The results of the experiments on the culture of flounder Platichtyhys flesus luscus (Pallas, 1811) and turbot Psetta maeotica (Pallas, 1811) at the Romanian Black Sea coast

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The results of the experiments on the culture of flounder *Platichthys flesus luscus* (Pallas, 1811) and turbot *Psetta maeotica* (Pallas, 1811) at the Romanian Black Sea coast

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SUMMARY – The paper includes the experiments carried out in 1994-1997 at RMRI Constanta concerning the culture of flounder and turbot, using populating material obtained through controlled spawning. The reproduction was made with wild animals bred in captivity, which released good quality seminal products by about six months from their catch for flounder and about nine months for turbot, without hormonal stimulation. The fecundation percentages varied between 10 and 80% for flounder and between 20 and 70% for turbot, and those of hatching between 20 and 50% for flounder and between 10 and 90% for turbot. The culture of turbot juveniles started from wild specimens weighing 2-4 g each (3-4 months old), and their mean weights were 100 g each at the age of 1.5 years, 350 g each at the 2.5 years and 750 g each at 3.5 years old, without providing them the best culture conditions.

Key words: Flounder, turbot, Black Sea, culture.

RESUME – *"Résultats des expérimentations d'élevage du flet* Platichthys flesus luscus *(Pallas, 1811) et du turbot* Psetta maeotica *(Pallas, 1811) sur la côte roumaine de la mer Noire". L'article présente les résultats des expérimentations déroulées en 1994-1997 à l'Institut Roumain de Recherches Marines de Constantza concernant l'élevage du flet et du turbot à l'aide des individus obtenus par reproduction dirigée. Pour la reproduction on a utilisé les animaux élevés en captivité, qui ont cédé les produits séminaux à environ 6 mois après leur capture pour le flet et 9 mois pour le turbot, sans stimulation hormonale. Les taux de fécondation ont varié entre 10 et 80% chez le flet et entre 20 et 70% chez le turbot, tandis que ceux d'incubation se situaient entre 20-50% chez le flet et 10-90% chez le turbot. Les jeunes du turbot ont été élevés avec les exemplaires sauvages, à partir de 2-4 g/individu (l'âge de 3-4 mois) jusqu'à 100 g/individu à 1,5 ans, 350 g/individu à 2,5 ans et 750 g/individu à 3,5 ans – sans leur assurer les conditions optimales d'élevage.*

Mots-clés : *Flet, turbot, mer Noire, élevage.*

Introduction

The flounder *Platichthys flesus luscus* (Pallas, 1811) and turbot *Psetta maeotica* (Pallas, 1811) are two flatfish species in the Black Sea seriously affected by the anthropogenic impact.

The interest for the culture of these two species at the Romanian littoral has increased simultaneously with the establishing of small ventures wishing to promote mariculture.

These species can be selected for aquaculture for biological reasons (they have a big growing potential in intensive conditions) and economic reasons (their commercial value is high, they are considered "festive" species; the culture fish is not going to compete with the wild one, as the demand is greater than the offer).

The first attempts of breeding these fish species in the Black Sea were made in the 70's, in the former URSS, and the technologies for controlled spawning were finalized in the 80's (Cepurnov, 1989). At the Romanian littoral, the experiments concerning the controlled spawning of these species have been carried out beginning with 1994, and initially they aimed at elaborating new ways and methods for rehabilitating natural populations, and subsequently they have been extended to finding possibilities for the culture of these species.

Materials and methods

Specimen collection

The initial experimental group included wild animals, caught from their natural environment:

(i) With beach net: flounder (juveniles and spawners) and turbot (juveniles).
(ii) With gill net (above the isobaths of 20-30 m): turbot (spawners).

Experimental conditions

The broodstock were bred separately, by species, in external concrete basins (10 x 5 x 1.3 m) with sandy bottom (abt. 10 cm), intermittently supplied with marine water and permanently with compressed air. The basins were partially covered by tarpaulin in order to prevent direct action of sun.

