**Mariculture Committee** **ICES CM 2000/F:04**

REPORT OF THE

# WORKING GROUP ON MARINE FISH CULTURE

**St. Andrews, New Brunswick, Canada**
**5–7 June 2000**



International Council for the Exploration of the Sea

Conseil International pour l’Exploration de la Mer

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## TABLE OF CONTENTS

**Section** **Page**

## 1 PARTICIPANTS

The Working Group on Marine Fish Culture (WGMAFC) convened its second meeting under its new remit at St. Andrews, Canada from 5–7 June 2000. The following representatives of ICES Member Countries participated in the meeting:

| | |
|---|---|
| Canada: | J. Castell (Chair), C. Clarke (Rapporteur), D. Martin-Robichaud, E. Trippel |
| Norway: | A. Mangor-Jensen |
| Belgium: | P. Dhert |
| Denmark: | J. Støttrup (by e-mail) |
| USA: | D. Bengtson, D. Perry |
| UK: | S. Wadsworth, I. Bricknell, S. Baynes |
| Spain: | T.B. Peleteiro, A. Garcia-Alcazar |

G. Kissil, B. Koven, and A. Tandler (Israel), and T. Blair, B. Chang, and K. Waiwood (Canada) also participated in the meeting. The meeting was chaired by J. Castell (Canada) and C. Clarke (Canada) acted as rapporteur with assistance from Tammy Blair and Blythe Chang. Affiliations and contact details of the participants and other correspondents who have been involved in the Working Group discussions over the past year are given in Annex 1.

## 2 TERMS OF REFERENCE

The following terms of reference were approved by the ICES Council at the 1999 Annual Science Conference in Stockholm, Sweden.

ICES C.Res. 1999/2:32

The **Working Group on Marine Fish Culture** [WGMAFC] (Chair: Dr J. Castell, Canada) will meet in St. Andrews, New Brunswick, Canada from 5–7 June 2000 to:

a) report on the current status of marine fish cultivation in Member Countries and on the factors that are likely to constrain 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) assess the prospects for establishing predictive criteria of juvenile quality;

e) report on the establishment of a nutrient database for larval feed composition and establish standard protocols for nutrient analysis;

f) review progress toward the identification of alternative protein and lipid sources for marine fish diets;

g) prepare a Theme Session for the 2000 Annual Science Conference on ‘New trends in fish feeding in aquaculture’;

h) encourage the use of an ICES standard weaning diet and standard enrichment emulsions as controls for research experiments and report on studies using them;

i) review the animal products used in fish feed formulations in ICES Member Countries, and possible contaminants (and their concentrations), contained in these fish feeds.

WGMAFC will report to the Mariculture Committee at the 2000 Annual Science Conference.

## 3 AGENDA

The meeting was hosted by the Department of Fisheries and Oceans and held at their Biological Station Conference Center in St. Andrews, New Brunswick, Canada. Discussion of the topics identified in the Terms of Reference occupied the first two days of the meeting (5–6 June). The final morning (7 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 Biological Station of the Department of Fisheries and Oceans and The Huntsman Marine Research Center on the afternoon of 6 June.

# 4 ACTIVITIES OF WGMAFC

## 4.1 Introduction

Participants received a warm welcome to the Biological Station on Monday, 5 June from the Station Director, Dr Tom Sephton. Dr John Castell, the Chair of WGMAFC, briefly explained the role of WGMAFC within the ICES organization and under the Mariculture Committee. With that WGMAFC began discussions related to the topics outlined in the Terms of Reference.

## 4.2 Marine Fish Production in 1999 [Term of Reference a)]

Summaries of the 1999 production of marine fish in ICES and observer countries are presented in Tables 1 and 2. These were based on submissions from WGMAFC members.

## 4.3 Constraints to Further Development [ToR a)]

### 4.3.1 Broodstock

In **Norway**, halibut egg production is limiting, not enough halibut eggs are available—need a more prolonged production period. There is a halibut fry shortage that limits the expansion of the on-growing stage of commercial farming. There is a low level of technology in many hatcheries and fry production is now mostly extensive, employing floating bags with natural live food organisms. There is a need for egg production units to supply hatcheries with good quality eggs from broodstocks that have been manipulated to spawn all year round, if commercial culture development is to become a reality. One sea cage farm claims to be making money. All cod fry are extensively produced at present. On-growth of cod has been done for several years, but the supply of juveniles is limiting. There is only one turbot hatchery left (Stolt). Most fry production goes for export (mainly to Spain); there is very little on-growing in Norway, because of the requirement for higher temperatures than can be provided at a reasonable cost.

In **Spain**, obtaining additional seabream, sea bass and other new cultured species broodstocks from the wild is a problem. There is a general concern regarding the need to maintain genetic diversity through selection of broodstocks, keeping of pedigrees and, where necessary, cryopreservation of gametes.

In the **UK**, numbers of halibut broodstocks are low for genetic diversity to be maintained. Broodstock nutrition needs to be improved. Larval survival is still low. Investment in new species is low.

In **Canada**, there is a limited genetic base for cultured halibut and haddock, therefore, careful consideration must be given to selection of breeding stocks and resulting performance. Efforts should be made to minimize in-breeding depression by using pedigree information when selecting F1s as future broodstock. There is a need for systematic selection of broodstock and keeping of records for new marine species being brought into production. There are now 3 years of haddock production, so selection of broodstock and f-lines is currently lacking and will be needed.

In the **USA**, there is a lack of large facilities available for research, e.g., broodstock nutrition is an important area of study but there is a need for several large tanks for experimentation. Also, there is a need for genetic research for selective breeding, but large facilities are required to experiment with different genetic crosses.

**Recommendation**

There is a need for systematic selection of broodstock and keeping of records for new marine species being brought into production.

**Table 1.** Production (1000s) of juvenile marine fish in ICES Member Countries and several other countries in 1999.

| Country<br>Species | Belgium | Canada | Croatia | Denmark | France | Greece | Iceland | Italy | Norway | Portugal | Spain | Turkey | UK | USA | TOTALS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sea bass (*Dicentrarchus labrax*) | | | | | | | 200 | | | | 7,300 | | | | **7,500.00** |
| Seabream (*Sparus aurata*) | | | | | | | | | | | 35,000 | | | | **35,000.00** |
| Turbot (*Psetta [Scophthalmus] maximus*) | | | | 505 | | | | | 150 | | 1,000 | | 50 | | **1,705.00** |
| Cod (*Gadus morhua*) | | 107.50 | | | | | | | 150 | | | | 34 | | **291.50** |
| Atlantic halibut (*Hippoglossus hippoglossus*) | | 46.10 | | | | | 300 | | 350 | | | | 160 | | **856.10** |
| Pacific halibut (*Hippoglossus stenolepis*) | | 0.05 | | | | | | | | | | | | | **0.05** |
| Flounder (*Platichthys flesus*) | | | | 73 | | | | | | | | | | | **73.00** |
| Winter flounder (*Pleuronectes americanus*) | | 0.50 | | | | | | | | | | | | | **0.50** |
| Yellowtail flounder (*Limanda ferruginae*) | | 1.00 | | | | | | | | | | | | | **1.00** |
| Summer flounder (*Paralichthys dentatus*) | | | | | | | | | | | | | | 306 | **306.00** |
| Lemon sole (*Microstomus kitt*) | | | | | | | | | | | | | 27 | | **27.00** |
| Red drum (*Sciaenops ocellata*) | | | | | | | | | | | | | | >500 | **>500** |
| Tautog (*Tautoga oritis*) | | | | | | | | | | | | | | | **0** |
| Sablefish (*Anoplopoma fimbria*) | | 1.60 | | | | | | | | | | | | | **1.60** |
| Haddock (*Melanogrammus aeglefinus*) | | 57.00 | | | | | | | | | | | | | **57.00** |
| Eel (*Anguilla anguilla*) | | | | | | | | | | | | | | | **0** |
| **TOTALS** | | **213.75** | **0** | **578** | **0** | **0** | **500** | **0** | **650** | **0** | **43,300** | **0** | **271** | **>806** | **45,668.75** |

Data were not available for Estonia, Finland, Germany, Ireland, Latvia, Netherlands, Poland, Russia or Sweden.

**Table 2.** Production (tonnes) of farmed marine fish in ICES Member Countries and several other countries in 1999.

| Country | Belgium | Canada | Croatia | Denmark | France | Greece | Iceland | Italy | Norway | Portugal | Spain | Turkey | UK | USA | TOTALS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | | | | | | | | | | | | | | | |
| Sea bass (*Dicentrarchus labrax*) | | | 1,000 | | 2,600 | 19,000 | 20 | 5,800 | | 1,000 | 1,670 | 6,500 | | | **37,590** |
| Seabream (*Sparus aurata*) | | | 1,000 | | 1,300 | 21,000 | | 5,100 | | 1,900 | 7,600 | 7,300 | | | **45,200** |
| Turbot (*Psetta [Scophthalmus] maximus*) | | | | | 2,000 | | 0 | | 100 | | 2,083 | | ~150 | | **4,333** |
| Cod (*Gadus morhua*) | | | | | | | 2 | | 30 | | | | | | **32** |
| Atlantic halibut (*Hippoglossus hippoglossus*) | | 1.5 | | | | | 12 | | 400 | | | | ~2 | | **415.5** |
| Pacific halibut (*Hippoglossus stenolepis*) | | 54[1] | | | | | | | | | | | | | **54** |
| Flounder (*Platichthys flesus*) | | | | | | | | | | | | | | | **0** |
| Winter flounder (*Pleuronectes americanus*) | | | | | | | | | | | | | | | **0** |
| Yellowtail flounder (*Limanda ferruginae*) | | | | | | | | | | | | | | | **0** |
| Summer flounder (*Paralichthys dentatus*) | | | | | | | | | | | | | | 9 | **9** |
| Lemon sole (*Microstomus kitt*) | | | | | | | | | | | | | | | **0** |
| Red drum (*Sciaenops ocellata*) | | | | | | | | | | | | | | >500 | **>500** |
| Tautog (*Tautoga oritis*) | | | | | | | | | | | | | | | **0** |
| Sablefish (*Anoplopoma fimbria*) | | | | | | | | | | | | | | | **0** |
| Haddock (*Melanogrammus aeglefinus*) | | | | | | | | | | | | | | | **0** |
| Eel (*Anguilla anguilla*) | | | | | | | | | | | 300 | | | | **300** |
| **TOTALS** | | **55.5** | **2,000** | **0** | **5,900** | **40,000** | **34** | **10,900** | **530** | **2,900** | **11,353** | **13,800** | **~152** | **>509** | **88,434** |

[1]Live-captured adults that were held in cages for up to nine months. Data were not available for Estonia, Finland, Germany, Ireland, Latvia, Netherlands, Poland, Russia or Sweden.

