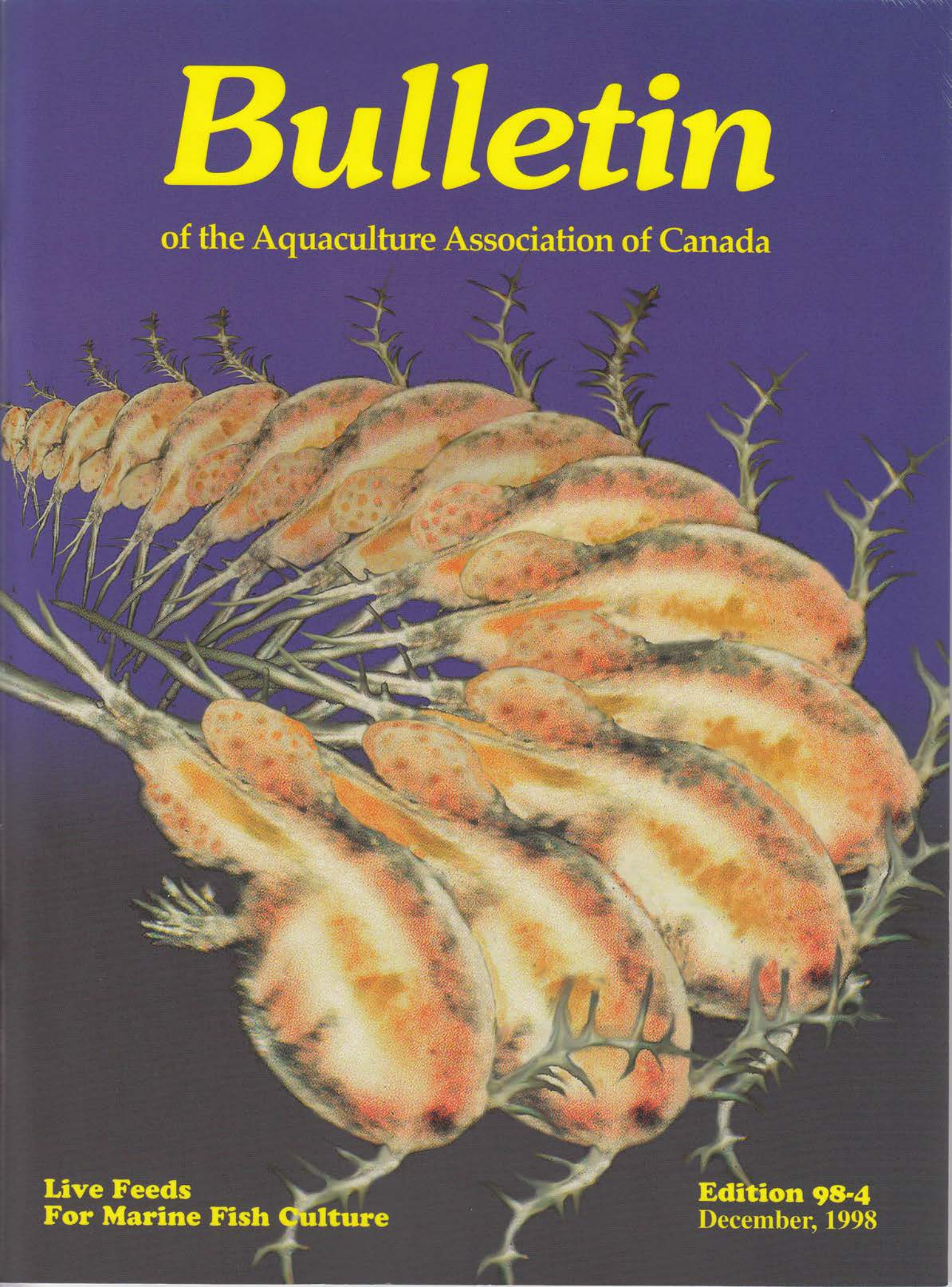
Bulletin

of the Aquaculture Association of Canada



Live Feeds
For Marine Fish Culture

Edition 98-4
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Aquaculture Association of Canada

