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Marine aquaculture finfish species diversification

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 16

1995
pages 9-19

Culture of *Solea* spp.

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SUMMARY - This paper deals with the broodstock management and larval rearing of *Solea senegalensis* Kaup obtained in a pilot-research project in Portugal since 1994. *Solea senegalensis* is a common species in the southern European countries which is well adapted to warm climates and is exploited under extensive aquaculture in earth ponds in Portugal and Spain. After 7 months in captivity a wild broodstock spawned naturally and the spawning season lasts from March until June, at temperatures ranging from 16.5±0.5°C to 22±1.0°C and salinities from 30 ‰ to 35 ‰. Eggs with 100% fertilization presented viability ranging from 100% to 90%. Larvae were reared in 200 l fibreglass cylindroconical tanks and the newly hatched larvae had a total length of 2.66±0.11 mm. As first feeding the larvae with a total length of 3.34±0.08 mm were stocked at an initial density of 100 larvae/litre. *Brachionus plicatilis* was given as first feeding for larvae from day 3 to day 5 along with newly hatched Artemia nauplii from day 4 onwards. During the larval rearing the environmental conditions were: normal photo period, 35‰ salinity and a temperature of 18°C. The benthic stage is achieved on day 19 when total length is 7.29±0.09 mm and the larvae were then transferred to 200 litre flat bottomed fibreglass tanks. Survival rate at this stage ranged from 68.6% to 87%. Juvenile rearing was performed in the laboratory to day 38 where a total length of 16± 0.84 mm was achieved and the juveniles went to ongrowing areas in commercial fish farms. Larval feeding strategies as well the weaning period will be discussed and the main problems will be identified. The ongrowing stage is still under progress, but the lastest results indicators will be presented. An analysis of the possibilities of *Solea senegalensis* as a new species for aquaculture in the Mediterranean area will be discussed.

Key words : Soleidae, broodstock, larvae, growth.

RESUMÉ *- Dans ce travail, nous présentons les résultats obtenus lors d'une étude pilote sur le maintient des géniteurs et élevage larvaire de Solea senegalensis Kaup qui est en cours au Portugal depuis 1994. S. senegalensis est une espèce bien adaptée à des eaux plus chauds et qui fait partie de la pisciculture extensive traditionelle dans les pays du sud de l'Europe. Après sept mois en captivité, des géniteurs sauvages ont pondu naturellement. Les pontes sont étallées pendant Mars à Juin dans des conditions de temperature et salinité variant entre 16.5±0.5°C et 22±1.0°C et entre 30 ‰ to 35 ‰ respectivement. Les oeufs fécondés à 100% ont presenté un taux de viabilité entre 100% et 90%. Les larves à l'éclosion mesuraient 2.66±0.11 mm et ont été élevés dans*

des bacs cylindro-coniques de 200 litres avec une densité initial de 100 larves/litre ayant atteint une longuer totale de 3.34±0.08 mm à la première nourriture exogène. Les conditions ambientales observés pendant l'élevage etaient photoperiodicité naturelle, salinité 35‰ et temperature 18°C. Le plan alimentaire des larves a été des Brachionus plicatilis entre le 3ème et le 5ème jour après l'éclosion, suivie de nauplii et metanauplii d'Artemia.. La periode benthique a été atteinte au 19ème jour quand la longuer totale était de 7.29± 0.09 mm, et les larves ont alors été transferrés dans des bacs au fond plat. à ce stade, le taux de survie variait entre 68.5% et 87%. L'élevage des juveniles a été faite en laboratoire jusqu'au 38ème jour, date à laquelle les larves ayant atteint une longueur totale de 16±0.84 mm, ont été expedieés dans les aquacultures commerciales locales. Les differents stratégies de nourriture des larves ainsi que la période de sevrage seront discutés, et les problèmes majeurs identifiés. Bien que des experiences de grossissement soit encore en cours, les résultats preliminaires seront presentés, et les possibiltés d'élevage de Solea senegalensis, en tant que nouvelle espèce pour la zone de la Méditerranée, seront discutés.

