International Council for the
Exploration of the Sea

C.M./1987/F:36

REPORT OF THE WORKING GROUP ON MASS REARING OF JUVENILE MARINE FISH TO THE MARICULTURE COMMITTEE OF ICES

Brest, France, 24-26 June 1987

This Report has not been approved by the International Council for the Exploration of the Sea; it has therefore at present the status of an internal document for Working Group review purposes only and does not represent advice given on behalf of the Council.

* General Secretary
ICES, Palaegade 2-4
DK-1261 Copenhagen K
Denmark

T A B L E   O F   C O N T E N T S

1. PARTICIPANTS

The Working Group convened its first meeting at IFREMER, Brest, France, on June 24-26th. Members present were: P. Sorgeloos, BELGIUM; K. Waiwood, CANADA; N.H. Norsker, H. Paulsen, J.G. Stoettrup, DENMARK; N. Devauchelle, J. Gatesoupe, J. Guillaume, J. Robin, FRANCE; S. Bolla, D. Danielsen, I. Huse, L. Joergensen, E. Kjoersvik, Y. Olsen, H. Rabben, H. Reinertsen, G. Rosenlund, S. Tilseth, NORWAY; J. Alonso, M.A. Rivas, SPAIN; B.R. Howell, UNITED KINGDOM.

See appendix 7.2. for addresses.

I. Huse, Norway, (chairman) and H. Paulsen, Denmark, kindly served as rapporteurs for the meeting.

2. TERMS OF REFERENCE

The working group meeting was held at IFREMER, Centre de Brest, France. June 24-26. 22 experts from 7 countries participated.

The meeting provided a forum for ICES Scientists studying larval fish rearing to compare the results of their studies and evaluate strategies for production of fish larvae to aquaculture. Discussions focused on the following terms of reference (ICES C.Res. 1986/2:39):

a) evaluate different rearing strategies and methods to aid in overcoming the barrier to large-scale intensive culture of marine fish posed by the lack of effective production methods for fry and other juvenile forms;

b) identify subject areas related to each strategy and method where lack of knowledge prevents development and availability of large numbers of low cost juveniles for intensive culture as well as coastal and ocean ranching programmes;

c) prepare findings in a form suitable for early publication;

d) recommend suitable action to overcome the problems.

3. STATUS REPORT ON SPECIES

3.1. TURBOT (Table 1)

3.1.1. Active interest in turbot rearing, in both research and commercial organisations, has now spread to 6 European countries. Commercial production of market size fish in 1986 is estimated to have been over 200 t shared between farms in Spain, France and the U.K.

3.1.2. A recent increase in both the number of hatcheries and the number of ongrowing sites indicates that production over the next few years is going to increase sharply. A significant consequence of the projected increase in the availability of fry is likely to be the development of a large number of ongrowing units, particularly on the Atlantic coasts of France and Spain.

3.1.3. In northern countries production of market size fish is limited to sites where warm water effluents are available and is unlikely to increase as fast as in more southerly countries. However, the concentration of hatcheries in these countries for the exports of juveniles, indicates that climatic conditions may be more favourable for this part of the production proces than at more southerly countries.

3.1.4. During the last 5 years there has been some diversification of production methods for juveniles with extensive pond methods, developed by the Norwegians for cod, being applied to the turbot both in Norway and Denmark. It is unknown whether these methods will be adopted commercially but the high quality of the juveniles raised by these methods provides a valuable standard against which juveniles produced by intensive methods can be compared.

3.1.5. With intensive methods the principal research effort is focused on the provision of nutritionally adequate feeds both by improving the quality of live feeds and by the development of artificial diets. This reflects the high and variable mortality during the first two weeks of larval development. In this context the value of bacteria is also being considered.

3.1.6. An additional, and perhaps neglected, cause of high mortality may be egg quality. Though this has long been recognized as an important area for research, work to date has largely been limited to evaluating the rates of fertilization in relation to ovulation and other relevant parametres. The study of other factors is currently restricted by the lack of objective criteria for defining quality. Approaches to solving these problems are currently being developed in Norway, and in UK.

Table 1

TURBOT
(* G = Government funded; P= Private)

Country	Organisation	Status*	Production in 1986 (Fry ('000s)	Market size (t)	Research Areas (and comment).
Denmark	1. Danish Institute for Aquaculture, Horsholm	G/P	-	-	Rearing Systems
	2. Danish Institute Fish.&Mar.Res., Hirtshals	G	9	-	1. Intensive rearing systems using copepods as live foods. 2. Extensive rearing systems in concrete tanks 3. Development of microencapsulated feeds for larvae.
France	1. Aquaturbot Tregnier	P	60	-	Hatchery only
	1. SOOAB Tregnier	P	-	5	ongrowing - development dependant on juvenile availability
	2. Ferme Marine du Donlet, Ile d'Oleron (Sea Farms)	P	15	25	up to 40 T
	3. Gravdines, Dunkirk	G	-	-	Intensive (experimental) systems for juvenile on-growing in warm water efficient?
	4. IFREMER, Brest	G	-	-	Nutrition, microbiology
	5. LNPAA, Brest	G	-	-	Pathology
	6. IFREMER. Aqualive Noirmontier	G	-	-	Ongrowing experimental in inland Sclive geothermal water
	7. Devasud, Montpelier	G	-	-	Pilot scate hatchery
	8. Several small on growing farms (Nantes to Biarritz also Brittany)	P	-	<5	Developing - dependant on juvenile availability
W. Germany	1. B.U.T.T. Kiel	P	-	-	New company
	2. Institute of Hydrobiology & Fisheries, Hamburg	G	?	?	No information

