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International Council for the
Exploration of the Sea

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

Palavas-les-Flots, 16-19 June, 1989

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1. PARTICIPATION

The Working Group convened its third meeting at Palavas-Les-Flots, France on June 16-19, 1989. Members attending the meeting were:

P. Sorgeloos, Belgium; W. Waiwood, Canada; H. Paulsen, J.G. Støttrup, Denmark; I. Fjallstein, Faroe Islands; G. Quantz, F. rep. Germany; S. Bolla, I. Huse, L. Jørgensen, E. Kjørsvik, T. Kleppe, A. Mangor-Jensen, T. Meeren, K.E. Naas, A. Skadsheim, Norway; J. Iglesias, A. Ortega, Spain; P.O. Larson, Sweden.

Also attending the meeting were:

Martin Daintith, Steve Nel, Australia; P. Leger, P. Maes, S. Corneillie, P. Van Sprang, Belgium; B. Chatain, C. Fauvel, J. Person, J.H. Robin, France; A. Tandler, Israel; J.A.J. Verreth, Netherlands; Y.G. Attramadal, D. Danielsen, R. Farestveit, K. Gravningen, J. Gulbrandsen, K. Hamre, I. Holmefjord, I. Lein, E. Moksness, J.R. Rainuzzo, Norway; M. Gillespie, Scotland; F. Amat, A. Esterez, M.J. Fernandes-Reiriz, G. Minkoff, J.B. Peleteiro, M. Planas, J.C. Navarro, M. Torre, Spain; P.O. Larson, Sweden; D. Bengtson, USA.

See Appendix I for addresses.

I. Huse, Norway, (chairman) and J.G. Støttrup, Denmark, kindly served as rapporteurs for the meeting.

2. TERMS OF REFERENCE

The ICES working group on "Mass Rearing of Juvenile Marine Fish" met to work according to the following terms of reference (ICES C. Res. 1987/2:43):

- a) evaluate quality criteria for eggs and larvae to standardize their application 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) evaluate the rotifer and Artemia production and enrichment process to identify standardized procedures, nutrient and environmental requirements to form the basis of a reproducible industrial scale production process;
- d) discuss the role of microflora in cultures of prey and larvae and develop strategies;
- e) identify the elements of a standardized production process for marine fish fry, and the need for further work.

3. DEVELOPMENT OF EGGS AND LARVAE

3.1. Quality criteria for eggs and larvae.

The discussion was based on the report by Kjørsvik et al.: "Egg quality in marine fish." The characteristics, which underlie good or poor egg quality are incompletely understood. The paper focuses on possible indicators of egg quality, and summarizes current knowledge.

Fertilization rate is the criterion most commonly used for the determination of egg viability, but several studies show that fertilization rate is insufficient, when used alone. The most reliable practical method of determining egg viability is probably observation of morphological characteristics in the early stages of development. Some biochemical parameters may also be promising as quality indicators, but these are not well documented at present.

Morphological and physical characteristics of eggs with low viability are often similar to those seen in the egg due to the over-ripening process. Environmental parameters (such as temperature) during gonad maturation, and stripping of eggs at the correct time after ovulation seem to be important determinants of egg quality.

Biochemical composition of the eggs is clearly correlated with the composition of the broodstock feed. Correlations between chemical composition of the eggs and egg

viability are, however, only observed when severe deficiencies have been induced in the eggs.

Also, M. Planas presented a report entitled "Biochemical factors affecting egg quality in turbot: A general approach." Turbot eggs from four different spawnings were examined at different stages of development in order to evaluate the possible value of the biochemical composition (ash, proteins, carbohydrates, glycogen, lipids and fatty acids) and enzymatic activities of newly spawned or developing eggs as quality criteria.

There seems to be a direct relationship between egg quality and low carbohydrate-high glycogen content. Fatty acid content is higher in good quality spawning while enzymatic activity is higher in over-ripe eggs. In good quality eggs there are higher levels of several fatty acids (mainly 16:0 and 22:6 n-3). The ash content of a batch of eggs also seems to be related with the posterior success of incubation.

3.2. Biochemical composition of marine eggs and larvae.

3.2.1. Fatty acid composition in turbot eggs.

Biochemical composition may be a possible criterion for egg and larval quality and a comparison of the biochemical composition of eggs from wild and domestic broodstock may provide useful information towards this end. Preliminary results on the fatty acid content in turbot eggs from wild and domestic broodstock were presented by D. Danielsen and revealed higher content of HUFA's and lower content of C16:0 in eggs from the domestic broodstock.