### 4.3.2 Disease

In the **UK**, disease outbreaks such as infectious salmon anaemia (ISA) and infectious pancreatic necrosis (IPN) have been a problem. There is a concern with diversification in the industry that species new to aquaculture may serve as carriers of viruses and diseases that might have an adverse impact on the salmon industry. This risk is best defined as the risk posed to established aquaculture species from the new species (i.e., does the new species harbour diseases, asymptomatically, that may cause serious damage to established species) or conversely are the new species susceptible to diseases that are manageable within the established industry? It is also important that wild stocks are not put at risk from aquaculture activities.

In **Norway**, disease is a major concern for halibut farming, particularly nodavirus that has had a drastic impact on hatcheries. Many hatcheries use the extensive rearing method involving floating enclosures for larval rearing, the results of which are unpredictable. Wild zooplankton are used, salinities and temperatures can fluctuate. It is not until the bags are collected and fish transferred to tanks that problems may become evident (triggered by the stress of handling). The level of technology in most hatcheries is rather low. In production, fry quality is a problem—there may be malpigmentation and incomplete eye migration for halibut. Some are related to diet—with wild zooplankton there can be differences in species available, it appears that particular species are of little problem (except a few). When the larvae are fed live organisms other than wild zooplankton, enrichments are required. For intensive methods, in rearing systems using *Artemia*, there is need for development work on improved enrichments to reduce the incidence of malpigmentation and incomplete metamorphosis.

In the **USA**, disease is also a constraint to aquaculture development, and the national Food and Drug Administration (FDA) regulates drug use. Only four therapeutics are available for use (one of which is sodium chloride), and only for specific species. So, if fish disease occurs, there is no legal way to treat fish except under special experimental permit. The FDA is trying to change these regulations so drugs can be more available for more species.

**Discussion**

The obvious example of new species being vulnerable to a manageable disease is the susceptibility of Atlantic halibut to infectious pancreatic necrosis virus (IPNV). This is considered to be a manageable disease in salmonid culture, but causes serious mortalities in halibut culture. This concern also extends to the risk of new species becoming infected by wild populations of the fish being cultured, as the new species is often being reared in waters where native populations of the same animal occur, as well as possibly providing a pool of infection which may spread back into wild populations. Although the epidemiology of this process is poorly understood, there is anecdotal evidence that it may have occurred in the past. It is also important that wild stocks are not put at risk from aquaculture activities.

These concerns are exacerbated because the availability of sites may be limited due to environmental constraints in some countries, causing co-culture to be practised. There is already evidence that co-culture of Atlantic salmon with gold sinney wrasse (used as a biological control of sea lice) caused an increased risk of atypical *Aeromonas salmonicida* infection as the gold sinney wrasse are susceptible to this infection and could act as a reservoir of infection in these systems.

**Recommendation**

It is the recommendation of this Working Group that the following points be addressed:

1) That an improved understanding of the interactions between farmed and wild fish is undertaken to fully assess the disease risk to established species from new species brought into aquaculture and the risk to new species from established animals.
2) That a series of studies should be initiated to assess the susceptibility of new species coming into aquaculture to the established diseases in that country’s aquaculture industry.
3) A series of risk assessments should be established to determine if diseases found in the new species can be transmitted to established aquaculture species.
4) That epidemiological studies are put in place to model disease outbreaks in wild populations of fish and the interactions, if any, between these outbreaks with aquacultured animals.
5) That studies should be undertaken to assess the risk of cross infection between species on aquaculture sites where co-culture or polyculture is practised.

### 4.3.3 Environment

Environment is a major constraint in the **USA**. In the *northeastern* USA, there is great interest in farming marine fish because the wild fishery is depressed. However, it is difficult to establish sites for net pens there because of user conflicts (environmental, recreational boaters, etc.). Fish farms are looking primarily to recirculation systems as the most probable area for mariculture industry expansion. In the *southeastern region*, culture of marine species is located in salt-water ponds. At present there are few regulations regarding effluent and waste output from pond sites, but the government is looking at developing regulations so these may be a future constraint.

In **Israel**, seabream is limited by environmental constraints—main production is in a tourist area for coral reefs so the government limits the amount of food that can be utilized. However, this same regulation results in greater interest in more efficient diets.

In **Denmark**, the major fish species cultured is rainbow trout, produced in fresh water, but because of very restrictive laws to protect the environment, this industry has been stagnant for almost a decade. The eel farming has been progressing well in the past decade, reaching 3000 tonnes last year. A global over-production of eel has caused prices to fall and this, coupled with a decreasing supply and higher prices for glass eels, may influence production this year and for the next couple of years. Thus, prices of eel products and the global availability of fry are becoming a constraint to the eel producers.

In **Norway**, a set of regulations has been developed which limits the amount of nitrogen, phosphate, and particulate material that can be released into the environment.

One of the main topics for all species is environmental interactions with mariculture (there is already a working group for this topic). We want to present the best examples of site management. From an environmental perspective, there is a great interest in moving towards land-based, closed-system culture, however, from an economic perspective, this is not yet feasible.

### 4.3.4 Environmental impact of marine aquaculture

**Statement of issues and constraints** (prepared by Simon Wadsworth)

Marine fish culture can benefit from lessons learned from the Atlantic salmon farming industry. Atlantic salmon production in the marine environment had reached approximately 1 million tonnes by 1999. Further expansion is planned over the next 5 years and there is increasing concern about the environmental impact of current and planned activities. A number of management techniques have been adopted that have ameliorated the environmental impact of large-scale production in some areas. Examples of some of these practices are listed below. They include:

**Single bay (biological area) management**

All farms operating within a specific area cooperate with each other in a number of areas:

- Stock single year class (and species) of fish
- Stocks are confirmed disease-free prior to stocking
- Vaccinated if appropriate, e.g., *Aeromonas salmonicida* in the UK
- Health surveillance conducted—information shared between sites
- Treatments for ectoparasites conducted on all sites in the biological area
- Treatments are synchronous and coordinated between all sites
- All fish harvested within a specific production cycle (22 months)
- No harvest waste is allowed to return to the environment
- Fallow period allowed (4–6 weeks)
- Environmental monitoring conducted on an annual basis to monitor impact.

### 4.3.5 Technology

In **Canada**, for haddock we have observed up to 50 % cannibalism at weaning. It is necessary to grade into uniform size classes frequently to reduce cannibalism. There is a need for improved grading procedures that are less labour intensive and less stressful to the fish. (More on technology will be found in the following discussions dealing with ToR b).)

### 4.3.6 Economic

In **Spain**, high production costs are mainly due to high feed costs and high feed conversion ratios. For seabream and sea bass, marketing problems occur because the market is almost saturated and prices are dropping. In addition, there is importation from Greece (half the price of Spanish products). A new strategic plan for aquaculture (government initiative) would provide assistance to private companies trying to develop aquaculture, especially of new species. About 20 species of interest (each has regional importance). Getting broodstock is a problem.

In **Canada**, there is a lack of financial commitment by fish farmers to purchase juvenile halibut for grow-out and marketing to demonstrate the economic potential of culturing species under various conditions (e.g., land-based versus cage grow-out). The government should consider the benefits of demonstration facilities to support the widespread dissemination of culture technology (e.g., not financially support one industrial participant who may not succeed and yet retains all knowledge of technological developments).

The main constraint to marine fish aquaculture development in **Norway** is the limited government financial support to do the development and research needed.

There are no large facilities available for research, e.g., broodstock nutrition is an important area of study but there is a need for several large tanks for experimentation. Also, genetic research for selective breeding is needed, but large facilities are required to experiment with different genetic crosses.

ICES should recommend that Member Countries endorse demonstration projects (as an example) to help promote aquaculture.

**Recommendation** (Dave Bengtson)

WGMAFC recommends that governments support or fund the construction and use of sufficiently large aquaculture facilities (i.e., large replicated structures) to conduct scientifically acceptable research in areas where such are required (e.g., broodstock nutrition, selective breeding); furthermore, governments, perhaps in association with industry, should support commercial-scale facilities for purposes of demonstration and training.

### 4.3.7 Legislative/regulatory

In **Spain**, there are different laws in different regions of the country, related to aquaculture regulations. A plan to standardise laws throughout the country is required.

**Discussion**

The problem of inconsistencies and lack of standardization in regulations affecting aquaculture occurs in many countries. This is a concern, rather than a constraint (different laws/regulations in different regions; the number of permits required; number of government agencies involved—this relates to disease transfer issues, transfer of stocks). The Commissioner for Aquaculture in Canada is reviewing the legal framework for aquaculture.

Discharge consents can control the levels of organic waste as well as the types and amounts of various chemo-therapeutants.

Government and industry should come to a consensus on feed quality and volume used and this could reduce the levels of nitrates and phosphates discharged as well as increase production efficiency, as seen in Israel and Norway.

Governments should assist with environmental monitoring strategies through a consensus in determining these strategies with industry and academia. The cost of this monitoring should be determined in discussions between government and industry. Governments should fund research on the effects of marine fish culture on the environment. Governments should establish environmental standards for industry to follow, but allow industry to implement strategies to meet those standards.

**Recommendation**

Governments should promote the use of Best Management Practices, identify successful new strategies for environmentally responsible aquaculture, and rely on science-based decisions to support the development of marine fish culture. Governments should work with industry to solve environmental problems in ways that increase the efficiency of aquaculture systems.

Lack of registered therapeutants is another constraint in the **USA**. Only four therapeutants are available for use (one of which is sodium chloride), and only for specific species. There is a need for more registered compounds for treatment of new species.

**Legislative and Regulatory Issues** (prepared by Ed Trippel)

A variety of legislative issues exist that can affect the success of fish culture in particular countries or regions within countries. This reflects the relative attention and concern for environmental quality, coastal habitat use, and use of fish in culture by different countries, states, and provinces. Concerns include (i) spread of diseases, (ii) importing certain strains of fish (eggs, larvae, etc.), (iii) use of therapeutants, (iv) density of cage sites, (v) co-habitation of different species within a site, (vi) slaughter method, (vi) licensing of sites, (vii) allowing sites to lay fallow, (viii) navigation around sites, and others. Certain regions within countries may be particularly weak in these efforts, perhaps as a means of attracting private investment in aquaculture. The lack of standards or poor harmonization of rules and regulations regionally, nationally, and internationally can jeopardise, in part, the successful cultivation of marine species.

