Sea bass (Dicentrarchus labrax) reproduction in captivity: gametogenesis and spawning

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Abstract

This paper describes the large-scale production of eggs and larvae of sea bass (Dicentrarchus labrax) and shows how spawning can be planned to produce viable eggs throughout the year by manipulation of photoperiod and temperature. The rearing temperature was 9.5-22°C during spawning and 9-24°C during the gametogenesis. The photoperiodic cycles tested were normal (i.e. the photoperiodic cycle observed at Palavas-les-Flots, Mediterranean coast, France, 1984/1987) advanced by 3 to 5 months and delayed by 2 months.

Keywords: Sea bass, Dicentrarchus labrax, spawning, reproduction, aquaculture.

INTRODUCTION

The rearing of marine fish requires a reliable supply of eggs and normal yolk sac larvae. Gametes from fish that have matured in the wild and been caught when ripe are not a solution for three main reasons: the annual productivity in the sea is variable from year to year; even with high productivity, sperm and oocytes of good quality are difficult to obtain; and the production of gametes in the wild is seasonal.

The control of spawning in captivity, on the other hand allows a better monitoring of the quantity and quality of eggs collected during or outside the normal spawning periods. Early results from freshwater salmonids were obtained by stripping and artificial fertilization (Huet, 1970). The first tests on marine fish were run later, at the end of the last century and especially during the sixties and seventies. These followed different strategies, depending on the species and the geographical area. In summary, flatfish such as plaice (Pleuronectes platesss), turbot (Scophthalmus maximus) and sole (Solea solea) traditionally spawned and fertilized their eggs naturally in tanks of running sea water (Shelbourne, 1968; Devauchelle et al., 1987, 1988). However, mullet (Mugil cephalus) spawned only after hormonal injections (Kuo et al., 1973), while eggs of sea bream (Sparus aurata) and sea
bass (Dicentrarchus labrax) were obtained naturally (Boulineau, 1969; Girin, 1979; Devauchelle, 1984) or with hormonal help, most often HCG (Human chorionic gonadotropin) (Barnabé, 1976a, 1976b; Bedier, 1979; Zanuy et Carillo, 1984) and LHRH (Luteinizing hormone releasing hormone) injections (Zohar et al., 1984; Barnabé et Barnabé-Quet, 1985; Suquet, 1986), sometimes followed by handstripping. Production of eggs outside the normal spawning season was obtained under artificial thermal and photoperiodic cycles (Girin et Devauchelle, 1978; Barnabé et Paris, 1984; Devauchelle, 1984; Coves, 1985; Zanuy et al., 1986) which have a major effect on gametogenesis under the temperature regimes found at this latitude (43.31 N).

Several works describe and compare the results of such experiments during the last 15 to 20 years (Barnabé, 1986; Devauchelle, 1984), all conclude that more fundamental research needs to be done. It is also necessary for commercial hatcheries to transfer to a large scale the techniques developed in laboratories.

Experiments with this latter aim have now been carried out on the gametogenesis and spawning of sea bass. These were successful in readily producing large number of eggs and yolk sac larvae at any season. The results are presented here.

MATERIAL AND METHODS

Stock management

This section describes the conditions used at the IFREMER Laboratory during the years 1984 to 1987. This laboratory is situated on the Mediterranean coast of France (at 43.31 N). The broodstock consisted of equal numbers of wild fish and fish reared in hatcheries. Fish from the wild were chosen without abnormalities, and hatchery reared fish were selected on the basis of highest growth rate. Each group of spawners contained several weight classes: five classes of females ranging from 1 to 4 kg and four classes of males weighing 0.7 to 2.5 kg. At the end of each spawning season, the highest weight class of fish was replaced by a young class to give an annual renewal of an average of 20% of the whole group. The sex ratio was 1 male to 2 or 3 females, i.e. female weight represented at least 75% of total biomass. In each group, stocking density varied from 4 to 13 kg/m³ without effect on fecundity or egg fertilization and hatching percentages.