Great Britain	1. Golden Sea Produce Scotland (G.S.P.)	P	200	100	
	2. Mannin Sea Farms Isle of Man	P	-	-	New company (fry production)
	3. Clearwater Isle of Man	P	-	-	1. Broodstocks/egg supply under contract to clear water. 2. Sex control
	4. Frippak Aberdeen	P	-	-	Larval feed development
	5. Sea Fish Industry Authority, Scotland	G/P	50		Under contract to G.S.P.
	6. Scottish Marine Biological Association	G	-	-	1. Egg quality 2. Larval nutrition (studentship)
Norway	1.Flodevigen Biol.Station	G	-	-	1. Extensive larval culture 2. Broodstock management
	2. Inst.Mar.Res.Austevoll	G			
	3. ØYE Sea Farm	P	25 (1.6.87)	-	Intensive and extensive culture systems (projected production of 100.000 juveniles per yr)
	4. LMC, Bergen	P	-	-	New Company (extensive juvenile production)
	5. Statoil		-	-	New company. Pilot scale assessment.
	6. Mowi, Bergen (Norsk Hydro)	P	-	-	New company.(Extensive juvenile production)
	7. Norsk Hydro	P	-	-	Larval nutrition. New company.
	8. Sea Farms	P	-	-	New comapny. Extensive juvenile production (and on-growing?)
Portugal	1. University of Porto	G	-	-	Limited ongrowing experiments
	2. Faro	G	-	-	Pilot scale integrated farm.
Spain	Min.Agriculture Inst.Esp.Ocean				
	1. Santander	G	-	-	Intensive Systems (hatchery)
	2. Vigo	G	-	-	Intensive Systems
	3. Plan Marisquero Norte, Vilaxoan	G	-	-	Pilot scale intensive systems
	4. University St.Jacques	G	-	-	Genetics and pathzoology
	5. Marfish Palmejra (Norsk Hydro)	P	-	50	Ongrowing only.
	6. Insuina, El Grone	P	4	25	
	7. Marcultura Esteiro	P	6	-	Developing sea cage culture
	8. Tinamenor Santander	P	50?	-	Hatchery only
	9. Cultipec El Grove	P	-	-	New company (hatchery and ongrowing)
	10. Ibergaliza El Grove	P	-	5	Ongrowing only.
	Several other ongrowing sites being established and one hatchery.				

3.2. HALIBUT (Table 2)

Rearing experiments started less than 10 years ago. Basic studies on eggs and larval stages are carried out and broodstocks are established.

Activities and Institutions involved.

Country	Activity	Participating Organisations
Norway	Natural history	UT
	Broodstock husbandry	IA,IMR,UT,LMC, MOWI, Sjølaks
	Broodstock diseases	IMR
	Egg production	IMR, IA, MOWI, LMC
	Spawning behaviour	IMR
	Reproductive endo-crinology	IMR
	Egg quality	IMR, IA, UT
	Incubation methods	IA,IMR,UT,
	Larval production systems, first feeding, larval nutrition, water quality	IA,IMR,UT,LMC,MOWI SINTEF
	Larval pathology	IA, IMR
	Larval physiology	IMR
	Larval behaviour	IMR
	Larval biochemistry	UT,FORUT, IMR
	Functional morphology of larvae	UT
	Nutrition in ongrowing phase	UT, IMR, SSF
	Husbandry in ongrowing phase	UT, IMR
	Cage development	IMR
Iceland	Production systems	MRI, IFL
Scotland	Broodstock husbandry	Ardtoe
	Larval and juvenile production, first feeding and larval nutrition	
Canada	Natural history	SA
	Broodstock development	MSRL, SA, FRD
	Reproductive endocrinology	MSRL
	Husbandry in ongrowing phase	SA

ADDRESSES

IMR:
Division of Aquaculture
Institute of Marine Research
Directorate of Fisheries
Bergen - Norway

IA:
Institute for Aquaculture Research
The Agricultural Research Council of Norway
Sunndalsøra - Norway

UT:
University of Tromsø
Institute of Biology and Geology
Tromsø - Norway

FORUT:
Research Foundation at the University of Tromsø
Tromsø - Norway

Ardtoe:
Marine Farming Unit
Sea Fish Industry Authority
ARDTOE - Acharacle Argyll PH36 4LD
UK

MRI; IFL:
Marine Research Institute
Islands Lax Inc.
Iceland

SJØLAKS:
Måløy - Norway

MOWI:
Bergen - Norway

LMC:
Lagune Management and Construction
Øygarden - Norway

SSF:
Norwegian Herring oil and meal Industry
Research Institute
Bergen - Norway

SINTEF
Center of Aquaculture
Trondheum - NTM - Norway

FRD:
Fisheries Resource Development
c/o Marine Sciences Research Laboratory
Memorial University
St. John's Newfoundland - Canada

MSRL:
Marine Sciences Research Laboratory
Memorial University
St. John's Newfoundland - Canada

RESOURCE — Biological and technical personnel engaged in halibut rearing

NORWAY: ~ 42
ICELAND: ~ 5
FAROE: ~ 2
SCOTLAND: ~ 5
CANADA: ~ 10

PRODUCTION

No commercial production yet.