The juveniles were bred in internal glass fibre basins (2 x 1 x 1 m and 3 x 1 x 1), with sandy bottom, intermittently supplied with marine water and permanently with compressed air.

The larvae kept in PVC vans (abt. 70 l), permanently supplied with marine water and compressed air, provided with neon light of 40 W.

The eggs were incubated in pelagic egg incubators (70 l), with upward circular current, permanently supplied with marine water and compressed air. We have also obtained good results in incubation in nytal fish pounds suspended in internal basins.

Feeding

The broodstock fed on small marine fish (fresh and/or frozen). The turbot spawners were experimentally fed with granulated food (prepared in our laboratory, using our own recipes).

The juveniles fed on small marine fish (fresh and/or frozen), and shrimp (*Palaemon adspersus*) and adults of *Artemia* sp.

For the larval stages we have prepared cultures of phytoplankton (*Tetraselmis sueccica, Nannocloris* sp., *Dunaliella salina)* and small marine invertebrates (larvae of mussel *Mytilus galloprovincialis*, rotifer *Brachionus plicatilis* and phyllopod *Artemia* sp.).

Working methods

The working methods for each stage have been taken from the French-IFREMER (Person-Le-Ruyet, 1989; Suquet *et al*., 1991; Person-Le-Ruyet *et al*., 1993) and Ukrainian (Cepurnov, 1989; Bityukova *et al*., 1990) technologies, and adapted to the conditions and possibilities of the Romanian littoral.

Results and discussions

Environmental conditions

The environmental conditions have been monitored by weekly determinations of the main physical and chemical parameters of the culture environment (pH, dissolved oxygen, salinity, organic substance, ammonia nitrogen). As the temperature is very important, especially for the maturation and incubation process, it was registered daily.

The increased value of some physical and chemical parameters have been caused by the supply water (non-filtered and without thermic control) and excess of food (live and/or inert).

Table 1 includes the variation of physical and chemical parameters of culture environment (extremes values) by technological stages, for the experiments period.

Table 1. Variations of the main parameters of culture environment in various technological stages in 1994-1997

Stage	Parameters (limits)					
	T (°C)	pH	Salinity (ppt)	Organic substance (mg O_2/l)	Dissolved oxygen (mg/l)	Ammonia nitrogen (µ gN-NH_4/l)
Maturation						
Flounder	2-8	7.6-8.9	12-18	1.02-6.0	5.78-14.50	10.2-588.0
Turbot	4-17	7.9-8.2	14-18.5	1.5-3.6	7.8-10.8	20.0-200.0
Incubation						
Flounder	4-14	7.9-8.5	12.7-19.0	1.4-12.2	5.67-14.2	50.0-300.0
Turbot	10.5-19.8	7.9-8.5	12.5-18.0	1.5-5.7	4.0-12.0	90.8-600.0
Larval development						
Flounder	8.2-16.5	7.9-8.5	12.2-19.2	1.8-11.75	5.78-11.20	15.2-1380
Turbot	14.7-22.3	7.8-8.6	12.5-18.0	3.8-10.54	5.50-10.25	20.2-1250
Juvenile breeding						
Turbot	–1.5-26.5	7.5-9.5	12.7-18.1	1.6-3.99	3.13-8.69	24.63-168.9
Spawner conditioning						
Flounder	–2-26.7	7.8-9.0	12.8-18.0	1.84-4.02	3.80-8.20	30.1-180.2
Turbot	–1.7-27.2	7.9-8.7	13.8-18.4	1.05-4.47	3.62-9.21	29.9-138.3

The organic substance and ammonia nitrogen caused sometimes mortalities especially for larvae, both of flounder and turbot.

Water temperature had also a negative influence both on the incubation and growth of flounder and turbot larvae (through big differences during a day), and on turbot spawners (in summer time, when it exceeded 27°C for a week, and in winter, when it was below 0°C for three weeks). In such cases the mortalities reached 100%.

