Mariculture Committee

ICES CM 1997/F:7

REPORT OF THE

WORKING GROUP ON MARINE FISH CULTURE

Murcia, Spain
23–26 June 1997

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International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

Palægade 2–4 DK–1261 Copenhagen K Denmark

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1 PARTICIPANTS

The Working Group met for its first meeting under its new name at Murcia, Spain from 23-26 June, 1997. The following representatives of ICES member countries participated in the meeting:

Belgium:	P. Coutteau, P. Lavens, K. van Ryckeghem
Canada:	J. Castell
Denmark:	J. Støttrup
France:	J. Robin
Norway:	S. Bolla, J. Holm, I. Holmefjord, I. Lein, T. van der Meeren, V. Øiestad
Spain:	E. Abellán, R. Flos, A. Garcia-Alcázar, A. Garcia Gómez, J. Iglesias, J. Peleteiro
U.K.:	R. Shields, B. Howell

A. Tandler and B. Koven (Israel) also participated in the meeting. The meeting was chaired by B. Howell (UK) and J. Støttrup (DK) acted as rapporteur. Affiliations and contact details of the participants are given in Appendix I.

2 TERMS OF REFERENCE

The following terms of reference were approved by the Council (C. Res. 1996/2:32) during the 1996 Annual Science Conference in Reykjavík, Iceland:

The Working Group on Marine Fish Culture [WGMAFC] (Chairman: Dr B. R. Howell, UK) will meet in Murcia, Spain from 23-26 June 1997 to:

a) report on the current status of marine fish cultivation in ICES Member Countries and on the factors which are likely to constrain the further development and sustainability of the industry;

b) report on research into the characteristics of reared fish and reappraise the potential for establishing quality assessment criteria;

c) report on the current and continuing availability of live foods for larval marine fish and review the prospects for their replacement with formulated feeds;

d) assess the impact of recent advances in the development of alternative on-growing systems for marine fish and plan for a Theme Session on this topic for 1998;

e) evaluate the effects on larval performance of alterations to biotic and abiotic environmental variables;

f) report on developments in fish welfare issues and assess their impact on marine fish cultivation practices.

The Working Group will report to the Mariculture Committee at the 1997 Annual Science Conference.

3 AGENDA

The meeting was hosted by the Centro Oceanografia de Murcia and held at their aquaculture research facilities at Carretera de la Azohia near to Puerto de Mazarrón. Discussion of the topics identified in the Terms of Reference occupied the first three days of the meeting (23-25 June). The final morning (26 June) was dedicated to completing a draft report and finalising the recommendations of the Working Group. Some relief was provided by a tour of the experimental facilities of the Institute on 24 June and by evening visits to nearby tuna cages and a sea bass and sea bream farm (CULMAREX) on 23 and 24 June respectively.

4 ACTIVITIES OF THE WORKING GROUP

4.1 Introduction

The meeting was opened on Monday, 23 June with a warm welcome from Dr. Julio Mas, the Director of the Spanish Institute for Oceanography in Murcia. Dr. Bari Howell, the chairman of the WG, expressed the WG's gratitude for the opportunity to meet in an area primarily concerned with the culture of sea bass and sea bream, the two marine species which continue to be the most important in ICES countries. He continued by explaining that the renaming of the now defunct WG on Mass Rearing of Juvenile Marine Fish reflected the continuing increase in commercial marine fish culture activities in ICES countries with an accompanying increase in importance of issues other than those concerned with the early developmental stages. The broader remit of the new WG on Marine Fish Culture provided an opportunity to address the full range of issues associated with this important commercial activity.

4.2 Marine Fish Production in 1996 [Terms of Reference a)]

Summaries of the 1996 production of marine fish in ICES countries are presented in Tables 1 and 2. These were based on submissions by WG members supplemented where necessary from other sources. Although these data may be subject to the inaccuracies common to all data of this type, they are regarded as being a true reflection of current trends.

Following a brief discussion of current production trends, status reports on the most important commercial species (sea bass, sea bream, turbot and halibut) were received. Progress toward the development of techniques for species not having yet attained commercial levels of production were subsequently discussed and a presentation received on the problems presented by one such species, yellowtail (*Seriola dumerillii*), considered to have potential for culture in southern Europe.

4.2.1 Production of juveniles

The production of both sea bass and sea bream continued to increase with the combined production in 1996 exceeding 60 million (Table 1). This represented 27% of the European production of these species (217 million). Production of turbot, the other commercially established species, was relatively stable but with northern European countries (Norway, Denmark and the UK) increasing their market share from 14 to 27%. This was attributable to a three-fold increase in production in Denmark coupled with almost a 50% fall in production in Spain. Halibut production remains modest but continues to show a gradual increase reflecting the steady progress that is being made towards the development of rearing techniques. The majority of the cod production was used to support stock enhancement trials and the reduction over the previous year reflected a decrease in the level of this activity. In addition to these species, large numbers (<250,000) of sole (*Solea senegalensis*) were produced by Spanish hatcheries to support pilot-scale growth trials both in intensive and extensive systems.

4.2.2 Production of market-size fish

The total production of marine fish in ICES countries in 1996 exceeded 12,000 t.p.a, an increase of about 20% on the previous year (Table 2). This increase was almost entirely attributable to large increases in the production of sea bass and sea bream, notably in Spain. The combined production of these species represented 24% of the total European production of about 38,000 t. As with juveniles, the production of turbot remained stable but is increasing at a low level in some northern European countries. The production of 35 t of cod in Iceland was based on wild-caught juveniles. A similar activity exists in Norway but production statistics are difficult to obtain. Production of halibut remains at a low level (<100 t) but is set to increase as juvenile supplies improve. In addition, small quantities (<50 t) of sole (*Solea senegalensis*) were produced in Spain and Portugal in extensive systems.

4.2.3 Species reports

Sea bass and sea bream
In Spain these species are produced in intensive pond systems but on-growing also takes place in cages. In Europe, sea bream production has reached a plateau, but production of the sea bass is still increasing. One of the problems is the market demand which effects selling price and hence profitability.

Juvenile production is relatively unproblematic, but there is concern over the spread of nodavirus (viral nervous necrosis in sea bass). There are no known cases of this disease in Spain but no law is in force to control the import of live fish. Although importing this species from France is not encouraged, it is up to the individual producer to protect their own disease-free status.

The production of market size fish is also limited by the availability of ongrowing sites (cage installations) and legal difficulties in obtaining the licenses. Reduction of feeding costs by optimisation of FCRs (less than 1.5) is also a priority. In sea bass vast differences in growth rates even within the same batch is a problem as well as the relatively long time required to reach the market size.

In Israel the production of sea bream now exceeds 1200 tons; a rapid increase from the origins of farming some 5-6 years ago. Six million sea bream fry are produced yearly and this production is increasing. Israel has two large hatcheries. There is also interest in many species rather than single species; in particular Sparid species which are easy to rear. Availability of ongrowing sites and legal problems are an increasing problem. At present the production is limited to the Red Sea area but expansion is severely limited because of conservation issues. The Mediterranean coast could be exploited but these sites are very exposed. Future constraints are investment initiatives in more exposed sites.