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Cover: A copepod isolated from Passamoquoddy Bay and maintained in culture by Dr. John Castell at the Biological Station, Department of Fisheries and Oceans, St. Andrews, is unique among the cultured copepod species. It has the highest concentration (35% of total fatty acids) of docosahexaenoic acid (DHA, 22:6n-3) of any cultured copepod for which compositional information is available. Even when fed a diet that contains no DHA it still synthesizes this fatty acid and maintains the concentration near 35%. Densities as high as 500 000 individuals can be maintained in a culture volume of 200 L. The copepods range in size from about 30 microns in length for the newly-hatched nauplii to over 400 microns for the adult copepods. The species has been tentatively identified as *Hemicyclops arenicolae* Gooding, 1960, by Dr. Ju-She Ho of California State University, Long Beach, California (JDC thanks Gerhard Pohle at the Atlantic Reference Centre, Huntsman Marine Science Centre, St. Andrews, for assisting in the identification.) *Photography and photocomposition by D.E. Aiken.*

Contents

Proceedings of the Live Feeds Session, Aquaculture Canada '98
Chris Hendry, guest associate editor

Introduction

There is currently a great deal of interest in the culture of commercially valuable marine fish species such as Atlantic halibut *(Hippoglossus hippoglossus)*, haddock *(Melanogrammus aeglefinus)*, winter flounder *(Pleuronectes americanus)*, yellowtail flounder *(Pleuronectes feregineus)*, Atlantic cod *(Gadus morhua)*, turbot *(Scophthalmus maximus)* and various species of sea bream. To date, the successful culture of the early larval stages of these fish has required the use of live feed organisms. One of the principal hurdles in the culture of marine finfish species is the difficulty in providing suitable live food for the early larval stages and problems with weaning fish onto particulate feeds. The objective of the Aquaculture Canada '98 Special Session on Live Feeds was to bring knowledge about the latest technologies and live feed species to researchers and those involved in the marine fish culture industry in Canada. Financial support for the invited speakers to travel to the conference was provided by The Canadian Center for Fisheries Innovation and the Atlantic Canada Opportunity Agency in St. John's, Newfoundland.

The Session focused on a number of different live food organisms and feeding strategies being applied to the culture of marine fish larvae. Some marine fish hatcheries rely wholly, or partly, on natural plankton, but collection of plankton can be time consuming, costly and unreliable. Also, larval fish are vulnerable to phytotoxins and parasites that are sometimes collected with the wild zooplankton. There has been a recent increase in the the use of rotifers *(Brachionus plicatilis)* and/or brine shrimp *(Artemia* sp.) which, although easier to supply, require enrichment because they are nutritionally inadequate for larvae of cooler-water marine fish. Single cell or micro-algae are also vital to most marine fish hatcheries, both as food for other organisms (rotifers, *Artemia*, etc.) and as a direct supplement ("green water") to the larval culture tanks. There were reports of commercially available, as well as experimental, enrichment media used to enhance the nutritional value of rotifers and *Artemia.* This is a topic of considerable interest in live feed research.

Invited speakers in the Live Feed Special Session held at Aquaculture Canada '98 in St. John's (l to r): Ms Tania De Wolf, Dr. Moti Harel, Dr. Josianne Støttrup, Dr. John Castell, Dr. Atsushi Higawara, Dr. Lesley McEvoy, and Dr. Phillippe Dhert.

Among other alternative live feed organisms discussed at this Special Session were cultured copepods and molluscan trocophores. Another topic covered in the Live Feed Session was the need to control the unusually high bacterial counts that are often associated with the live feeds used in larvae culture. Ms. Tania DeWolf from Belgium introduced information on a new product from INVE that disinfects *Artemia* nauplii at the same time that it enriches it with essential fatty acids. It is certain that both the disinfection techniques and the use of probiotics (adding good bacteria to crowd out the harmful bacteria) introduced at this meeting will be the subject of considerable attention in the near future.