Controlled spawning

Flounder

The eggs and sperm were collected by the stripping method (Zaharia and Bilal, 1994a).

The spawners were at a satisfactory maturation level for about eight weeks (half of February-half of April), the flounder (like turbot) is among the fish species releasing seminal products in portions.

The maturation percentage of flounder spawners was of 80-100% for females and 100% for males.

We must mention that during the experimental period it was not possible to ensure an optimum sex ratio owing to the insufficient number of males we had, because when catching them from their natural environment about 80% of specimens were females.

The seminal products were collected through stripping twice a week, beginning at a water temperature of 7°C up to 12℃.

The prolificacy of flounder females was of 250,000-700,000 eggs/kg body female.

The fecundation was usually made by the semi-wet method, using about 0.3 ml sperm/100 ml

eggs, with a 10 minutes waiting time, during which the spermatozoa penetrate the eggs (Person-Le-Ruyet, 1989), the fecundation percentages reached 30-80%, and those of hatching 20-50%.

The flounder eggs are pelagic, they have a spherical shape, without oil globule. The vitellus is homogenous, the membrane is even and transparent, the vitelline space is very narrow. Egg diameter varies between 1.04 and 1.30 mm (900-600 eggs/cm^3).

The embryonic development lasted for 5-6 1/2 days, meaning 1400-1600 °h covering the six characteristic stages (Svetovidov, 1964; Dehnik, 1973) which have been described in an already published paper (Zaharia, 1994) (Fig. 1).

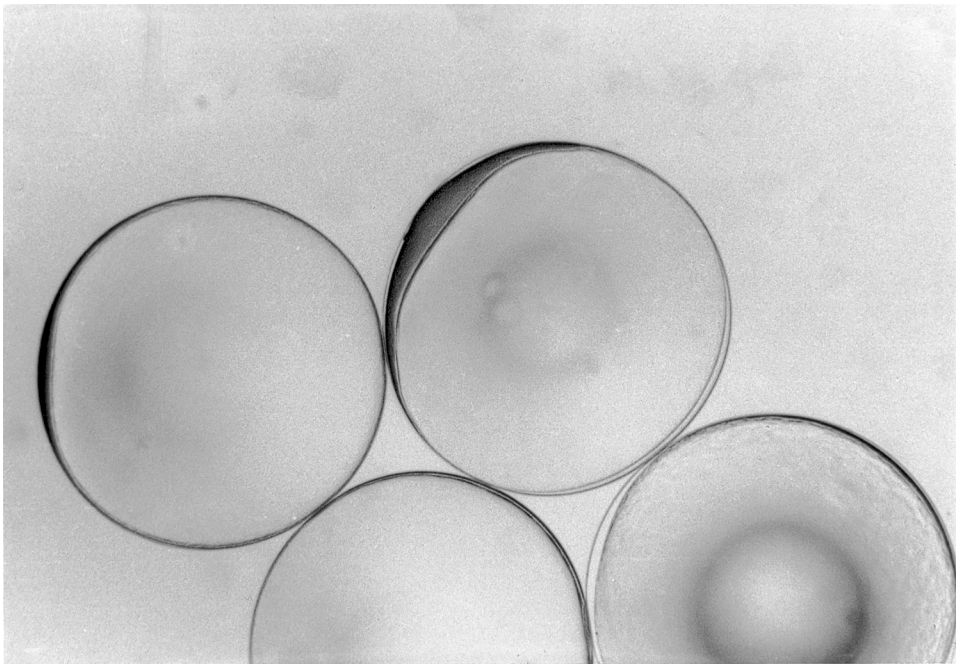

Fig. 1. First stage of embryonic development of flounder – 2 hours after fecundation (5 x 1.5 x 3) (original photo).

At hatching, the flounder larvae are 2.47-3.50 mm long. The body is slim, yolk sac is big and oval, just detached from the head. The intestine tract is linear. The anus opens immediately after the yolk sac, in the fin zone. The body is brown-yellow coloured, the melanophores are spread over the head, along the spinal side of body, on the intestine. Pigment spots occur in the middle of the caudal zone. The eyes are black-grey.