3.3. COD

3.3.1 Activities

3.3.1.1. Scientific Activities.

Mass rearing of juveniles are for the time being only carried out in Norway in the ICES countries. The institutions involved in these activities are:

- Institute of Marine Research
  Directorate of Fisheries, Bergen and Flodevigen
- University of Bergen
- University of Tromso
- FORUT research foundation, University of Tromso

The research and development activities are mainly focused on using natural sea water basins/ponds for mass rearing of cod fry. A system has been developed for fry production, which includes:

- Brood stock management, natural spawning system in 175 m^3 enclosed pens with automatic egg collection.
- Egg incubation systems.
- Pond/basin management system and larval release strategy.

The research activity are concentrated upon:

- Pond ecosystem analysis, hydrography/chemistry primary and secondary production and ecosystem modeling.
- The study of larval start feeding strategy, predator prey relationships as well as natural history studies.
- Quantitative and qualitative analysis of larval gut content during the early start feeding periods and comparative analysis of larval biochemistry.
- Study of larval morphological development, histology.
- Study of larval/fry immunology and development of vaccines and vaccination procedures. (dip, bath and oral vaccination).
- Broodstock spawning behaviour.
- Broodstock disease investigation.
- Egg quality investigations.

The aim of mass rearing of juvenile cod is to produce fry for food fish farmers and for restocking experiments. In this respect the method of producing cod fry in pond systems is analysed on a cost benefit basis.

Intensive rearing experiments with larval cod are also carried out in Bergen, Norway. The experiments are a joint programme between the Institute of Marine Research, Department of Aquaculture and the University of Bergen. The work is focusing on:

- Larval behaviour, feeding behaviour and physical stress investigations.
- Development and testing of dry feed diets.
- Bacterial grazing by flagellates in the culture systems.

3.3.1.2.Commercial activities.

This year the pond production method of cod fry is being commercialized. The expected production of juveniles will probably be in the order of 1-2 million.

Three private companies came in operation in spring 1987 and two of these are doing development work towards brood stock management, optimization of hatching systems and pond production strategies. The three companies in cod fry production are:

LMC (Lagoon Management and Constructions)
Bergen,
Sea Farm Ltd., Bergen,
Strandabø/Clearwater, Bergen.

3.3.2. Resources/Production.

A total of 8 pond production systems are in operation in 1987. Four of these ponds are operated by research institutions, three in the Bergen area and one in Tromsø.

The private companies have four ponds in operation, and each company has its own brood stock and hatchery, as well as the research institutions. A total of 1 to 2 million fry are expected to be produced in 1987.

Several salmon fish farms have had pilot cod plants in operation since 1985 and the production in 1987 is expected to be in the order of 300 - 500 tons.

3.3.3. Key Problems

The production of cod fry in the two pond systems operated by the Institute of Marine Research in Austevoll, has been tested and to a certain extent shown reproducible results.

All implications, however, of using ponds or basins for cod fry production are not at present fully understood. The most important areas of investigation are:

- Ecosystem studies and evaluation of the pond carrying capacity related to primary production secondary production and the succession of species in the two trophic levels. The effect of the top predator (fish larvae) stocking density on growth rate and survival. In order to develop methods to control production.

The particles, which are selected by the cod during the early start feeding period is unknown. Effort in research should therefore be put on:

- Identifying early larval fish feeding particles
- Evaluating the nutritional composition and requirement for larval cod essential for growth and survival.

The experience so far indicates that the heavy mortality occurs close to and shortly after memtamorphosis. The mortality is caused by cannibalism, necessitating:

- Reducing cannibalism by increasing food production/weaning by developing better dry feed and feeding procedures.
- Develop capture and size-sorting systems.

Fry production from intensive rearing is still not possible. The reasons for this are not fully understood, but is most likely related to early nutrition and cannibalism.

Artificial diets for startfeeding has yet to be developed. Such experiments are now being carried out with diets based on cod roe.

3.4. SEA BASS AND SEA BREAM

3.4.1. Activities

Unfortunately Dr. Beatrix Chatain, IFREMER/Palavas les Flots, who had agreed to present a status of research and commercial activities, was not able to participate in the meeting. The status report on these very important forerunners in commercialization of marine fishes is therefore not exhaustive.

3.4.1.1. Research activities.

The basic research effort to domesticate these species was carried out in France and Italy from the early seventies. Presently a number of institutions in France, Italy, Spain, Yugoslavia, Greece, UK, Denmark, Turkey, Tunisia and Israel are engaged in R & D to improve the production process for these species.

With a commercial production in operation in several countries for both species, the demand for R & D is more related to optimation and problem prevention rather than innovation. The R & D requirements mentioned are:

- Improvement of intensive rearing technology (sea bream more than sea bass).
- General nutrition

- General pathology

4.3.1.2. Commercial activities.

Tables 3 & 4 give a more or less tentative presentation of the production of juveniles and market size fish the last years.

The numbers are very uncertain, and must be considered as minimum estimates. The production will, however, increase substantially in 1987, and a total fry production of well above 12 million for both species together will be reached.

This tendency seems to continue, and the full market impact will be reached in -88-89.

No doubt the development of the sea bass and sea bream industry will be decisive for other marine species like turbot and sole. The problems and solutions are likely to be similar, and technology and concepts will also be transferable.