3.2.2. Fatty acid composition in halibut larvae.

José Rainuzzo presented results on individual 5-day old halibut larvae, showing very low variance between samples. In general, over the first 48 days, the results showed a gradual consumption of saturated (especially 16:0) and monoenoic acids, and decrease in larval fatty acids from ca. 150 ug to 90 ug. The content of HUFA's remained unchanged during this period, although 22:6 n-3 showed a slight decline.

3.2.3. Phosphate metabolites in halibut larvae.

L. Jørgensen and H. Grasdalen presented their results on phosphate metabolites in halibut larvae. In Vivo ³¹P-NMR was used to characterise phosphate metabolites in yolksac larvae of halibut at three different stages of development. The major contributors to the NMR spectrum were inorganic phosphate (Pi), phospholipids (PL), sugarphosphates (SP), phosphocreatine (P-Cr), and ATP.

From day 10 to day 50 after hatching the relative amount of sugarphosphates (SP), phosphocreatine (P-Cr), and ATP increased by 450, 445 and 242%, respectively, while that of phospholipids (PL) decreased by 62%. The marked increase in P-Cr coincides with the increased activity of the larvae and that the swimming muscle gets loaded with chemical energy. The position of the inorganic phosphate resonances indicated a mean internal pHi of 7.4 in the larval tissue and 5.6 in the yolk.

3.3. Development of the digestive system.

E. Kjørsvik presented a paper entitled "Development of the digestive system in marine fish larvae, with special reference to the cod (Gadus morhua L.)."

From enzymatic studies trypsin was found to be present in fish larvae even before hatching, and the tryptic content in the larva was dependent on its "feeding status". The secretion of this enzyme from the pancreatic tissue into the intestine in response to ingestion of live as well as artificial food particles was also studied.

Morphological and enzymatic studies give good evidence for digestion and absorptive patterns in the gut of larval fish. Morphological data offer information on both the absorptive process in the larval gut and on nutritional quality of the diet. Such studies should therefore be crucial in the attempts to develop optimal feed for intensive rearing of larval fish.

3.4. Conclusions

The presentations and discussions in this session had mainly bearing on points a) and b) in the Terms of Reference. Although not exhaustive, the presentations and discussions shed much light on the question of quality criteria for eggs and larvae, and also on the relationships between broodstock management/condition and egg quality. Morphological examination of the eggs still seems to be the most reliable indicator for egg quality. Biochemical characterization seems promising, but needs further verification. Over ripening due to too late stripping seems to be an important cause of bad egg quality. It was also demonstrated that broodstock feeding regime was reflected in the chemical composition of the egg (turbot).

4. LARVAL NUTRITION AND LIVE FOOD

4.1. Fatty acid requirements in larval fish

During the previous ICES Working Group meeting, several participants committed themselves to participate in an intercalibration study on the HUFA requirements for marine larval fish. The aim of this study was to better identify the qualitative and quantitative HUFA requirements in marine larval fish. Several countries have responded and requested the various emulsions which were prepared and made available by the Artemia Reference Center for enrichment of rotifers and Artemia.

J.G. Støttrup presented results from one trial on first-feeding larval turbot, using rotifers enriched with an emulsion containing 3 levels of HUFA. The results showed an effect of the enrichment on the HUFA and lipid content of the rotifers. However, the poor and highly variable results on growth and survival in turbot larvae feeding on rotifers in this experiment appear unrelated to the fatty acid content or distribution in the rotifer. Rotifers have often been proved to be an inadequate diet for marine fish larvae, but high variation in larval growth and survival is encountered in both intensive and extensive systems, independent of the type and origin of the food organisms. Thus, other factors related to larval "quality", water quality, or quality of the food organisms may, at times, play a more important role in larval nutrition.

Preliminary results on larval sea bass using Artemia nauplii with different fatty acids (J.C. Navarro et al.) have shown that, there are no significant differences between treatments when comparing Fulton condition factor (Weight/Length E3). Survival and the variation of weight and length were better indicators. Significant regressions can be found when plotting the percentage of moisture content against the survival rates of the larvae fed different levels of essential fatty acids. RNA:DNA ratio gives only clear variations as long as there are detectable amounts of essential fatty acids in the live prey. Variations in the oxygen consumption can be found among animals affected in varying degrees by the essential fatty acid deficiency syndrome.