**Recommendation**

WGMAFC recommends that the governments of ICES Member Countries make an effort to reach a consensus among countries (regions) such that harmonisation occurs in regulations governing aquaculture.

### 4.3.8 Access to sites

**Israel**

There are limited sites available for sea cages due to the lack of sheltered areas on the Mediterranean coast. Seabream culture is limited by environmental constraints—the main production is in a tourist area for coral reefs, so the government limits the amount of food that can be utilized. However, this same regulation results in greater interest in more efficient diets and has led to significantly improved feed conversion ratios and reduced nutrient loading in the environment.

**Belgium**

Constraints to marine fish culture including the short coastline, the polluted water (North Sea), the scale of tourism within the country which makes property very expensive for land-based culture. However, Belgium has developed other products (live feeds) which are essential for the global mariculture industry.

**United Kingdom**

Development of commercial marine aquaculture is constrained by the limited number of suitable sites where the environmental impact is not great. There is a need for more land-based sites, but competition for the most effective use of coastal land is considerable. Recent availability of grant funding within Wales, in particular, has fostered the establishment of two new farms that make use of recirculation technology.

**Spain**

In Spain there is competition for land use with tourism.

**Canada**

There are restrictions on new site allocations due to salmon cage congestion and disease problems in the Atlantic region and to user conflicts in the Pacific region.

### 4.3.9 Larval culture and nutrition

In **Canada**, for halibut, problems are unique for each hatchery. Problems associated with transporting eggs from government laboratories to commercial hatcheries were a constraint in 1999 that appears to have been overcome in 2000 as companies have become self-sufficient.

**Belgium** has developed other products (live feeds) which are essential for the global mariculture industry. Regarding the ***Artemia* problem**, people have to pay a very high price for *Artemia*, so they are looking for alternatives, but they are not all there at the moment. With regard to rotifers, there seems to be seasonal pressures in rotifer production and there is very little information on the species available, the biology of different species/strains or where they come from. Ester Lubzens has a rotifer reference center. WGMAFC may recommend that ICES endorse the idea of Member Countries providing support to a reference center such as Esther's to help her continue the maintenance of the reference center.

The shortage of *Artemia* and the resulting high prices present a serious constraint for farmers in Spain and other Mediterranean countries with large production hatcheries for species such as seabream and sea bass. In Norway, there are problems related to diet comprising wild zooplankton; it appears that particular species present little problem (with a few exceptions). For intensive rearing systems using *Artemia*, work is needed to develop enrichments that reduce the incidence of malpigmentation and incomplete metamorphosis of flatfish such as halibut.

**Live Food Constraints and Further Developments of the Industry** (prepared by Philippe Dhert)

It is obvious that the supply of cysts from the Great Salt Lake (for a long time, the only commercially available supply of *Artemia* cysts) cannot, at least for the time being, cope with the rapidly growing aquaculture industry. This leads to a growing concern by the aquaculture industry to find appropriate solutions for diversification of the *Artemia* resources, for optimising the use of the available quantity of *Artemia* cysts at different locations, and finding appropriate complementary measures for replacing the present excess in demand.

Aside from the extensive efforts that have been carried out in the field of diversification of *Artemia* sourcing and harvest efficiency, it is obvious that at least an equal amount of attention should be dedicated to a more efficient use of the available *Artemia* supply, as well as to non-*Artemia* alternatives.

Today, a 100 % replacement is possible, but always at the cost of culture time, yield, health status, and quality condition of the cultured animals. A fast, realistic and beneficial approach today is the installation of a number of procedures that focus on:

1) a more efficient use of the available *Artemia*;
2) a partial replacement by artificial diets (co-feeding).

A more efficient use of available *Artemia* could be obtained by the use of prolonged enrichment and rearing techniques which result in larger and more energy-rich *Artemia* and, subsequently, more biomass while hatching fewer cysts. On the other hand, the onset of *Artemia* feeding could be delayed by the extension of the rotifer feeding period.

However, if rotifers need to partially replace *Artemia* their production will have to be improved in order to allow further industrialisation of the rearing process. Today the expansion of the size of commercial hatcheries has increased to such an extent that they can no longer rely on low density cultures with low predictability. However, relatively few efforts have been made towards identification and selection of interesting rotifer strains for the industry. In this regard, the establishment and further support of existing reference centers for rotifer strains deserves full support.

Besides serving the industry, which could rely on high quality reference strains, the reference center would also help researchers to set up interlaboratory nutritional studies with various marine species. Standard experiments starting with resting eggs could alleviate the problem of variation due to rotifer quality and, moreover, allow bacterial control in comparative larval rearing experiments. Large- and small-sized rotifer strains are also listed high on the demand list for first feeding and delay of *Artemia* feeding.

Alternative mass production systems for rotifers operating at low cost are the ultimate goal for commercial hatcheries. Demonstrations on the use of environmentally friendly culture techniques as, for instance, the closed recirculation technique for rotifers could help the industry to adopt more efficient and reliable live food production systems.

**Recommendation 1**

In order to ensure that the supply of juvenile fish for on-growing continues to meet the growing demand, ICES Member Country governments are urged to continue to support research on methods for enhanced efficiencies and new technologies for production of live food organisms and research on developing alternative live food organism species.

**Recommendation 2** (Dave Bengtson)

Given the great international importance and utility of reference centers for the maintenance and distribution of reference biological materials, the Working Group applauds the governments of Belgium and Israel for their support of the *Artemia* Reference Center (Ghent University) and the Rotifer Reference Center (Israel Oceanographic and Limnological Research), respectively, and in the strongest terms urges their continued support; furthermore, the Working Group urges ICES Member Countries to support the *Artemia* and Rotifer centers by whatever means possible (including financial) and to establish new reference centers for other live food species.

**Copepods**

The development within the use of copepods for rearing marine species can be characterized as divergent and rather sporadic. Their use in extensive systems has been established and copepods are the primary live food basis within commercial hatcheries producing small yet stable numbers of juvenile marine fish. The species normally identified in these systems are calanoid species belonging to genera such as *Acartia*, *Temora*, and *Centropages*. Harpacticoids abound in these systems but because of their benthic habits and monitoring procedures, these species are generally overlooked. Nauplii of benthic harpacticoid species, however, have been identified in the stomachs of fish larvae from extensive systems.

In intensive systems, copepods are seldom used, apart from hatcheries, which are able to provide copepods collected from outdoor tanks or from the wild. Intensive cultures of copepods similar to those employed for other live prey such as rotifers and *Artemia* are not found in commercial hatcheries although culture techniques have been developed at a research scale. The most likely candidates for intensive cultures are species that can be maintained at high densities and have relatively short generation times. Several harpacticoid species make ideal candidates, such as species of the genera *Tisbe*, *Nitochra* or *Tigriopus*.

### 4.3.10 Feeds

For halibut farming, broodstock nutrition needs to be improved. Performance of haddock in Canada is limited by the development of fatty liver syndrome (hepatosomatic index as high as 40 %) due to a limited ability to utilize high levels of dietary lipid. There is a requirement for research on juvenile nutrition and grower diets to avoid development of fatty liver syndrome.

Local feed manufacturers will not commit to developing and manufacturing formulated feeds specific to the needs of new cultured species because quantities required are too small.

**Alternate protein and lipid sources**

An important issue is the development of alternative sources of dietary protein and lipid, both for health concerns (the possible presence of anti-nutritional factors or toxins) and from a social acceptance standpoint, and finally from the perspective of the depletion of the traditional marine sources.

**Investment/funding**

In the **USA**, there is difficulty in getting private investment for marine fish farms because the government is not sharing the risk. Also, in **Israel** subsidies are limited to 30 % or 40 % of the investment, which makes entrepreneurs reluctant to get into mariculture.

### 4.3.11 Public perception

- Developing environmentally friendly foods
- Human health aspects

**Recommendation on public perception**

Governments should promote public education about aquaculture, its health benefits and sustainability, based on scientifically valid information.

### 4.3.12 Biological constraints/concerns (general discussion)

The following is a list of the topics that were discussed:

- Lack of understanding of the relationship between stress and mortality. What constitutes stress, how to reduce stress, environmental and nutritional factors—at each life stage? Biotic and abiotic factors.
- Weaning.
- Genetic strain improvement—selective breeding for desired characteristics.
- Preservation of genetic diversity in wild stocks for future use in aquaculture (e.g., sea bass).

**Recommendation**

We should encourage cooperation on relevant research between industry/academia/governments. Similar programmes exist in the UK and the USA.

## 4.4 Review of Technological Developments [ToR b)]

1) Techniques for better estimation of growth in cages (e.g., image analysis with video cameras)
2) Fish counting systems for biomass estimation
3) Feeding systems with positive feedback loop to reduce pollution and improve food conversion
4) Sorting and grading (e.g., mechanical separations and need for passive grading systems for sensitive species)
5) Seawater recirculation
    a) Halibut must be kept onshore up to at least 100 g, recirculation is needed for nursery stage
    b) Ozone technology for disinfection can be purchased off the shelf. System must include activated charcoal to remove bromides produced by the ozone. May also want to use ozone for effluent treatment to meet environmental requirements for discharge (also in processing facilities to prevent disease transfer).
    c) Increased efficiency through high density production of live food organisms (e.g., rotifers) in recirculation systems
6) Harvesting technology, including killing methods (e.g., $CO_2$, cold shock, electrocution, clove oil), to improve flesh quality, effluent containment for disease control and reduced environmental impact
7) New tank designs
    a) including self-cleaning tanks (especially for early larval stages and particularly during weaning)
    b) shelves for flatfish culture—being developed for use in tanks or cages (modified salmon cages)
    c) automated algal production systems (e.g., grow fence and Craigie-NRC light box)
8) Photomanipulation to improve fish growth and to advance or delay spawning

9) Fish health development of vaccines, immunostimulants, and probiotics, to minimise the use of antibiotics and other chemotherapeutants
10) Animal welfare (related to tank design, grading, harvesting, stress)
11) Occupational safety for aquaculture workers must be considered
12) Live food production
    a) automation, improved efficiency, scaling up to commercial scale, improved quality, bacterial control
    b) greater diversity of *Artemia* resources
    c) alternatives to rotifers and *Artemia* (e.g., nematodes, copepods), improved algal culture systems, lower cost algal pastes.