Turbot

For spawning purposes, we have used:

(i) Matured spawners, caught from heir natural environment. This method was used over all those experiment years, and we had problems in maintaining the spawners alive after being caught with gill nets above the isobaths of 20-30 m. Usually the sex ratio was unsatisfactory, the females were predominant (70% females in the catch from their natural environment). In many cases (50%) it was necessary to sacrifice the males and use the entire gonad for fecundation, and the fecundation percentages were good (up to 80%).

(ii) Spawners coming from their natural environment which had matured by administering of hypophysis suspension from sexually matured carp, a unique dose, depending on the maturation degree, as follows:

- Females: 2.0-3.0 mg/kg body.
- Males: 2.5-3.5 mg/kg body.

The females matured 100%, the males 50% (with a small quantity of sperm, so that in some cases it was necessary to sacrifice the males and use the whole gonad for fecundation).

(iii) Spawners coming from their natural environment, bred in captivity and matured after 8-9 months of breeding in captivity.

Irrespective of the method of collecting seminal products, the spermatozoa motility was estimated at a microscope, after being activated with seawater in a dilution 1:100. According to the four degrees arbitrary scale (Suquet *et al.*, 1991), the turbot spermatozoa were classified between classes I (20% of ground) and 4 (80% of ground).

Movement duration of the spermatozoa belonging to class 4 varies between 10 and 15 minutes (Zaharia and Bilal, 1994b).

The artificial fecundation was carried out with the dry and semi-wet methods, taking into consideration the great survival capacity of the turbot spermatozoa in seawater, as well as the high concentration of spermatozoa (0.7 – 11 x 10^9 spermazoons/ml) (Suquet *et al.*, 1991). For fecundation it was enough to mix manually 0.3 ml sperm at 100 ml eggs, in 50 ml seawater. Ten minutes later, the eggs were washed and placed in the incubators (Person-Le-Ruyet, 1989).

The fecundation percentages varied between large limits (0-90%) and were mainly determined by the sperm quality. The hatching percentages varied between 10-90%.

The turbot eggs are pelagic and have a spherical shape. The membrane is thin and transparent. The cytoplasm is homogenous and the perivitelline space is very narrow. While the embryo develops, it increases a little. The egg diameter varies between 1.10 and 1.33 mm, the oil globule between 0.17 and 0.23 mm. The oil globule is always in the upper part of the eggs.

The embryonic development took 4-5 days, meaning 1300-1500 °h, and all six characteristic stages were covered (Dehnik, 1973). Their description made the object of a published paper (Zaharia and Alexandrov, 1997) (Fig. 2).