Turbot

In Spain, turbot production has increased rapidly during the past 10 years and is now around 2000 t. Production has been stable during the last 2-3 years and seems to satisfy market demand. During the past 1-2 years, the number of farms has also stabilised, and the surviving farms all operate their own hatcheries. Among these farms a few large farms produce the majority of the fish. The main problems continue to be low larval survival (5 to 10%) with accompanying high production costs. High costs during on-growing has led to research into alternative methods for on-growing such as cage systems or re-cycling systems. Wild turbot attract higher prices than cultured turbot, partly because wild turbot are sold at a larger size (3-5 kg) than farmed fish (0.8-1.5kg). In France, production is increasing by around 10% per year mainly through increasing production in individual farms. New sites are difficult to obtain and this contributes to the stability of production. The development of alternative (e.g. recycling) technology would alleviate this constraint.

Halibut

Halibut larval rearing in Iceland based on an *Artemia*-feeding strategy has met with success. This is a significant development reflecting the general trend towards intensive rearing away from the semi-extensive approach which relies on seasonally-dependent copepod production. The Icelandic halibut hatchery has access to a very large broodstock population and turbot broodstock husbandry methods have been successfully applied. Photoperiod control of spawning has resulted in the year-round availability of eggs.

In contrast, in the UK, one company has adopted the semi-extensive copepod based system successfully applied to turbot production by the Danish company Maximus. Other UK companies are continuing to develop *Artemia*-based systems.

Expected survival in commercial operations in Norway is reportedly around 10% from fertilised egg to 5g. Survival figures of 40% or even higher have been obtained, so the techniques remain highly variable. Survival is less variable in the intensive systems but further improvements are required before economic viability is achieved.

In Canada, one hatchery was operational in 1996 following transfer of the Norwegian rearing technology. Pigmentation problems were prevalent and related to the lack of copepods. A second hatchery started operating in 1997, again involving the transfer of Norwegian technology.

Interest in establishing halibut production in the Faroe Islands and Chile has also been reported.

Disease risks are restricting the transfer of eggs and fish and therefore the rate of development of the industry in areas where stocks are not well established.

The selling price for reared halibut is higher than for the wild market. This is due to different target markets (mainly the fresh catering market) and the relatively high variability in the quality of wild fish.

New species
A wide range of 'new' species are being evaluated both in response to market saturation of well established species and as a way of exploiting new (particularly cold-water) environments. In northern countries, the species being evaluated include the witch flounder (*Glyptocephalus* sp), the yellowtail flounder (*Limanda* sp), black cod (*Anoplopoma* sp.) and wolfish (*Anarrichus* sp). Rearing of the spotted wolfish (*A. minor*), based on artificially fertilised eggs, has been proceeding in Norway since 1994. This species performs extremely well in culture in cold-water environments attaining a size up to 5 kg (mean 3 kg) after three years at temperatures between 4 and 8°C.

Among the new species being evaluated in warmer-water environments are the sparids such as common dentex (*Dentex dentex*), common sea bream *Pagrus pagrus*, and sharpsnout sea bream *Diplodus puntazzo* and a *Carangid,* the yellowtail *Seriola dumerilii.* The need for diversification is particularly evident in southern European countries because of the recent sharp decline in prices for those species already farmed.

In Spain, the Mediterranean yellowtail, *Seriola dumerilii,* is considered to be a particularly promising species. It grows much faster than either sea bream, bass or turbot attaining a weight of 1000-1100 within 1 year, 3 kg within 2 yrs and 5 kg within 3 yrs. This rate of growth is similar to that of the Japanese yellowtail. The fish have been successfully grown on both moist and soft-dry pellets. These growth trials have been based on wild-caught juveniles because it has not yet proved possible to rear the species in captivity, the main obstacles being reproduction and larval rearing. Captive broodstocks have, however, been established and work is in progress on the control of reproduction. Commercial production, based on wild-caught juveniles, was about 13-20 t in 1995, but this has decreased to 1 t produced by one farm because of the low availability of juveniles. The market demand and the value of the species is high and so the potential for farming is high and conditional on the solution of early rearing problems.

The tuna, *Thunnus thynnus,* is another very fast-growing species with potential for culture. Since around 1985 fattening plants for commercial sale of tuna have been common. Fish are caught with seines and fattened in sea cages for a few months (4-5 months) before being sold. This activity is mostly supported by Japanese investors and the fish are destined for the Japanese market.

In conclusion, both production levels and the number of species being farmed is steadily increasing. The principal constraints on the further development of the industry are:

a) low and variable survival during the larval stages of some species (e.g. halibut and turbot);
b) availability of suitable sites in combination with legal constraints and environmental pressures;
c) food costs and growth variation;
d) disease, particularly nodavirus in sea bass and halibut;
e) market saturation;
f) technical problems of rearing the early stages of some novel species (e.g. yellowtail, tuna).

4.3 Characteristics of reared fish [ToR b)]

Morphological abnormalities of reared fish, such as skeletal deformities, swimbladder deficiency and pigmentation abnormalities, have been well documented and extensively studied. There is increasing evidence, however, that physiological and behavioural defects may be of at least equal importance and these have been somewhat less extensively studied. In addition, sex ratios of reared fish may deviate appreciably from a 1:1 ratio with male domination being common in several species including sea bass and sole. Recent work in these areas was reviewed.

4.3.1 Physiological defects

Recent work on sole (*Solea solea*) has shown that dietary lipid quality during the larval stages may have a prolonged physiological effect, as exemplified by cold-tolerance, on later developmental stages even though there may be no detectable effect on other performance indicators such as larval survival, weaning success and juvenile growth rates. It has also been shown that such nutritional deprivation may have a general effect on hardiness influencing tolerance to other environmental factors (e.g. oxygen concentration) as well as possibly having an effect on disease resistance.

Such work illustrates that growth and survival, though clearly important, are alone inadequate criteria for assessing rearing methodology. The use of short-duration 'stress tests' as a measure of juvenile quality may proof useful but extreme caution must be exercised in interpreting the results. In the case of the sole, differences in response to a standardised exposure to low temperature which were evident while the fish were still being fed diets of differing quality rapidly diminished once the groups had been weaned onto a common formulated feed. However, more prolonged exposure to low temperature some 6 months later revealed that physiological differences attributable to larval diet quality had persisted. Further work is required not only to elucidate the full impact of events during development on later developmental stages but in the development and application of predictive tests of juvenile quality.

4.3.2 Behaviour

Studies of the behavioural attributes of reared fish have assumed increasing importance in recent years particularly in relation to the use of reared fish to enhance natural populations. Evidence of behavioural deficits among reared fish has been reinforced by recent work undertaken as part of an EU-funded project on the enhancement of flatfish stocks. This has clearly shown that post-release survival may be prejudiced by deficiencies in cryptic behaviour (colour adaptation, burying), feeding behaviour and predator recognition and avoidance. The demonstration of a learned component to these behaviours suggests that improved performance may be induced by changes to rearing methodology and the development of pre-condition techniques.

4.3.3 Sex ratios

Sexual maturation of fish is of economic importance in fish culture because of the suppressive effect on growth rate. In marine fish, males are usually less desirable because they mature at least one growing season before females and the consequent growth rate suppression often occurs before market size is reached. While methods are now being developed to obtain all female broods of marine fish, there are numerous documented and anecdotal reports of unexplained male domination among reared populations. These include sea bass (*Dicentrarchus labrax*), turbot (*Scophthalmus maximus*), sole (*Solea solea*) and halibut (*Hippoglosus hippoglossus*). There are also some reports of similarly skewed sex ratios in natural stocks.