Fish were transported to the experimental facilities in small tanks (0.5 to 1 m³) at variable densities (10 to 200 kg/m³) depending on transport duration (maximum 6 hours) and water temperature (9.25°C). Anaesthesia is recommended only for excited fish. At their arrival at the station, fish were stocked in a tank for 1 to 6 months. On arrival, the fish underwent sanitary treatment at ambient temperatures (9-25°C). On the first day, fish were placed in a furazolidone bath (20 to 30 g/m³) for 30 minutes. The following day, a second treatment was administered with formalin (350 g/m³) for 30 minutes. These two treatments were repeated respectively on the third and the fourth day. During the first month of their acclimatization the fish were sexed with special scissors (Devauchelle, 1985).

The maturation tank capacity was 12 m³. Its organization described (fig. 1) was based on a completely open seawater circulation system. The flow rate was adjusted to give an average total volume renewal of 10% per hour for stocking densities ranging from 4 to 6 kg/m³. In case of higher densities, the flow rate was doubled to keep the dissolved oxygen up to 80% of saturation. Each tank was aerated. Eggs were collected in a plankton net placed under the outflow. Lighting was provided by fluorescent lights giving a maximum 1500 lux at the surface of the tank.

The fish were fed daily ad libitum (with a diet consisting of 60 to 80% artificial pellets (Aqualim-7 mm) and 20-40% trash fish of various species which were fresh or frozen for a maximum of 2 months. The tanks were cleaned once a week to once a month, depending on season and stocking density.

Long term retardation of spawning

Prolongation of the spawning season was caused mainly by photoperiod modifications. Since natural ovulations are only obtained in the thermal range 9-17°C, the light cycles were adjusted to induce spawning seasons at the period when the natural temperatures reach these levels, most often from October to June. At the beginning of the experiments, the fish were all in non-breeding condition. The fish were divided into three groups (table 1). The control group (tank 1) was subjected to natural photoperiodic and thermal cycles ranging from 8 hrs. 30 min to 15 hrs. 30 min of light per day and from 9 to 26°C. Tank 2 was advanced by 3 months in 1985-1986, and by 5 months in 1986-1987. This was obtained by concentrating the decreasing photoperiod into 3 months instead of 6 months in 1985-1986 and 4 months instead of 6 in 1986-1987. Then the period corresponding to the increasing of photoperiod was divided into two phases. The first consisted of photoperiods less than 10 hours of light per day until most females had spawned at least once. Then the photoperiod is quickly increased to 16 hours of light per day. The period between two spawning seasons lasted 12 months. The temperature was that of ambient seawater. Tank 3 was delayed. In 1984-1985 the decreasing photoperiod is spread over 8 months instead of 6. During the following 12 months the variations of the natural cycle delayed by 2 months were reproduced. In 1986-1987 a supplementary delay of 2 months was tested by spreading the increasing and decreasing photoperiods over 7 months instead of 6 months. The temperature cycles were natural. These changes are shown by figure 4.
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Table 1. — Characteristics of the three groups of spawners.

<table>
<thead>
<tr>
<th>Tank</th>
<th>Spawning season</th>
<th>Control</th>
<th>Advanced</th>
<th>Delayed</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Stocking density kg/m³</td>
<td>3.6</td>
<td>10.3</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>Number of females</td>
<td>16</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Number of males</td>
<td>8</td>
<td>19</td>
<td>16</td>
</tr>
</tbody>
</table>

Spawning collection

During gametogenesis, biopsies were made with scissors (Devauchelle, 1985) to estimate the growth stages of the oocytes. When oocyte diameters were greater than 650 μm, the females were injected with LHRH₄ (10 to 20 μg/kg fish) and placed in a spawning tank where the eggs were spawned naturally and fertilized naturally at a minimum 13°C, 24 to 96 hours after injection. Then, each female was subjected to another biopsy. If they still contain oocytes over 650 μm diameter, they were again injected with LHRH₄ as before. A third examination and injection was made if necessary.

The hormonal treatments accelerated the final maturation and ovulation processes. As a consequence, it can be used for short term acceleration of spawning, especially for getting a large quantity of eggs in a short period. When synchronization is not necessary, the fish are left in the stocking tank where they may ovulate and spawn naturally. The fish subjected to hormonal treatments were identified by colored ink injected at the base of the pectoral fin.