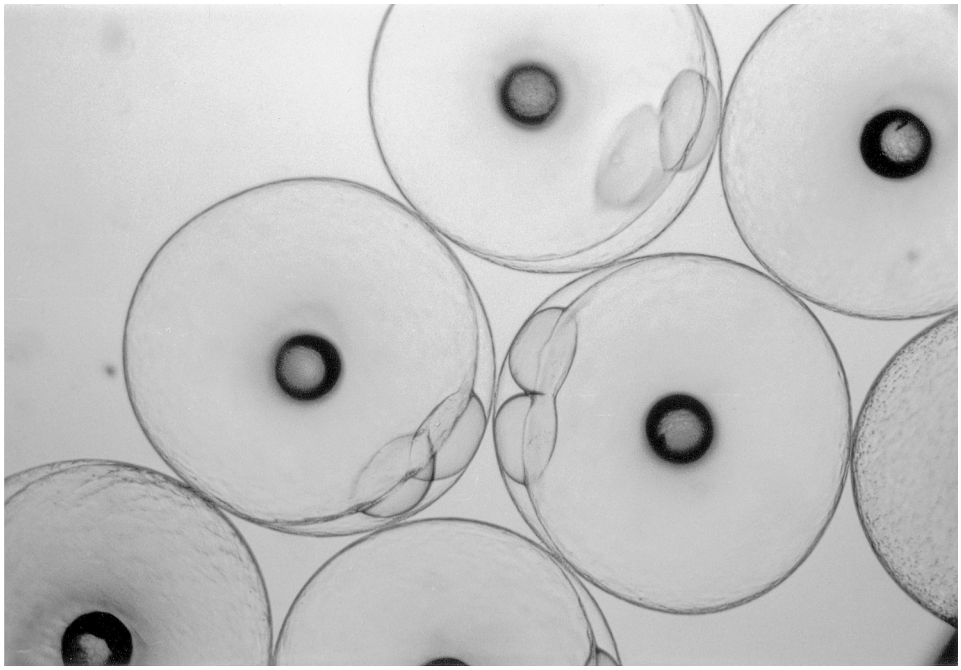

Fig. 2. Stage of embryonic development of turbot – 4 hours after fecundation (5 x 1.5 x 3) (original photo).

After hatching the turbot larvae had a length of 2.80-4.00 mm. The yolk sac is big and oval. The oil

globule is in the lower part, that is why the larvae swim horizontally, belly upside. The body is brilliant pink, with branched out melanophores. In the middle of the caudal zone, pink and black pigments are placed on the fin edge.

Both for flounder and turbot, the incubation survival was influenced by inadequate environmental conditions and poor quality of seminal products (too young or overmature spawners).

As far as spawners maturation is concerned, we don't recommend hormonal stimulation (which we used only as an extreme method), until all mechanisms of hormonal stimulation are well known for those species (Suquet *et al.*, 1991). For spawning purposes, it is more certain to use sires matured in captivity, with a yearly renewal of the broodstock.

The maturation of turbot spawners in captivity is something new for the Black Sea basin, the Ukrainian technology for obtaining turbot juveniles has been based on using wild spawners caught at the maturation stage by the fishing vessels and fecundation is carried out on board the vessels (Bityukova *et al.*, 1990).

Larval rearing

Flounder

The 14 days old larvae have a length of 4.18-4.82 mm, mouth opening 165-198 µm, food searching instinct and ingested food in the intestine can be noticed (Fig. 3). The flounder larvae were reared up to 60 days old (early metamorphosis).

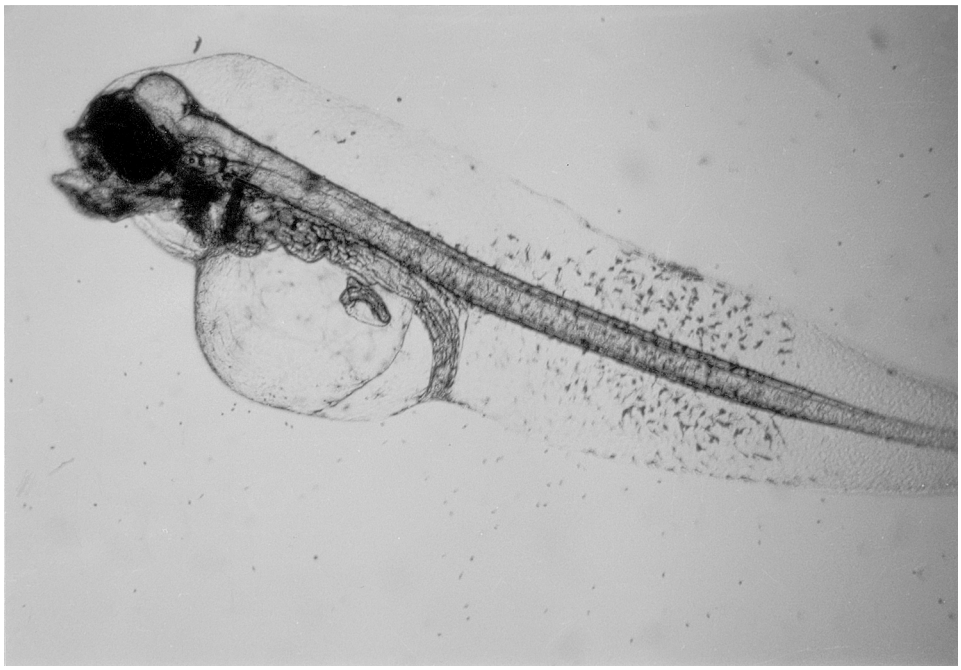

Fig. 3. Flounder larva, 4 days old at the initial mixed feeding (5 x 1.5 x 3)
(original photo).