P. Sorgeloos, Ph. Leger, S. Corneillie and S. Nel reported results on improved larviculture success when feeding HUFA-enriched Brachionus and/or Artemia in the culture of the Asian seabass Lates calcarifer, the freshwater prawn Macrobrachium rosenbergii, the European seabass Dicentrarchus labrax, respectively the dolphin fish mahi-mahi Coryphaena hippurus. In the case of Lates an attempt has been made to quantify HUFA requirements in pre-metamorphosing larvae: HUFA enrichment in the Artemia diet improved

survival at metamorphosis and resistance to salinity shocks (in stress tests). In Macrobrachium, HUFA enrichment resulted in earlier and more synchronous metamorphosis. Increased HUFA-levels in the diet of Dicentrarchus improved larval growth; HUFA deficient diets resulted in spinning behaviour ("whirling disease"), enlarged gallbladder and mortality. Coryphaena has extremely high requirements for HUFA's, increased phospholipid levels in the enriched Artemia appeared to result in the production of healthier larvae.

4.2. Conclusions

This session had bearing on point c) in the Terms of Reference. Although the main emphasis was on larval nutrition, rotifers and Artemia nauplii were used in most of the work presented. Nutrition in marine fish larvae, and consequently live prey composition, has had a tendency to be considered equivalent to HUFA requirements. It was agreed that this is an important factor, but that other requirements e.g. free amino acids and the balance between lipid classes should also be more closely investigated.

5. ZOOTECHNIQUE

5.1. Fatty acid intercalibration exercise.

P. Léger oriented on the progress of this project, also a development from the last ICES Work Group meeting. The results from a previous comparative exercise using Artemia, showed that differences in results on fatty acids and distribution, between laboratories may be high due to the high variation of methods for extraction, esterification and instruments used. Even though these differences were not dramatic, comparison of data would be facilitated if an intercalibration exercise is performed.

The Artemia Reference Center has now prepared a document comparing the commonly used methodologies and their combinations, in terms of accuracy, precision and simplicity. Based on these results, a method is proposed for lipid extraction, esterification and GC-analysis. This method will be evaluated first by SINTEF (Norway) and Dr. Kissil (Israel), and later by Dr. Cowey (UK) or Dr. Ackman (Canada) for final appraisal.

5.2. Standardised larval rearing technique.

A consequence of the difficulty in rearing certain species of marine fish larvae is that the variation between and within experiments may often exceed that resulting from the parameter/s being tested. Among the recommendations made during the last meeting of this Working Group was the development of a standard laboratory test-system with high reproducibility for the rearing of marine fish species for use in nutritional requirement studies; that is, all non-nutritional parameters should be standardised. J.G. Støttrup, Denmark, agreed to prepare a proposal. The aim is to increase the number of comparative observations. Alternatively, it can be used as a reference for comparison.

A table was compiled from the data at hand to provide a basis for discussion, since a choice of physical and biological parameters for a test system for turbot larvae is inevitable. This table was submitted as part of a paper entitled "Outline for a standard laboratory test-system for turbot larvae."

5.3. Role of microflora in larval rearing.

The use of antibiotics in aquaculture was discussed, especially in relation to possible environmental effects from effluents containing antibiotics.

G. Minkoff, Spain, reported that growth of *Vibrio* type bacteria was controlled in rotifer mass cultures using antimicrobial agents. It was found that a 7.5ppm dose of Furazolidone on the day of innoculating the culture was sufficient for maintaining the bacterial levels at 0-100 CFU/ml during the subsequent 5 culture days. Furazolidone did not seem to have any residual effects once the cultures were rinsed and reinoculated in fresh medium. Rotifers from mass cultures treated in this way had low bacteria levels following an overnight enrichment in Frippak booster.

Furthermore, J. Robin reported a positive effect of yeast on bacterial levels in rotifer and Artemia cultures.

5.4. Conclusions

This session had bearing on points c), d) and e) in the Terms of Reference. The fatty acid intercalibration is in progress and will eventually provide a good basis for comparison of experimental results. An effort to standardize the experimental set up has been initiated. The pathological effects of microflora was still considered to be the largest threat to the development of marine fish aquaculture as it has proved to be for penaeid shrimps. The use of antibiotics was much discussed on the

basis that it is widely used today. The lesson learned from shrimp culture is that it is no commendable way. Disinfection and drying out of the whole plant for e.g. one month each year seems to be a possible solution.