Whatever the spawning technique, the eggs of each spawn were automatically collected and concentrated (fig. 1) in a plankton net. Their total number and percentage viability (number of eggs normally segmented/total number of eggs collected) were estimated. Only viable eggs with the highest buoyancy were selected and placed in open water circulation incubators (fig. 2) at densities up to 10,000 eggs per liter.

RESULTS

Mortality of broodstock was observed at two different times, just after fishing and during the acclimatization period. Mortalities occurred in fish, injured
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**Figure 2.** Diagram of incubators used for large number of eggs at densities up to 10,000 per liter (W = sea water; A = air; I = incubators).

by successive manipulations, and later in the maturation tank, especially when manipulations linked to hormonal treatments were too brutal or too long. In both cases the mortality rate reached 5% per year.

Mortalities also occurred if fish weighing less than 0.5 kg were introduced into the maturation tanks, when larger fish sometimes attacked and killed them. In some cases 100% of such fish were killed in a few days. During the conditioning, the main problems were parasites: copepods (Caligidae) were treated by $300 \times 10^{-6}$ formaldehyde once a week until eradicated. Triconemia and metacercaria were suppressed with formaldehyde (37%) baths at $200 \times 10^{-6}$; plathelminthes were treated with Mebinoxadole 3% incorporated into the fish food at 4 g/kg of diet.

The food intake varied between 1.5 and 11% (dry food kg/kg fish x 100) (fig. 3). The lowest food consumption occurred at the lowest temperatures and at spawning periods, when oocytes measured over 500 μm. Both factors may explain why in 1986-1987 the fish in the delayed tank ate small quantities over a longer time than the control 2nd advanced tanks, because the spawning season began just after the cold period at the start of the increase in temperature.

The spawning periods (fig. 4). — Biopsies during the whole gametogenic cycle showed that ovulation was not well synchronized between fish. However, mean oocyte diameter in a particular tank increased with decreasing photoperiod. The oldest females (2.5-3 kg) responded most quickly to stimulation by light. Oocytes longer than 650 μm were first observed 4 to 5 months after maximum photoperiod or after the beginning of decreasing photoperiod in tank 1 (control) and 2 (advanced). In tank 3 (delayed season), oocyte diameter increased before the decreasing photoperiod. Females with 650 μm diameter oocytes were observed only 1 month after the decrease. Thus the delay was not strictly respected in this treatment: instead of 4 months delay we obtained 2 months.

In tanks 1 and 2, the spawning periods which followed hormonal injections began during the end of the decreasing photoperiod, while natural spawns were collected at increasing photoperiods. In tank 3, natural spawns were obtained even during the decreasing photoperiod. The maturation processes were observed at temperatures ranging from 9 to 24°C and mostly under decreasing photoperiod from 16 to 10-15 hours of light per day. In any case, viable eggs were obtained between 9.5 and 22°C and at photophases ranging from 8 hrs. 30 min to 15 hours of light per day. When temperatures rose above 17°C, the use of LHHRH became necessary to induce spawning. The total fecundity ranged from 146,000 to 502,300 eggs per kilogram of fish (table 4). Those of groups 1 and 2 were similar while the fecundity in group 3 was somewhat lower. Similar results were obtained for egg viability, while hatching percentages were not clearly different between treatments (table 3).