There is evidence that skewed sex ratios in different species may be attributable to a variety of factors such as water temperature, pH, growth rate and stock density. It has been known for some time that the sex of several fish species such as the Atlantic silverside (*Menidia menidia*) and some tilapia can be influenced by water temperature during early rearing. More recently it has been shown that the Japanese flounder (*Paralichthys olivaceus*), is similarly affected. In all these cases a rise of four or five degrees Celsius may make the difference between a 50:50 sex ratio and 100% male.

A further environmental influence on sex control that should be considered is pollution by water borne contaminants that mimic fish reproductive hormones. The majority of the published information on the effect on sex of water-borne contaminants is associated with oestrogen-mimicking compounds such as nonyl and octyl phenols, which are used in detergents, and phthalates used in the manufacture of plastics. The presence of such xenobiotics in natural waters has been associated with feminisation, but as yet there is a lack of experimental evidence to link such substances with masculinisation. This, however, remains a distinct possibility. There is evidence, for example, that freshwater plant sterols can be degraded into androgenic compounds by bacterial action. These have been shown to cause several fish species, downstream of paper mills, to develop male secondary sexual characteristics. Further work is clearly needed in this area.

4.4 Availability of live food and development of formulated feeds [ToR c)]

The quality and availability of live food for marine fish larvae has long been of concern but the continuing increase in global demand is accentuating these problems and threatening its long-term sustainability. The current status of the use of live foods and their improvement and the prospects for replacing them with formulated feeds are reviewed below.

4.4.1 Current status of *Artemia* availability

The total world consumption of *Artemia* cysts is now around 3000 t.p.a. The main source of *Artemia* being exploited is that from the Great Salt Lake (GSL), USA (> 2000 t.p.a of processed material). There was a sharp decline in production two years ago but production levels have now returned to normal. In 1996 around 500 t were obtained from novel sources (e.g. central Asia) and an additional 20 t from sources already exploited in the past such as San Francisco Bay, Canada, Brazil and Vietnam. Production from alternative sources is expected to be higher than current levels but an increase in the demand for *Artemia* is also envisaged.

The decline in harvest in the GSL over that two-year period resulted in serious price increases up to 65$/kg with consequent financial problems to consumers. Over-exploitation is no longer considered the main reason for that low production. Climatic effects and/or genetic selection towards viviparity are now considered to be more important factors affecting cyst exploitation. Selective harvesting of the cyst-producing component of the population will favour the viviparous component and result in a progressive lowering of cyst production.

The present *Artemia* production system cannot be effectively managed or manipulated. The failure to identify the main cause for production collapse causes concern. There is a fear of a future decline in salinity in the southern arm of the GSL which will result in a lower production of *Artemia*. However, the northern part is currently unproductive because salinity is too high, and so a salinity drop may merely result in a shift in production from the southern to the northern part.

Quality differences among strains of *Artemia* may cause hatching problems. For example, diapause in *Artemia* results in differences in hatching rate. Diapause deactivation may not be effective and so high variability in hatchability may be evident. Such differences in hatching rates may cause problems within the hatcheries. For example, if cysts require more than 22-24 hours to hatch, additional tankage would be required. In addition, there is often insufficient data on the nutritional value of new strains and there is a high variability in the effectiveness of the decapsulation methods which are often applied in southern European hatcheries.

New *Artemia* products have been developed which address the problems in culture tanks which may arise from the introduction of bacteria with the live food. The problem arises from the bacteria which contaminate the cysts as well as the proliferation of bacteria during the enrichment process. New cyst products have been introduced into the market which are pre-treated in certain ways to prevent this bacterial development. In addition, viable decapsulated cysts are now becoming available which are better able to tolerate storage.

4.4.2 Current trends in rotifer production

There is a significant shift towards the use of artificial diets for culturing rotifers resulting in a more stable production than is usually achieved. Higher rotifer densities are expected to become the norm in future cultures (>1,500-2,000/ml). In Greece, densities at harvesting of around 10,000 rotifers/ml are achieved using Japanese technology which uses commercially available algal paste as a food source. Further, the use of rotifer cysts may increase in the future because it allows better strain selection and improved microbial management.

Different strains have different sensitivities to culture conditions. A rotifer reference centre has been established in Israel (Dr. Lubzens - IOLR, Haifa). However, the future of this activity is unsure because of lack of funds. A similar reference centre is being established in Canada (Stewart Johnson, Institute for Marine Biotechnology, Halifax).

4.4.3 Other live foods

Trochophores.
The use of trochophores, which are small in size (~50 µm) and can be stored in liquid nitrogen, may be a valuable and convenient food organism for species with very small larvae at first feeding. Comparative trials, however, have shown that their use confers no advantage over the use of rotifers, either offered alone or as a mixture, for species able to ingest this organism.

Copepods.
Copepods are the most dominant natural food of marine fish larvae and their nutritional value may therefore be regarded as optimal. Wild copepods are used in some commercial hatcheries in northern European. A Danish turbot hatchery, for example, has produced stable numbers of juveniles based on extensively reared copepods. However, the production cannot be based entirely on copepods and relies on *Artemia* nauplii to meet the food requirements of the later developmental stages. Nevertheless, the use of copepods during the early stages may confer lasting benefits as manifested in, for example, normality of pigmentation in flatfish. Culturing copepods intensively has met with limited success only with harpacticoid species whose nauplii are too small for most species of first feeding marine fish. Thus, harpacticoids at best can only replace rotifers in larviculture and here the labour requirements are comparable to those required for rotifer cultures.

It has become evident that further attention needs to be given to energetic requirements in larval feeding studies. For example, in feeding trials with halibut larvae high mortalities around metamorphosis were attributable to a shortage of metabolic energy. This indicates the need to determine optimum protein/energy ratios, not just lipid quality.

4.4.4 Enrichment status

The use of microalgae ('greenwater technique') to enhance the nutritional value of rotifers maintains protein, lipid and energy levels. Adequate protein levels can be obtained by maintaining high food rations, egg ratios and growth rates during production or by supplying >0.4µg/rotifer of an artificial diet for 24 h before offering to the fish larvae. High growth rates result in increased protein/lipid ratios but in decreased HUFA levels and DHA/EPA ratios. It also proved possible to manipulate the ratio of n-9/n-3 fatty acids.

HUFA content following enrichment depends on the dosage used, the duration of enrichment and the type of enrichment emulsion. The ICES reference emulsions provide a wide range of HUFA content for nutritional experiments on fish larvae (see Appendices II and III).

A comparative study on the distribution of lipids in copepods and *Artemia* showed large differences in that the lipids in *Artemia* are primarily in the form of neutral lipids. This may have important dietary consequences for larvae. Samples of live food from commercial hatcheries showed that the requirement for n-3 HUFAs in terms of % dry matter decreased with increasing DHA/EPA ratio. In this study, the n-3 HUFA content and DHA/EPA ratio varied greatly both between and within hatcheries. It was not possible to relate this observation to the success of the hatchery.

Different strains of *Artemia* may respond differently to enrichment procedures despite the use of similar techniques and enrichment emulsions. This can have important consequences. For example, differences in the incidence of malpigmentation among flatfish juveniles has been associated with changes in the strains of *Artemia* used. These aspects should be studied further to elucidate the variations between batches of *Artemia* and their reaction to enrichment techniques.