### 4.5 Behavioural Criteria to Evaluate On-growing Systems and Operational Procedures [ToR c)]

1) Latency time to feeding after transfer / feeding rate: widely accepted as valid index of how suitable the conditions are for the fish. Needs observation, unless trained to demand feeders.
2) Growth rate is normally measured; doesn't provide same information as feeding and activity level observations.
3) Activity levels (either abnormally high or low, but it is difficult to obtain a standard as the control).
4) Aggression (as indicated by eye and fin damage, reduced growth and survival) and cannibalism as indicators of need for grading, sorting, reduced density.
5) Need "control" measure.
6) Feeding behaviour.
7) Flashing related to parasites such as *Trichodina*.
8) Coloration and swimming behaviour to evaluate stress levels in flatfish.

### 4.6 Predictive Criteria for Juvenile Quality [ToR d)] (information from Stephen Baynes)

It would be valuable to have a simple assay to test "quality." One has been proposed for scallops to assess "quality" after transportation, i.e., righting time (time delay between being turned upside down to flipping right side up). However, there does not appear to be anything similar in fish. Other indices might include:

Physiological

- Low stress response (cf. T. Pottinger and Carrick, 1999)
- High stress tolerance (cf. Howell *et al*., 1995)

Morphological

- Flatfish pigmentation (recent review by Bolker and Hill, 2000). Good predictor as obvious indicator of nutritional and environmental problems. Also often associated with other abnormalities.
- Sense organs
- Interocular distance: unpublished data show high COV, and difference to wild fish: indicative of problematic metamorphosis? (Ellis, pers. comm.)
- Lateral lines: indicative of problematic metamorphosis? (Ellis *et al*., 1997)

Behavioural (danger that assessment will be very time consuming)

- Phototactic behaviour of halibut larvae: proposed for assessing fitness (Karlsen *et al*., 1995)
- Activity levels of halibut larvae: suggested for early detection of bacterial infection (Skiftesvik and Bergh, 1993)
- Weaning: acceptance of novel prey items presumably depends upon fast rate of learning and low fear response to novel items in environment (Bromley and Howell, 1993)

Immunological—disease challenge?

It appears that the focus topics of 7) and 8) are on-growing rather than stock enhancement. However, there is some work on behavioural predictors of quality for stock enhancement, which is loosely connected.

- Jumping behaviour in *Plecoglossus altivelis* related to upstream swimming (for negotiating waterfalls) and used as predictive index for stocking effectiveness (Tsukamoto *et al*., 1999)
- Tilting behaviour (tendency to show a fright response) in *Pagrus major* used as index for cautiousness = predator avoidance (Tsukamoto *et al*., 1999)
- Schooling behaviour in *Seriola quinqueradiata* (Masuda *et al*., 1998)
- Feeding rate (after handling stress) (Masuda and Ziemann, 2000)
- Rate of learning (of conditioned response) (Masuda and Ziemann, 2000)

It is unlikely that a simple behavioural index could so easily act as a predictor, due to the limits of a single test. For example, cautiousness (reduced risk taking) may reduce vulnerability to predation, but may be associated with a lower motivation to feed (Huntingford *et al*., 1988).

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### 4.6.1 Additional criteria that need to be considered in evaluating juvenile fish quality

1) disease status
2) metamorphosis
3) timing, duration, and synchrony
4) proportion of incomplete metamorphosis (incomplete eye migration, malpigmentation)
5) growth rate (size at age and temperature; use thermal growth coefficient to standardise growth measurement, facilitate comparison among hatcheries)
6) morphometrics
7) interocular distance
8) liver size, etc. (compare hatchery fish with wild)
9) deformations (spine, jaw, operculum)
10) length-weight ratios (condition factor)
11) disease screening
12) corticosteroid treatment for detection of carriers
13) lethal sampling for known bacterial and viral pathogens
14) feeding rate and behaviour
15) weaning success and time to completion
16) stress tests—salinity, handling, corticosteroid
17) cortisol levels in tank water may be worth investigating, but may not be feasible
18) phototactic behaviour—may be correlated with feeding behaviour
19) swimming stamina (counter-current)—but may not be feasible to set up tests
20) Standard Rearing Protocols
    a) There is a need to develop standard protocols for raising each species. It is known that factors, including nutrition and rearing environment (temperature, etc.) at early larval stages, have effects on juvenile quality. Need to determine predictive criteria.
    b) Past performance of individual hatcheries. Hatcheries should be able to provide a record of standard rearing protocols for their fish (growth rates, survival at various stages, nutritional history).
    c) Genetic strains.

## 4.7 Nutrient Database for Larval Feed Compositions and Standard Protocols for Nutrient Analysis [ToR e)]

1) J. Castell is in the process of setting up an Oracle database on live feed nutrient analysis (Annex 4) and asked if it would be useful to pursue this initiative.
2) This database will provide more detailed information on fatty acids used (compared to what is generally reported in publications).
3) Members agreed that this is a worthwhile initiative.
4) Members were asked to review the proposed database and provide input on additional information that should be included (or excluded). Also, should this Working Group endorse this initiative?
5) Is there concern with potential misuse of such a database? There could be different levels of access (e.g., for contributors versus others).
6) J. Castell suggested setting up a live feed database subgroup (Bill Koven, Philippe Dhert). WGMAFC members were asked to submit names of individuals with data to input.
7) Initial estimate of cost to set up and operate the database for one year is approximately CDN $100,000.

## 4.8 Review Progress toward Identification of Alternative Protein and Lipid Sources for Marine Fish Diets [ToR f)]

1) At present, it may not be economical to use plant protein concentrates as alternatives. There is some use of these products for larval feeds which is justified because of the high value of these products.

2) Advantages of plant protein sources
   a) theoretically unlimited production
   b) avoid potential transmission of disease from animal protein sources
   c) avoid inclusion of contaminants in fish feeds (e.g., dioxins, PCBs, pesticides often associated with animal protein and lipid sources)
3) Research at Eilat has been done on an experimental canola protein concentrate and commercially available soy protein concentrates using seabream as a model species. The ultimate aim is for complete substitution of fishmeal. So far, results show that as the amount of plant protein increases, there is a reduction in growth, related to reduced feed intake. Need to improve voluntary feed intake—palatability enhancers may help. There may be differences in soy protein concentrates from different manufacturers (some are more palatable to fish). Digestibility for protein and NPU were as good as for fishmeals but there was a decrease in energy retention probably related to the reduced feeding.
4) Have also tested Australian lupine (methionine enhanced form) developed for use with sheep and chickens. Got good voluntary feed intake with seabream.
5) Will probably need to use protein concentrates, rather than vegetable meals, because plants contain a lower protein percentage than do fish meals.
6) Poultry oil can partially replace fish oils in high energy diets for seabream.
7) Simon Wadsworth reported some evidence of increased gut lesions in salmonids with increased soy substitution.
8) Expect price of fishmeals and oils to increase in future due to expected world-wide shortage.
9) Also experimental work on development of high DHA or high ARA sources from heterotrophically grown algae (see Annex 2).
10) Other possible protein sources that are being considered include fava bean, pea concentrates, potato concentrates, corn gluten meals, and bacterial protein sources grown on petroleum-based products.
11) J. Castell suggested a shared bibliography on this topic.

**Recommendation**

Given the almost certainty that fishmeal and fish oil will become more scarce and more expensive in the long term, WGMAFC encourages governments to research in the area of replacement protein and lipid sources.

### 4.9 Prepare a Theme Session for the 2000 and 2001 Annual Science Conferences [ToR g)]

#### 4.9.1 2000 (Brugge, Belgium)

**"New trends in fish feeding in aquaculture"**

**Recommend** that there is an exception to the submission deadline for abstracts, and that Moti Harel is asked to present information about new products from Martek.

**Recommend** that in the future, the conferences become more widely publicised, such as on web-sites for the European Aquaculture Society and the World Aquaculture Society, and in aquaculture magazines and bulletins. To facilitate advertising, a list of potential advertising sites should be drafted and kept for reference.

#### 4.9.2 2001 (Oslo, Norway)

**"Diversification of aquaculture"**

Possible co-conveners for session:

- Malcolm Gillespie (Ardtoe, UK)
- Pascal Divanach (Crete, Greece)
- Niall Bromage (Stirling, UK)
- Muki Shpigel (Eilat, Israel)

- Yves Harache (France)

This would include topics within the use of new technologies and new species (such as use of alternative culture methods or polyculture). The focus, however, will remain related to marine fish culture: interactions of species related to disease and disease management, use of other culture organisms to reduce net/cage fouling.

"Diversification of aquaculture, especially, 1) development of new marine fish species, and 2) polyculture to reduce environmental impacts and improve economics of marine fish culture."

**"Long-term effects of culture conditions on juveniles"**

Possible co-coveners for session:

- Patrick Sorgeloos (Ghent, Belgium)
- Dave Bengtson (Kingston, RI, USA)
- Bill Koven (Eilat, Israel)
- Elin Kjørsvik (Tromsø, Norway)
- Karin Pittman (Bergen, Norway)

Recommend changing the topic title to "Improvements in juvenile quality" to reflect a broader interest base.

Include topics on: broodstock diets (nutrition), broodstock holding conditions, genetic factors and broodstock selection, probiotics, effects of early larval nutrition and conditioning, microbial factors—abiotic and biotic factors, diseases, early rearing culture conditions, predictive criteria of juvenile quality for on-growing in aquaculture or for stock enhancement purposes.

"Session would be devoted to genetic and environmental factors involved in the improvement of juvenile quality, especially: 1) selective breeding, 2) rearing conditions including both abiotic and biotic factors such as nutrition, microbiology, and 3) methods to assess the quality of juveniles and their subsequent performance in on-growing and as it relates to stock enhancement purposes."

### 4.9.3 2002 (Denmark)

If one of the suggested Theme Sessions for 2001 is rejected, the Working Group recommends that it could be a possible session for 2002.

**"Use of immunomodulators in marine fish feeding: Possible beneficial and detrimental effects on the immune systems of the fish"**

Antibiotics are ineffective, vaccines are effective short-term, working towards natural disease resistance whether by genetic selection or other means will be important. The use of immuno-modulators in marine aquaculture has the potential to provide many benefits to the industry. They can, in theory, improve fish health by up-regulating the immune system, reducing the requirement for intervention with immuno-therapeutics and improving animal welfare. They also offer the potential to improve larvae and fry survival as judicious use of these compounds could protect larvae from endemic pathogens in the hatchery. It is proposed that the Theme Session examine this topic and investigate the potential benefits and possible detrimental effects that the use of immuno-modulators may have on marine larvae aquaculture.

