in 1993-94 with the collection of juvenile fish from the wild. Fish were reared in tanks supplied with surface seawater, and were fed a mixture of trash fish, squid and industrial sea bream/sea bass feed. Fish did not reproduce spontaneously and the first attempts to hormonally induce spawning were made when the fish were three years old. Injection with gonadotropin-releasing hormone agonist (GnRHa) induced spawning in a few fish, resulting in the production of a small number of fry and the development of an F1 cultured broodstock. In subsequent years, the cultured three-year-old broodstock was treated with sustained-release GnRHa-microspheres, which induced two consecutive tank spawns, with fertilization success reaching 43%. Eggs were stocked in 500-l tanks supplied with water at 23-28°C, phytoplankton was supplied 2 days after hatching, and one day later mixed-size rotifers were provided. Illumination was maintained on a 24-h basis and the tanks were fitted with a surface-oil remover. After day 10-12, artemia or dry feed were also offered along with the rotifers, while weaning on industrial feed was completed by day 33 post-hatching. At days 41 and 203, mean fry weight was 2.0 and 65 g, respectively. Our studies demonstrated that the shi drum has a good survival and growth rate in culture, although natural spawning is still problematic.

**Key words:** *Umbrina cirrosa*, shi drum, reproduction, larvae, aquaculture.

**RESUME** – "Données préliminaires sur la biologie reproductive et la production en écloséries de l'ombrine côtière (*Umbrina cirrosa*) à Chypre". L'ombrine côtière (*Umbrina cirrosa*) est une espèce marine très commune de haute valeur marchande en Méditerranée. L'évaluation de ses potentialités de culture a commencé en 1993-94 avec des juvéniles pêchés en mer. L'élevage a été réalisé en bassins alimentés avec de l'eau de mer superficielle, et une nourriture constituée de poissons, calmars et granulés de type daurade/loup. L'espèce ne pondant pas spontanément, des essais de reproduction artificielle ont été réalisés sur individus de trois ans stimulés avec des implants hormonaux (GnRHa). Ces essais ont donné quelques alevins à l'origine d'un stock de géniteurs. Au bout de 3 ans, ce stock a été traité avec des implants hormonaux (microsphères de GnRHa) à relargage continu, qui ont conduit à deux pontes (à 1 jour d'intervalle) présentant un taux de fécondation moyen de 43%. Les œufs ont été stockés dans des bassins de 500 l alimentés avec de l'eau à 23-28°C sous photopériode de 24 heures. Du phytoplancton a été ajouté deux jours après l'éclosion, des rotifères le troisième jour, des artemia et/ou de la nourriture inerte au bout de 10-12 jours en mélange avec les rotifères. Le sevrage sur aliment inerte était achevé à 33 jours. A 41 et 203 jours, les poids moyens étaient respectivement de 2,0 et 65 g. Ces résultats montrent que l'espèce présente une bonne survie et une bonne croissance, mais que la reproduction reste problématique.

**Mots-clés :** *Umbrina cirrosa*, ombrine côtière, reproduction, élevage larvaire, aquaculture.

### Introduction

The shi drum (*Umbrina cirrosa*, Linnaeus 1758), is a member of the Sciaenidae family, which includes 270 species from around the world (Nelson, 1994), and five in the Mediterranean Sea (Fischer *et al.*, 1987). It has a typical Sciaenid, fusiform body and has marked diagonal gold stripes on a light brown to olive green background. Juveniles can be found in the estuaries, while the adults frequent coastal waters less than 100 m in depth and prefer sandy or rocky bottoms. The shi drum has a subterminal mouth and feeds on fish and benthic invertebrates, but can also consume aquatic vegetation. In the wild, the shi drum has been shown to have a fast growth during the first three years

in life (Fabi and Fiorentini, 1993). Given the concern for the development of a diversified aquaculture production in the Mediterranean region and the high market price of the shi drum, there has been an interest in recent years for the evaluation of this species as a potential candidate for commercial aquaculture (Melotti *et al.*, 1995; Barbaro *et al.*, 1996; Libertini *et al.*, 1998). In Cyprus, efforts at evaluating the aquaculture potential of the shi drum were initiated in 1993 by Aquaculture Technologies Ltd., with the collection of juveniles from the wild. A captive-reared broodstock was then developed at the facility, and in 1996 a small population of the first F1 progeny of the wild fish was transferred to the Marine Aquaculture Research Station of the Cyprus Fisheries Department. This paper describes our collective experiences at controlling the reproduction, and rearing the larvae and fry of the shi drum.

## Materials and methods

Wild-caught juvenile shi drum were acclimatized in tanks supplied with flow-through surface seawater, exposed to a natural photoperiod (latitude 35°N) and thermal regime (Fig. 1), and fed on a mixture of trash fish, squid and industrial feed. The fish consumed industrial pellets readily, although they were not very aggressive in their feeding behavior. Similar to other cultured fishes, squid was a preferred food item, especially for the adult fish. For sampling and manipulation purposes, fish were crowded using a soft-net seine or radial dividers, removed using a dip net and placed into an anaesthetic bath of 0.25 ml/l 2-Phenoxy-ethanol.