6. PRODUCTION STATISTICS

Data on the production of juveniles of some marine fishes during 1988 plus expected production for 1989 for some countries are given in the following table:

	Sea bass		Sea bream		Turbot		Cod		Halibut	
	88	89	88	89	88	89	88	89	88	89
France	1000?	1000?	?	?	200	200				
Spain			3000	5500	70	250				
Norway					350	900	300	800	2	20
U.K.					250	300				
Portugal	200	400		400						
F.R.Germany					20	50				
Italy	1000	1500	1100	2000						
Greece			1200	1500						
Yugoslavia	2000	2800	500	700						
Israel	20	60	600	750						
Turkey			500	1000						
Cyprus		500								
Tunisia	100	120								
Morocco	50	50								
Total	4370	6430	6900	11850	890	1700	300	800	2	20
Sum 88:	12,462			Sum 89: 20,800						

Numbers in thousands.

7. RECOMMENDATIONS

The following recommendations were identified and agreed upon:

A. Egg quality and larval development.

General recommendations:

The Working Group noted with concern the general lack of information in several areas related to egg quality and larval development. These areas included:

1. The relationship, particularly in temperate and cold water species, between broodstock nutrition/health and egg and sperm quality, including egg viability and larval malformations. Also basic biology of the maturation cycle of all captured species including when chemical components are incorporated into the eggs. Studies on feeding in wild stocks could provide insight into nutritional requirements of broodstock and chemical characterization of eggs.
2. The biochemical and physical processes related to this phenomenon, particularly for those species requiring stripping.
3. Standardization in the criteria used to assess sperm, egg and larval quality (e.g. fertilization rate, cleavage, hatching rate, deformity rate, lipid and amino acid composition) and the development of new criteria for egg and larval quality based on biochemical, histological and physiological characteristics.
4. Basic information on the developmental morphology and physiology of fish larvae. This includes enzymatic activity and digestive capabilities in relation to various feeds and environmental conditions.
5. Standardization of methods for the disinfection of eggs and the transport of eggs and larvae. Current procedures are reported to be highly variable, and substantial losses occur in some cases.

The Working Group recommends that research in these areas be continued and encourages participants to present their findings at future meetings.

Specific recommendations:

Several studies are being conducted on the effect of broodstock nutrition on egg viability and the nutritional requirements of wild vs captive broodstock. It is recommended that these results be presented at the next meeting together with recommendations for future research.

A standard treatment/response test for larval quality should be devised and it is recommended that the

usefulness of a salinity challenge test should be investigated for different species.

The Working Group recommends that chemical content of eggs should be presented both on a per egg and weight basis preferably at the morula stage.

It is recommended that chemical analysis of larval fish only be conducted on samples without stomach contents. If this is not possible, the gut content should be estimated.

Definition of a standard rearing system for species currently being cultured has been initiated, and a first draft was presented at this meeting. Standardization will permit comparisons among research groups. It was realized that further development is required before a standard can be adopted. The Working Group recommends that this initiative be followed up and a final report be presented at the next meeting.

The Working Group also recommends that the following be presented at the next meeting:

- a review of procedures for determining variability in sperm quality (presenter?).
- result from current studies on variability in chemical content of eggs and larvae (presenter?).
- a review of egg disinfection methods (presenter?).
- a descriptive list of larval deformities for turbot, sea bass, sea bream, halibut and cod (presenter?).

B. Nutrition.

General recommendations:

The Working Group recognized several areas requiring further research, including larval requirements with regard to energy, HUFAs, amino acid composition and vitamin E and C; differences in availability, composition and digestability between live versus inert diets and the use of analytical data on wild eggs and larvae as a reference for the above considerations. The Working Group recommends that research in these areas be continued and encourages participants to present their findings at future meetings.

Specific recommendations:

There should be better reporting of enrichment protocols in terms of temperature, type and composition of diets, concentrations of food organisms, timing and storage. When possible, test diets should be isocaloric, isolipidic and isonitrogenous.

The interlaboratory study on HUFA requirements in fish larvae and the intercalibration study on HUFA analysis should be continued.