Several variants have been tried in order to ensure a satisfactory feeding regime, which included especially the following organisms (Alexandrov *et al.*, 1997, 1998):

(i) Microscopic algae (especially flagellate *Tetraselmis sueccica*), for the entire experiment duration.

(ii) Larvae of mollusc *Mytillus galloprovincialis* (trocophores and primary veligers), from 5 days to 16 days old (Fig. 4).

(iii) The rotifer *Brachionus plicatilis* from 13 days to 20 days old.

(iv) The phyllopod *Artemia* sp. (nauplii and metanauplii) from 18 days to 60 days.

The attempts of replacing live food by inert food failed.

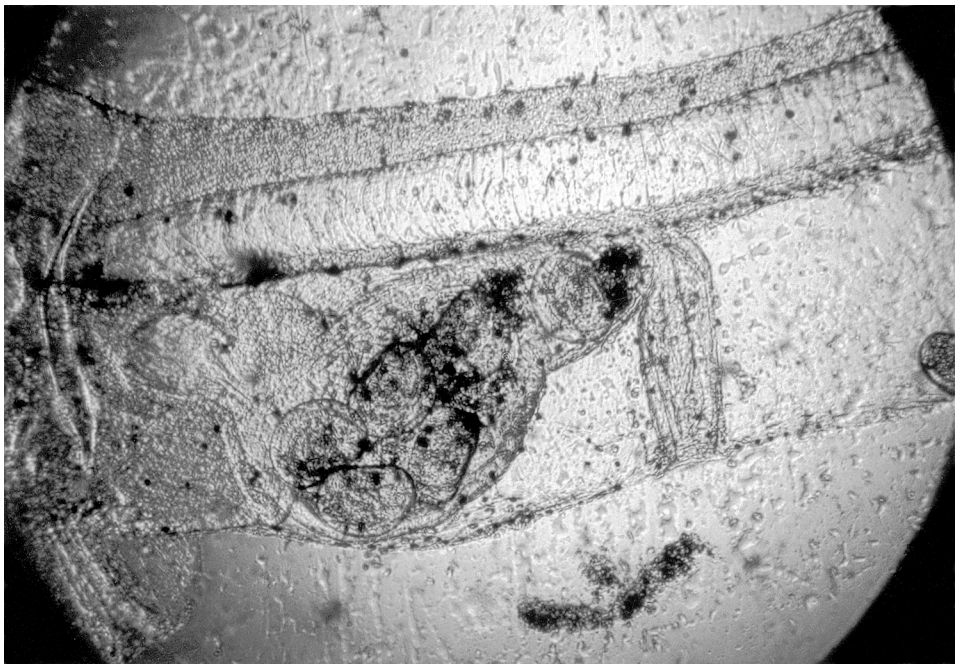

Fig. 4. Intestine content of flounder larva fed on mussel primary veligers (5 x 1.5 x 3) (original photo).

Turbot

The six days old larvae have the yolk sac entirely resorbed, oil globule still present, ingested food can be noticed in the intestine, mixed feeding has begun. The seven days larvae have the oil globule entirely resorbed and well co-ordinated movements. At the nine days larvae the predatory instinct occurs, they are feeding actively.

The turbot larvae were reared up to 30 days old (early metamorphosis).

Several variants were tried in order to establish a feeding regime. The larval preferences were as follows:

(i) Microscopic algae (especially the flagellate *Tetraselmis sueccica*), from 3-4 days old, when the feeding becomes exclusively exogenous.