The National Research Council, Industry Research Assistance Program, provided funding for a Live Feed Workshop Demonstration on June 4 following the Aquaculture Canada conference. The five invited speakers, Dr. Lesley McEvoy, NERC, University of Stirling, Scotland, Dr. Josianne Støttrup, Danish Institute for Fisheries Research, Dr. Moti Harel, University of Maryland, Dr. Philippe Dhert, University of Ghent, and Dr. Atsushi Higawara, Nagasaki University, as well as Ms. Tania De Wolf, INVE, and Mr. Phil Boeing, Aquafauna Bio-Marine, Inc., participated in this workshop and demonstrations held at the Ocean Sciences Centre, Memorial University of Newfoundland. In adidtion, there were over 40 participants from industry, university and government research programs. Attendees indicated that this format was most useful for technology transfer and was their favourite part of the 1998 AAC meeting. The format was informal and after approximately 20 minutes of introductory comments from each speaker, the discussion was largely driven by questions from the participants. INVE provided 8 kilograms of *Artemia* cysts that were used in demonstrating proper decapsulation procedures and newly hatched nauplii were used to demonstrate enrichment techniques. Phil Boeing of Aquafauna Bio-Marine, Inc. provided free samples and introduced a number of new live feed enrichment products including Algamac-3010, a spray-dried heterotrophic algal product that has very high DHA content and a high DHA/EPA ratio. Aquafauna Bio-Marine also sponsored the delicious lunch that was supplied to all workshop participants.

This live feed workshop was enthusiastically received by the participants who felt that it was a more useful and informative format than the usual conference approach that has short presentations, limited time for questions, and no time for discussion. There was unanimous agreement that another workshop should be included in the program of Aquaculture Canada '99 being held in October, 1999, in Victoria, BC.

— John Castell and Joe Brown
organizers of the Live feeds Special Session
and Post-Conference Workshop

AQUACULTURE CANADA '99

16th Annual Meeting of the Aquaculture Association of Canada
October 26-29, 1999 Victoria Conference Centre, Victoria, BC

Special Session for Aquaculture Suppliers and Vendors

The ***Suppliers and Vendors Technical Session*** will be a forum for suppliers to exchange technical ideas and display innovative technology and services. Presentations will be 15 minutes in length. To participate in this session, submit a short summary paper to the address below, outlining the product or service, the key points of the presentation, and the name of the presenter. The summary will be published in the meeting program. Presentations in the ***Suppliers and Vendors Technical Session*** must be non-promotional; therefore, orders and sales discussions are not permitted. For information on this session, please contact: Virginia Eccleston, #206-3185 Barons Road, Nanaimo, British Columbia, Canada V9T 5T3 (tel 250-756-3845, fax 250 755-8749, e-mail: veccleston@hotmail.com).

The Nutritional Quality of Live Feeds for Larval Fish

Moti Harel and Allen R. Place

Live feed organisms do not meet the nutritional requirements of larval fish for both essential amino acids (EAA) and essential fatty acids (EFA). Rotifers and *Artemia* nauplii, which are widely used as live feed organisms in hatcheries, supply only half of the methionine and tryptophan requirements for most fish larvae. Furthermore, these live feeds are lacking in docosahexaenoic acid (DHA, 22:6n-3), an essential fatty acid required for normal embryonic development of neural and visual tissues. Moreover, recent studies have suggested that several fish species also require arachidonic acid (AA, 20:4n-6), and that diets should be carefully formulated with a species-specific dietary DHA to EPA to AA ratio. To overcome these live feed deficiencies, commercial hatcheries are applying various enrichment techniques to help increase survival, improve growth, enhance metamorphosis and swim bladder inflation, reduce skeletal deformities, and enhance pigmentation and stress resistance in many fish larvae. However, further nutritional improvements are essential for the successful culture of additional marine and cold-water fish species. This paper addresses nutritional factors that affect larval performance and the application of live feeds enrichment technology, including the use of algal sources of DHA- and AA-rich phospholipid extracts.

Introduction

One of the major drawbacks to the development and intensification of sustained marine aquaculture is the fact that most of the commercially important marine fish species require live organisms at first feeding. These first feeds tend to be either various species of microscopic algae, rotifers (*Brachionus plicatilis*), or brine shrimp (*Artemia salina*) nauplii. Nevertheless, the nutritional quality of these organisms in terms of protein and lipid composition is insufficient for optimal larval growth and survival of many commercially important fish species. On a commercial production scale, reduced growth and survival translates into additional tank space, extended larval rearing periods, and associated operational costs.

Fish larvae hatch with an endogenous store of nutrients which, in several species such as salmonids and halibut, can last for 3 or 8 weeks, respectively. The larvae of these species are well-developed upon hatching and are large enough to accept large prey and even formulated feeds. However, most of the warm-water marine fish larvae hatch with very limited yolk reserves that last only a couple of days. In this case, the nutritional content of the yolk-sac is crucial for the successful development of high quality embryos and larvae.

