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

Vigo, Spain, 6-8 June 1988

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1. PARTICIPATION

The Working Group convened its second meeting of Instituto Espanol de Oceanografia (IEO), Vigo, Spain, on June 6-8, 1988. Members present were: P. Sorgeloos, P. Lavens, S. Corneillie and W. Verdonck, Belgium, K. Waiwood, Canada, J. Stottrup, N.H. Norsker, H. Paulsen, Denmark, J.L. Nicolas, J. Robin, France, J.A.J. Verreth, Netherlands, I. Huse, A. Mangor-Jensen, K. Naas, S. Tilseth, D.S. Danielssen, J. R. Rainuzzo, T. Kleppe, S. Devesa, L. Jorgensen, Norway, M. Gillespie, Scotland, J. Iglesias, I. García de la Banda, J.C. Navarro, M. Olmedo, A. Ortega, M. Planas, F.J. Sánches, M. Torre, Spain, G. Quantz, W. Germany, B.R. Howell, U.K.

Further participated G. Kissil, H. Mordechay, A. Tandler, Israel.

See appendix 1 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 conducted as a joint ICES/EIFAC workshop. 34 Experts from 11 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. 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 brood stock 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. QUALITY CRITERIA FOR EGGS AND LARVAE.

3.1 Description

The survival of the early development stages of most marine fish in culture systems is generally low and highly variable. Though this may, at least in part, be due to a failure to consistently provide optimal rearing conditions, the viability of eggs at the time of fertilisation is undoubtedly an additional contributory factor. Fertilisation rates, which may vary from 0 - 100%, provide direct evidence of fluctuating egg quality. Additional indirect evidence is provided by observed variations in a range of physical, chemical and biological characteristics of eggs, though their association with subsequent viability has not been unequivocally demonstrated.

This observed variability of eggs may be due to:

a. Changes in the properties of eggs between ovulation and fertilisation. It is a well established fact for some species (e.g. trout) that post-ovulatory ageing has a marked and rapid effect on the viability of eggs. This is likely to be an important factor in species where the gametes are hand-stripped, though of a little or no importance in species which spawn naturally in captivity (e.g. sole) where good synchrony between ovulation and oviposition would be expected.

b. The inherent quality of eggs, defined as their potential for normal development to beyond metamorphosis at the time of ovulation. This may be influenced by various aspects of broodstock management through nutrition, and its possible effect on yolk composition, may be expected to be of particular importance.

Limited progress in this field has been made during recent years, but further research is required to identify objective and readily applied criteria for assessing the viability of eggs at the time of fertilisation and to define optimum procedures for managing broodstocks, and securing fertilizating the eggs.

3.2 Strategies

The principal research objectives should be to:

a. Establish any association between the observed or other characteristics of eggs and viability. Consideration should be given to the development of challenge or "stress" tests similar to those, which have been valuably applied to other aquatic organisms. Such investigations would require the development of standard rearing conditions to eliminate the interferring effects of environmental variables.

b. Establish the causes of the variability in egg viability. It is important to distinguish effects due to overripening from those related to aspects of the environment of the parent fish. A systematic investigation of the effects of post-ovulatory ageing on the characteristics of eggs is, therefore, a prerequisite of any systematic investigation. With regard to parental effects, studies of the variability of the composition of yolk should be initiated.

3.3 Recommendations

Immediate research priorities should include: -

a. The establishment of any association between observed variations in the characteristics of eggs at the time of fertilisation and their subsequent performance under standard conditions.

b. The determination of the extent to which the variable characteristics of eggs at the time of fertilisation depend on their post-ovulatory age.

c. The determination of the extent to which yolk composition (free amino-acids, fatty acids, vitamins etc) varies, its dependence on parental history (age, nutrition etc) and its relationship to subsequent performance. Analysis of eggs from natural stocks would provide a valuable standard for comparison.

Progress towards the attainment of these objectives would be accelerated by the pooling of data from as many stocks of each species as possible. Standardization of procedures would therefore be essential to ensure comparability of the data from different souces.

4. BROOD STOCK/EGG QUALITY

4.1 Egg quality studies in the common sole, solea solea (L)

Interest in the study of egg quality has primarily been stimulated by the highly unpredictable survival of marine fish larvae during the early stages of rearing. This poor performance may be due to variable conditions at the time of early feeding but may also be contributed to by conditions during egg incubation as well as to variable viability of eggs. The latter has been shown to be greatly influenced by the post-ovulatory age of the eggs at the time of fertilization, a factor likely to be of considerable importance in species, where artificial fertilizations are necessary (eg turbot) but, perhaps, of minimal importance in species, which spawn readily in captivity (eg sole).