(ii) The rotifer *Brachionus plicatilis* from 8-9 days old (at the beginning only eggs, then only adults specimens) up to 20 days old.

(iii) The phyllopod *Artemia* sp. (nauplii and metanauplii) from 10-12 days old (simultaneously with *B. plicatilis*) up to 30 days old (end of experiment).

The attempts of feeding turbot larvae on inert food failed, they didn't accept but live food like the flounder larvae.

Taking as a guide the six stages scale (Cepurnov, 1989), we have succeeded to rear the larvae up to the 6th stage (its ending for flounder being 60 days and its beginning for turbot 30 days). We have made observations on their behaviour up to the before mentioned ages in order to establish the adequate conditions for each stage separately.

We think we haven't succeeded in obtaining a complete metamorphosis both for flounder and for turbot owing to the inappropriate food – it is well known the role of fat acids n-3 HUFA (highly unsaturated fatty acid) (Person-Le-Ruyet *et al*., 1993) and optimum conditioning.

Growth of turbot juveniles

As we couldn't obtain juveniles through controlled spawning, we used wild specimens, caught from their natural environment only for turbot which had a higher growing rate capacity and we have started from specimens of 2-4 g each (3-4 months old).

The specimens obtained had weights of 100 g at 1.5 years, 350 g at 2.5 years and 750 g at 3.5 years; they grew more rapidly than in their natural environment (150 g at 3 years, 250 g at 4 years) (Svetovidov, 1964).

The food has included fresh and/or frozen small marine fish, *ad libitum* (with a poor food conversion index: 8.5) and live food (juveniles of shrimps *Palaemon adspersus* and adults of *Artemia* sp.) added for low ages.

The survival was of about 90%, with no differences between ages.

Spawners conditioning

Both flounder (300 g each) and turbot (2500 g each) spawners have adapted themselves to the breeding in captivity very well, the adaptation period was shorter for the flounder than for turbot, and more rapid for the subsequently brought specimens than for the previously caught ones.

They fed on small marine fish, *ad libitum*, with an experiment of granulated food for the turbot (including 55% small fish and 45% vegetal mixtures, as well as mineral and vitamin premix). For a short period (10 days) they were given granules including antibiotics (chloramphenicol and streptomycin) for the treatment of vibroses.

The energetic value of granules was of 5663.5 cal/kg (as against 2414.6 cal/kg for small fish) and protein content 63.7%.

The spawner survival percentage for a year (except of the spawning technologic losses) has varied between 0 and 90% for turbot and 80-90% for flounder. We think the high mortalities in turbot spawners are due to the temperatures exceeding 27°C which lasted for a rather long period.

The culture systems experimented in breeding juveniles and spawners were rudimentary, we used the simplest ways and methods. It is necessary to extend the experiments on utilization of granulated food, with a as complex as possible biochemical composition.

Taking into account the occurrence of vibroses especially in summer time, both spawners and young stages must be vaccinated.

Conclusions

On the basis of the technologies elaborated in France and former URSS for the culture of flatfish, in 1994-1997 we carried out experiments for the culture of flounder and turbot at the Romanian littoral, aiming at the rehabilitation of natural population and development of mariculture.

The experiments have proved:

(i) The possibility of obtaining the biological material for the fish culture through controlled spawning, starting from wild broodstocks which are yearly renewed.

(ii) Controllable culture systems for rearing of larvae and feeding organisms (microalgae, small

invertebrates) are necessary in order to obtain a complete metamorphosis of flounder and turbot larvae.

(iii) In captivity, the growing rhythm of turbot is obviously improved in comparison with that in the natural environment. We think it can be higher in optimum breeding conditions.

(iv) Taking into consideration the conditions offered by the Romanian littoral, our experience in that field and the consulting of a great amount of specialized literature, we think these two valuable species – flounder and turbot can successfully be objects of mariculture in Romania.

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