Possible co-conveners:

- Ian Bricknell (UK)
- Simon Wadsworth (UK)
- Olav Vadnstein (Norway)
- Jorunn Skjerno (Norway)
- Tony Ellis (UK)

## 4.10 Encourage Use of an ICES Standard Weaning Diet and Standard Enrichment Emulsions [ToR h)]

1) J. Castell distributed a handout of the information ICES Standard Reference Weaning Diet and Reference Emulsions available from the Ghent University website: http://allserv.rug.ac.be/~jdhont/rend/rend.htm. [Please note: This material is attached as Appendix 1 and Appendix 2 to this report.]
2) A bibliography of publications which have cited the use of these diets is available (Annex 2).
3) J. Castell suggested that the Working Group endorse the use of reference diets.

## 4.11 Review the Animal Products Used in Fish Feed Formulations in ICES Member Countries, and Possible Contaminants (and their Concentrations) Contained in these Fish Feeds [ToR i)]

Increased vigilance and effort should be placed on analysis and protection to ensure that unsafe contaminants are not present in feed ingredients.

Dioxins have been one of the major concerns as a feed ingredient contaminant. All of the blood-meal products have been taken out of aquaculture feeds in the UK due to the human health scare; meat meal production has also ceased due to mad cow disease (BSE). Poultry meal is also being avoided in the UK, but not in Canada. Canada is still using blood and bone meals.

Prions (BSE) are of concern in EC countries.

Public perception plays a significant role in determining which products are used as feed ingredients and which products are avoided.

In the UK, there is a scientific committee addressing research into feed contaminants such as aflatoxins, pesticide residues, and heavy metals. In general, the industry is responsible for regulating the safety of the products.

In Canada, there has been very little work done on dioxins in wild fish. Environment Canada has studied pulp mill effluents in fish used for feeds. Since this study, the pulp mill waste treatment protocols have undergone various changes. Contaminants (organochlorines, PCBs, etc.) in fish meal are now at very low levels. In sediments, the organochlorines and PCBs seem to be coming from the feeds, but PAHs are below background levels found in other areas away from aquaculture sites. It seems that the feed dilutes the "normal" levels of PAHs. Additional information may be available in 2001 or 2002.

In Israel, no aquaculture firm can market its product without permission from the Department of Heath. The Department will monitor the fish (not the food) for levels of heavy metals and dioxins.

Tributyltins are no longer allowed for use as anti-fouling agents in fish cages. They have switched largely to using copper arsenates.

**Recommendation**

For 2001, investigate and collect information on the agencies and regulations for the monitoring of contaminants in feed ingredients, as well as on the monitoring programme itself in each country.

Dave Bengtson will volunteer to retrieve some information. John Castell will collect information by contacting feed companies.

The Department of Fisheries in Brugge (Bruges), Belgium may have a lot of information on PCBs.

# 5 CONCLUSIONS

The following conclusions and recommendations emerged from the discussions:

a) **Broodstock**

There is a need for the systematic selection of broodstock and keeping of records for new marine species being brought into production.

b) **Disease**

It is the recommendation of this Working Group that the following points be addressed:

- That an improved understanding of the interactions between farmed and wild fish is undertaken to fully assess the disease risks to established species from new species brought into aquaculture and the risk to new species from established animals.
- That a series of studies be initiated to assess the susceptibility of new species coming into aquaculture to the established diseases in that country's aquaculture industry.
- A series of risk assessments should be established to determine if diseases found in the new species can be transmitted to established aquaculture species.
- That epidemiological studies be put in place to model disease outbreaks in wild populations of fish and the interaction, if any, between these outbreaks with aquacultured animals.
- That studies be undertaken to assess the risk of cross infection between species on aquaculture sites where co-culture or polyculture is practised.

c) **Economics**

- ICES should recommend that Member Country governments endorse demonstration projects (as an example) to help promote aquaculture.
- Recommend that governments support or fund the construction and use of sufficiently large aquaculture facilities (i.e., large replicated structures) to conduct scientifically acceptable research in areas where such are required (e.g., broodstock nutrition, selective breeding); furthermore, that governments, perhaps in association with industry, support commercial-scale facilities for purposes of demonstration and training.

d) **Legislative/Regulatory**

- Governments should promote the use of Best Management Practices, identify successful new strategies for environmentally responsible aquaculture, and rely on science-based decisions to support the development of marine fish culture. Government should work with industry to solve environmental problems in ways that increase the efficiency of aquaculture systems.
- WGMAFC recommends that ICES Member Country governments make an effort to reach a consensus among countries (regions) such that harmonisation occurs in regulations governing aquaculture.

e) **Live Foods**

- In order to insure that the supply of juvenile fish for on-growing continues to meet the growing demand, ICES Member Country governments are urged to continue to support research on methods for enhanced efficiencies and new technologies for the production of live food organisms and research on developing alternative live food organism species.
- Given the great international importance and utility of reference centers for the maintenance and distribution of reference biological materials, the Working Group applauds the governments of Belgium and Israel for their support of the *Artemia* Reference Center (Ghent University) and the Rotifer Reference Center (Israel Oceanographic and Limnological Research), respectively, and in the strongest terms urges their continued support; furthermore, the Working group urges Member Countries to support the *Artemia* and Rotifer centers by whatever means possible (including financial) and to establish new reference centers for other live food species.
- WGMAFC should continue to endorse and support the development of an International Live Food Nutrient Database and Standard Analyses Protocols to be used by the laboratories that provide input to the database.

f) **Public Perception**

- Governments should promote public education about aquaculture, its health benefits and sustainability, based on scientifically valid information.

g) **Feed Contaminants**

- For next year, investigate and collect information on the agencies and regulations for the monitoring of contaminants in feed ingredients, as well as on the monitoring programme itself in each country.

h) **ICES Annual Science Conference**

- Recommend that in the future, the ASC conferences become more widely publicised, such as on websites for the European Aquaculture Society and the World Aquaculture Society, and in aquaculture magazines and bulletins. To facilitate advertising, a list of potential advertising sites should be drafted and kept for reference.

i) **Annual Science Conference Theme Session**

- Recommend that there is an exception to the submission deadline for abstracts, and that Moti Harel is asked to present information about new products from Martek.
- Recommend that in the future, the conferences become more widely publicised, such as on websites for the European Aquaculture Society and the World Aquaculture Society, and in aquaculture magazines and bulletins. To facilitate advertising, a list of potential advertising sites should be drafted and kept for reference.

j) **Working Group Meetings**

- For 2002, it was proposed to meet in Denmark (Hirtsals) or Portugal (Faro) between March and June. Additional suggestions for the next meeting include Weymouth, England (Bari Howell) and Ghent, Belgium in conjunction with Larvi '01 in September 2001 (Patrick Sorgeloos). David Bengston was nominated as rapporteur for the 2002 meeting.

## 6 RECOMMENDATIONS FOR FUTURE ACTIVITIES

The **Working Group on Marine Fish Culture** [WGMAFC] (Chair: Dr J. Castell, Canada) will work by correspondence in 2001 to:

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

b) graph and evaluate historical trends for major species and predict future development;

c) review technological developments in relation to fish production and their application to various species;

d) report on alternative sources of protein and lipid, including electronically available bibliography;

e) encourage development of standard reference diets;

f) support development of an international research programme in support of the development of micro-diets including exchange of test micro-diets among laboratories and conducting feeding trials among different species;

g) support research programmes on fish health research and report on existing and emerging diseases of cultured marine fish, including treatments used;

h) compile complete list of procedures and methods for monitoring of feeding regimes;

i) review fish welfare in relation to marine fish culture;

j) work with WGAGFM in developing standard culture conditions under which strains, stocks or species might be tested to evaluate their performance.

| | |
|---|---|
| **Priority:** | This is an important aspect to the future development of mariculture in the sea and in land-based operations. |
| **Scientific Justification:** | a) This provides a continuing mechanism for focusing the WG activities.<br>b) In addition to providing data on most recent years' production by country for each marine fish species being cultured, it would be useful to have a multiyear graphical perspective to more clearly see trends that are developing.<br>c) The continued expansion of marine fish culture is dependent on new technological developments such as improved methods for sorting larval and early juvenile fish to prevent cannibalism, improved land-based recirculation systems and the need for better methods to estimate growth.<br>d) The increasing demands for fishmeal and fish oil by aquaculture and agricultural industries has resulted in increases in prices. The high prices and the lack of opportunities to expand the capture fishery make it imperative that alternative protein and lipid sources be developed for use in feeds for aquaculture. Information on alternative sources of protein and lipid is vital to the continued |

| | |
|---|---|
| | expansion of the aquaculture industry.<br>e) In order to facilitate comparison of growth, survival, and other performance criteria of fish reared by various culture facilities and research groups, it is necessary to standardize various aspects of the experimental design and to incorporate reference protocols. The ICES Standard Reference Weaning Diet and enrichment emulsions are tools that can help in this regard. All members of WGMAFC are encouraged to utilize the ICES Reference Diet or enrichment emulsions in their own research programmes and to encourage other researchers to do the same.<br>f) It is still necessary to provide live food organisms at first feeding for most marine fish species that are utilized in aquaculture. There are, however, very encouraging recent research results in the development of micro-diets that might shorten the period of time that live feeds are required and, perhaps, eventually permit larval marine fish to be fed prepared feeds and avoid live feeds. As a number of members of WGMAFC are involved in such research, we propose to develop a collaborative micro-diet research programme.<br>g) As the marine fish culture industry grows, there will be an increasing need to develop disease control procedures such as new vaccines, improved monitoring, and other aspects of fish health. It is very important to have knowledge about the most recent developments in marine fish health research. Each newly identified disease poses a threat to the growth of the marine fish culture industry. Knowledge and the proper technique to isolate and identify each disease organism are vital tools in the fight to control these diseases. WGMAFC will review existing and emerging diseases of cultured marine fish and report on the most effective treatments to control these diseases.<br>h) To ensure that fish culture is economical and to reduce contamination of the aquatic environment, it is vital to reduce the amount of feed that is wasted and not consumed by the fish. It is thus necessary to make available a complete list of procedures and methods for monitoring feeding regimens and indicate which are the most effective in improving efficient feed utilization and reducing waste.<br>i) By culturing fish, as opposed to harvesting from the wild, we make a commitment to proper care of the cultured animals. There is increasing pressure to ensure that cultured animals receive humane treatment. WGMAFC will review the aspects of fish welfare in relation to marine fish culture.<br>j) Though genetics is the topic for WGAGFM, WGMAFC proposes that the two groups work together to develop standard culture conditions under which strains, stocks or species might be tested to properly and consistently evaluate their performance. |
| **Relation to Strategic Plan:** | Responds to Objectives 1 (d), 2 (a, d), 3 (b), 5 (a), and 7 (b, e). |
| **Resource Requirements:** | None required, other than those provided by the host institute. |
| **Participants:** | WGMAFC members |
| **Secretariat Facilities:** | None required |
| **Financial:** | None required |
| **Linkages to Advisory Committees:** | There are no direct linkages to the advisory committees. |
| **Linkages to other Committees or Groups:** | WGPDMO, WGAGFM |
| **Linkages to other Organisations:** | |

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## ANNEX 2: LIST OF DOCUMENTS WITH REFERENCE TO ICES DIETS OR EMULSIONS


### Published Manuscripts

Caers, M., Coutteau, P., and Sorgeloos, P. 2000. Dietary impact of algal and artificial diets, fed at different feeding rations, on the growth and fatty acid composition of *Tapes philippinarum* (L.) spat. Aquaculture, 170: 307–322.