Mots-clés: *Soleidae, géniteurs, larves, grossissement.*

INTRODUCTION

Solea vulgaris and *Solea senegalensis* are two common soles of Atlantic and Mediterranean waters, and they have been used as alternative species in marine aquaculture. Morphologically these two species are extremely close and larvae are very difficult to distinguish (Lagardère *et al.*, 1979). *Solea senegalensis* represents 95% of the sole catch in the southern part of Portugal, and it is a species which ranges from temperate to subtropical waters.

Cultivation of *Solea vulgaris* has been achieved in France and the UK since the seventies (Shelbourne, 1975) but *Solea senegalensis*, a species which is well adapted to warm climates and is commonly exploited in the extensive coastal aquaculture utilising earthen ponds along the south coast of Portugal and Spain, has been little studied (Drake *et al.*, 1984; Rodriguez, 1983; Dinis 1986, 1992). However the life cycle is similar to that of the better known *Solea vulgaris* (Lockington)(Russel, 1976).

Both species have a high market price, and growth rate of *Solea senegalensis* under extensive policulture conditions are higher than *Dicentrarchus labrax.* Only *Sparus aurata* presents a better growth (Drake *et al.*, 1984).

Both Soleidae are gonochoric and the first maturation for the females is achieved at age 2^+ for *S. senegalensis* and 3^+ for *S. vulgaris*, when the total lengths are 32 cm and 30 cm respectively (Dinis, 1986). However Ramos (1982) reported refers 27 cm as the total length at first maturation for *Solea vulgaris* in the Mediterranean area. The spawning season is late winter for *S.*

vulgaris (January - March) and spring for *S. senegalensis* (April- June)(Ramos, 1982; Dinis 1986; Andrade, 1990). Fecundity for both species is very similar, with 509 oocytes/g fish for *S. senegalensis* and 530 oocytes/g for *S. vulgaris*.

The reproduction in captivity of *Solea senegalensis* has been the subject of research in Spain and Portugal since the early eighties (Rodriguez and Pascual, 1982; Rodriguez, 1984; Dinis, 1986; Dinis, 1992). Due to bad results concerning weaning and growth of juveniles to the marketable size (Metailler *at al.*, 1983; Dinis, 1992) there was a decrease in the importance and potential for aquaculture, subsequently resulting in a decrease in the research studies.

However, due to a decrease in the profitability of sea bream and sea bass cultivation resulting from over production in the Mediterranean area research efforts have been renewed.This paper deals with the possibilities of the cultivation of *S. senegalensis* in the Mediterranean area as well as with the production systems. Some preliminary results obtained during the larval and juvenile phase will be addressed.

MATERIAL AND METHODS

Broodstock Management

The reproduction of marine species in captivity depends on numerous environmental factors, and the induction of spawning is quite often hormonal dependent or by manual stripping. In Soleidae experiments with HCG (Girin, 1979) in *S. vulgaris,* hypophyses extracts of carp and tuna (Rodriguez and Pascual, 1982) and LH-rH by Dinis (1986) in *Solea senegalensis,* did not give positive results, either because the fish did not show any gamete emission, or the quality of eggs was very poor. Concerning stripping although the good results have been obtained for other flat fish such as the turbot, the technique is not feasible in *Solea spp*.

Thus, the natural spawning was the only way to obtain viable eggs. Quality of the broodstock, methods of capture and the methods conditioning (environmental factors and nutrition) were found to be essential for obtaining viable gametes.

Capture

The adults of *S. senegalensis* are very sensitive to methods of fishing, because they are easily damaged by nets. Devauchelle (1980) recommended "la capechade", but we have obtained good results with net traps during the night. Trammel nets should be avoided, but short trawls or beach seines may be used.

Adaptation to captivity

Prophylactic baths of antibiotics are recommended as well for the control of ectoparasites. Doses and therapeutics are dependent on the health of the fish (Menezes and Dinis, 1987).

During the first week the fish should be kept in tanks with a thin bottom sand layer and in dark, in order to improve adaptation, since this species is active nocturnally .

The use of already mature broodstock is not possible, because either the mature females have a low survival or reabsorved the gonads. So it is advisable to set up the stock during the interspawning period, which is between July and December. The identification of sexes is difficult during this period because there is no external differentiation, but with some experience it is possible to identify sexes. Stock density should be 1- 1.5 kg/ m^2 surface.