Table 3 Production of juvenile sea bass and sea bream

Country	Sea Bass juveniles x 1000		Sea Bream juveniles x 1000	
	1985	1986	1985	1986
France	1150	1500	700	120
Italy	1200	2000	800	1000
Spain	100	1000	30	
Yugoslavi	2000	≤ 2000	-	
Greece	100	600	100	1000
U.K.	5	≤5	5	≤5
Denmark	5	≤5		
Turkey	N.E.		N.E.	
Tunesia	N.E.	500	N.E.	
Israel	-	-	-	300
Total	4560+	7610+	1635	2425

N.E. Newly Established

Table 4 Production of sea bass and sea bream

	Sea Bass		Sea Bream	
Country	1985	1986	1985	1986
France	100	100	15	20
Italy	100	100	50	60
Spain	50	50	10	50
Yugoslavia	–	developing	–	
Greece	20	20	20	30
U.K.	–			
Danmark	–		–	
Turkey		developing	–	30
Tunesia		–	–	
Israel			100	150
Total	270	270	190	340

3.5. OTHER SPECIES

3.5.1. Dover Sole

There are limited brood stocks in England, France, (Italy) and Norway, but none of them are producing larvae for aquaculture purposes, except in Norway, where some ongrowing experiments with dry food is going on.

The Dover Sole seems to have been given up as an intensive aquaculture organism, but has been considered as a candidate for extensive aquaculture ranching. This is, however, not related to rearing problems, but rather to ongrowing problems. It is very difficult to make a diet for commercial production, which gives an acceptable growth and survival rate. Palability and smell as effects of chemical attractants are probably the most important factors to get a better growth rate.

In addition to the nutritional problems, there is also severe disease problems in aquaculture of Dover Sole, especially the black patch necrosis (BPN).

3.5.2. Plaice

Activities:

Due to low market prices, plaice seems to be of no commercial interest at the moment.

Though it is of great value as a "model species", and aquaculture related activities on plaice are carried out by the following institutions in Norway:

- The Institute of Marine Research, Division of Aquaculture, are rearing brood stock plaice, studying the effect of photoperiod on maturation and growth.

- The Center of Aquaculture, SINTEF, (The Foundation for Scientific and Industrial Research at the Norwegian Institute of Technology).

One main object is to test and develop intensive rearing systems for flatfishes including the processes concerning live feed production. The plaice activities are focused on broodstock rearing and management, including conditioning nutrition studies and controlled spawning.

Another objective is to optimize the physico-chemical environment during the incubation period of eggs and larvae, by doing energetic studies.

Emphasis is also put on optimizing the start feeding conditions of the larvae and the composition and quality of the live feed.

Genetechnology experiments are performed as a cooperative work between the Institute of Biotechnology at the Technical Highschool (NHT) SINTEF and the Institute of Medical Biochemistry at the University of Oslo.

Northland Research Foundation, Bodø, is starting a project in cooperation with a private firm, "Norsk Havbruk", on plaice fry production. Their plan is to do searanching experiments on the northern coast of Norway.

3.5.3. Catfish (Anarhicas sp.)

Norway:

Broodstocks of catfish were established at Flodevigen Biological Station during the last part of 1986 and first half of 1987. Some preliminary work was done both to get natural spawning and stripping without any success.

Some ongrowing experiments with juveniles are going on, and the growth rate seems promising.

In addition, some newly hatched larvae from naturally spawned eggs in the sea was fed directly on ordinary dry salmon food with success. Although, there was some mortality, it seems possible to feed catfish larvae on dry food without addition of live food in the first period.

The project is partly privately (BP) and partly governmentally financed.

The main problem is to get spawning products from the broodstocks to get a production of yolk sac larvae.

3.5.4. Other

- Japanese red seabream in Yugoslavia (CENMAR Cie), first commercial production in Europe. Eggs imported from Japan, hatchery production 1986; growout ongoing.

- Puntazzo puntazzo (European bream species) in Yugoslavia (CENMAR) and Greece (CEPHALONIAN Fisheries Ltd.). First commercial production in Europe; hatchery production in 1987; growout ongoing.

- Several tropical species in the USA:

  mullet (Mugil cephalus)
  milkfish (Chanos chanos)
  red seadrum (Scian)
  mahi mahi(Dorado)
  siganid (Siganus siganus)

- Mugil hatchery <u>trials</u> in S. Europe (Italy, Portugal).

4. REPORT ON PROBLEM AREAS

4.1. BROODSTOCK/SPAWNING

4.1.1. Methods for egg production for all species have been developed empirically and there is a lack of quantitative information on factors affecting egg production both in terms of quantity and quality. For example ration levels and food quality are factors likely to be of particular importance, whose effects are poorly understood. In addition, investigation of the effects of enrivonmental factors, such as temperature on the maturation cycle are important.

4.1.2. Techniques for endocrinological manipulation of marine fish are poorly described as compared to freshwater fish. This is an important area for future study.

4.1.3. Methods employed for fertilization vary with species and problems of variable quality are much less important with species that spawn naturally (sea bass, sea bream, cod, sole) than with those where artificial fertilisation is practiced(turbot, halibut). Natural spawning of turbot has been accomplished but optimum conditions, particularly in relation to tank size & shape, stocking density and sex ratio are not known.

4.2. EGG/LARVAL QUALITY

4.2.1. Description of problems

Varying egg quality is likely to be one major cause of differences in the "survival potential" for different larval groups. Increased knowledge of these problems will therefore contribute to optimization of survival and functionality of larvae, and will be important for selection of broodstock and the evaluation of husbandry procedures. Many of these problems are due to lack of basic knowledge concerning for instance physiology, biochemistry, biophysics, microbiology and broodstock nutrition.

The most pertinent problems concerning quality are:

4.2.1.1. Lack of objective criteria for characterization of egg quality.

Such criteria should preferably be applied at early cleavage stages, i.e. as soon as possible after fertilization. Ideal criteria should be applicable to several species, and tests for quality should be as simple and standardized as possible.