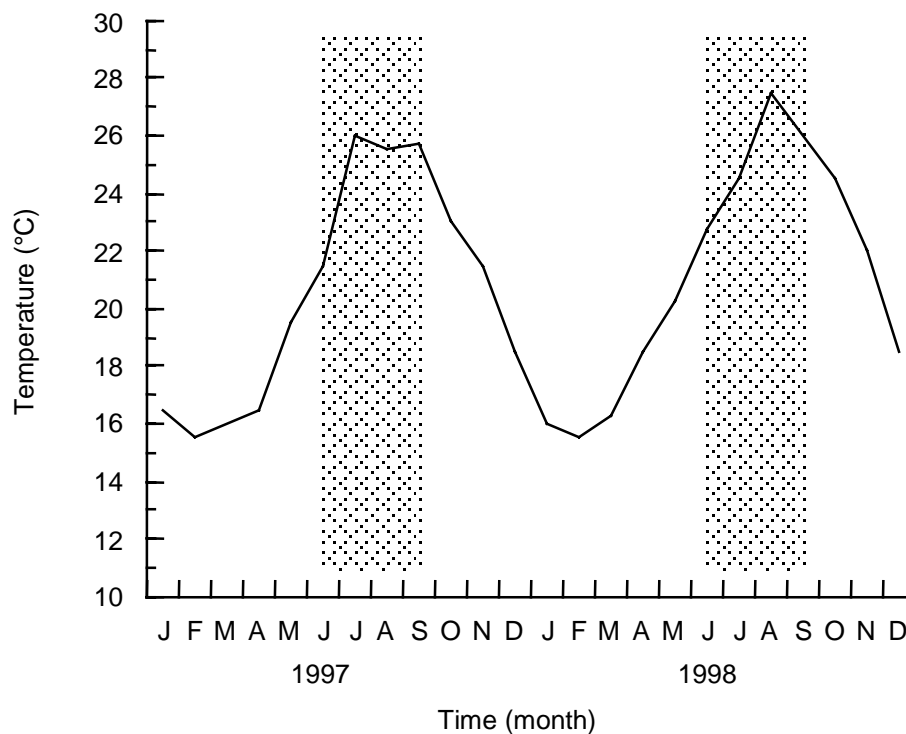


Fig. 1. Annual fluctuation of surface seawater temperature used for the spawning, larval rearing and grow-out of the shi drum (*Umbrina cirrosa*) in Cyprus. Shaded areas indicate the natural spawning season.

## Reproduction

As spontaneous spawning was not observed during the spawning season, our attempts to obtain eggs from the shi drum necessitated the use of hormonal treatments. Three spawning induction trials were undertaken using either injections of a gonadotropin-releasing hormone agonist ([D-Ala<sup>6</sup>-Pro<sup>9</sup>-

NEt]-mGnRHa, GnRHa), or GnRHa-loaded sustained-release microspheres (Mylonas *et al.*, 1995). Female fish were selected based on a gonadal biopsy collected using a plastic or glass catheter, and only fish with maximum oocyte diameter of 500 µm were chosen. Male fish were chosen, if they were expressing milt upon gentle abdominal pressure. Both the GnRHa injections and the GnRHa microspheres were administered intramuscularly. After treatment, fish were placed separately from the rest of the broodstock and were allowed to spawn spontaneously in 5 m<sup>3</sup> outdoor tanks fitted with overflow egg collectors. The tanks were supplied with surface seawater (Fig. 1).

During the first spawning trial on 18 July 1995, wild three-year-old females (mean weight = 1450 g) at the Aquaculture Technologies Ltd. facilities were injected with 5 µg GnRHa/kg, were placed in a separate tank with spermiating males and were allowed to spawn. The same females were re-injected with 5 µg GnRHa/kg after four days, and after nine days were injected again with 15 µg GnRHa/kg, at which time the males were also injected with the same dose of GnRHa. The collected fertilized eggs were stocked in the larval tanks for hatching and rearing.

The second spawning experiment was carried out on 7 July 1997 at the facilities of the Cyprus Fisheries Department, using two-year-old cultured broodstock. These fish were the F1 progeny from the wild fish spawns described above. Nine males and three females were injected with 8 µg GnRHa/kg, put together in an outdoor tank and allowed to spawn. The released eggs were collected daily from the egg collector, and their fertilization success was evaluated under a stereoscope.

The final spawning induction trial was undertaken on 8 July 1998 at the facilities of the Cyprus Fisheries Department using the same broodstock as in the 1997 experiment, which was then three-years-old. Nine females and 12 males were selected from the broodstock population, treated with GnRHa-loaded microspheres at a dose of 40 and 24 µg GnRHa/kg, respectively, and allocated into two spawning tanks. All spawned eggs were collected daily, fertilization success was evaluated, and the eggs were stocked in larval tanks for hatching and rearing.