Evidence for the existence of variations in egg quality that are attributable to parental factors is inconclusive. This is limited to observed variations in certain characteristics of eggs at the time of fertilization, but their association with egg viability has not been established.

The principal objectives of the studies of sole egg viability at Conway are:

a. To define standard procedures for incubating eggs/larvae until the onset of exogenous feeding to obtain an objective measure of variations in viability.

b. To identify characteristics of eggs and pre-feeding larvae, which may be indicative of their viability.

c. To determine the existence of any association between viability and such characteristics and ultimately to parental factors.

The following progress has been made.

a. An artificial seawater has been identified which supports the development of eggs and larvae through to the completion of yolk resorbtion with a survival equal to that in natural seawater.

b. Samples of eggs from natural stocks are being accumulated to assess the constancy of their biochemical composition and to provide a standard for comparison with eggs from captive stocks.

c. RNA:DNA ratios are being evaluated as a possible indicator of larval viability at the onset of exogenous feeding. The analytical procedure tested has proved sufficiently sensitive to distinguish between larvae fed for 1-2 days and unfed controls.

5. LARVAL NUTRITION - ARTEMIA/ROTIFER PRODUCTION

The discussion on Rotifer/Artemia production and larval nutrition concluded in the following:

5.1 Recommendations with regard to larval nutrition studies

a. Need to catalogue all information with regard to the requirements for highly unsaturated fatty acids (HUFA's) of marine larval fish.

Mr. Juan Carlos Navarro (Instituto de Acuicultura de Torre de la Sal, Castellon, Spain) has accepted to prepare a preliminary draft of literature information on HUFA requirements in larval fish (both freshwater and marine) that will be circulated among the participants of the Vigo-conference, who will add their available information as to allow

Mr. Navarro to finalize a working document for presentation and discussion at the next Working Group meeting.

b. Need to better identify the qualitative and quantitative HUFA-requirements in marine larval fish.

The Artemia Reference Center (State University of Ghent, Belgium) has accepted to prepare three enrichment emulsions (low in HUFA, average HUFA composition, very high HUFA-levels) for use in an intercalibration study with different marine larval fish species: several participants at the Vigo-workshop comitted their participation in this study and will use the three reference enrichments for Brachionus and/or Artemia in larviculture tests with seabass (Belgium, France, Spain), seabream (Israel), turbot (Norway, Denmark, Spain, France) and halibut (Norway, UK).

The following criteria will be evaluated: survival, growth and vitality of the fish larvae at the end of the experiment as well as their HUFA-composition.

The same and other laboratories will try to collect larvae from wild populations and from extensive cultures of turbot, halibut and cod for HUFA-analysis.

Results should be available for presentation and discussion at the next meeting of the Working Group.

c. Need for analytical data on the biochemical composition of eggs and larvae (stage to be identified) from wild fish species and of their natural diet (copepods, cladorecans, etc.): HUFA's, free amino acids, pigments, vitamins, minerals, etc.

d. Need for the development of a standard laboratory test-system with high reproducibility for the larviculture of marine fish species for use in nutritional requirement studies.

Mrs. Josianne Stottrup (Danish Institute for Fisheries and Marine Research, Hirtshals, Denmark) has accepted to prepare a proposal

for a standard test with turbot, which will be made available to interested participants for comments and eventual application.

e. Need for the development and application of a so-called vitality test or stress test to evaluate the quality (performance) of fry produced in hatcheries, produced in extensive (pond) systems, or harvested from the wild.

Several participants agreed to evaluate different procedures (e.g. 5 to 20 seconds dry shock followed by 24 to 48 hrs starvation as applied with Japanese red seabream, European gilthead seabream, respectively; temperature and/or salinity shock as applied with mysid and penaeid shrimp) with different species (bream, bass, turbot, halibut and cod) and submit their results for discussion at the next meeting of the Working Group at which occasion fine-tuning and standardisation of procedures can be proposed.

f. Need for the development of other methods to evaluate culture success (growth): RNA/DNA ratio's (spectrophotometric technique), histological analysis (ultrastructure of digestive tube and other organs), definition of growth potential, bioenergetical data, physiological criteria, etc.

g. Need for further study on selection and use of live feeds as alternative for Brachionus, i.e. better meeting the nutritional requirements for the predator, giving less problems for cleaning and handling; e.g. Tisbe.

h. Need to better evaluate the quality of the fish larvae at the start of the larviculture: better definition of what is a normal larva (e.g. deformities, pigmentation, etc.)