4.2.1.2. Causes for egg quality variations.

Egg quality might be influenced by broodstock management or techniques used for obtaining the eggs (stripping/natural spawning), but there is a general lack of basic knowledge in this field.

4.2.1.3. Larval quality estimation

Effects of egg quality on the larval functionality and survival potential are unknown, and should be investigated. Effects from varying incubation conditions might also effect the larval quality. Tests for evaluation of larval quality should therefore be developed.

4.2.1.4. Basic knowledge

Data from (field observation of) the natural range of egg quality in different species are unknown, and should be considered.

4.2.2. Strategies

Little is known about causes and effects of egg and larval quality, and few systematic investigations have been carried out in this area.

Investigations concerning quality have used different criteria, such as fertilization rate, eggs- and larval survival, morphology, mechanical resistance of eggs, buoyancy, hatching success, chemical composition, chromosomal aberrations, relative size and "ripeness" of eggs.

These criteria has not yet been synthesized, which should be done. It should also be considered to what extent data from freshwater fishes are applicable to marine fishes.

4.2.3. Recommentations

- Quality criteria for fish eggs and larvae should be synthesized and evaluated for consistency and applicability for several species. New quality criteria should be sought based on morphology, physiology, biochemistry and behaviour.

- Tests for egg- and larval quality should be developed, and be as simple and standardized as possible.

- Broodstock management effects on egg quality should be investigated, and the quality criteria must be correlated with broodstock management and larval survival. Such methods should be evaluated for several species.

- The natural range of egg quality in different species should be investigated by field studies.

4.3. LIVE FOOD PRODUCTION

Since suitable artificial diets are unlikely to be available in the very near future, it is necessary to intensify the present work with live food organisms in order to support existing and new fish hatcheries.

4.3.1. Description of problems (not in order of priority)

- lack of stability in production systems,
- no precise definition of nutritional requirements,
- nutritional variation,
- incompatibility of fry rearing results,
- lack of knowledge of adequate prey characteristics,
- control of hygienic quality of the live food.

4.3.2. Strategies

- optimisation and standardisation of production techniques,
- enrichment of Brachienus and Artemia with defined or crude products,
- evaluate alternative species biologically and chemically,
- broad chemical characterisation of live feeds,
- assessment of the importance of microbiological conditions in live feed cultures.
- development of standard test for quality evaluation of fry,
- feeding behaviour studies,
- improved operational procedures,
- evaluation of the industrialization potential of new developments.

4.3.3. Recommendations for increased efforts

Improved dialogue between researchers and farmers through the organisation of (regular) joint meeting(s) through ICES-EIFAC

- Encouragement of joint international research programmes through ICES-EIFAC
- International intercalibration of biological and biochemical methods through ICES-EIFAC
- Efforts in automation research and development
- Increased efforts in applied microbiological, histological and fundamental biological work
- Need for improved aquaculture data bases.

4.3.4. Live food

Over the last years outputs of commercial hatcheries of seabass, seabream and turbot have significantly improved in terms of survival rates and quality of the produced fry (healthier larvae more resistant to stress, less deformities, better swim bladder formation and filling, better pigmentation, easier weaning onto prepared feeds). The commercial hatcheries report more consistent hatchery outputs and a reduction of the fry production costs.

This has been achieved by feeding the larvae a better live prey, i.e.

- Application of improved techniques for the production of live feeds:

  * Brachionus: use of small and large strain
  * Artemia: use of selected strains and batches (size of nauplii, HUFA content)
  * Artemia: application of improved and standardized methods for cyst desinfection (decapsulation), hatching (constant temperature, pH, light), nauplii separation and cleaning, cold storage of freshly hatched nauplii.

- Application of improved techniques for HUFA enrichment for Brachionus and Artemia: use of algae, algal substitutes and especially emulsified diets,

- Application of new feeding strategies: more size differences of live prey, more frequent feedings,

4.3.5. Suggestions for future developments/improvements

- gradual supplementation up to complete substitution of live feeds by formulated dry feeds (function of fry production economics)
- development/use of simple stress-test to evaluate fry quality
- use of histological analysis for tracing nutritional deficiencies
- as more is learned about the qualitative/quantitative food requirements of marine fish larvae, adjust the bioencapsulation/enrichment diets for Brachionus and Artemia: HUFA's (esp.22:6ω3) free amino acids, phospholipids, minerals, etc..
- need for better analytical methodology and reporting (especially quantitative methods).

4.4. LARVAL NUTRITION, MICROBIAL ENVIRONMENT AND WEANING

4.4.1. Description of problems

The period of endogenous feeding after hatching is very short in marine fish larvae. The gut is poorly differentiated at the start of exogenous feeding with a short transit time and a poor enzymatic activity. Pinocytosis of proteins and intra cellular digestion may play an important part for nutrient intake. In larval nutrition there is a lack of information concerning both qualitative and quantitative aspects of nutritional requirements.

The role of bacteria is suspected to be very important especially for the mass production of live food, their dietary value and palatability. During stress periods, the susceptibility of fish larvae to opportunistic bacteria seems to be increased.

Zooplankton, which is eaten by wild larvae is not easy to produce. In extensive rearing there are technological problems for the control of phyto-and zooplankton production, but there is no true nutritional problem since larvae feed on their natural diet. In intensive rearing the production of artificial food - either food organisms or prepared diets-needs further knowledge about larval nutrition.

Rotifers, Artemia and perhaps monospecies cultures of copepods have a biochemical composition which is different from the natural diet, mainly in terms of fatty acids and proteins or free amino acids.

Compound diets are quite different from the natural food, but their use is ineluctable 1 or 2 months after hatching. The main question is: When should larvae be weaned for obtaining the best compromise between production cost and quality or fingerlings?