Proteins are the main yolk constituent of newly hatched fish larvae and are used for energy needs during the period of endogenous feeding and completion of development of body tissues.[1-3] Of total yolk protein content, the free amino acids (FAA) pool constitutes 20 to 50%. Approximately 20 to 30% of this pool is synthesized into new body proteins and the remainder is used for energy needs for activity and homeostasis.[4-6] The main energy expenditures for maintenance are protein turnover and ion transport. Protein turnover, which is the dynamic steady state between protein synthesis and degradation, accounts for about 40% of the total energy cost for maintenance.[1,7]

Amino Acid Requirements for Optimal Growth

The growth rate for fish larvae is the highest among all vertebrates.[8] Both freshwater and marine species larvae can exhibit mass gains as high as 50% body weight per day. Growth is an energy demanding process, estimated at around 87% of the total energy expenditure in juvenile fish.[9] How fish larvae manage to accommodate the cost for both high growth and maintenance at the limited capacity for oxygen uptake is unclear. It has been suggested that fast growth rate in fish is attained through high rates of protein resynthesis at minimal energy costs.[8,10] In fish larvae, the rate of protein synthesis is greater than 100% of whole body protein/day, compared with only 1 to 3% in adult fish.[11-15] In addition, energy costs of protein

synthesis are inversely correlated with growth rate, and decrease to a minimum biochemical cost of about 50 mmol ATP/g protein at above 16% relative daily growth rate.[11,16-18] This means that the energy cost of protein deposition for tissue growth decreases at higher growth rates. The explanation for the high synthesis efficiency at faster growth rate is not known. However, it has been proposed that the production and maintenance of several protein translation factors, such as a constant activation of tRNAs and synthesis of rRNA, decreases significantly at higher growth rate and hence permits additional energy for synthesis and deposition of tissue proteins.[7,19]

The free amino acid (FAA) pools are relatively small in fish larvae and account for only a few percent of the whole body amino acids.[4,5] Amino acids which are not incorporated into proteins are used either for energy production, transaminated to other amino acids, used for lipid and/or carbohydrate production via gluconeogenesis and lipogenesis, or used in the synthesis of other nitrogen-containing molecules. The dynamic relationship between the FAA pools and protein pools is maintained by a turnover of proteins through constant degradation and re-synthesis. In mammals, alanine, glutamine, and aspartate are preferentially used for energy production, while other nonessential amino acids (non-EAA) and all 10 EAA are spared for protein synthesis.[20] In fish embryos and larvae, however, depletion rates of both non-EAA and EAA are comparable,[4] indicating no sparing selectivity between the metabolism of EAA and non-EAA. Therefore, larval fish dietary proteins need to contain higher proportions of EAA in order to compensate for their loss during metabolism.

Another important aspect in determination of the quality of dietary proteins is the EAA profile in relation to the profile in fish larvae. Dietary imbalances even in one of the EAA can lead to a significant decrease in food conversion efficiency and to an increase in oxidation of EAA.[21] In fact, the two main live feed organisms (rotifers and newly hatched *Artemia* nauplii) do not meet the EAA requirements of fish larvae (Table 1). They contain about half the methionine and tryptophan proportion of fish larvae. Moreover, *Artemia* nauplii are also lacking in threonine. Such EAA imbalances can lead to slower growth if larvae are fed solely rotifers or *Artemia*. *Artemia* nauplii also contain low levels of taurine compared with levels found in wild zooplankton and fish larvae.[7] Taurine is produced from methionine via cysteine. Its role in larval nutrition is not yet clear, but it has been shown that taurine is associated with bile salt production in teleost fish.[22] In addition, taurine is an important osmolyte involved in cell volume regulation of fish.[23,24] Therefore, taurine-deficient larvae may be more susceptible to environmental changes.

Fatty Acid Requirements for Optimal Structural and Metabolic Functions

Synthesis of structural components of cell membranes is another major process associated with larval growth and development. Long-chain polyunsaturated fatty acids (PUFA) are used in cellular membrane phospholipids. The properties of cell membranes are largely those of their component fatty acids, especially the essential fatty acids (EFA), namely linoleic acid (LA, 18:2n-6) and linolenic acid (LNA, 18:3n-3) families, which cannot be synthesized by animal cells and hence must be provided in the diet.