A relationship between survival and lipid content in fish has been demonstrated in larval winter flounder. In turbot, protein level in rotifers was found to be important for rearing success. Survival of halibut larvae has been shown to depend on the *Artemia* enrichment regime used with lipid level being of particular importance. Also, normal pigmentation was shown to be related to the provision of high-lipid *Artemia* for specific periods of time during the larval stage. Further studies are required to find the minimum length of time that high-lipid diets are required for normal development.

With regard to vitamin enrichment, standardised procedures are now used for vitamin analysis. The vitamin C content of algae was found to depend on culture conditions as well as species. Vitamin C content in rotifers and *Artemia* can be increased but a stabilised form of ascorbic acid is required. Ascorbyl palmitate, which is lipophyllic, is used in enrichment diets because it is stable and can be incorporated as ascorbic acid in high concentrations in the target organism. Vitamin enrichment in *Artemia* is feasible and is maintained in the organism at the enrichment level for at least 24 hours. The benefits of vitamin C enrichment was primarily displayed in catfish as growth characteristics, whereas in marine fish the effects were more subtle and manifested in the form of increased resistance to stress and disease.

Enrichment with alfa-tocopherol acetate with slow conversion into tocopherol in the live food is feasible. Again, Vitamin E levels in algae vary widely and the levels in the rotifers and *Artemia* can be highly manipulated.

Phospholipid enrichment in rotifers is considerably less effective than HUFA enrichment because of a breakdown of the phospholipid. In *Artemia*, slight enrichment may be possible and soya-PC was shown to protect PUFA levels after enrichment.

There is a possibility of overdosing with vitamin A and other lipid soluble vitamins in cases when diets contain high oil levels.

4.4.5 Live food replacement diets

It was acknowledged that the total replacement of live foods with formulated feeds is not a short-term aspiration, though significant progress towards this goal is being made. Recent research, particularly in Israel, showed that the presence of live food increased assimilation in sea bass larvae. Highest growth rates where found where microdiet was co-fed with live

Artemia, ingestion of the particulate feed depending on the concentration of the *Artemia*. The effects of *Artemia* were found to be both visual and chemical. The chemical stimulation was attributable to four amino acids (glycine, alanine, betaine and arginine). Polar lipid fractions, such as PC and LPC (Phosphatidylcholine and LisoPC), were also shown to increase assimilation rate. *Artemia* was also shown to increase digestive performance by improving digestive enzyme activity and the hormonal control mechanism.

Rapid progress in this area requires the adoption of a multidisciplinary approach incorporating behaviour, nutrition, physiology and endocrinology.

4.4.6 The use of reference diets in nutrition research

Strong endorsement was given to the principle of using standard reference diets (live food, enrichment media, weaning diet, microdiets, or juvenile on-growing diets) among scientists studying nutrition, culture, disease resistance and other aspects of marine fish culture. The existing ICES reference emulsions and weaning diets (see Appendices II and III) continue to be made available to scientists within ICES and other countries so that the comparability of research results is enhanced. Variations of abiotic and biotic culture conditions as well as species and strains of fish will influence relative growth, survival, and other performance results. This emphasises the need to adopt standard protocols and experimental designs in nutrition studies.

4.5 Recent advances in the development of alternative on-growing systems for marine fish (ToR d)]

The development of marine fish farming is constrained by the availability of sites for conventional on-growing systems (tanks, cages). In the case of pump-ashore tanks systems cost is also a major consideration. This section reviews some alternative approaches which may alleviate this constraint.

4.5.1 Land-based farms

The main types of tanks used in on-growing installations are either circular or octahedric. Octagonal tanks with an additional "bottom" have been tested for halibut to increase carrying capacity. Despite this, these systems do not utilise the production space optimally so that the high initial investment costs results in a high production cost.

Shallow raceway systems which can be stacked utilise space more effectively for flatfish species. Most commonly the width is 2 m and the length may be 50 m. To date, these systems have been tested on a pilot scale (4 x 20 m). The advantages are that they provide excellent water exchange close to the fish, permit high fish densities and are self-cleaning. The distribution of food is easy because the current aids the distribution of pellets. In addition, the system can easily be automated and is suitable for standardisation. The use of racks would save space and reduce initial investment for any given production level. This system has been tested with turbot and halibut with good survival rates. In such systems, the fish spend more time on the bottom and swim less. This may or may not be an advantage.

A disadvantage of such systems would be the rapidity with which oxygen would be depleted in the event of a plant failure. Clearly, this is an area of concern but one which may be surmountable with flatfish, for example, by the use of sprinkler systems to maintain a moist well oxygenated atmosphere. This is, however, yet to be tested.

The more intensive use of water was assayed on a pilot scale on turbot in shallow raceway systems. The removal of particles by floatation, adjustment of pH and addition of oxygen at regular intervals enabled the water to be re-used 20 times without detriment to fish performance. With only 5% water exchange, the growth rate of 200 g turbot was similar to that obtained in open systems. Growth was impaired when the water was re-used more often (i.e. with a lower water exchange).

The almost complete recycling of water enables the fish farm to be established inland. This may be advantageous especially in countries where the issuing of new licences are a major constraint. Water recycling is dependant on biofilters. Aerobic and anaerobic biofilters are in use in combination in Israel in freshwater systems and this system is presently being adapted for marine systems. Alternative methods such as water stripping using multicrop systems has been assayed in Israel, using oysters and/or clams to remove algal particles and macroalgae to remove dissolved nutrients, thus reducing nutrient loading in the effluent.

4.5.2 Sea-based farms

Conical net pens are in use for sea bass and bream. In Norway, net pens with taught 'trampoline' bottoms have been introduced for halibut. this is known as the 'AMY pen'. Outdoor culture renders halibut susceptible to UV-irradiation which damages fat tissue. Consequently, halibut behaviour in these pen types was investigated and revealed higher activity at low light intensities. Social interactions were also observed with fin nipping, eye injuries and even 'irrational behaviour' requiring the removal of these individuals. In relation to feeding, 'scramble' behaviour and lack of interest in the food items have been observed. Undesired surface activity has been observed and appears to be related to climatic conditions. Higher growth was observed when adapting the feeding regime to halibut behaviour.

4.5.3 Extensive or semi-extensive culture

Research needs in extensive or semi-extensive systems are several and contrast with those of intensive systems. The carrying capacity must be known for the individual system as well as a detailed knowledge of the ecology. Water management is an important issue and is tied to the suitability of the site and adaptability to the form of culture. Hydrological studies are also needed. Some species may be better suited to these methods because of specific characteristics which make it difficult to rear them in current intensive systems, though improved technology may overcome some of these limitations. In developed countries extensive or semi-extensive aquaculture can play a role in development of wetlands or semi-protected zones linked to other social or economical activities, such as ecotourism. It is important therefore that any appraisal of potential should consider an integrated socio-economic development for the benefit of the overall activity.

4.5.4 Proposed theme session for the 1998 Annual Science Conference

The title proposed for this theme session was:
Farming marine fish beyond the year 2000: Technological solutions for biological challenges

It was further proposed that the session should embrace the following topics:
- Biological needs and mechanisms
- Technology used for fish farming
- Methods and management regimes reducing the risk of disease
- Socio-economic and geographical constraints
- Ethics and animal welfare

4.6 Biotic and abiotic environmental variables (ToR e)]

Control of the microflora is suggested to be one of the main elements in generating stable and predictable rearing systems. Three general approaches may be considered:

a) Non-selective reduction of bacteria, achieved through surface disinfection of eggs, reduction of input of organic matter, removal of organic matter and/or grazer control of bacterial biomass;
b) Selective enhancement of bacteria by the addition of selected bacteria to rearing tanks or to the feed;
c) The improvement of larval resistance to bacterial infections by stimulation of the general immune system or modulation of maternal immunity.