Feeding regime

Studies on stomach contents of *Solea senegalensis*, showed a dominance of Polychaetes (*Hediste diversicolor*) but some Anphipods, Copepods and Isopods were also identified (Bernardo, 1990). The results of Dinis (1986) based on the work of Fluchter and Trommsdorf (1974), showed the importance of Polychaetes in the feeding regime in captivity of *S. senegalensis*. Thus the feeding regime was based on Molluscs *(Loligo vulgaris*) and Polychaetes *(Hediste diversicolor) .*

Pathology

The ectoparasites of *Hemibdella solea* (Hirudinae) (Burneson, pers. comm.) was identified. This parasite seemed not to affect the fish, and although the density of the parasite may reach around 20/cm^2 on the spawners, no harmful were noticed on the spawners.

Eggs and Larvae

Incubation

The pelagic eggs were collected using surface collectors and incubated in the same circuit of the broodstock in order to maintain the same temperature and salinity of the spawning.

After hatching, the larvae were transferred to cilindroconical 200 litres fibreglass tanks, in a closed system, were salinity was maintained at 35‰ and temperature ranged from 16.5 ± 0.5°C to 22 ± 1.0°C.

Larval development

The larvae were fed on day 3 using Rotifers *(Brachionus plicatilis)* as first prey followed by *Artemia* nauplii, and metamorphosis was achieved on day 19, when they were transferred to flat bottom 200 litre fibreglass tanks. Density of the larvae were 100 larvae/litre. Total length was accessed daily as well as mortality. Poslarvae remained in these tanks until they were transferred to outdoors earth ponds for ongrowing at day 38.

The weaning period for this specie produces high mortalities, because the benthic fish is not very active and may resist to long periods of starvation. The use of rehydratable pellets incorporated with atractants substances was essayed (Dinis *et al.*, 1987; Metailler *et al.*, 1983), but survival and growth was very poor, around 33%.

Ongrowing

The experimental ongrowing was done with the collaboration of private fish farms, and two situations were chosen. In monoculture, without supplemented food and continuos running water from an adjacent reservoir, and in polyculture with sea bream , taking into account the benthonic macrofauna identified in this type of tanks were Polychaetes represents one of the most important groups (Pousão-Ferreira, pers. comm.).

RESULTS

Broodstock

A wild broodstock (Table 1) obtained from a natural population of an extensive area was set up in a 2.5m^3 fibreglass flat bottom circular tank, in a closed system at the University of Algarve, in August 1993. Fish was caught using traps, during night and transported to the University in tanks provided with aeration.

A prophylactic bath of Furaltadone (15 p.p.m.) during 24 hours to prevent necroses from the fishing was done. The mortality to adaptation was 7% but the dead fish corresponded to the more damaged and smaller fish

Fish were fed ad libitum with Mollusc and Polychaetes, three times a week, following the regime proposed by Dinis (1986). Environmental parameters ranged from 12 to 20°C temperature, salinity 30 -35 ‰ and with a normal photo period.

Table 1. *Solea senegalensis* - Broodstock

Total Length (mm)	Total Weight (g)	Sex
420	937.1	M
355	471.1	M
540	1019.8	F
375	646.8	M
425	821.5	F
470	1340.0	F
346	436.3	M
480	1268.3	F
415	875.8	F
450	1062.9	F
495	1582.9	F
385	659.4	M
445	982.8	F
472	1192.5	F

Eggs

After 8 months in captivity, in the beginning of April 1994 the first batch of eggs was emitted, and to June a total of 1,56 Kg of eggs was produced. The spawning season of 1995 started one week prior to that of 1994, and to the end of May a total of 2,40 Kg of eggs were obtained. Fig.1 shows the results of 1994 and 1995 spawning season.