Caers, M., Coutteau, P., and Sorgeloos, P. 2000. Incorporation of different fatty acids, supplied as emulsions or liposomes, in the polar and neutral lipids of *Crassostrea gigas* spat. Aquaculture. In press.

Caers, M., Coutteau, P., Cure, K., Morales, V., Gajardo, G., and Sorgeloos, P. 1999. The Chilean scallop *Argopecten purpuratus* (Lamarck, 1819): II. Manipulation of the fatty acid composition and lipid content of the eggs via lipid supplementation of the broodstock diet. Comparative Biochemistry and Physiology (B), 123: 97–103.

Caers, M., Coutteau, P., Lombeida, P., and Sorgeloos, P. 2000. The effect of lipid supplementation on growth and fatty acid composition of *Tapes philippinarum* spat. Aquaculture, 162: 287–299.

Han, K., Geurden, I., and Sorgeloos, P. 2000. Enrichment strategies for *Artemia* using emulsions providing different levels of n-3 highly unsaturated fatty acids. Aquaculture, 183: 335–347.

### Submitted Manuscripts

Cavalli, R.O., Vanden Berghe, E., Lavens, P., Nguyen, T.T., Wille, M., and Sorgeloos, P. 2000. Ammonia toxicity as a criterion for the evaluation of larval quality in the prawn *Macrobrachium rosenbergii*. Submitted to Comparative Biochemistry and Physiology.

Helland, S., Triantaphyllidis, G.V., Fyhn, H.J., Evjen, M.S., Lavens. P., and Sorgeloos, P. Modulation of the free amino acid pool and protein content in populations of the brine shrimp *Artemia*. Submitted to Marine Biology.

### Manuscripts In Preparation

Howell, B.R., and Logue, J.A. In prep. Long-term consequences of larval diet quality on the phenotype of later developmental stages of the sole, *Solea solea* (L.).

League, J.A., Howell, B.R., Bell, G.Y.J., and Cossins, A.R. In prep. Effects of dietary polyunsaturated fatty acid deprivation upon tissue composition and stress tolerance in juvenile Dover sole (*Solea solea* (L.)).


### Projects

FAIR-CT96-1852 GIGANUGA: Breeding improvement of *Crassostrea gigas* by nutritional and gametogenesis control.

INCO ERBIC18CT970188 INCO Scallop: Improvement of scallop production in rural areas.

INCO mud crab: Requirements for n-3 HUFA and vitamins of mud crab larvae.

Vlir – South Africa: Mud crab.

## ANNEX 3: HIGH DHA (DOCOSAHEXAENOIC ACID) ENRICHMENT OF LIVE FOOD ORGANISMS USING NOVEL DHA-RICH PHOSPHOLIPIDS

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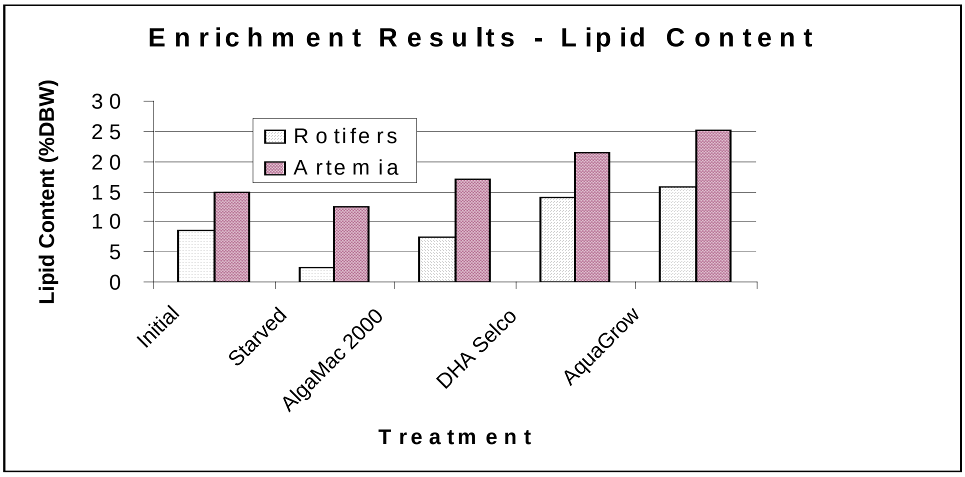
Eggs of marine fishes contain markedly high levels of DHA (docosahexaenoic acid), ranging from 10–40 % of total fatty acids, and generally a ratio of DHA:EPA (eicosapentaenoic acid) greater than 2:1. This suggests that the early development of the larvae requires high levels of DHA for optimal growth and survival. Standard enrichment procedures for rotifers and *Artemia* nauplii, however, do not provide the required levels of DHA and DHA:EPA ratios. We report that this goal has now been achieved by using a spray-dried phospholipid extract of DHA-rich *Crypthecodinium sp.* algal biomass (Martek Biosciences Corporation, Columbia, MD, USA), of which the total fatty acids are 49 % DHA and less than 0.5 % EPA.

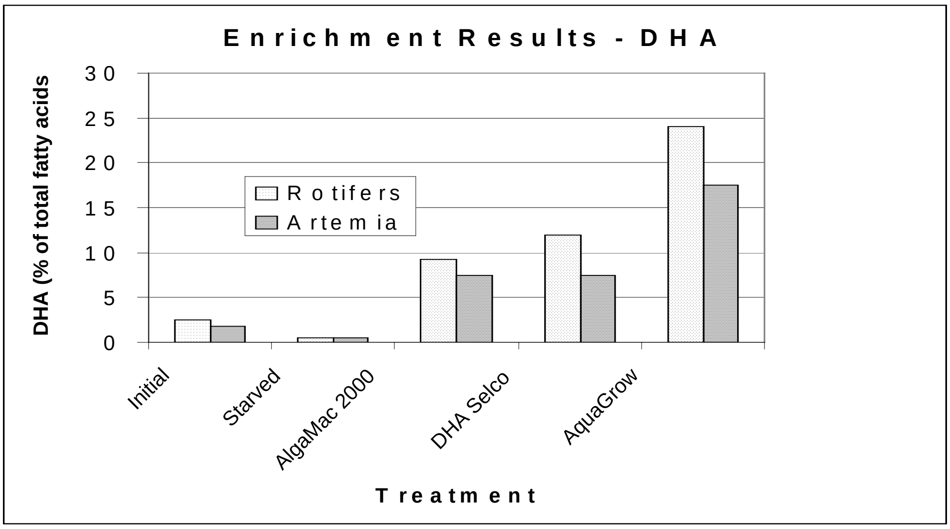
Enrichment of stage II *Artemia* nauplii was carried out at 28–30ºC, in aerated 20 ppt artificial sea water (200,000 nauplii/liter). Two equal portions of 0.3 g DHA-rich phospholipids/liter were given at time 0 and after 8 hours. A significant increase ($p < 0.05$) in nauplii lipid content from an initial level of 16.3 % to 23.7 % dry weight (DW) was obtained after 16 hrs. DHA increased from undetectable levels to 41mg/g dw (17.2 % of total fatty acids), while EPA content increased only slightly from 5 % to 6.2 % of total fatty acids. Similar enrichment patterns were also observed with the rotifer, *Brachionus plicatilis.* Using this process of DHA-rich phospholipids enrichment, feeding experiments with seabream *(Sparus aurata)* larvae demonstrated better growth and swimbladder inflation rates when fed on high DHA-enriched food organisms relative to larvae fed on food organisms enriched with other commercial preparations.

In an effort to determine the most effective molecular carrier of DHA for larval food organism enrichment, we tested the hypothesis of whether DHA-ethyl esters or DHA-containing phospholipids could improve *Artemia* enrichment. Different enrichment treatments having varying proportions of phospholipids and ethyl esters at a constant level of DHA were fed to the nauplii. A significant ($p < 0.05$) higher absorption of 3l % DHA by *Artemia* was obtained during the first 8 hrs at 10 % dietary phospholipids level compared to 5 %, while no further improvement in absorption was obtained at higher phospholipid percentages or with the addition of any level of free fatty acids. The triacylglycerol: phospholipid ratio in the nauplii was independent of this ratio in the enrichment diets ($p < 0.05$). Further results show that *Artemia* survival was not affected even at dietary phospholipid levels of 40 %. These results imply that the phospholipid extracts of DHA-rich *Crypthecodinium* sp. algal biomass provide an effective DHA enrichment of food organisms for the enhancement of growth and survival in marine fish larvae culture.

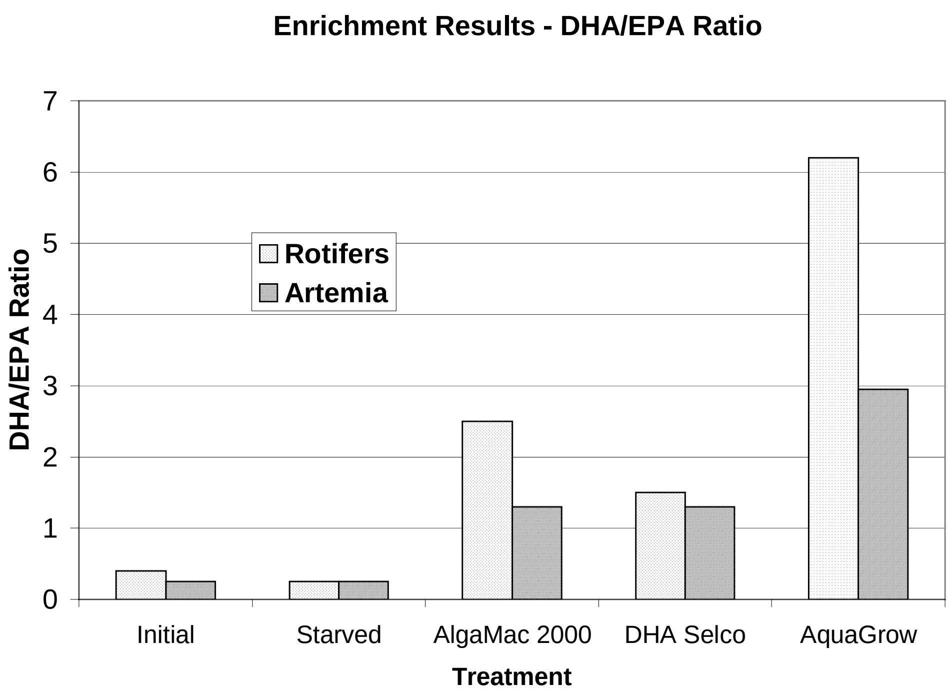
(Abstract presented at the 1998 World Aquaculture Society meeting in Las Vegas, NV.)