4.4.2. Alternative feeding strategies for the food fequence

A - LIVE FOOD

Classical method: *B. plicatilis* + *Artemia*

Alternative live food: Copepods
Ciliates and marine rotifers for start feeding of sea bream.

B - COMPOUND DIET

Classical method e.g. day 30 (turbot), day 45 (sea bass)

Earlier weaning: between day 10 (sole) to day 15 (turbot) or 20 (sea bass).

Compound diet at start feeding.

4.4.3. Evaluation of the different strategies

Copepods are the live food organisms the composition of which is the closest to that of natural food and therefore they may serve as a tentative reference for the nutritional requirements of larvae. Their content of essential fatty acids is high with a good ratio between 22:6 n-3 and 20:5 n-3. The repartition of organic nitrogen according to Hayashi et al. (1986) is 50% or more as free amino acids or peptides, 10-20 % sarcoplasmic proteins, 30-40 % insoluble proteins. This could be the reason why compound diets give so bad results at start feeding. Until now compound diets cannot be given but after day 15 - 20 after hatching. It may be supposed they could be used before that period with a technique allowing the retention of high level of free amino acids in the artificial particles. However, the cost of live food organisms is not so high during the first month of larval rearing due to the small amounts required per larva.

The improvement of the dietary value of rotifers and *Artemia* with essential fatty acids is now well investigated and several reliable methods for enrichment do exist. However, the proportion of free amino acids is not as high as in copepods (30 % in rotifers). The technique for improving their dietary value from this point of view is not established. Recent data seem to indicate mineral deficiencies in mass-produced rotifers (Robin, 1987). Vitamin deficiencies may also occur (for instance their role is suspected in the dietary value of different strains of *Artemia* for the pigmentation of flatfish - Seikai and Nakamura-).

The use of copepods themselves or other alternative live food (ciliates and marine rotifers) is conditioned by an improvement of their mass production.

The shortening of the period of feeding on live food is linked to an improvement of the composition of the prepared diet and food and feeding technology.

Bacteria associated to fish larvae and zooplankton are much more numerous in plate counts (1000 and 10000times higher than in natural environment). The composition of microflora is also different and variable. However, the quantity of *Vibrio/Aeromonas* and active bacteria generally increases. Except by frequent cleaning and water renewals, it is difficult to control bacterial development. Therefore, these bacteria cause variability in rotifer production, and growth and/or survival of fish larvae. Bacteria occur and act in water, on the body surface and in the gut. They could give some growth factors. They could be eaten by rotifers and help to digest the nutrients in gut. However, the role of the total microflora for fish larvae seems negative or neutral. On the other hand, selected bacteria could improve rotifer production if they are continuously added (probiotic) or if rotifers are cultured in chemostats.

4.4.4. Recommendations for increased effort

A - BASIC RESEARCH ON LARVAL NUTRITION

- Energy budget of larvae in relation to dietary composition.

- Development of digestive system and enzymatic activities, especially for recently-investigated species (halibut). Morphological and functional aspects.

- Identification of specific requirements of larval stages (phospholipids, nucleotides, other growth factors).

- Quantitative determination of dietary requirements and their evolution during development.

- Role of microflora in larval nutrition

- Feeding behaviour.

B - IMPROVEMENT OF THE DIETARY AND SANITARY VALUE LIVE FOOD

- Culture techniques
- Enrichment
- Diversification and choice of species
- Control of microflora.

C - COMPOUND DIETS

- Composition (nutrients, attractants, pigments, physical properties)
- Technology of microcapsules and microbound diets.
- Feeding techniques.

D - FEEDING STRATEGIES

- Food sequence and time of weaning
- Feeding frequency and duration of meals
- Use of automatic feeding systems.

4.5. INTENSIVE AND EXTENSIVE REARING

4.5.1. Intensive culture systems There are in fact two kinds of intensive culture systems:

- green water system(semi intensive): in which indoor tanks are inoculated with algae and rotifers to create a confined ecosystem before larvae inoculation, after which rotifers and Artemia were daily added to maintain a food concentration during larval development. This system is intermediate between extensive culture systems and intensive clear water systems. This technique is used for sea bream, mainly in Japan.

- clear water system: in this technique larvae are introduced in indoor tanks in clear water, and food, such as rotifers, Artemia or otheris daily provided to the tank. Daily amount of food is determined by a general pattern adjusted each day by a survey of the prey remaining in the tank. In this technique it is possible to control environmental factors and food.

Disadvantages: Intensive culture systems need high sophistication of facilities to be able to control factors and equires much manpower. It needs high level of labour in larval rearing control and living prey production. Therefore the technique is expensive and requires high levels of investment and trained personnel. Pathological problems, mainly infectious, can be encountered due to the high levels of biomass.

The fry obtained from intensive culture systems seems for the moment to have a lower physiological status than the animals obtained by extensive culturing, and this also seems to affect weaning and ongrowing success. Some abnormalities also occur, sometimes at high rates. However, these problems will probably be solved by improvements of nutritional and environmental factors.

Advantages: In practice, when the larval culturing process is sufficiently established, it is possible to have

- a good predictibility of the production

- high quantity of fry per hatchery

- fry provided all over the year by using spawning control

- a predictable cost of fry

All the difficulties encountered and the studies needed to define the production process of a species induce new knowledge in biological sciences and can be used and adapted for other species.

Techniques used are also useful for more fundamental studies.