## Larval rearing

Fertilized eggs were counted (volumetrically) and were placed in 0.5 to 3 m<sup>3</sup> cylindro-conical tanks, where incubation, hatching and larval rearing took place. The tanks were supplied with surface seawater of ambient temperature and were exposed to natural or manipulated photoperiod. Surface skimmers to remove the surface oil film were activated for the first 10 days after hatching. Phytoplankton was supplied to the tanks during the early stages of rearing, and the larvae were offered, progressively, rotifers (*Brachionus plicatilis*), freshly-hatched or enriched artemia nauplii (*Artemia* spp.), and industrial dry feed. Adjustments on the type and quantity of food items offered to the larvae were made daily, based on a visual examination of the feeding behavior of the larvae and the content of their digestive tract. After the nursery phase, fish were stocked in circular, flat-bottom tanks for grow-out and their growth was evaluated periodically. Another population of 70,000 fry were moved to an off-shore facility for trials of their adaptation to cage culture, and for growth evaluations.

## Results

### Reproduction

All males were reproductively mature at two years of age and produced small amounts of expressible milt during the spawning season (June-August), while a very small number of males produced expressible milt as one-year-old at a size of 200 g. On the other hand, only a small percentage of two-year-old females had well developed ovaries with vitellogenic oocytes. Ovarian examination suggested that female gonadal development is of the group-synchronous type (Tyler and Sumpter, 1996), with multiple batches of oocytes recruited from a vitellogenic pool and undergoing final oocyte maturation (FOM) at different times during the reproductive season. There was no sexual dimorphism in this species, but there was a noticeable size difference between the two sexes, which increased with age (Fig. 2). At two years of age, mean ( $\pm$  standard deviation) total length and body weight, respectively, of males were 38  $\pm$  2 cm and 654  $\pm$  102 g, and those of females were 39  $\pm$  1 cm and 715  $\pm$  79 g. At three years of age, mean total length and body weight, respectively, of males were 45  $\pm$  3 cm and 1089  $\pm$  190 g, and those of females were 48  $\pm$  3 cm and 1433  $\pm$  212 g.