Recent research on halibut and turbot in Norway has demonstrated improved survival of larvae through surface disinfection of eggs and stimulation of the non-specific immune using a range of immunostimulants. Work is in progress aimed at determining the point during development at which the immune system can be stimulated. In addition, the use of matured water to favour slow-growing harmless bacteria to out compete opportunistic and potentially harmful species demonstrated improved survival. This may be a promising method for reducing seasonal problems with water quality. This parallels work in scallop hatcheries in China where survival rates of 30% in filtered water can be increased to 80% through the use of water 'matured' in the dark for 4 days.

Research on the effects of abiotic variables has been similarly neglected. Information on the effects of temperature, for example, has been largely limited to considerations of effects on feeding and growth though it has been recently shown that temperature during the larval stages can have an important influence on the recruitment of muscle stem cells as well as on sexual differentiation. Both these effects can have a major influence on the characteristics of reared fish.

4.7 Fish Welfare issues [ToR f)]

4.7.1 Background/Status

For some time, the Council of Europe has been in the process of producing a Recommendation of the Welfare of Farmed Fish. In 1992, the then UK Minister for Agriculture Fisheries and Food, John Gummer, asked the Farm Animal Welfare Council (FAWC), an independent advisory body, to provide advice for UK Ministers on this subject. Following extensive consultation in Great Britain and, to a lesser extent, other countries which are party to the Council of Europe Farm Animal Welfare Convention, FAWC published their report in September 1996. It concentrates almost exclusively on salmon and trout farming and makes 131 recommendations. It has since been circulated for consultation to interested organisations in the UK and has been broadly welcomed. The UK Government is shortly to issue its response to the report. A draft Council of Europe Recommendation document covering salmon, trout and carp is under consideration by a drafting group comprising representatives from the UK, France, Italy and Norway; formal consideration is expected to begin later this year.

4.7.2 Brief Summary of the FAWC Recommendations

Recommendations are made in the following areas; Sites, Equipment and Environmental Control; Stockmanship and Inspection; Feeding; Production Life Cycle; Handling, Grading and Transport; Disease and Parasitism; Availability of Veterinary Medicines; Mutilations; Predators; Genetics and Killing; and Slaughter.

Most reflect good practice and their implementation should cause few problems. Those recommendations which deal with the frequency of mortalities removal and food deprivation periods prior to slaughter may be more difficult to implement, and those which deal with stocking densities and slaughter techniques are more contentious. An Animal Welfare Coordinating Committee on Fish Welfare has been established to advise on further research requirements. It has concluded that novel methods of stunning and slaughter, stocking density and social behaviour, and the wider availability of medicines, should be the highest priority areas.

4.7.3 Implications for Marine Fish Cultivation

Although marine species are not covered by the current draft Recommendation, pressure for them to be included is likely to increase as the industry expands. Most current marine fish cultivation practice should be consistent with the vast majority of the recommendations made for trout and salmon. However, the lengthy and highly intensive early rearing phases of marine fish and the manipulation, by hormone injection, of reproductive cycles in some species may require special welfare consideration. Slaughter techniques of the larger flatfish may also require unique solutions. Preferred practice for the control of marine fish diseases should develop as the industry expands. The influence of stocking density on growth rates and yields and the importance of social behaviour will be as deserving of research in marine fish as in salmonids.

The WG recommends that the aquaculture community should be attentive to welfare practices. Therefore, a code of practice should be formulated by representatives of the aquaculture community to address the public concern for animal welfare. The aquaculture community should further ensure their full participation in the formulation of any regulatory measures, to ensure that working practises are considered.

5 CONCLUSIONS

The following conclusions and recommendations emerged from the discussions:

1. There is a need to further encourage the closure the gap between the commercial and research sectors by the further development of strategic instruments that will promote the mutual exchange of information.
2. Value was perceived in the establishment of a 'disease task force'; an inter-European scientific service which would observe, follow and identify solutions to "new" diseases.
3. There is a need for more basic and large scale research in order to solve technological challenges.
4. Research on fish behaviour should be promoted in order to provide a sound basis for the evaluation of rearing technology and management procedures in relation to animal welfare.
5. The scope of larval nutritional studies should be expanded to consider the full range of macro- and micro-nutrient requirements, with greater reference to the composition of natural zooplankton for guidance.
6. It was concluded that live foods will not be eliminated in the near future. However, the organisms currently widely used (rotifers and *Artemia*) will not be sufficient to develop feeding protocols for potential new species and alternate live food organisms should be investigated.
7. Microdiet development should continue and special emphasis should be placed on attractiveness, impact on digestive physiology, microdiet particle structure, nutritional composition and studies of their use in rearing systems.
8. The WG encourages the use of standard protocols and experimental designs in nutritional studies.
9. The feasibility of establishing a nutrient database for larval feed compositions should be investigated. In this respect, Dr. Castell (Canada) has volunteered to serve as a centre for input of ideas and information. The principles followed will include the use of standardised analytical techniques and insistence that all authors include standard reference samples with submission of new data.
10. Further research is needed to better understand the quantitative and qualitative effects of microbial development in larval tanks on larval performance. The effectiveness of mature water should be further investigated and a standard protocol established.
11. Endorsement should be given to the maintenance of a rotifer reference centre having the facility to make available all rotifer strains commonly used and those considered as suitable candidates for the culturing of marine fish larvae. This centre will also include a database of protocols for the culture and maintenance of these strains.

12. The need for further research to establish predictive indicators of juvenile quality was recognised.
13. Research is needed to identify the factors implicated in sexual differentiation.

6 FUTURE ACTIVITIES

a) It was agreed that the Working Group should continue to review the current status and problems of marine fish cultivation activities. This provides a continuing mechanism for focusing its activities.

b) The continued expansion of marine fish cultivation is dependent on the development of novel systems which will reduce production costs and site and species constraints. Such developments will be reviewed and the extent to which they are applied across species will be evaluated.

c) Recent studies of fish behaviour in culture systems suggest the importance of behavioural criteria in evaluating both systems and operational procedures. Progress towards the establishment of such criteria will be evaluated.

d) It is becoming increasingly evident that the phenotype of reared fish is highly dependent on events during early development. Such events may influence physical characteristics, physiological processes (e.g. thermotolerance and disease susceptibility), behaviour and gender. This increasingly active field should be reviewed and the prospect for establishing predictive criteria of juvenile quality re-assessed.

e) There is a need to establish a nutrient database for larval feed compositions which takes adequate account of protocols for nutrient analysis. This will serve to facilitate both intra- and inter-specific comparisons of nutritional requirements.

f) Feed cost, determined largely by that of its major components of protein and lipid, is one of the most important determinants of the economic viability of intensive rearing methods. The influence of the future availability of these materials and the progress toward the identification of suitable substitutes will be assessed.