Recommandations and perspectives

Work should continue in both system types.
comparison and cooperation between researchers working in the two system types is vital. For example:

- Biochemical analysis of the fry produced by semi extensive method could be used as reference for improvements of intensive method.

- Surveys easily carried out in intensive culture systems give approach to understanding larval evolution in semi extensive culture.

The two rearing methods can be coordinated for a commercial development of a species: the first fry maybe produced by semi extensive technique to study ongrowing and to begin commercial production, after which the intensive systems will be able to produce fry on a larger scale for increased production.

4.5.2. Extensive culture system

Extensive rearing systems are defined as rearing of aquatic organisms in an environment where food/prey organisms are naturally produced.

There are three types of extensive rearing systems:

1) Artificial, constructed ponds/basins in concrete or earth, inoculated with sea water.

2) Natural sea water ponds or estuarine areas closed by dam(s), or small fresh water lakes filled with sea water through pump systems.

3) Open small landlocked fjord or estuarine areas where artificially produced frys are released to feed on naturally produced prey.

The first two methods are being tested out by research institutions in Norway and Denmark. The second method has been commercialized in Norway. The third method is well established for anadromous fish in several countries and also for marine species like the red sea bream in Japan. Recently a major research program on sea ranching of cod (Gadus morhua) has been launched in Norway to strengthen local stocks. This programme is a combination of method 2) for fry production and method 3) for the release program.

Disadvantages

Closed marine ecosystems can so far only be controlled to a small extent. This is mainly due to lack of sufficient ecological knowledge about the species and transfer of energy between the different trophic levels. The disadvantages are mainly connected to:

- The level of primary and secondary production is unpredictable in time
- The release of marine fish larvae in the pond has to be closely timed with the secondary production in the pond.
- The succession of species in the different trophic levels varies between years and ponds with different geographical locations
- The production of fry is seasonally limited
- Larval/fry diseases and/or parasites are difficult to control

Advantages

Some of the disadvantages will definitely be overcome when more knowledge about closed marine ecosystems is gained. Diseases may be controlled by development of vaccines and vaccination methods. The experience so far with extensive rearing of marine fish fry is:

- Predators can easily be removed

- The released larvae find adequate nutrients in the diet of naturally produced prey organisms to overcome the difficult first feeding period.

- The system seems well suited for mass rearing of juveniles both for ongrowing in fish farms and sea ranching purposes.

- Large numbers of high quality fry are produced in pond/basin systems (fully pigmented, low frequency of malformations)

- The investment costs can be low

- The production is not labour intensive and consequently labour costs are low.

4.5.3. Recommandations

Comparative studies and cooperation between researcher working with intensive systems.
For example:

- fry produced by extensive method could be analyzed to be used as reference for improvements of intensive methods.

- the surveys easily made in intensive culture systems can be used to improve extensive methods.

The use of the two rearing methods can be coordinated for the commercial development of a species.

Extensive technique may produce the first fry to begin development and to study ongrowing, after which intensive systems will be able to produce fry at a higher scale for an increased production .

5. RECOMMENDATIONS

The following recommendations are based on evaluation of the key probems identified by the working group.

Research should continue both in intensive, semi intensive and extensive systems as investigations in all these system categories supplement each other in providing information about the organism studied.The ultimate objective with this combined effort should, however, be to gain sufficiant information to establish an intensive and predictable production line for marine fish fry.

Quality criteria for fish eggs and larvae should be synthesized and evaluated for consistency and general applicability. New quality criteria should be sought out based on morphology, physiology, biochemistry and behaviour.

Broodstock management and nutrition effects on egg quality should be investigated and correlated with egg and larval quality criteria.

Natural range of egg quality in different species should be investigated in field studies.

The use of live prey is and will continue to be vital in culturing of marine fish. A synthesis of the Artemia and rotifer preparation and production process should be carried out in order to optimize, stabilize and standardize production conditions as well as enrichment procedures and diets.

International intercalibration of biochemical and biological procedures should be organized through ICES - EIFAC.

Morphological and biochemical development of the digestive tract of larvae of new species in culture should be investigated to evaluate ability and time for start feeding.

Specific nutritional requirements of different larval stages should be established, especially concerning different lipid components, as well as proteins, peptides and amino acids.

The role of bacteria in larval culturing should be given special attention as it may be the most important single factor governing production.

Weaning diets should be improved based on new information from nutritional, physiological, and biochemical studies to shorten the live prey period in order to avoid prolonged use of expensive Artemia.

With farming of marine fish species now on the verge of becoming an important commercial activity, the working group should continue its work. An especially active area is Galicia, Spain, and next year's meeting should be held in conjunction with an EIFAC meeting on nutrition of fish larvae chaired by Dr.s Konrad Dabrowski and Patric Sorge-loos, in Vigo June. The working group members members houls participate in the EIFAC meeting and then have a subsequent three day working group meeting with participants also from the EIFAC meeting. The working group meeting should be chaired by Ingvar Huse.

6. TERMS OF REFERENCE FOR NEXT MEETING

At the working group meeting 1988 the following terms of reference are sugggested:

The group should meet to:

a. Evaluate quality criteria for eggs and larvae in order to standardize the application of such criteria in science and commerce.

b. Demonstrate possible correlations between egg and larval quality criteria and the broodstock condition in terms of management procedures, nutrition, and the chemical composition of oocytes.

c. Synthesize the rotifer production and enrichment process in order to identify standardized procedures, and nutrient and environmental requirements, which will form the basis of a reproducible industrial scale production process.

d. Discuss the role of microflora both in prey cultures and in larval cultures, and to discuss strategies to overcome possible related problems.

e. Identify the elements of a standardized production process for marine fish fry, identify elements where further work is required, and recommend action and priorities on this basis.