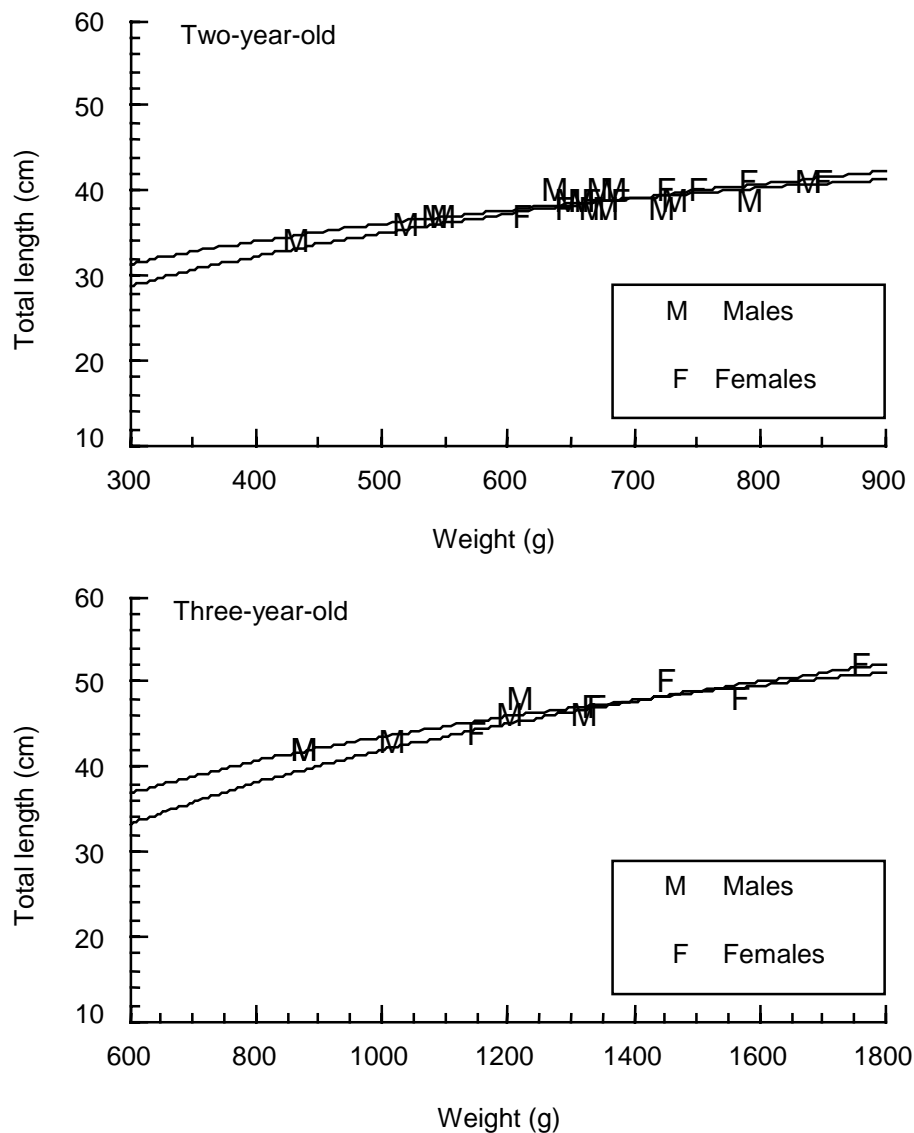


Fig. 2. Length and weight relationship of cultured shi drum (*Umbrina cirrosa*) at the beginning of the spawning season (July 1997 and 1998), maintained at the Cyprus Fisheries Department, Marine Aquaculture Research Station.

On the first spawning experiment, a small number of dead, infertile eggs was collected two days after each injection of GnRH $\alpha$ . After the third injection, a small batch of fertilized eggs was collected and was stocked in the larval rearing tanks for incubation. This was the first F1 progeny produced from wild, captive-reared shi drum, and was used for the development of a larger cultured broodstock.

During the second spawning experiment using two-year-old cultured fish, only three females were found to have vitellogenic oocytes of a large enough diameter, thus making them eligible for hormonal induction. Even these females, however, had a relatively small number of such large vitellogenic oocytes in their ovary during the spawning season (data not shown). After GnRH $\alpha$  injection, a small volume of dead, infertile eggs was released daily from day 14 to 42. Then, more than six weeks after the GnRH $\alpha$  injections, fish spawned 7 to 18 ml of fertilized eggs on three consecutive days.

At the final spawning induction trial, spawning started two days later and continued for three days, although the last spawn produced only infertile eggs. Assuming that all females in each tank participated in spawning, fish produced between 10 and 25 ml of eggs per kg of body weight, with fertilization success during the first two spawns ranging between 20 and 43% (Fig. 3). A very small amount (<1 ml) of infertile eggs was collected for a few days from the large holding tank where the remaining of the broodstock was kept.

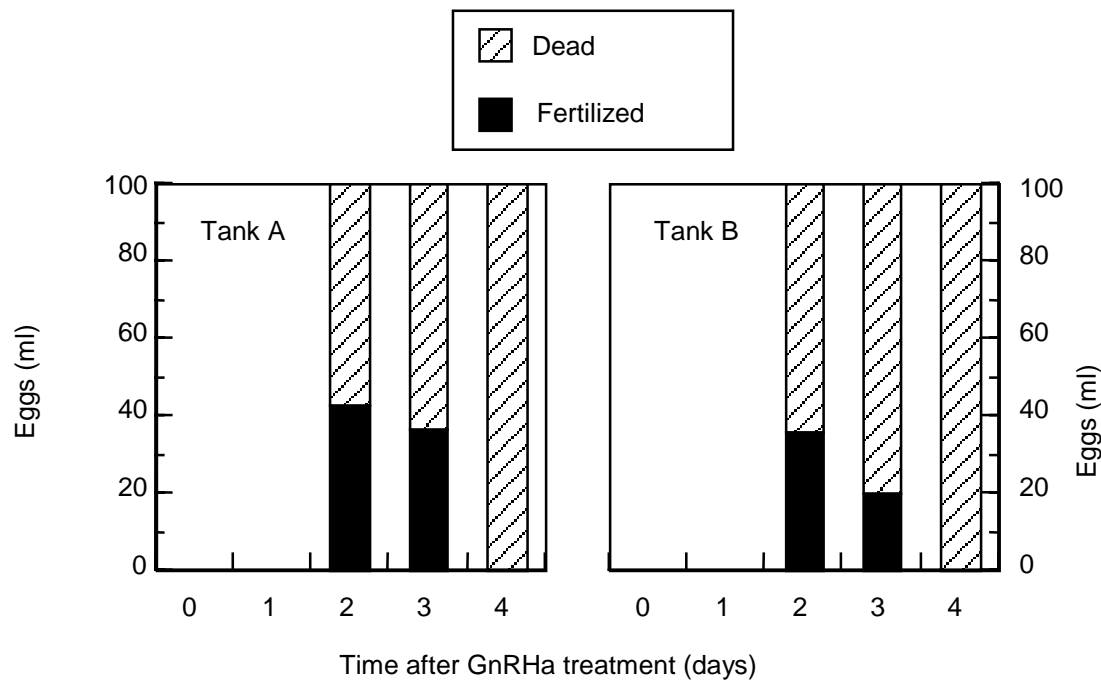


Fig. 3. Fertilization percentage of cultured shi drum (*Umbrina cirrosa*) maintained at the Cyprus Fisheries Department, Marine Aquaculture Research Station and induced to spawn using GnRHa-loaded microspheres (8 July 1998).