Fatty acid analysis was identified to be a major problem in comparison with nutritional studies and the following recommendations for an intercalibration exercise were made:

The following recommendations are made on the basis of the results of last years intercalibration exercise using Artemia. They should serve as a framework for establishing

intercalibration exercises for any other type of analysis that is deemed necessary in the future by the ICES working group (such as a nucleic acid analysis, CHN analysis or even proximate composition).

The results of last years exercise with Artemia suggests that participants used different procedures for hatching, lipid extraction and probably fatty acid identification. It is therefore recommended that all participants use the exact same procedures for all parts of the exercise in order to ensure similar results.

The following specific recommendations are made:

a. Exact sampling and storage procedures be provided to be followed by all participants.

b. An expert in the field of lipids (recommendations by the committee = J.B. Sargent or C.P. Convey) be asked to provide exact procedures for lipid extraction and fatty acid methylation of different types of samples, (animal tissues, feeds, algae and invertebrates) to be followed by all participants.

c. The use of an internal standard in the samples to allow for quantification of the component fatty acids be established for all extractions & lipid analysis.

d. A set of fatty acid ester standards be made and distributed to all participating laboratories for routine reference to all analysis made. A gas chromatograph readout of the standard can be provided all with the standard for cross reference.

e. All data reported in calibration exercises or all future reports be given in quantification terms, i.e. total lipid/dry weight of sample, weight of fatty acid/dry weight of sample.

It is recommended that these exercises continue to be coordinated by a central organisator such as the Artemia Reference Center.

Commercialization Aspects

Rotifer

a. Need of small strains for particular species. Small strains exist but most people use the bigger sized Brachionus. Small strains can be very useful for several species: bream, siganids, etc.

b. Variability of the "standard"-food used, which interferes with culture success, especially with regard to culturing the smaller strains.

c. Harvesting and cleaning rotifers after enrichment with formulated diets is still causing problems.

d. Hygienic problem: transfer bacteriae from big rotifer tanks into the fish culturing tanks.

Artemia

a. Problems with the standardization of hatching techniques, enrichment procedure and cleaning.

b. Problems of proper transfer of the standard procedures for disinfection, hatching, cold storage, etc., (difficulties with regard to routine procedures).

c. Standardization of a culturing technique for ongrowing Artemia.

Copepods

a. Upscaling technique for the culture of copepods.

Strategies

a. Set-up of rotifer bank from where suitable strains can be obtained.

b. Evaluation of different types of yeasts or algal substitutes or other diets for rotifers.

c. Assessment of the importance of microbiological problems in rotifer cultures.

d. Organization of regional workshops or demonstrations of optimal techniques for the farmers.

e. Adaptation/integration of existing laboratory culturing techniques to commercial scale. Set-up of feeding tests during nursery stage, especially for seabream, (cannibalism).

f. Performing feeding trials with copepods for different species of fish larvae to investigate if it does contribute significantly to the improvement of larval survival in the early larval stages.

Recommendations

a. ICES should stimulate the creation of a rotifer bank.

b. At the next meeting delegates should report on the culture procedures applied at their farm(s) as to allow the finalisation of standard procedures.

c. ICES should consider the organisation of a 1-day workshop on practical aspects of live food culture (Brachionus and Artemia).

d. Algae culturing should be added to the terms of reference of future meetings of the working group.

5.2. Standardization procedures

Recommendations of Group Work on Intensive and Extensive Rearing System.

a. Definitions - last years definitions accepted.

b. Recommendations and perspectives: Work should continue in both system types with special emphasis on:

- The significance of green water on larval growth and survival taking into account effects on prey and environmental conditions.

- Standardised evaluation of larval conditions using stress test based on such factors as: temperature, salinity and other environmental factors as determined for each species.

- Investigations of optimum features of holding systems such as: light level, colour, size, shape and water flow.

6. ROLE OF MICROFLORA IN LARVAL REARING

6.1 Turbot

a. Description

A rapidly increasing mortality appears frequently in larvae rearing tanks, due to starvation. Different mortality percentages are observed, although the culture conditions are standardised and larvae from the same spawning and batch are used. The stress factor, which causes these high mortalities is thought to be the high bacterial level, since the survival rate for the turbot larvae is directly related to the origin of the Brachionus and the amount of bacteria present in the Brachionus culture.

b. Strategy

The best strategy is to keep the bacterial concentration at an acceptable level both in larval rearing tanks and prey cultures through a reduction of available substrate.

c. Recommendations

All the media, which can serve as a source of bacteria for the larvae have to be analysed.