Table 1. Comparison of essential amino acid profile of some live food organisms and marine fish larvae (% of total essential amino acids). The results for live feed organisms are from Watanabe and Kiron.[59]

Amino acid	Live feed organisms			Marine fish larvae	
	Rotifers	*Artemia* nauplii	*Tigriopus japonicus*	Sea bream	Striped bass
Isoleucine	8.74	8.35	7.55	8.58	12.66
Leucine	16.63	19.56	15.17	14.85	19.53
Methionine	2.56	2.86	3.45	4.3	5.06
Phenylalanine	10.66	10.33	10.57	7.42	6.12
Threonine	9.81	5.49	11.49	9.57	9.30
Tryptophan	3.84	3.3	3.45	–	6.15
Valine	11.09	10.33	10.11	12.56	13.87
Lysine	18.2	19.56	17.24	15.84	13.20
Arginine	14.07	16.04	15.86	12.38	8.86
Histidine	4.48	4.17	4.83	5.61	5.60

All the members of these two independent families derive from their respective precursors, LA and LNA.

The conversion of LA and LNA precursors to longer-chain PUFA requires an alternating sequence of chain elongation and desaturation.[25] Elongation takes place in the endoplasmic reticulum and is not considered as rate-limiting step, while several desaturations take place in the cell microsomes and are considered rate limiting. The first desaturation step in the biosynthesis of PUFAs is by a Δ6-desaturase. The same enzyme is involved in the desaturation of LA and LNA, and is a rate-limiting step in the synthesis of arachidonic acid (AA, 20:4n-6) and eicosapentaenoic acid (EPA, 20:5n-3). Since both LA and LNA are substrates of the same desaturase, competition can occur. A second desaturase, the Δ5-desaturase, completes the formation of AA and EPA. This enzyme represents a second regulatory step in PUFA biosynthesis.[27] Additional elongation and desaturation steps are necessary to form docosahexaenoic acid (DHA, 22:6n-3). Recently, it has been suggested that conversion of EPA to DHA occurs through two successive elongations followed by Δ6-desaturation of 24:5n-3 to 24:6n-3 in cell microsomes and completed by peroxysomal retroconversion to 22:6n-3.[28]

EFAs are major components of membrane phospholipids; they play a key role in the integrity and fluidity of intracellular and plasma membranes as well as being key second messengers. In this regard, they modulate activity of membrane-bound receptors, enzymes, molecular carriers, and ionic channels.[28] In addition, several fatty acids, namely dihomo-γ-linolenic (DGLA, 20:3n-6), AA, and EPA can be easily converted into a wide range of eicosanoids including prostaglandins, thromboxanes, prostacyclines, and leukotrienes. AA catabolism in mammals leads to the production of the 2-series prostaglandins (PG_2), while in fish, AA an 20:3n-6, and particularly EPA, are involved in eicosanoids production yielding 1- and 3-series prostaglandins (PG_1 and PG_3), respectively. Eicosanoids, including prostaglandins, have many physiological roles in fish. They stimulate ionic transport in gills and kidneys,[29,30] and testosterone production in testes.[31] Prostaglandins also modulate neural transmission and hypothalamic function as well as regulating cerebral blood circulation.[28] Bearing in mind the significant role of AA as an eicosanoid precursor, it seems very likely that dietary deficiency of AA at early developmental stages may affect normal function of the brain and neural system.

In the absence of dietary n-6 and n-3 fatty acids, larval growth, learning, and visual acuity are impaired and severe structural and metabolic disorders can take place. Dietary supplementation of n-3 highly unsaturated fatty acids (HUFA) is often associated with improved performance in terms of food utilization efficiency, survival, growth, and swim-bladder inflation. To date, the quantitative dietary EFA requirements of fish larvae have been established for only a few species. The minimum dietary requirements (% dry weight) for n-3 HUFA are 2% for larval red sea bream (*Pagrus major*), 1.3% for larval turbot (*Scophthalmus*

Table 2. DHA and EPA content of rotifers and Artemia nauplii after enrichment[1] with different sources of lipids.