### Larval rearing

Hydrated eggs had an average size of 780  $\mu\text{m}$  and hatched in two days at 24-26°C. At hatching, shi drum larvae have a likeness to sparid larvae and exhibit a similar behavior. Two days after hatching, the mouth which is terminal at this stage, was large and well-developed, and the larvae were ready to feed. Between days 5 and 8, swimming activity slowed down and the larvae aggregated at the surface. Swim-bladder inflation took place between days 3 and 8. After day 13, larvae began to darken and by day 18 became benthic and swam actively around the tank. The mouth became subterminal after day 24 and the larvae were feeding exclusively on industrial feed after day 31-33. Throughout the larval rearing period, shi drum were not distributed uniformly in the tank, but tended to aggregate on the surface or bottom of the tank, depending on stage of development. Cannibalism was not observed during the larval rearing phase, even though size-grading was not done.

The various feeding protocols followed are shown in Fig. 4. Phytoplankton (*Chlorella* spp., *Tetraselmis* spp. or *Nanochloris* spp.) was added to the rearing tanks two days after hatching, in order to develop a pseudo-green water, and enriched rotifers of mixed size were supplied as food items at a density of 4 individuals/ml. In the protocol used at the Cyprus Fisheries Department, phytoplankton and rotifers were provided for a relatively long period of time (36 days post-hatching), while artemia was not offered at all. In the Aquaculture Technologies Ltd. protocol, artemia was offered between days 11 and 40. In both hatcheries, dry feed was offered at an early stage since the shi drum accepts readily industrial feed.

Shi drum growth was very fast and the larvae reached a total length of 25 mm in 17 days, and a weight of 2 g in 41 days (Fig. 5a and b). A fast rate of growth was continued during the nursery and grow-out phase, even though the fish were maintained in tanks (Fig. 5b and c). One year after hatching, shi drum had an average weight of 250 g and two years after hatching had a weight of 650 g (Fig. 2). Feed conversion efficiency increased from a value of 1.0 when the fish were 15 g, to 2.1 when they were 233 g in weight (Fig. 5c). The fish were very docile and easily crowded and netted, without much struggling. Although some scale loss occurred during handling and grading of fry and juveniles, non-anaesthetized shi drum could be graded, weighed and transported without many post-handling mortalities. On the other hand, significant mortalities occurred after anaesthesia of fry with 2-Phenoxy-ethanol (data not shown). There was a delayed onset of anaesthesia, and when the fish

recovered they appeared to be suffocating and trying to gulp air from the surface. The occurrence of skeletal deformities was low, the most prevalent of which being a "twisted" mouth. However, this abnormality did not appear to interfere with feeding or damage the appearance and, hence, commercial value of the fish. A problem with appearance, however, is posed by the extreme frailty of the fin rays, which resulted in a partial to complete loss of the dorsal and caudal fins.