**DISCUSSION**

The methods tested for spreading the spawning season of sea bass gave interesting results: in 1986-1987, during 8.5 months, 111.9 x 10⁶ eggs were collected from three tanks controlled mainly by light and containing a total weight of 291 kg fish. 92.4 x 10⁶ eggs were viable (82.5% viability and 66% gave normal larvae. As observed by several scientists (Billard, 1979; Bromage et al., 1982; Wooten, 1982; Wooten, 1982; Lam, 1983; Duston and Bromage, in press; Bye, 1984), the major control of the initiation of maturation is photoperiod. However, even in our temperate climate, the duration of gametogenesis seems to be closely linked to the temperature levels as well as to the spread of thermal variations, as observed for subtropical species (Lam, 1983). In our experiment, use of LHHRH induced the maturation processes and spontaneous egg release under subnormal temperatures, but it was not efficient in stimulating any stage of the gametogenesis at any temperature. Control of temperature also appeared necessary in order to fully complete oocyte development. Thus, to delay the spawning season more efficiently than we did, we suggest that temperature levels should be kept high (16°C<T°C<22) or at least to reduce their decrease less rapidly when photoperiod decreased and, as a consequence, initiated gametogenesis. In the case of advanced seasons, the role of temperature does not seem to be so crucial. The delay of 15 days to 1 month
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Figure 3. — Changes in food intake and oocyte diameters over the 1986-1987 spawning periods for the three treatments (Control, Advanced and Delayed tanks); vertical bars: min.-max. diameters.

Figure 4. — Variations of temperature and photoperiod in the Control Advanced and Delayed tanks over the period April 1984-April 1987.

Table 2. — Light (L) and temperature (T) conditions at the beginning of increasing oocyte diameter, at the 650 μm point which is the lower limit for efficient hormonal injections, and when at least 50% of the oocytes have reach 650 μm.

<table>
<thead>
<tr>
<th></th>
<th>Beginning of gametogenesis</th>
<th>Oocyte diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First 650 μm</td>
</tr>
<tr>
<td>Control</td>
<td>L 16.00 to 10.15 to 9.45</td>
<td>10.15 to 9.45</td>
</tr>
<tr>
<td></td>
<td>T (°C) 20-24</td>
<td>17</td>
</tr>
<tr>
<td>Advanced</td>
<td>L 16.00</td>
<td>9.00</td>
</tr>
<tr>
<td></td>
<td>T (°C) 13</td>
<td>23</td>
</tr>
<tr>
<td>Delayed</td>
<td>L 14.00 to 16.00 to 15.30</td>
<td>15.30</td>
</tr>
<tr>
<td></td>
<td>T (°C) 21</td>
<td>12</td>
</tr>
</tbody>
</table>

L: Hours. minutes of light per day.
Table 3. — Spawning seasons and characteristics of eggs collected in the Advanced, Delayed and Control groups.

<table>
<thead>
<tr>
<th>Year</th>
<th>Control</th>
<th>Advanced</th>
<th>Delayed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1984-1985</td>
<td>1212000</td>
<td>2750000</td>
<td>4370000</td>
</tr>
<tr>
<td>1985-1986</td>
<td>9690</td>
<td>85</td>
<td>89</td>
</tr>
<tr>
<td>1986-1987</td>
<td>280000</td>
<td>284000</td>
<td>369000</td>
</tr>
<tr>
<td>Total number of eggs collected</td>
<td>1212000</td>
<td>2750000</td>
<td>4370000</td>
</tr>
<tr>
<td>Viability %</td>
<td>96</td>
<td>89</td>
<td>85</td>
</tr>
<tr>
<td>Number of viable eggs per kg female</td>
<td>280000</td>
<td>284000</td>
<td>369000</td>
</tr>
<tr>
<td>% induced spawnings</td>
<td>39</td>
<td>65</td>
<td>0</td>
</tr>
<tr>
<td>% natural spawnings</td>
<td>61</td>
<td>35</td>
<td>100</td>
</tr>
<tr>
<td>% viable yolk sac larvae*</td>
<td>67</td>
<td>78</td>
<td>57</td>
</tr>
<tr>
<td>% abnormal yolk sac</td>
<td>11</td>
<td>11</td>
<td>7</td>
</tr>
</tbody>
</table>

* percentage of viable eggs.

Table 4. — Variation in fecundity (average number of eggs collected per kilogramme of female) in each tank over the period 1984-1987.