7 RECOMMENDATIONS

The Working Group on Marine Fish Culture recommends that it works by correspondence in 1998 to plan a meeting in 1999 to:

a) report on the current status of marine fish cultivation in ICES countries and on the factors which are likely to constrain the further development of the industry;
b) review technological developments in relation to fish production and their application to various species;
c) report on the establishment of behavioural criteria which can be used to evaluate on-growing systems and operational procedures;
d) review studies of the effect of events during early development on the phenotype of the juvenile stages and re-assess the prospects for establishing predictive criteria of juvenile quality;
e) report on the establishment of a nutrient database for larval feed compositions and establish standard protocols for nutrient analyses;
f) review progress toward the identification of alternative protein and lipid sources for marine fish diets.

Table 1 Production ('000s) of juvenile marine fish in ICES countries in 1996. Data for 1995 are given in italics.

COUNTRY	HALIBUT *Hippoglossus hippoglossus*		COD *Gadus morhua*		TURBOT *Scophthalmus maximus*		SEA BASS *Dicentrarchus labrax*		SEA BREAM *Sparus aurata*	
Canada	12	*0*	12	*0*	-	-	-	-	-	-
Iceland	35	*3*	7	*0*	-	-	150	*110*	-	-
Norway	104	*85*	60	*162*	210	*250*	-	-	-	-
Denmark	-	-	0	*11*	750	*250*	-	-	-	-
UK	10	*15*	-	-	100	*100*	-	-	-	-
Ireland	-	-	-	-	-	-	-	-	-	-
Portugal	-	-	-	-	-	-	?	*5343*	?	*2396*
France	-	-	-	-	2000	*1800*	16500	*14900*	10500	*5200*
Spain	-	-	-	-	850	*1500*	3854	*4380*	28419	*28240*
Total*	161	*103*	79	*173*	3910	*4155*	20504	*19390*	38919	*33440*
Change (%)	+56		-54		-6		+6		+16	

* Totals for sea bass and sea bream exclude data for Portugal because of missing values for 1996.

Table 2. Production (tonnes) of farmed marine fish in ICES countries in 1996. Data for 1995 are given in italics.

COUNTRY	HALIBUT *Hippoglossus hippoglossus*		COD *Gadus morhua*		TURBOT *Scophthalmus maximus*		SEA BASS *Dicentrarchus labrax*		SEA BREAM *Sparus aurata*	
Iceland	1	*10*	35*	*33*	-	-	1	*0*	-	-
Norway	90	*80*	?	*?*	30	*50*	-	-	-	-
Denmark	-	-	-	-	80	*5*	-	-	-	-
Holland	-	-	-	-	0	*11*	-	-	-	-
UK	-	-	-	-	5	*0*	-	-	-	-
Ireland	-	-	-	-	32	*0*	-	-	-	-
Portugal	-	-	-	-	?	*82*	310	*254*	605	*419*
France	-	-	-	-	850	*800*	1500	*1350*	1000	*900*
Spain	-	-	-	-	1890	*2010*	900	*650*	4700	*3300*
Total	91	*90*	35	*33*	2887	*2958*	2711	*2254*	6305	*4619*
Change (%)	+1		+6		-2		+20		+37	

* Based on wild-caught juveniles

Appendix I Affiliations and contact details of the participants

NAME/ADDRESS	TEL/FAX/E-MAIL
Abellan, E, Centro Oceanografia de Murcia, Carretera de la Azohia s/n, Puerto de Mazarrón (Murcia), Spain	Tel: +34 68 15 33 39 Fax: +34 68 15 39 34 Email: emilia.ieomz@mx2.redestb.es
Bolla, Ms S, Richard Withs vei 4, N-7041 Trondheim, Norway	Tel: +47 73 92 02 18 Fax: +47 73 92 02 18 Email: solvieb@internet.no
Castell, Dr J Dept of Fisheries and Oceans, PO Box 550, 1707 Lower Water Street, Halifax, Nova Scotia B3J 2S7, Canada	Tel: +1 902 426 6270 Fax: +1 902 426 1862 Email: j_castell@bionet.bio.dfo.ca
Coutteau, Dr P INVE TECHNOLOGIES N.V., Oeverstraat 7, B-9200 Baasrode, Belgium	Tel: +32 52 331320 Fax: +32 52 334531 Email: p.coutteau@inve.be
Flos, Prof. Rosa Institut de Ciències del Mar, CSIC, Passeig Joan de Porbó s/n, 08039 Barcelona, Spain	Tel: +34 3 221 4328 Fax: +34 3 221 7340 Email: roflos@icm.csic.es
Garcia-Alcázar, Ms A, Centro Oceanografia de Murcia, Carretera de la Azohia s/n, Puerto de Mazarrón (Murcia), Spain	Tel: +34 68 15 33 39 Fax: +34 68 15 39 34 Email: alicia. ieomz@mx2.redestb.es
Garcia-Gómez, Mr A, Centro Oceanografia de Murcia, Carretera de la Azohia s/n, Puerto de Mazarrón (Murcia), Spain	Tel: +34 68 15 33 39 Fax: +34 68 15 39 34 Email: antonio. ieomz@mx2.redestb.es
Holm, Prof J C, Institute of Marine Research, Austevoll Aquaculture Research Station, N-5392 Storebø, Norway	Tel: +47 5618 0342 Fax: +47 5618 0398 Email: jens.christian.holm@imr.no
Holmefjord, Dr I, Akvaforsk, Institute of Aquaculture Research, N-6600 Sunndalsøra, Norway	Tel: +47 71 69 25 77 Fax: +47 71 69 02 92 Email: ivar.holmefjord@akvaforsk.nlh.no
Howell, Dr B, CEFAS Conwy Laboratory, Benarth Road, Conwy LL32 8UB, UK	Tel: +44 1492 593883 Fax: +44 1492 592123 Email: b.r.howell@cefas.co.uk
Iglesias, Dr J Instituto Español Oceanografia, Gabo Estay-Canido, Apdo 1552, 36280 Vigo, Spain	Tel: +34 86 492111 Fax: +34 86 492351 Email: Jose.Iglesias@vi.ieo.es
Koven, Dr B Israel Oceanographic & Limnological Research Ltd National Centre for Mariculture, PO Box 1212, Eilat 88112, Israel	Tel: +972 7 6361420 Fax: +972 7 6375761 Email: Koven@agri.huji.ac.il
Lavens, Dr P, Universiteit Gent, Rozier 44, 9000 Gent, Belgium	Tel: +32 9 2643754 Fax: +32 9 2644193 Email: patrick.lavens@rug.ac.be
Lein, Dr I, Akvaforsk, Institute of Aquaculture Research, N-6600 Sunndalsøra, Norway	Tel: +47 71 69 98 00 Fax: +47 71 69 98 01 Email: ingrid.lein@akvaforsk.nlh.no
Meeren, Dr T van der, Institute of Marine Research, Austevoll Aquaculture Research Station, N-5392 Storebø, Norway	Tel: +47 5618 0342 Fax: +47 5618 0398 Email: Terje.van.der.Meeren@imr.no