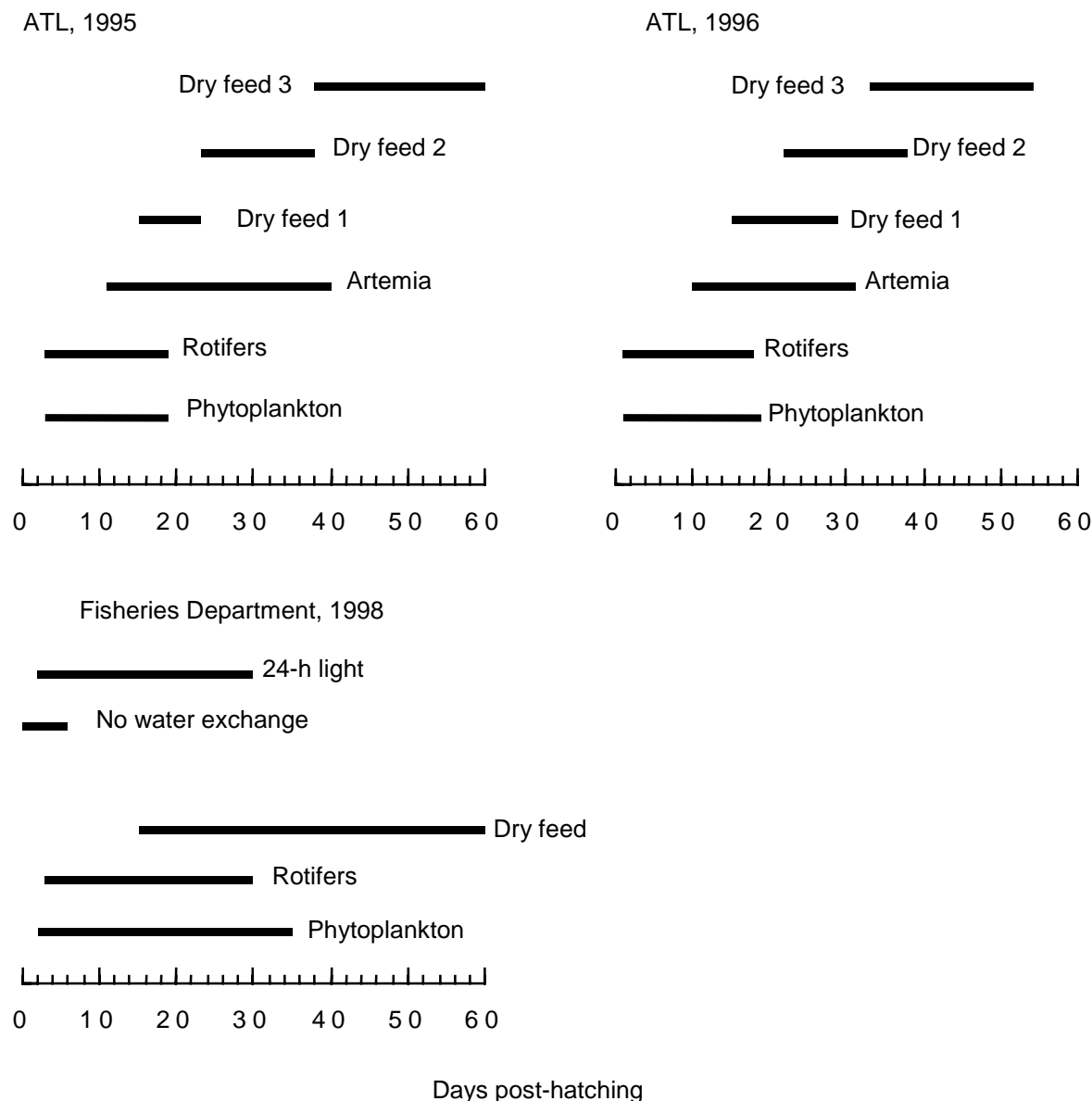


Fig. 4. Feeding scheme of shi drum (*Umbrina cirrosa*) larvae at the hatcheries of Aquaculture Technologies Ltd. (ATL) and the Cyprus Fisheries Department, Marine Aquaculture Research Station at different years. Fish were exposed to a natural thermal regime using ambient surface seawater (Fig. 1).

Shi drum fry adapted well to the sea cage and fed actively when offered extruded, sinking feed. The fish continued to occupy mostly the sides and bottom of the cage, and moved to the center only when feed was offered. Unfortunately, this cage-distribution behavior of the fish proved detrimental in the exposed and highly turbulent waters off the coast of Cyprus. During storms, the young fish which frequented the vicinity of the cage net, became abraded on its surface, resulting in heavy losses during the following days. As a result, the fry were moved to a smaller cage inside the service harbor



and the growth monitoring experiment was discontinued. Therefore, data on the growth of the shi drum to market size were obtained only from tank-reared fish.