<table>
<thead>
<tr>
<th>Year</th>
<th>Control</th>
<th>Advanced</th>
<th>Delayed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1984-1985</td>
<td>292700</td>
<td>—</td>
<td>164600</td>
</tr>
<tr>
<td>1985-1986</td>
<td>319000</td>
<td>328000</td>
<td>146000</td>
</tr>
<tr>
<td>1986-1987</td>
<td>434600</td>
<td>502300</td>
<td>441000</td>
</tr>
</tbody>
</table>

on the expected results still remains unexplained. As suggested by Richter et al. (1987), Devauchelle (unpubl. data), some developmental stages of oocytes might be subjected to an endogenous rhythm and are of constant duration. The conclusions concerning the role of temperature and photoperiod are very similar in most countries. In practice, however, different thermal strategies are necessary, depending on the areas considered. In Brittany, for instance, the natural temperatures range from 6-7°C to 17-18°C. Thus the levels must be raised in winter (Devauchelle, 1986) to obtain spawning of D. labrax, while at other seasons eggs of this species are spawned naturally or with hormonal help and without thermal control at all. By contrast, along mediterranean and Adriatic coasts or in subtropical areas, precautions are necessary mainly during summer, to keep temperatures below 24-26°C during the gametogenesis and between 9-22°C during spawning. Unfortunately, acclimation of sea bass at high temperatures has not yet allowed the modification of the conditions required for gametogenesis or spawning (Fuchs; Devauchelle, unpublished data). The worst results obtained during the delayed spawning season may be improved by more care with temperature levels. Previous works (Zanuy et al., 1986; Coves, unpublished data) related similar observations under unadapted thermal conditions during gametogenesis or thermal and photoperiodic cycle contractions. On the other hand, hatching success calculated from viable eggs depends more on incubation conditions than on the pattern of external factors to which the broodstock is subjected.

For sea bass, it must be stressed that the injection of LHRH$_4$ on a large scale during normal or shifted spawning seasons gives as good a performance as natural spawning (these data) or as results obtained by LHRH$_4$ on an experimental scale (Barnabé et Barnabé-Quet, 1985). Similar observations have been made on other marine fish: Chanos chanos (Lee et al., 1986), Mugil cephalus (Lee et al., 1987) or Sparus aurata (Zohar, 1986) for instance. Two major facts explain this success: firstly, the direct effect on the hypothalamo-hypophysis axis, as demonstrated by Donaldson (1986) on salmonids or Zanuy and Carillo (1987) on both marine and freshwater fish; in comparison, those of HCG which induces an immunity effect. Secondly, through prior biopsy, only those females with large oocytes (diameter over 650 pm) are selected for hormonal treatment. This avoids the forcing of immature females. The special cisors used for biopsies, already tested on flatfishes and round-fishes (Devauchelle, 1985) were frequently used (once a week) in these experiments without damage to fish or to their productivity.

In conclusion, the combined use of LHRH$_4$/biopsies and natural spawns, whatever the spawning season, is well suited to the large scale production of eggs. Excessive manipulation is avoided. If necessary, large batches of yolk sac larvae can be produced on a chosen day. Preliminary observations did not show a significant difference in larval survival between broodstock spawning normally and broodstock spawning out of season.

CONCLUSION

Broodstock management is not now the major problem in the development of sea bass aquaculture. Work carried out on nutrition should simplify the maintenance conditions and probably increase larval survival rates. This is of particular interest in the case of extreme rearing conditions. The high cost of temperature control could be reduced when the roles of temperature and photoperiod have been defined...
more precisely. The use of hormones also requires further examination with regards to the developmental stage of oocytes considered for treatment. However, the results obtained enable the production of yolk sac larvae at the average price of 0.01 centime. This is a minor part of the price of 100-150 day-old juvenile which is sold FF 3.50-4 (1987) along the Mediterranean coast. An average of four yolk sac larvae are necessary to produce one juvenile. Finally, the progress made these last 10 years on sea bass broodstock and in larval rearing make sea bass a possible candidate for further research on marine fish production, especially in the fields of genetics, nutrition, reproductive physiology, and aquaculture. This may also help to improve broodstock management and larval rearing of several other species; in particular, problems affecting the development of eggs of species with larvae with high growth or survival rates are now important in the development of aquaculture.

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REFERENCES


Bedier E., 1979, Production à l'échelle pilote d'alevins de loup (Dicentrarchus labrax L.) présenté à The Early Life History of Fish. Symp. Woods Hole, USA, 2-5 April 1979, 17 p.