The Working Group recommends that the following be presented at the next meeting:

- results from a study on the relationship between changes in lipid classes and amino acids in plaice larvae (J.Rainuzzo and R.Farrestvedt, SINTEF)
- a litterary review of lipid class presence and function in marine fish (J.Rainuzzo, SINTEF)
- a review of the role of lipids in marine organisms (J.Sargent, Sterling University, to be invited)
- a review of protein metabolism in fish (Kaushik, INRA, to be invited)
- a review of amino acid biochemistry and the involvement of amino acids in the biochemistry of fish larvae (H.J.Fyhn, Bergen University, to be invited)
- a collection of litterature on nutrition and histology (D.Bengtson, Rhode Island University)

C. Microbiology

General recommendations:

The Working Group recommends that research in the following areas be continued:

- development of standard procedures for ensuring sanitary conditions at the commencement and during commercial or experimental larval rearing.
- evaluation of methods to reduce the susceptibility of larvae to bacterial infections and the use of alternate microbial organisms to exclude or reduce harmful bacteria from the larval rearing systems.

Specific recommendations:

At least one routine microbiological survey should be made during the course of a commercial or experimental production cycle.

The Working Group recommends that ICES should discourage the indiscriminate use of antibiotics in juvenile marine fish production, and encourage the treatment of effluents to destroy antibiotics whenever it is used.

D. Next meeting

The Working Group recommends that the group should continue its work and meet in Oban, Scotland 29 June to 2 July 1990 with Ingvar Huse as Chairman.

The following terms of reference were suggested by the group for the Working Group meeting 1990. The group should meet to:

a) describe nutritional requirements, primarily in terms of fatty acid and amino acid content, in various species of marine fish larvae; furthermore to collect information on the function of these compounds in the organism.

b) develop criteria, in addition to growth and survival, for the evaluation of quality of marine eggs, fish larvae and juveniles.

c) evaluate properties and differences in live and inert diets, for the purpose of the development of adequate inert diets for marine fish larvae.

d) determine alternative strategies to to the use of antibiotics for the reduction of microflora in the culture of prey and larvae.

e) Further describe a standardized production process for marine fish fry.

8. DESCRIPTION OF THE FORM OF THE NEXT WORK GROUP MEETING

An alternative approach to the form of the Working Group meeting was suggested for next year, and was unanimously accepted. The new approach is intended towards a more structured programme to allow more time for discussions.

The meeting in 1990 will be divided into 3 topics:

Nutrition
Larval development
Zootechnique

- Nutrition includes topics concerning live prey organisms, fish larval nutritional requirements, enrichment techniques, and work on inert diets. P. Sorgeloos and J. Verreth have accepted to convene this topic.

- Larval development includes larval physiology, anatomy, histology, criteria for - and factors related to or affecting egg- larval and juvenile

quality. E. Kjørsvik. has accepted to convene this topic.

- Zootechnique includes microbiology, environmental factors, behaviour and the standardization of techniques and rearing systems. B. Chatain and G. Minkoff have accepted to convene this topic.

The conveners will also suggest keynote speakers and topics, and draft recommendations.

One or two lectures on specific topics may serve to introduce each topic. During the discussions short presentations on relevant results (1-2 overheads) can be presented.

Results related to the topics should be made available to the other participants in the form of a short report, including detailed materials, methods, results sections and a brief summary and/or abstract.

Furthermore, an activity report from each country will be requested for future Working Group meetings.

9. VOTING FOR CHAIRMAN CANDIDATURE

Ingvar Huse was elected as the Working Group's candidate for chairman.

APPENDIX I

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APPENDIX II

Written presentations:

- Buzzi, M: Preliminary results on the survival and metamorphosis rate of the freshwater prawn Macrobrachium rosenbergii fed Artemia nauplii enriched with different contents of n-3 polyunsaturated fatty acids.
- Corneillie, S. et al: Influence of n-3 HUFA on the growth, survival and morphological development of the larvae of the sea bass Dicentrarchus labrax.
- Daintith, M: Culture of the Tasmanian Whitetail (Lovettia sealii).
- Devresse, B et al: Essential fatty acid (n-3 HUFAs) requirement for the freshwater prawn Macrobrachium rosenbergii during the larval rearing cycle.
- Dhert, Ph. et al: Improved larval production of Asian seabass (Lates calcarifer) using HUFA-enriched live food.
- Dye, J.E. et al: Halibut broodstock and hatchery results.
- Jørgensen & H. Grasdalen: Phosphate metabolites in larvae of halibut.
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