	Initial content	Algae *Isochrysis galbana*	DHA-Selco[2]	DHA-PL[3] extract
Rotifers				
Lipid (% dry weight)	9.0	7.5	13.9	15.4
DHA (% of total FA)	1.6	9.0	12.2	23.7
DHA:EPA	0.3	2.3	1.4	6.1
***Artemia* nauplii**				
Lipid (% dry weight)	16.3	17.7	20.6	25.3
DHA (% of total FA)	0	7.3	7.5	17.2
DHA:EPA	0	1.4	1.4	2.7

[1]Enrichments were carried out in artificial seawater at 28°C (25 ppt) in 1-L conical containers provided with vigorous aeration and constant illumination by fluorescent light. Rotifers or *Artemia* nauplii (instar II) were stocked in each enrichment container at a density of 500 000 or 200 000 individuals/L respectively. Rotifers were enriched for 8 h with two portions of 0.1 g/L of each enrichment material given at times 0 and 4 h, and *Artemia* nauplii were enriched for 16 h with two portions of 0.3 g/L of each enrichment material given at times 0 and 8 h.

[2]DHA-Selco — Commercial enrichment emulsion (INVE Aquaculture NV, Baasrode, Belgium)

[3]DHA-PL — Phospholipids extract of *Crypthecodinium* sp. algal biomass.

maximus),[32] 1.7% for juvenile striped jack (*Longirostris delicatissimus*),[33] and 1% for white perch fingerlings (*Coregonus lavaretus*).[34] In the n-6 family, LA is the major EFA; 1 to 2% of diet is required to relieve symptoms of n-6 EFA deficiency,[35] but to ensure the biological functions of EFA, intake of up to 6% is generally recommended. Intake of LA probably decreases the need for AA, but both qualitative and quantitative AA requirements of fish larvae have received little attention.

Most of the studies related to the role of EFA in fish have focused on the n-3 series while the n-6 series has received little attention, despite the fact that a dietary source of n-6 fatty acids was found to be essential in marine fish.[45] Studies have reported that dietary inclusion of AA improved larval growth and pigmentation.[45-47] AA is incorporated preferentially into the phosphatidylinositol (PI) fraction of both mammalian and fish retina and brain.[48-51] Another important role of n-6 fatty acids is their involvement in intravascular and cellular metabolism of cholesterol in plasma and in adrenals.[25]

DHA is a key fatty acid component in membrane phospholipids of the cerebral cortex, heart, and photoreceptor membranes of the rod outer segment of the retina.[36] Therefore, the need for DHA has been estimated to be higher during the developmental period of neural and visual tissues at early stages of life. In fact, n-3 HUFA accounts for most of the egg fatty acids. In striped bass (*Morone saxatilis*) they account for 40% of egg total fatty acids,[37] 45% in Atlantic cod (*Gadus morhua*),[38] and approximately 30% in sole (*Solea solea*),[39] turbot (*Scophthalmus maximus*)[40] and Gilthead sea bream (*Sparus aurata*).[41] DHA accumulates at a faster rate in lipids of fish eggs than other EFA. For example, DHA accumulates over 70% faster in sea bream egg lipids over 70% faster than EPA or LA in response to a dietary increase.[42] Elevated egg n-3 HUFA content is also associated with improved egg viability, larval growth rate, and swim-bladder inflation rate.[41] It is interesting to note, however, that DHA content decreases during embryonic development. In cod, DHA content falls sharply to 7% of the total FA, in sea bream to 3 to 4%, and in striped bass to levels less than 1%. Although most of the DHA in egg lipids is used for energy production, its high initial content is probably essential mostly during the rapid proliferation of membranes and neural tissues in the developing embryo and larvae.[43] In this regard, keeping the dietary DHA:EPA ratio above 2 is important for maintaining optimal membrane function while unbalanced DHA:EPA ratios can lead to poor larval growth and survival.[44] This ratio in commonly used larval live feeds is still below 2, even after enrichment with currently available commercial products.

Freshwater and marine animals differ significantly from land animals with respect to fatty acid content and requirements. The n-6 series are the predominant