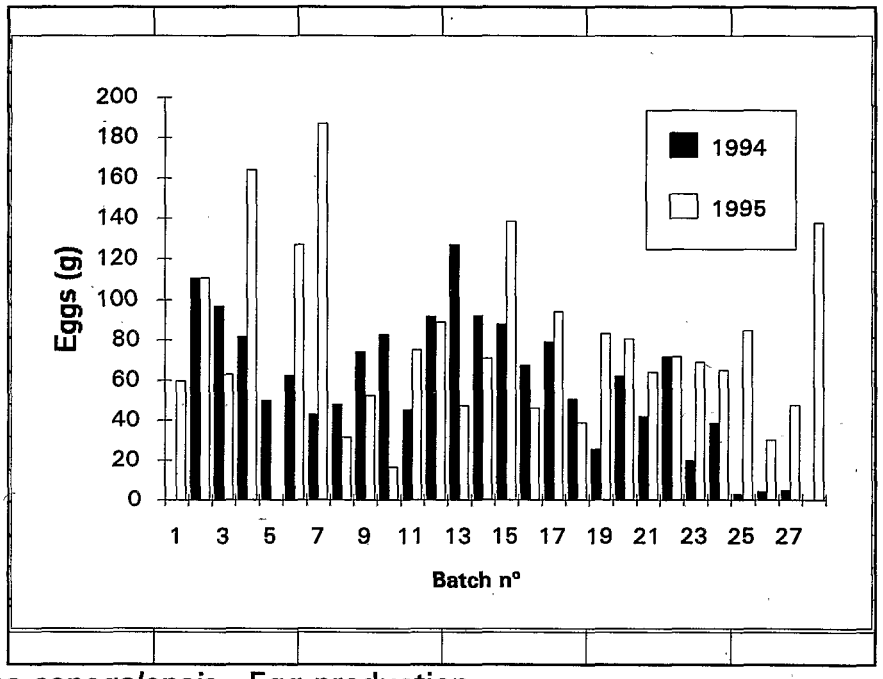

Fig. 1. *Solea senegalensis* - Egg production.

Eggs with a diameter ranging from 975µ to 1013µ and 100% fecundity presented a viability ranging from 87% to 98% .

During 1994 no decrease in the size and quality of the eggs was found. However, during 1995, the size of the eggs had decreased slightly at the end of the season to a diameter of 890±0.02µ and viability deceased to 75% .

At 17°C, incubation period was 48 hours, but at 20°C this period was reduced to 36 hours and the eclosion ranged from 82% to 90%. Quality of newly hatched larvae was not affected by the decreased of the incubation period.

Larvae

The larvae hatch with 2.66±0.11 mm total length and at first feeding size increased to 3.34±0.08 mm. They were fed on *Brachionus plicatilis* (5br/ml) enriched on 50% *Isochrysis galbana* and *Chlorella spp.* from day 3 to day 5 post hatch, and newly hatched *Artemia* nauplii from day 4 to day 15. Enriched *Artemia* metanauplii were given "ad libitum" from day 12 to day 30. The growth curve for the larval period is shown in Fig. 2.

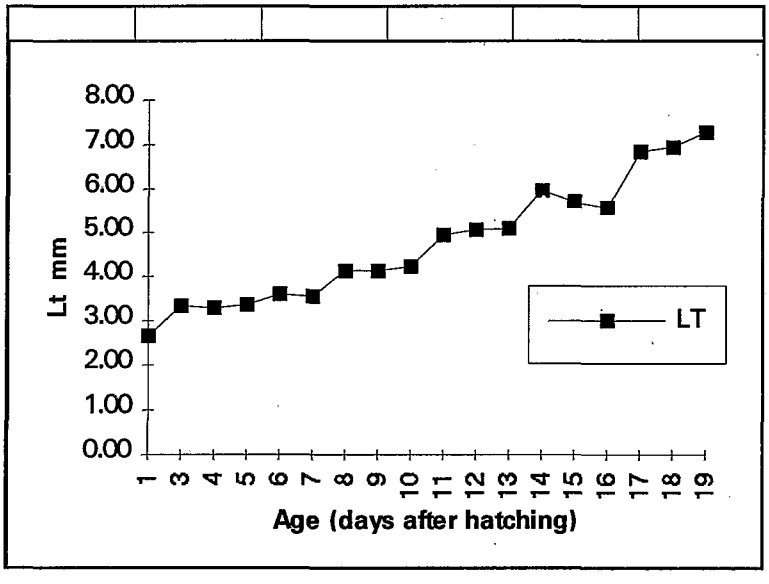

Fig. 2. *Solea senegalensis*- Larval growth length.