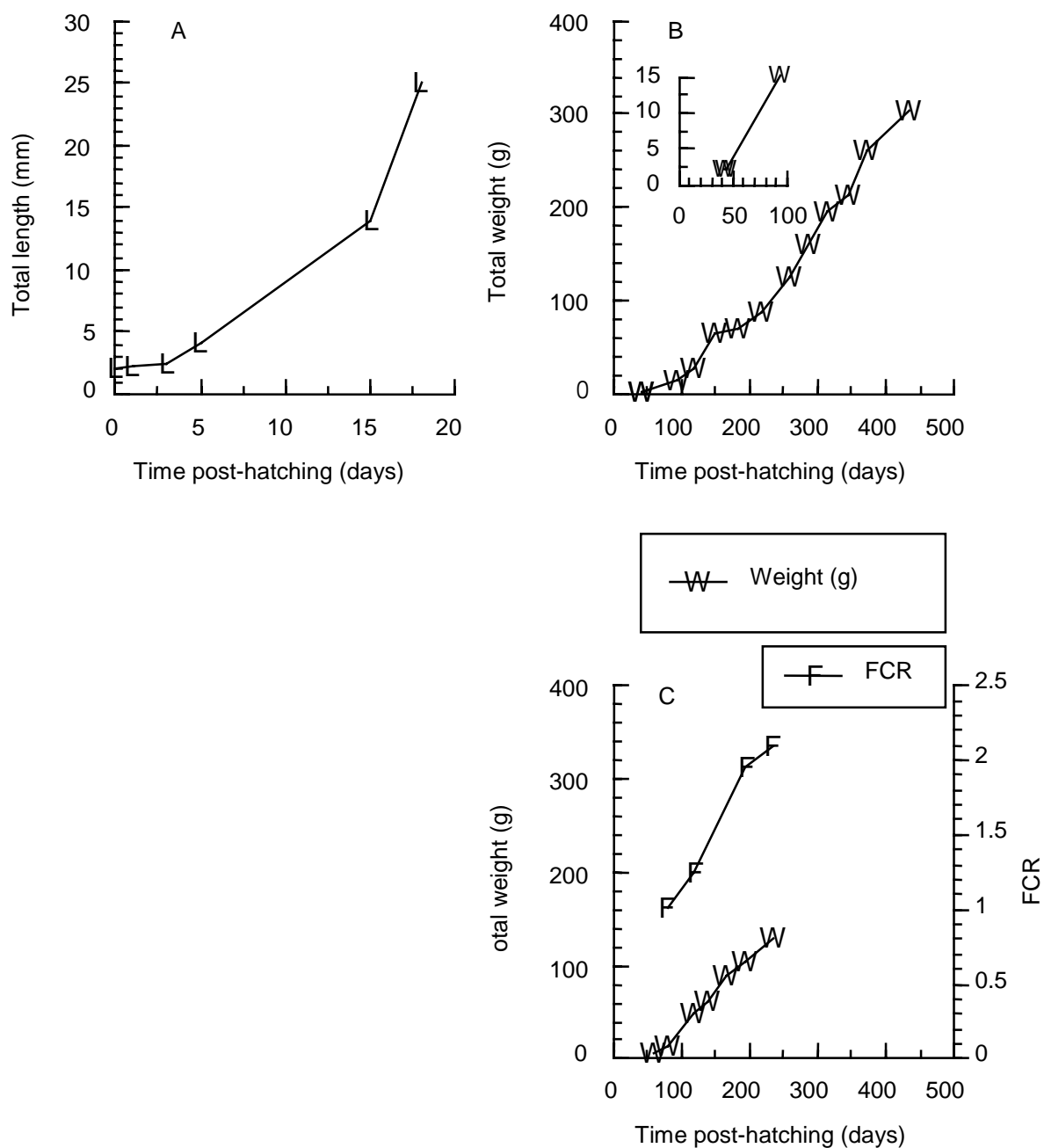


Fig. 5. Growth performance of shi drum (*Umbrina cirrosa*) during the larval rearing and grow-out phase at the Aquaculture Technologies Ltd. facilities. Mean length (A) and weight (B) increase during the 1995 season, and mean weight increase and feed conversion ratio (FCR) during the 1996 season (C). Fish were exposed to a natural thermal regime using ambient surface water (Fig. 1), and once weaned were fed on an industrial dry diet.

## Discussion

The shi drum was a relatively easy fish to acclimatize to tank-rearing conditions, and accepted dry industrialized feed readily. Although nothing is yet known on the nutritional requirements of this



species and the fish were reared mainly on a sea bream/sea bass diet, growth was fast and there was no sign of nutritional deficiency, i.e. skeletal deformations, behavioral abnormalities, sensitivity to stress or susceptibility to diseases. The problem with partial loss of the dorsal and caudal fins may be due to a high stocking density, or intrinsic water quality problems of one of our facilities. We observed that transfer of affected individuals to larger tanks in other facilities resulted in partial restoration of the fin rays. This problem should be a point of concern, however, since even mild deformations of this sort can have a proportionally high impact on the commercial value of the fish.

As reported for wild shi drum (Fischer *et al.*, 1987) first reproductive maturity may occur at two years of age. However, some males can mature precociously at one year of age, as reported for other species (Holland *et al.*, 1996), while most females mature at three years of age. Although females do initiate reproductive development during their second year of life, vitellogenesis does not appear to be completed and the oocytes do not reach their full size. This phenomenon of the first reproductive cycle of the females being an incomplete one has been described also in other fishes, and is referred to as the "dummy run" (Holland, 1999). In the striped bass (*Morone saxatilis*), this "dummy run" cycle is characterized by very low plasma levels of  $17\beta$ -estradiol, and absence of vitellogenin from the blood, as well as the developing oocytes. Hormonal measurements in the plasma or histological evaluations of the developing oocytes were not done in the present study, so it is not known whether the same situation exists in the shi drum. In terms of the fact that male shi drum mature at least one year earlier than females, this is a common phenomenon in the reproductive biology of gonochoristic fishes. Male shi drum produced relatively small volumes of expressible milt during the spawning season, which can be one of the explanations for the low fertilization success. Many fish species exhibit reduced milt volume when reared in captivity (Suquet *et al.*, 1992; Clearwater and Crim, 1998) and although GnRH $\alpha$  treatments have been shown to enhance milt production (Garcia, 1993; Sorbera *et al.*, 1996; Mylonas *et al.*, 1997a), further studies are needed to establish whether GnRH $\alpha$  treatments can enhance spermiation in the shi drum.