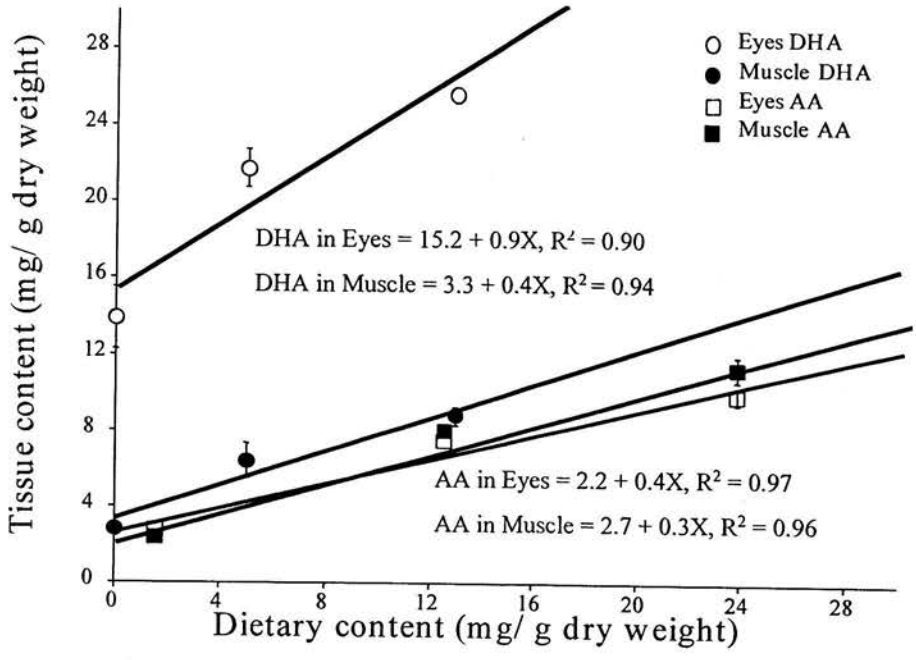

Figure 1. Accumulation of DHA and ARA of eyes and muscle tissues in hybrid striped bass larvae in response to dietary increase (n=6).

fatty acids in terrestrial animals, whereas n-3 series are the major fatty acids in freshwater and marine animals.[52] A number of warm and freshwater fish, such as common carp (*Cyprinus carpio*) and eels (*Anguilla japonica*) require a mixture of both n-6 and n-3 fatty acids. Other fish species, such as *Tilapia zillii* prefer n-6 series over n-3 series as EFA. On the other hand, rainbow trout (*Oncorhynchus mykiss*), have higher preferences for n-3 series,[53-55] probably because of the greater degree of unsaturation required to maintain membrane fluidity and permeability in a cold-water environment.

The requirements of n-3 HUFA are more critical for marine fish than for freshwater fish, because marine fish have limited ability to chain elongate and desaturate dietary LNA.[56,57] Assuming the conversion efficiency in rainbow trout is 100%, the relative conversion efficiencies in the marine fish ayu (*Plecoglossus altivelis*), red sea bream (*Pagrus major*), globefish (*Fugu rubripes rubripes*) and rockfish (*Sebasticus marmoratus*) are 38%, 15%, 13%, and 7%, respectively.[58] Furthermore, the capacity of each specific tissue to synthesize n-6 and n-3 long-chain PUFA from their precursors depends on the presence of active desaturases. If the desaturation activity in the liver is taken as 100, activity in adrenals, testes, heart, kidneys and brain are 142, 45, 8, 12, and 14, respectively.[25] Thus, important organs such as brain, heart, and kidneys greatly depend on the liver for the synthesis and supply of long-chain PUFA. For example, muscle tissue lipids of hybrid striped bass accumulate both DHA and ARA at equal levels and rates in response to dietary increases (Fig. 1) indicating no specific role or preference for either DHA or ARA with regard to function in muscle tissue. In eyes, however, DHA accumulation was 4 times greater and twice as fast than observed with muscle tissue, which suggests a specific physiological role of DHA in the function of this light-sensitive organ.

Hybrid striped bass larvae (striped bass x white bass, *M. chrysops*) are unable to convert LNA to n-3 HUFA sufficiently for successful growth and survival, thus dietary supplements of n-3 HUFA are necessary through feeds of enriched *Artemia* (Fig. 2). The best larval performance was achieved when fed a combination of 25% DHA- and 25% ARA- (of total fatty acids) enriched *Artemia*. We do not suspect that all species of fish larvae require such high amounts of ARA in their diet. The exact DHA:EPA:ARA ratio is probably species-specific, but more attention needs to be paid to addressing ARA requirements of larval fish.