Øiestad, Prof. V. NFH-University of Tromsø, NFH-Uitø, N-9037 Tromsø, Norway	Tel: +47 776 46000 Fax: +47 776 46020 Email: victor@nfh.uit.no
Peleterio, Mr J, Instituto Español de Oceanografia, Gabo Estay-Canido, Apdo 1552, 36280 Vigo, Spain	Tel: +34 86 492111 Fax: +34 86 492351 Email: tito.peleteiro@vi.ieo.es
Van Ryckeghem, Karla, Universiteit Gent, Rozier 44, 9000 Gent, Belgium	Tel: +32 9 2643754 Fax: +32 9 2644193 Email: karla.VanRyckeghem@rug.ac.be
Robin, Dr J, IFREMER, Centre de Brest, B.P. 70, 29280 Plouzané, France	Tel: +33 298 22 43 87 Fax: +33 298 22 45 47 Email: jrobin@ifremer.fr
Shields, Dr R, Seafish Marine Farming Unit, Ardtoe, Acharacle, Argyll, PH36 4LD, UK	Tel: +44 1967 431 213 Fax: +44 1967 431 343 Email: R_Shields@fc.uhi.ac.uk
Støttrup, Dr J, Danish Institute for Fisheries and Marine Research, North Sea Centre, PO Box 101, DK-9850 Hirtshals, Denmark	Tel: +45 9894 4500 Fax: +45 3396 3260 Email: jgs@dfu.min.dk
Tandler, Dr A, Israel Oceanographic & Limnological Research Ltd National Centre for Mariculture, PO Box 1212, Eilat 88112, Israel	Tel: +972 7 6361420 Fax: +972 7 6375761 Email: tandler@agri.huji.ac.il

Appendix II ICES ENRICHMENT EMULSIONS

Following recommendation 3 of the International Council for the Exploration of the Sea (ICES) Working Group on Mass Rearing of Juvenile Fish (Bergen, Norway 21-23, 1993; ICES 1994), two series of experimental emulsions have been prepared to study (n-3) Highly Unsaturated Fatty Acid (HUFA) requirements in marine fish larvae through the bioencapsulation technique.

A first series consists of emulsions differing in the concentration of (n-3) HUFA (0, 10, 30 or 50%) with a constant ratio between eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The latter series could be used to manipulate overall (n-3) HUFA concentrations in live feed.

A second series, which could be used to study the effect of DHA/EPA ratio in live food on the production success, contains a level of 30% (n-3) HUFA with the DHA/EPA ratio equal to 0.6, 2, and 4, respectively. The emulsions are of the Selco type and contain lipid (62% on wet weight basis), vitamins, antioxydants, preservatives, emulgators and water.

The present set of ICES Reference Emulsions are now available for an interlaboratory nutritional study with different marine species. Limited quantities (up to a few kg) can be supplied at cost. It would be most appreciated if the results would be exchanged in order to report briefly on the application of the emulsions at the next WG meeting.

In addition to the formulations of the ICES emulsions (coded as ICES ./.C/.), two Experimental ICES emulsions (i.e. ICES 30/0.6/E/1 and 50/0.6/E/1) have been made available which do not contain any formulated vitamins. This would allow to add vitamins (e.g. E, C) to the emulsions as required for nutritional studies using live food enrichment.

<u>Approximate fatty acid profile of ICES emulsions series 1: (n-3) HUFA content variable, DHA/EPA constant</u>

Label	Sum (n-3) HUFA (% of total fatty acids)	DHA/EPA ratio	Major lipid class*
ICES 0/-/C	0	-	TG
ICES 10/0.6/C	10	0.6	TG
ICES 30/0.6/C	30	0.6	TG
ICES 50/0.6/C	60	0.6	EE

*: TG=triglycerides, EE=ethylesters
Fatty acid composition is available per batch. Batch number (#) and production date (. / . / .) are indicated in the label, for example "ICES 30/2/C/# . /. / ."

<u>Approximate fatty acid profile of ICES emulsions series 2: (n-3) HUFA content constant, DHA/EPA variable</u>

Label	Sum(n-3) HUFA (mg/g DW)	DHA/EPA ratio	Major lipid class*
ICES 30/0.6/C	300	0.6	TG
ICES 30/2/C	300	2	TG
ICES 30/4/C	300	4	TG

*: TG=triglycerides
Fatty acid composition is available per batch. Batch number (#) and production date (. / . / .) are indicated in the label, for example "ICES 30/2/C/# . / . / ."

ICES STANDARD WEANING DIET

The establishment of a standard reference diet has been considered by various working groups dealing with the standardization of nutrition research as one of the most important steps in facilitating direct comparison of results among laboratories, experiments and species (Castell et al., 1989; ICES, 1993) and has recently been recommended by the International Council for the Exploration of the Sea (ICES) - Working Group on Mass Rearing of Juvenile Fish (since 1996: WG on Marine Fish Culture) during its 6th meeting in Conwy, UK on June 22-24, 1995 (ICES, 1995).

In this context, the Laboratory of Aquaculture & Artemia Reference Center makes available a batch of a reference diet for performing an interlaboratory study of fatty acid requirements of marine fish during weaning and first ongrowing. The diet is available in a range of particle sizes as an extruded nucleus containing low levels of lipid and n-3 HUFA (respectively, 6.4% and <0.35% of dry diet). The essential lipid fraction of the diet is added by coating with a concentrated emulsion in a blender followed by drying. Development of the diet is described for European sea bass (Dicentrarchus labrax) in Coutteau et al. (1995, 1996) and similar studies for turbot (Scophthalmus maximus) are being prepared for publication.

The standard diet may be used for two purposes:

1. as a **reference diet** for a series of experiments (ie to compare growth under various experimental conditions). This would imply the selection of a standard, fixed formulation for the coated fraction. At present, we recommend the diet formulation containing 2.5% of n-3 HUFA.

2. as a **standard diet** to study lipid requirements (fatty acids, but also other lipophilic compounds such as vitamin E, phospholipids). The composition of the diet can be modified by varying the composition of the coated fraction.

The diet is available at cost as

1. extruded nucleus (for composition see Table 2), allowing the participating laboratory to apply its own formulation/technique to add the coated fraction.
2. specific formulations prepared at ARC; for example diets ranging in content of n-3 HUFA can be made available upon request.

Characteristics of batch ICES895

ICES895 is the most recent batch of extruded nucleus of the ICES reference diet (stored under N2 in the freezer)

1. Composition

Dry matter	94.6
(% dry wt.)	
Total lipid (Folch)	6.4
Protein (Kjeldahl)	63.0
Ash	6.6
Background total (n-3) HUFA	<0.35

2. Water stability

After 1 h leaching test at 25°C at 1 g/40 ml aq. des.
Insoluble dry matter: 77.5 %

3. Available fractions

Size range (mm)	Total Quantity (Jan 5, 1995; kg)
200-300	20
300-500	65
500-800	70
800-1200	10
>1200	40

Preparation of the diet at ARC

Example of formulation of diet containing 2.5 % (n-3) HUFA

1. Formulation (g per 100 g of final diet)

Extruded diet	92.465 dry nucleus (ie 97.76 product)#
Total coated fraction	7.533
Phospholipids[1]	2
Oil 50[2]	5
Hydrogenated coconut oil[3]	0
Emulgator blend[4]	0.5
Ethoxyquin[5]	0.015
Vitamin E[6]	0.02
Aq. deion. to make emulsion	20

(1), (3), (4), (5), (6): see formulation of the standard diet; (2) concentrate of ethyl esters with approximately 50% (n-3) HUFA (INVE Aquaculture N.V., Belgium); (#) correction for water content in extruded nucleus.