7. APPENDIXES

7.1. AGENDA

ICES Working Group on Mass Rearing of Juvenile Marine Fish.

W.G. Meeting IFREMER Centre de Brest 24-26th June 1987.

Wednesday 24th

0900 Opening - "Salon Rouge"
Presentation
Appointment of rapporteur
Terms of reference

Brief status reports:

Turbot/Sole
Sea bass/sea beam
Halibut
Cod
Other Species

National status reports

Lunch

Brood stock management, conditioning, and nutrition.
Spawning/stripping
Egg quality
Incubation

Visit to aquaculture facilities at Centre de Brest.

Thursday 25th

0900 Larval rearing:
Strategies
Technology
Environmental factors
Microbiology

Lunch

Larval nutrition
Live prey production
Artificial diets for larvae and prey
Weaning

Friday 26th

0900 to 1300
Evaluation in accordance with Counsil resolution
Identification of problem areas
Recommendations
Conclusion

7.2. LIST OF PARTICIPANTS

Belgium:
Lab. de Recherches biol.
de la Pollution
de l'Environnement
Rue J. Plateau 22
9000 Gand

Patrick Sorgeloos, live prey

Canada:
Dept. of Fisheries & Oceans
Biological Station
St. Andrews, N.B. EOG 2XO

Kenneth Waiwood, halibut, cod, haddock

Denmark:
Danish Institute
for Fisheries and
Marine Research
North Sea Center
P.O.Box 101
9850 Hirtshals

Josianne G. Støttrup, live prey, art. feed
Helge Paulsen, turbot
Niels H. Norsker, live prey

France:

IFREMER
Centre de Brest
BP 337
29273 Brest Cédex

Nicole Devauchelle, broodstock, turbot, shellfish
Joel Gatesoupe, fish larvae, turbot, rotifers
Jean Guillaume, nutrition
Jeanine Person, fish larvae, weaning
Jean Robin, algae, rotifers

Norway:

Flødevigen Biological Station
4800 Arendal

Didrik Danielsen, turbot, sole

Institute of Marine Research
Austevoll Marine Aquaculture Station
5392 Storebø

Ingvar Huse, halibut, turbot, cod
Håvard Rabben, halibut

Dept. of Aquaculture
C. Sundtsgt. 37
5004 Bergen

Snorre Tilseth, cod

SINTEF
Division of Applied Chemistry
Aquaculture Group
7002 Trondheim - NTH

Sylvie Bolla, live prey
Leif Jørgensen, plaice
Yngvar Olsen, live feed, algae
Helge Reinertsen, nutrition
Grethe Rosenlund, art. feed

University of Tromsø
Tromsø Biological station
9000 Tromsø

Elin Kjørsvik, development of eggs & larvae

Spain:

Laboratorio Oceanografico
Apartado 1373
38080 Santa Cruz de Tenerife

Maria Rivas, sea bass, sea bream

I.E.O.
Cabo Estay
Apartado 1552
36280 Vigo

José Alonso, turbot, sea bass, sea bream

United Kingdom:
Fisheries Laboratory
Lowestoft NR 33 OHT
Suffolk (now Conway)

B.R. Howell, Dover sole, cod, turbot

7.3. SUBMITTED PAPERS

BELGIUM

V. Franicevic, D. Lisac, J. Buble, Ph. Léger, P. Sorgeloos: International Study on Artemia. XLII. The effect of the nutritional quality of Artemia on the growth and survival of sea bass (Disentrarchus labrax L.) larvae in a commercial hatchery.

P. Leger, D.A. Bengtson, P. Sorgeloos, K.L. Simpson and A.D. Beck: The nutritional value of Artemia: A review.

P. Sorgeloos and P. Leger: Optimized larval nutrition (seabass, seabream, turbot).

DENMARK

N.H. Norsker: Production of harparcticoid nauplii for the first feeding of marine fish larvae.

H. Paulsen, N.G. Andersen: Extensive rearing of turbot larvae (Scophthalmus maximus L.)

J. G. Støttrup: Artificial diet for marine fish larvae.

J.G. Støttrup: The cultivation of the calanoid copepod, Acartia tonsa, for use as a live food organism for marine fish larvae.

FRANCE

N. Devauchelle: Four marine fish spawners in European hatcheries.

N. Devauchelle: Brood stock management, conditioning and nutrition, spawning, stripping, egg quality, incubation.

J. Gatesoupe: Introduction of live bacteria into the food chain for the larval rearing of turbot.

J. Robin: The quality of living preys for fish larval culture: preliminary results on mineral supplementation.

NORWAY

D.S. Danielsen and K.E. Gulbrandsen: Growth rate of turbot (Scopthalmus maximus L.) and sole (solea solea L.) based on dry pellets.

E. Kjørsvik: Egg quality in marine fish.

K. Naas and S. Tilseth: Present status of the poll and basin method for marine fish fry production.

I. Olsen, J. Rodrigueq, O. Vadstein: Changes in fatty acids and total lipid content in _Brachionus plicatilis_ upon changes in diet and starvation.

I. Opstad: Norwegian report of Activities.

H. Rabben: Cultivation of Atlantic halibut in Norway.

G. Rosenlund, L. Jørgensen: A study on the effects of the lipid composition in enrichment and weaning diets for marine fish larvae.

SPAIN

J. Iglesias: The current situation of turbot aquaculture in Europe.

UNITED KINGDOM

B.R. Howell: U.K. National report.