The total culture period for Brachionus must be shortened, by starting new cultures within regular time periods, since it has been shown that the quantity of bacteria (e.g. vibrio sp) increases rapidly in older cultures due to accumulation of organic material.

No use of antibiotics should be allowed for industrial purposes, but only for controlled research, this to prevent resistance building up for different bacterial strains.

Because it is still unknown exactly how the bacteria are affecting the fish, (due to poisoning, taste affection, infection) we must try to prevent rapid growth by altering culture conditions or by using bacteriostatics. Results obtained from the "altering conditions" can be applied directly in fish culture while the use of bacteriostatics must be limited to research.

To control the bacterial growth a rapid detection and identification system has to be developed. The substrates to be used are TCBS and marine Agar, which enables us to compare results from different laboratories.

6.2 Sea bass and Sea bream.

a. Description

Mortalities directly related to bacteria are not as high as in turbot in the early larval stage, although mortality in sea bass and sea bream larvae remains high. It is probable that bacteria are involved also in these undefined high mortalities.

b. Strategy

As with turbot, high levels of bacteriae should be avoided through the reduction of available substrate.

c. Recommendation

It is necessary that precautions are taken in sea bass and sea bream, because extremely high concentrations of bacteria in rotifers possibly cause digestive problems.

6.3 Cod and Halibut

a. Description

During the yolk sac stage, mortalities were extremely high, but recently rapid improvements in culture systems have been made, which will result in higher survival rates for these species. At this stage first trials on bacterial research can be taken into consideration.

b. Strategy

Before starting research on bacteriology good culture systems must exist, which can supply sufficient eggs and larvae. The bacterial chain from parent fish to larvae should be broken.

c. Recommendation

For research, antibiotic use during the long egg incubation period (halibut) can be tested to investigate the influence of antibiotics on hatching percentages and survival.

General remarks.

Pathological and histological investigations in all tissues should be performed routinely.

General observation is necessary and information should be exchanged between different research groups, since there is a lack of information in the available literature.

Axenic production of the different live feed can be tested and can provide more information about the impact of bacterial growth on larval survival.

Besides axenic conditions, decontaminating agents and bacteriostatics can be tested to lower the bacteria concentration.

Attention must be paid to sterilizing methods; because necessary bacteria, responsible for the maintenance of the system can be destroyed, which can cause e.g. ammonia problems. For this reason filtration is more suitable than u.v. treatment.

7. GENERAL RECOMMENDATIONS

The following recommendations are based on evaluation of the key problems identified by the working group and in accordance with the terms of reference for this meeting.

Stable high output intensive production lines for juvenile marine fish are not yet established either in research or commercially. Research should therefore continue in extensive as well as intensive systems to provide a further understanding of relevant mechanisms and processes.

Egg quality remains a problem. Further research should be carried out in order to relate egg quality to both pre and post ovulation parameters of possible relevance.

Standardized challenge (stress) tests for different species and developmental stages of larvae should be worked out and applied in order to evaluate larval quality.

Start feeding, including live prey production and quality remains the single most important problem area. Further work should be encouraged regarding HUFA-requirements in different species and stages, as well as the potential for incorporation of HUFA in live prey, especially Artemia and Brachionus. ICES should stimulate the creation of a Rotifer bank, and a 1-day ICES workshop on practical aspects of live food culture should be considered.

Bacteriae are among the main causes for the high variability experienced in marine fish fry production. The need for more research in this field is strongly emphasized. No commercial use of antibiotics should be allowed as part of a production procedure.

The working group has provided an important forum for analyses, standardization and strategy for the rapidly developing marine fish farming industry, as well as for the basis of coastal fish ranching programs. It should therefore continue its work. The next meeting should be held in Palavas-les Flots, Sete or Montpellier, France, 16-19 June 1979, with Ingvar Huse as chairman.

8. TERMS OF REFERENCE FOR NEXT MEETING

For the working group meeting in 1979 the following terms of reference are suggested:

The group should meet to:

a. Establish and analyse the present status of the industry in order to improve focus on bottlenecks and recommend research priorities.

b. Evaluate the results of the live prey enrichment standardization tests, and recommend action on the basis of these results.

c. Analyse collected information on HUFA requirements, and provide recommendations on HUFA levels in live prey for the different species.

d. Evaluate different systems and procedures for live prey production/enrichment and larval rearing, focusing on recommendable ways of avoiding negative effects of microflora.

Annex

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