Spontaneous spawning was rear in the shi drum, which exhibited the most commonly observed reproductive dysfunction in cultured marine species, namely unpredictable occurrence or absence of FOM, and hence ovulation or spawning. Some examples of species which have, or had at some stage of their domestication, this problem include the gilthead sea bream (*Sparus aurata*) (Zohar *et al.*, 1995), various flatfishes (Berlinsky *et al.*, 1996; Larsson *et al.*, 1997) and many members of the Serranidae family (Tucker, 1994). Studies in the gilthead sea bream and striped bass showed that the failure of captive fish to undergo FOM in captivity is due to the absence of pituitary gonadotropin (GtH) II release during the spawning season, (Zohar, 1988; Mylonas *et al.*, 1997b, 1998) even though GtH II accumulates continually in the pituitary during oogenesis. Fish exhibiting this type of dysfunction undergo normal vitellogenesis, but with the onset of the spawning season the developing oocytes fail to initiate FOM; instead they undergo atresia. Treatment with exogenous GnRH $\alpha$  at the completion of vitellogenesis stimulates gonadal steroidogenesis, FOM and ovulation. A single GnRH $\alpha$  injection did not induce ovulation reliably in the shi drum, while GnRH $\alpha$ -loaded microspheres, which release GnRH $\alpha$  for many weeks, were successful in inducing ovulation and spawning of viable eggs on three consecutive days. Most probably, the failure of the single injection protocol was due to the short residence time of GnRH $\alpha$  in circulation, which ranges from a few hours to a few days depending on the GnRH $\alpha$  initial dose, fish species and water temperature (Crim *et al.*, 1988; Zohar *et al.*, 1995). The greater effectiveness, compared to a single GnRH $\alpha$  injection, of another type of GnRH $\alpha$ -loaded microspheres to induce spawning in the shi drum has been recently demonstrated (Libertini *et al.*, 1998). In the latter case, a greater percentage of fish treated with the GnRH $\alpha$  microspheres spawned after treatment and produced more eggs per kg body weight, compared to the GnRH $\alpha$  injected females. Further experiments are necessary to optimize this technology for the control of reproduction of the shi drum and establish minimum effective GnRH $\alpha$  doses and appropriate application times.

As far as the larval rearing stages are concerned, the shi drum appears to fulfil many of the prerequisites for a potential aquaculture species. For example the larvae have a large mouth, grow at a very fast rate, accept industrial dry feed early, have high survival rates, do not exhibit aggressive cannibalistic behavior and can be handled easily without anaesthesia. The relatively large mouth of the shi drum means that the larvae can begin feeding as early as two days after hatching. Also, probably due to the intrinsic fast growth rate of this fish, the larvae are able to accept industrial dry feed at a very early stage, on day 15 post-hatching. This characteristic is similar to other species with fast growing larval stages, such as the common dentex (*Dentex dentex*), white sea bream (*Diplodus sargus*) and the red porgy (*Pagrus pagrus*) (P. Divanach, pers. comm.). For comparison, species like

the European sea bass (*Dicentrarchus labrax*) and gilthead sea bream are first offered dry feed around 25 to 30 days after hatching, depending on the quality of the diet. The use of artemia in the present study was reduced from 30 days during the 1995 season, to 20 days in 1996, and was completely abandoned in the 1998 season. The latter protocol was implemented in order to avoid problems with digestion, which was our experience with species like the sharpnout bream (*Puntazzo puntazzo*) and the red porgy (data not shown). Even without the use of artemia, larvae were completely weaned on industrial diets in less than 33 days, which is a very desirable characteristic for aquaculture. Independence from the use of artemia and early weaning on industrial diets reduces the cost and increases the efficiency of production.

Shi drum larvae and fry exhibited a very fast growth rate. In 41 days after hatching, fry have an average weight of 2 g, and can be moved to on-land pre-fattening facilities, or can be transported directly to sea cages for grow-out. For comparison, European sea bass and gilthead sea bream fry under optimal conditions require at least 90 and 120 days, respectively, to reach this stage. Therefore, the turnover rate of a shi drum hatchery can be very high, since more fry can be produced within a given period of time. Also, the fact that shi drum are not cannibalistic means higher survival and less need for repeated size grading, a common operation in marine hatcheries. One area of modification would be the size and shape of the rearing tanks for the shi drum. Since larvae and fry do not occupy the whole volume of the tank and prefer the surface, bottom or sides of the tank, depending on developmental stage, perhaps shallower tanks with a higher side/surface area to volume ratio should be used in a shi drum hatchery.

The fast growth observed during the larval rearing stages continued during the nursery phase. From an initial size of 2 g, fish grew to 15 g in about seven weeks. During the grow-out phase, however, growth was probably not maximal in fish maintained in tanks. Still, fish reached a mean weight of 250 g one year after hatching, and 680 g two years after hatching, which is the market size for wild-caught shi drum. Although still unverified, we believe that a higher growth can be achieved, if shi drum are reared in cages, and once a diet specific to the fish's own requirements is developed. Development of the appropriate diet can also result in decreases in the FCR, which in the present study, it was already well within the currently observed values in gilthead sea bream and European bass culture. It is obvious from our experiments that shi drum is not an appropriate species for cage culture in offshore or exposed areas with substantial wave action. The tendency of the fish to distribute themselves next to the cage net results in prohibitively high mortalities after storms, and reduces the maximum stocking density of the cage, in relation to gilthead sea bream or European sea bass culture. The use of facilities located in protected sites which are exposed to very little wave action, and of cages with a higher net-surface to volume ratio, can ameliorate these problems and make shi drum cage culture a profitable alternative to the existing species.

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