2. Preparation protocol

- emulsify coated fraction in 20 g of deionized water per 100 g of final product
- coat extruded nucleus homogeneously with emulsion (by nozzling or slowly dripping the concentrated emulsion in a planetary mixer)
- rinse the beaker and / or tubing of nozzle with a few ml of water and add the rinsing water to the diet
- dry the diet (in fluidized bed at incoming air 70°C, product temperature initially 20 °C, 94% dry matter reached after approximately 25 min when the product temperatures reaches 60 °C)

Formulation of the standard diet (batch ICES895)

COMPOSITION	% of diet
extruded basal diet	92.465
codfish powder1	24
egg white albumin2	11
whey protein concentrate3	11
isolated soy protein4	11
haemoglobin powder5	4
wheat gluten6	3
a-cellulose7	2.225
starch8	13
hydrogenated coconut oil9	4
emulgator blend10	0.4
vitamin premix11	2
vitaminC12	0.4
choline chloride13	1
mineral premix14	2
attractant premix15	3
asthaxanthine16	0.1
calcium propionate17	0.3
BHT18	0.005
BHA19	0.005
additional coated fraction	7.533
de-oiled soya lecithin19	2
oil mixture20	5
emulgator blend 10	0.5
ethoxyquin21	0.015
vitamin E22	0.02

(1) code 0271, Rieber & Son A/S, Norway; (2) type HG/LW, Orffa Belgium N.V., Belgium; (3) LACPRODAN-80, Orffa Belgium N.V., Belgium; (4) SUPRO 500E, Protein Technologies International, Belgium; (5) VEPRO 95 PHF, Veos N.V., Belgium; (6) BIOGLUTEN, Amylum N.V., Belgium; (7) Sigma C8002; (8) SNOWFLAKE 03401, Orffa Belgium N.V.; (9) cocos 32/34, Vandemoortele N.V., Belgium; (10) glycerol mono-oleate/sorbitan monostearate (1:1); (11) according to Coves et al. (1991), Roche, Belgium; (12) MG-L-ascorbyl-2-polyphosphate, STAY-C, Roche, Belgium; (13) 50% purity, INVE Aquaculture N.V., Belgium; (14) according to Coves et al. (1991); (15) according to Kanazawa et al.(1989); (16) CAROPHYL PINK, Roche, Belgium; (17) Orffa Belgium N.V., Belgium; (18) Federa, Belgium; (19) EMULPUR N, Lucas Meyer N.V., Belgium; (20) see preparation of the diet at ARC for example (21) 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinolin, Sigma E8260; (22) dl-a-tocopherol-acetate, Roche, Belgium;

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Appendix III Overview of the use of ICES EMULSIONS and the REFERENCE DIET. List of publications

A. ICES EMULSIONS

1. *FISH*

SPECIES	RESEARCH	INSTITUTE	PUBLICATION
Scophthalmus maximus* (eggs and larvae) via *Artemia	Egg and larval quality	Lab. of Aquaculture & ARC[1], B	Dhert *et al.* (1995)
Scophthalmus maximus* (larvae) via *Artemia	Effect DHA/EPA ratio of life feed on turbot larvae	Lab. of Aquaculture & ARC,[1] Be - ULL[2], Chili	Cure *et al.* (1995)
***Solea solea* (later development stages)**	Effect of larval diet quality	CEFAS Conwy[3], UK	
Marine fish	Larval requirements	DFO Halifax[4], Ca	
Dentex dentex	Larval requirements	SEAMASA[5], Mallorca, Es	
Hipoglossus hipoglossus	Larval requirements	Dunstaffnage Marine Lab.[6] , UK	

2. *MOLLUSCS*

SPECIES	RESEARCH	INSTITUTE	PUBLICATION
Placopecten magellanicus	Dietary FA requirements	Lab. of Aquaculture & ARC[1], B	Coutteau *et al.* (1996)
Mercenaria mercenaria	Effect of lipid supplementation	Lab. of Aquaculture & ARC[1], B	Coutteau *et al.* (1994)
Crassostrea gigas	Testing of filtration and ingestion	Lab. of Aquaculture & ARC[1], B -Ifremer[7], Fr	
Tapes philipinarum	Dietary FA requirements	Lab. of Aquaculture & ARC[1], B	

3. *CRUSTACEA*

SPECIES	RESEARCH	INSTITUTE	PUBLICATION
Penaeus vannamei **(larvae and postlarvae) via *Artemia* and *Brachionus plicatilis***	Effect of n-3 HUFA	Cenaim[8], Ec	Wouters *et al.*(1997)
Penaeus monodon **(postlarvae) via *Artemia***	Effect of DHA/EPA ratio	Lab. of Aquaculture & ARC[1], B	Kontara *et al.* (1995)
Penaeus vannamei **(postlarvae)**	Dietary n-3 HUFA requirements	Cenaim[8], Ec	Naessens *et al.*(1995)
Penaeus vannamei **(larvae)**	Effect of n-3 HUFA and DHA/EPA ratio in life feed	Cenaim[8], Ec	Naessens *et al.*(1995)

4. *ZOOPLANKTON*

SPECIES	RESEARCH	INSTITUTE	PUBLICATION
Artemia franciscana	Lipid classes and their n-3 HUFA	Lab. of Aquaculture & ARC[1], B - Universidad de Cadiz[9], Es	Coutteau & Mourente (1997)
Artemia franciscana Artemia sinica	Stability of DHA	Sintef[10], N	Evjemo et al.(1997)
Artemia franciscana	Enrichment strategy	Lab. of Aquaculture & ARC[1], B	
Artemia	Enrichment	NERC Unit of Biochemistry, Stirling[11], UK	
Daphnia galeata	n-3 HUFA requirements	Center of Limnology, Niewersluis[12], Nl	Weers & Gulati (1997)
Daphnia	n-3 HUFA requirements	Max-planc-Institut fur Limnologie[13], D	
Daphnia	n-3 HUFA requirements	Univ.California[14] Davis, CA, USA	
Copepods	n-3 HUFA requirements	Nederlands Inst. For Sea Research[15] Texel, Nl	

B. REFERENCE DIET

1. *FISH*

SPECIES	RESEARCH	INSTITUTE	PUBLICATION
Dicentrarchus labrax **L. (juveniles)**	Role of phospholipids	Lab. of Aquaculture & ARC[1], B	Geurden *et al.* (1997)
Scophthalmus maximus **(juveniles)**	Role of phospholipids	Lab. of Aquaculture & ARC[1], B	Geurden *et al.* (1997)

***Dicentrarchus labrax* L. (postlarvae)**	Role of phospholipids	Lab. of Aquaculture & ARC[1], B	Geurden *et al.* (1997)
***Dicentrarchus labrax* L. (weaning and first ongrowing)**	Fatty acid requirements: comparison of diets	Lab. of Aquaculture & ARC[1], B	Coutteau *et al.* (1996)
***Dicentrarchus labrax* L. (weaning and first ongrowing)**	HUFA requirements: selection of the basal diet	Lab. of Aquaculture & ARC[1], B	Coutteau *et al.* (1995)
***Scophthalmus maximus* (postlarvae)**	Incorporation of FA from dietary neutral lipids	Lab. of Aquaculture & ARC[1], B	
***Scophthalmus maximus* (newly-weaned)**	Role of phospholipids	Lab. of Aquaculture & ARC[1], B	
Anarhichas lupus	HUFA requirements	Fisheries and Marine inst.[16], Ca	

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