Survival at day 19 ranged from 68.6% to 87% when the metamorphosis had finished and the fish were moved to flat bottom tanks.

From day 19 till day 25, fish were fed on *Artemia* metanauplii while deep frozen *Artemia* was given "ad libitum", providing for the fish already with a benthic activity the first inert food. The growth curve during this period is shown in Fig. 3.

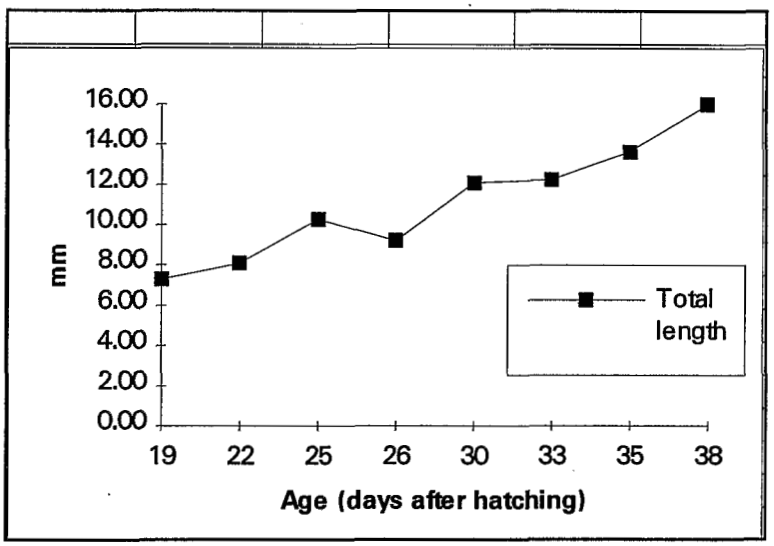

Fig. 3. Growth in length of posmetamorphosed *Solea senegalensis.*

During this period it was decided not to use a bottom sand layer, although McVicar (1980) and Campbell and Buswell (1982) had identified a bacterial disease, the black patch necrosis of *S. vulgaris*, which is easy controlled by a sand substrate. Such a disease was not registered in *S. senegalensis.*

At day 38 when the fish attained 16±0.84 mm they were transferred to outdoors earthponds for ongrowing.

Ongrowing

The available data is still very scarce. The results obtained so far should be considered as preliminary data.

For the ongrowing period, and taking into account the difficulties during the weaning period, it was decided to stock two types of earthponds. One, a monoculture one with a total area of 1000 m^2 with nets at the water entrance. No suplemented food was given, and fish only fed on the natural production. A total of 2000 fish was stocked in this pond in late July 1994. In June 1995 the pond was fished and a survival of 20% was registered. Fish attained 16.64±2.05 cm total length and 40.32±2.45 g weight. Another, a policulture one with *Sparus aurata* fed with pellets, and with a benthic macrofauna very rich in Polichaets. Fish in this conditions attained 35.25±1.79 cm total length and 456.1±3.61 g weight.

DISCUSSION

Good acclimation and appropriate food regime seem to be prerequesites for a correct broodstock management and the obtention of natural spawning with good quality eggs. The "Polychaetes effect" as Dinis (1986) had already mentioned, was vital and after an 8 month period natural spawning was obtained. The water temperature following the annual cycle also seemed to be

important. Similar results were reported by Vasquez *et al.* (1995). Broodstock management has clearly improved during the last three years, and the natural spawning in captivity no longer seems to be a problem.

Larval development showed very high survival, but the weaning period is still the bottleneck of fry production. Adequate food and pellet stability in the water and nutritional demand is not still clear. The study of the organogenesis of the digestive system (morphology and enzymatic identification) is often regarded as a key factors to an adequate identification of criteria for larval feeding.

Ongrowing seemed to be appropriate in policulture with seabream, however relative size for the restocking of the species and relative density should be addressed as an important objective for further studies. Artificial diets should also be developed because sole is the only marine specie which uses carbohydrates as a source of energy (Cadena-Roa, 1983), providing the possibility of lower costs of food due to a lesser needs for protein.

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