Enrichment Results - Lipid Content
Lipid Content (%DBW)
30
25
20
15
10
5
0
Rotifers
Artemia
Initial
Starved
AlgaMac 2000
DHA Selco
AquaGrow
Treatment

Enrichment Results - DHA
DHA (% of total fatty acids
30
25
20
15
10
5
0
Rotifers
Artemia
Initial
Starved
AlgaMac 2000
DHA Selco
AquaGrow
Treatment

Enrichment Results - DHA/EPA Ratio
DHA/EPA Ratio
7
6
5
4
3
2
1
0
Rotifers
Artemia
Initial
Starved
AlgaMac 2000
DHA Selco
AquaGrow
Treatment

**AquaGrow™ - Feed 15** is a spray dried nutrition product that is 15 % DHA by weight and is suitable for either aquaculture or animal feeds. It contains cells of the heterotrophically-grown alga *Crypthecodinium*, a natural aquaculture food source. The alga is grown under sterile and controlled conditions to insure a high quality, consistent product.

- Contains 30 % fat and 50 % DHA, with no EPA.
- DHA content is 15 % of the dry weight.
- Suitable as a source of DHA for aquaculture feeds (enrichment, larval and broodstock diets, finishing feed, etc.).
- Suitable as a component of animal feeds.

**AquaGrow ™ - AA** is a spray dried aquaculture nutrition product with a high arachidonic acid content. It is a unique source of arachidonic acid that is produced by a microbial fermentation process. Production under sterile and controlled conditions provides a consistent, high quality product.

- Contains over 30 % fat and 40 % AA.
- A unique source of arachidonic acid that increases stress tolerance in some larval species.
- Ingested readily by rotifers and *Artemia.*
- Suitable as a component of larval and broodstock diets.

**AquaGrow ™ – Chlorella** is a spray dried aquaculture nutrition product with a high DHA content. It is produced from a blend of two algae *(Crypthecodinium* and *Chlorella)* to produce a product that is high in DHA and contains the natural nutrients found in *Chlorella.* Both algae are grown under sterile and controlled conditions to insure a high quality, consistent product.

- Contains over 45 % fat and 30 % DHA, with no EPA.
- Ingested readily by rotifers and *Artemia.*
- Produces the ideal DHA:EPA ratio in zooplankton for improved larval growth and development.
- Contains the vitamins and nutrients found in *Chlorella*.
- Assimilated rapidly due to the high level of polar lipids.

**AquaGrow™ Advantage** is a spray dried aquaculture nutrition product with a very high DHA content. It contains cells of the heterotrophically-grown alga *Crypthecodinium,* a natural aquaculture food source. The alga is grown under sterile and controlled conditions to insure a consistent, high quality product.

- Contains over 20 % fat and 50 % DHA, with no EPA.
- Ingested readily by zooplankton.
- Provides a high level of DHA for optimal larval growth.
- Produces the ideal DHA:EPA ratio in zooplankton for improved larval growth and development.
- Stays suspended in water and does not foam.
- Suitable as a component of larval and broodstock diets.

## AquaGrow™ Product Compositions

| Proximate Analyses | Feed 15 | AA | Chlorella | Advantage |
|---|---|---|---|---|
| Fat | 30% | 34% | 46% | 23% |
| Protein | 20% | 9% | 19% | 7% |
| Carbohydrate | 25% | 43% | 18% | 5% |
| Ash | 8% | 5% | 10% | 53% |
| Moisture | 7% | 5% | 4% | 3% |
| Fiber | 8% | 1% | 1% | 8% |

| Fatty Acid Profile (% of fat) | Feed 15 | AA | Chlorella | Advantage |
|---|---|---|---|---|
| 14:0 (myristic) | 16 | 0 | 10 | 17 |
| 16:0 (palmitic) | 16 | 15 | 18 | 16 |
| 18:1 (oleic) | 8 | 10 | 29 | 8 |
| 20:4n-6 (AA) | 0 | 40 | 0 | 0 |
| 20:5 (EPA) | 0 | 0 | 0 | 0 |
| 22:5 (DPA) | 0 | 0 | 0 | 0 |
| 22:6 (DHA) | 50 | 3 | 30 | 50 |
| **Vitamins and Pigments** | **Feed 15** | **AA** | **Chlorella** | **Advantage** |
| Vitamin A (U/lb.) | 5,200 | 3,400 | 15,000 | 3,300 |
| Vitamin C (ppm) | 0 | 250 | 20 | 0 |
| Vitamin D (U/lb.) | 0 | 0 | 900 | 0 |
| Vitamin E (mg/lb.) | 0 | 0 | 110 | 0 |
| Carotene (mg/lb.) | 8 | 1.6 | 30 | 6 |
| Xanthophyll (mg/lb.) | 6 | 2.7 | 630 | 11 |
| **Major Minerals** | **Feed 15** | **AA** | **Chlorella** | **Advantage** |
| Calcium | 0.1% | 0.1% | 0.1% | 0.3% |
| Potassium | 0.65% | 0.4% | 0.6% | 0.56% |
| Phosphorus | 0.26% | 0.1% | 0.8% | 0.2% |
| Sodium | - | 1.0% | 2.7% | - |
| Magnesium | 0.14% | 0.1% | 0.1% | 0.09% |
| Sulfur | 0.96% | 0.5% | 0.4% | 0.53% |
| Chloride | - | - | 0.2% | - |
| **Trace Minerals** (ppm) | **Feed 15** | **AA** | **Chlorella** | **Advantage** |
| Iron | 48 | 55 | 52 | 24 |
| Manganese | 45 | 25 | 20 | 22 |
| Zinc | 15 | 7 | 18 | 5 |
| Copper | 3 | 3 | 9 | 3 |
| Cobalt | < 0.5 | < 0.5 | < 0.5 | < 0.2 |
| Iodine | 2 | 1 | 1 | 6 |

**Amino Acid Profile** (% of protein)

| Amino Acid | Feed 15 | AA | Chlorella | Advantage |
|---|---|---|---|---|
| methionine | 3.3 | 3 | 4 | 3 |
| cystine | 1.8 | 2 | 2 | 2 |
| lysine | 6.4 | 3 | 3 | 6 |
| phenylalanine | 3.3 | 5 | 4 | 3 |
| leucine | 6.5 | 7 | 7 | 7 |
| isoleucine | 4.1 | 3 | 4 | 4 |
| threonine | 5.4 | 5 | 5 | 5 |
| valine | 5.1 | 6 | 5 | 5 |
| histidine | 2.5 | 2 | 2 | 3 |
| arginine | 6.3 | 4 | 10 | 6 |
| glycine | 6.2 | 6 | 5 | 6 |
| aspartic acid | 13.2 | 10 | 9 | 13 |
| serine | 6.0 | 5 | 4 | 6 |
| glutamic acid | 12.9 | 16 | 12 | 13 |
| proline | 5.4 | 5 | 5 | 5 |
| hydroxyproline | 0.1 | 1 | 1 | 0 |
| alanine | 7.7 | 9 | 11 | 8 |
| tryptophane | | 6 | 3 | |
| tyrosine | 3.8 | 2 | 4 | 4 |

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## ANNEX 4: PROPOSED FIELDS FOR ICES LIVE FEED NUTRIENT ANALYSES DATABASE

| Field Name | Data Type | Format | Description |
|---|---|---|---|
| Source of Data | Text | | In this case, source of the data. Lab where analyses were done. |
| Contact person | Text | | Name, Address, phone fax and e-mail of contact from data source |
| ID Code Original | Text | | Sample Id from original source of data: alphanumeric |
| ID Code ICES Database | Text | | Sample Id in Nutrient Database Recorda. alphanumeric |
| Source of Sample | Text | | Site where sample was collected, either wild or cultured etc. |
| Date collected | Date | MMDDYYYY | Date Sample was Collected |
| Date Analyzed | Date | MMDDYYYY | Date(s) sample was analyzed |
| Species | Text | | Full Latin Name of Species |
| Tissue Type | Test | | Whole animal or specific tissue or organ |
| Category | Text | | Sample Category, Copepod, algae, mollusc, rotifer, etc |
| Age | Integer | 999 | Culture Age of sample in days |
| Weight | Float | 99.99999 | Weight of sample in grams (wet tissue or live weight) |
| Cell Density | Integer | 9999999 | (Algae, Rotifers, *Artemia*, Copepods etc.) cells or individuals per ml at harvest. |
| Tank or culture volume | Integer | 999 | Volume in litres of culture tank |
| Salinity | Float | 99.9 | Salinity of culture water in parts per thousand |
| Temperature | Float | 99.9 | Culture temperature ºC |
| Photoperiod | Number | 99:99 | Hours of light and hours of darkness per day |
| Light Source | Text | | Source of light (tungsten, fluorescent, natural, etc) |
| Light Intensity | Number | 999 | Light intensity in foot candles, Lux; Whatever is the standard accepted unit |
| Feed | Text | | Description of feed or media used |
| Feed History | Text | | Description of past feeding history. |
| Feeding Regimen | Text | | How often and how much was fed per day |
| Experimental treatment | Text | | Notes or comments on treatment of sample |
| **Proximate Analysis Data** | | | |
| Dry Matter Weight % | Float | 99.99 % | Percentage of original weight after drying |
| Ash % | Float | 99.99 % | Percent ash after muffling at 550 ºC |
| Lipid % | Float | 99.99 % | Percent lipid (Bigh & Dlyer or Folch extraction). |
| Crude Protein % | Float | 99.99 % | 6.25 x total nitrogen Kjeldahl procedure or C, H, N analyser. |
| Crude fiber % | Float | 99.99 % | Percentage of crude fiber (mild acid mild base digestion dry matter minus ash) |
| Starch or glycogen % | Float | 99.99 % | NaOH digestion, ethanol precipitation, sulphuric acid phenol colorimetric technique |
| Energy | Float | 99.99 | Kcal/g dry matter or kjouls/g dry matter |
| **Fatty Acid Analyses** | | | |
| 12:0 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 14:0 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 14:1n-7 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 14:1n-5 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 15:0 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 15:1n-8 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 15:1n-6 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| DMA 16:0 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 16:0 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 16:1n-9 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 16:1n-7 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 7Me-16:0 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 16:1n-5 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 16:2n-9 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 16:2n-6 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 16:2n-4 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 16:2n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 16:3n-6 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 16:3n-4 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |

| | | | |
|---|---|---|---|
| 16:3n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 16:3n-1 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 16:4n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 16:4n-1 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| DMA 18:0 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 18:0 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 18:1n-11 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 18:1n-9 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 18:1n-7 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 18:1n-5 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 18:2n-9 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 18:2n-6 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 18:2n-4 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 18:2n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| NMI 18:2 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 18:3n-6 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 19:0 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 18:3n-4 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 18:3n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 18:4n-6 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 18:4n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 18:4n-1 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 20:0 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 20:1n-13 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 20:1n-11 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 20:1n-9 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 20:1n-7 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 20:1n-5 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 20:2n-9 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 20:2n-6 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 20:2n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| NMI-20:2 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 20:3n-9 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 20:3n-6 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 20:3n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 21:0 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 20:4n-6 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 20:4n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 20:5n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 22:0 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 22:1n-13 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 22:1n-11 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 22:1n-9 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 22:1n-7 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 22:2n-9 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 22:2n-6 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 22:2n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| NMI22:2 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 22:3n-9 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 22:3n-6 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 23:0 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 22:3n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 22:4n-6 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 22:4n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 22:5n-6 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 22:5n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 24:0 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 22:6n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |

| | | | |
|---|---|---|---|
| 24:1n-13 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 24:1n-11 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 24:1n-9 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 24:5n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| 24:6n-3 | Float | 99.99 % | Fatty acid as percentage of total fatty acids |
| **Lipid class analyses** | | | |
| Total Neutral Lipid | Float | 99.99 % | Percentage of total lipid as neutral lipid |
| Total Polar Lipid | Float | 99.99 % | Percentage of total lipid as polar lipid |
| TG | Float | 99.99 % | Percentage of total lipid as triglyceride |
| Fatty acids % | Float | 99.99 % | Percentage of total lipid as free fatty acid |
| Total Sterols | Float | 99.99 % | Percentage of total lipid as sterols |
| Free sterols | Float | 99.99 % | Percentage of total lipid as free sterols |
| Sterol esters | Float | 99.99 % | Percentage of total lipid as sterol esters |
| Cholesterol | Float | 99.99 % | Percentage of total lipid as total cholesterol |
| Wax esters | Float | 99.99 % | Percentage of total lipid as wax ester |
| PC | Float | 99.99 % | Percentage of total lipid as phosphatidylcholine |
| PE | Float | 99.99 % | Percentage of total lipid as phosphatidylethanolamine |
| PS | Float | 99.99 % | Percentage of total lipid as phosphatidylserine |
| PI | Float | 99.99 % | Percentage of total lipid as phosphatidylinositol |
| Cardiolipin | Float | 99.99 % | Percentage of total lipid as cardiolipin |
| sphingomyelin | Float | 99.99 % | Percentage of total lipid as sphingomyelin |
| Total carotenoid | Float | 99.99 | mg/g of total lipid as carotenoid |
| Astaxanthin | Float | 99.99 | mg/g of total lipid as astaxanthin |
| **Amino Acid Analyses** | | | |
| Arginine | | | |
| Cystine | | | |
| Histidine | | | |
| Isoleucine | | | |
| Leucine | | | |
| Methionine | | | |
| Threonine | | | |
| Tryptophane | | | |
| Valine | | | |
| Alanine | | | |
| Aspartic Acid | | | |
| Glutamic Acid | | | |
| Glycine | | | |
| Phenylalanine | | | |
| Proline | | | |
| Serine | | | |
| Tyrosine | | | |
| Taurine | | | |
| Betaine | | | |
| **Macro Elements** | | | |
| Sodium | | | |
| Potassium | | | |
| Calcium | | | |
| Phosphorus | | | |
| Magnesium | | | |
| Chlorine | | | |
| **Trace Elements** | | | |
| Iron | | | |
| Copper | | | |
| Cobalt | | | |
| Iodine | | | |
| Manganese | | | |
| Zinc | | | |

| | | | |
|---|---|---|---|
| Selenium | | | |
| Molybdenum | | | |
| Fluorine | | | |
| Silicone | | | |
| Tin | | | |
| Vanadium | | | |
| Nickel | | | |
| Silver | | | |
| **Fat-soluble Vitamins** | | | |
| Vitamin A | Floating | 99.99 | mg/kg dry matter or (International units per kg diet) |
| Vitamin E | Floating | 99.99 | mg/kg diet of Alpha Tocopherol (or International Units per kg dry matter) |
| Vitamin D2 | Floating | 99.99 | mg/kg or International Units per kg dry matter |
| Vitamin D3 | Floating | 99.99 | mg/kg or International Units per kg dry matter |
| Vitamin K | Floating | 99.99 | mg/kg or International Units per kg dry matter |
| **Water-soluble Vitamins** | | | |
| Vitamin C (Ascorbic acid) | Floating | 99.99 | Ascorbic acid equivalents mg/kg dry matter |
| Thiamine | Floating | 99.99 | Thiamine mg/kg per kg dry matter |
| Riboflavin | Floating | 99.99 | Riboflavin mg/kg per kg dry matter |
| Nicotinic Acid or Niacin | Floating | 99.99 | Niacin mg/kg per kg dry matter |
| Pyridoxine | Floating | 99.99 | Pyridoxine mg/kg per kg dry matter |
| Pantothenic Acid | Floating | 99.99 | Pantothenic acid mg/kg per kg dry matter |
| Biotin | Floating | 99.99 | Biotin mg/kg per kg dry matter |
| Folic Acid | Floating | 99.99 | Folacin or folic acid mg/kg per kg dry matter |
| Vitamin B12 (Cyanocobalamin) | Floating | 99.99 | Cobalamin mg/kg per kg dry matter |
| Choline | Floating | 99.99 | Choline mg/kg per kg dry matter |
| Inositol | Floating | 99.99 | Inositol mg/kg per kg dry matter |

## APPENDIX 1: 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 Marine Fish Culture (WGMAFC) (before 1996, the Working Group on Mass Rearing of Juvenile Fish) during its sixth meeting in Conwy, UK from 22–24 June 1995 (ICES, 1995).

In this context, the Laboratory of Aquaculture & *Artemia* Reference Center makes available a batch of a reference diet (Annex 1) 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 (Annex 2). 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.

**REFERENCES**


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## ANNEX 1: CHARACTERISTICS OF BATCH ICES895

ICES895 is the most recent batch of extruded nucleus of the ICES reference diet (stored under $N_2$ 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 (5 January 1995; kg) |
|---|---|
| 200–300 | 20 |
| 300–500 | 65 |
| 500–800 | 70 |
| 800–1200 | 10 |
| >1200 | 40 |

## ANNEX 2: 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 (i.e., 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 (Annex 3); (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)

**ANNEX 3: FORMULATION OF THE STANDARD DIET (BATCH ICES895)**

| COMPOSITION | % of diet |
|---|---|
| **extruded basal diet** | 92.465 |
| codfish powder[1] | 24 |
| egg white albumin[2] | 11 |
| whey protein concentrate[3] | 11 |
| isolated soy protein[4] | 11 |
| hemoglobin powder[5] | 4 |
| wheat gluten[6] | 3 |
| a-cellulose[7] | 2.255 |
| native corn starch[8] | 13 |
| hydrogenated coconut oil[9] | 4 |
| emulgator blend[10] | 0.4 |
| vitamin premix[11] | 2 |
| vitaminC[12] | 0.4 |
| choline chloride[13] | 1 |
| mineral premix[14] | 2 |
| attractant premix[15] | 3 |
| asthaxanthine[16] | 0.1 |
| calcium propionate[17] | 0.3 |
| BHT[18] | 0.005 |
| BHA[19] | 0.005 |
| **additional coated fraction** | 7.533 |
| de-oiled soya lecithin[19] | 2 |
| oil mixture[20] | 5 |
| emulgator blend [10] | 0.5 |
| ethoxyquin[21] | 0.015 |
| vitamin E[22] | 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;

## APPENDIX 2: 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 set (Table 1) consists of emulsions differing in the concentration of (n-3) HUFA (0, 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 set (Table 2), 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 and 4. The emulsions are of the Selco type and contain lipid (62 % on wet weight basis), vitamins (Table 3), 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 (around 5000BEF/kg, shipment not included). 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.

## REFERENCES


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## TABLE 1: APPROXIMATE FATTY ACID PROFILE OF ICES EMULSIONS

| Set 1 | sum (n-3) HUFA (% of total fatty acids) | | DHA/EPA ratio | | major lipid class* |
|---|---|---|---|---|---|
| ICES 0/-/C | 0 | variable | - | constant | TG |
| ICES 30/0.6/C | 30 | | 0.6 | | TG |
| ICES 50/0.6/C | 60 | | 0.6 | | EE |
| Set 2 | sum(n-3) HUFA (mg/g DW) | | DHA/EPA ratio | | major lipid class* |
| ICES 30/0.6/C | 300 | constant | 0.6 | variable | TG |
| ICES 30/4/C | 300 | | 4 | | TG |

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

## TABLE 2: APPROXIMATE FATTY ACID PROFILE OF ICES EMULSIONS

| Set 1 | sum (n-3) HUFA (% of total fatty acids) | | DHA/EPA ratio | | major lipid class* |
|---|---|---|---|---|---|
| ICES 0/-/C | 0 | variable | - | constant | TG |
| ICES 30/0.6/C | 30 | | 0.6 | | TG |
| ICES 50/0.6/C | 60 | | 0.6 | | EE |
| Set 2 | sum(n-3) HUFA (mg/g DW) | | DHA/EPA ratio | | major lipid class* |
| ICES 30/0.6/C | 300 | constant | 0.6 | variable | TG |
| ICES 30/4/C | 300 | | 4 | | TG |

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

## TABLE 3: VITAMIN COMPOSITION OF ICES ENRICHMENT EMULSIONS

| added vitamins (background levels in used fish oils is low) | mg/kg Wet Weight |
|---|---|
| vitamin C | 800 |
| vitamin E | 3600 |
| vitamin A | 200000 |
| vitamin D3 | 20000 |