In conclusion, the most widely used larval live feeds — rotifers and *Artemia* nauplii — do not meet the EFA requirements of larval fish. They are lacking in DHA, and their DHA:EPA ratio is low compared to that in fish larvae and normal prey. Therefore, the n-3 HUFA content of live feed must be supplemented to meet larval requirements. For this purpose, several enrichment products and protocols have been developed. Examples of enrichment results of rotifers and *Artemia* with two different enrichment materials (commercial enrichment emulsion and DHA-rich phospholipids extract of *Crypthecodinium* sp. algae, Martek BioSci. Inc., Columbia, MD.) are given in Table 2. Significantly higher DHA enrichment of both rotifers and *Artemia* was obtained with DHA-rich phospholipids extract of *Crypthecodinium* sp. algal biomass. Moreover, feeding trials of halibut larvae with *Artemia* enriched with DHA-rich phospholipids extract resulted in 75% eye migration and 100% pigmentation at metamorphosis, as compared with only 15% eye migration and 50% pigmentation when fed on commercial marine oil emulsion-enriched *Artemia*. These encouraging results with the improved live feeds enable hatcheries to successfully culture more challenging marine and cold-water species.

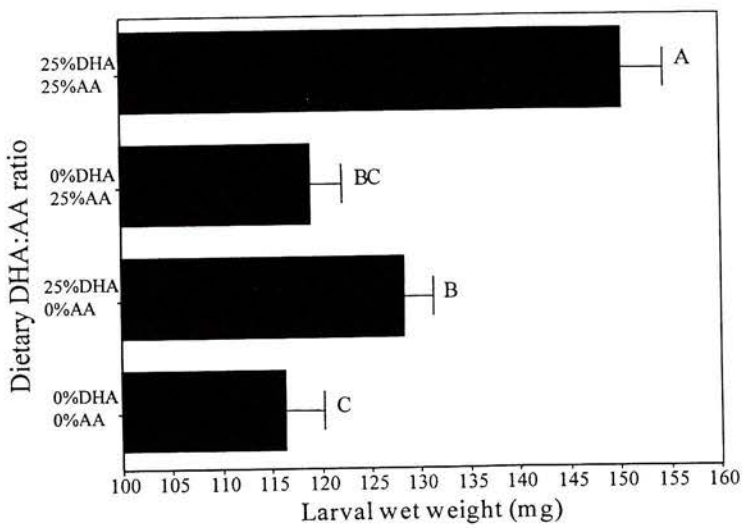

Figure 2. Mean larval wet weights of hybrid striped bass fed equivalent live feed rations (*Artemia*) enriched with different lipid emulsions for 21 days. Survivorship was identical in all treatments (n=6). Treatments sharing similar letters do not significantly differ ($P>0.05$).

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Problems and Techniques in Live Prey Enrichment

Lesley Ann McEvoy and John R. Sargent

Marine fish are generally unable to synthesise sufficient amounts of essential polyunsaturated fatty acids (EFAs) from dietary precursors and require them to be supplied, preformed, in their diet. Since the most convenient and commonly used live prey organisms, *Artemia* nauplii and rotifers, are deficient in these EFAs, it is necessary to "enrich" polyunsaturated fatty acid (PUFA) levels, particularly docosahexaenoic acid, prior to feeding the prey to fish larvae. Traditionally, this has been carried out by allowing the prey to filter-feed on microalgae, modified yeast, or micellar emulsions of PUFA-rich marine oils, but recently the use of preparations such as liposomes, spray-dried algae/protists, microcapsules, and silages has been reported. Because copepods, the natural prey of marine larvae, tend to be rich in polar lipids and there is evidence these lipids are easily digested and assimilated, attempts are being made to increase the polar lipid content of live prey or to increase the content of PUFAs in polar lipid form. Both PUFA and polar lipid enrichment is made more difficult because of problems inherent in the enrichment process: a) autoxidation and instability of enrichment diets, b) metabolic lipid class conversions by live prey, c) retroconversion of bioencapsulated docosahexaenoic acid (DHA, 22:6n-3) to eicosapentaenoic acid (EPA, 20:5n-3), and d) post-enrichment loss of PUFAs from *Artemia* nauplii prior to their ingestion by larval fish. The relative merits of the different techniques of live prey enrichment will be discussed, together with protocol modifications such as duration and frequency of enrichment, which may help to ameliorate some of the problems listed above.

Introduction

The phrase "live prey enrichment" in this paper will refer to the raising of levels of essential polyunsaturated fatty acids in live prey destined for cultivated marine fish larvae. Although we will primarily consider problems and techniques for enriching *Artemia* nauplii, many of the points raised are also applicable to rotifer enrichment.

Essential Fatty Acid Requirements of Marine Fish Larvae

Figure 1 is a simplified diagram showing the basic metabolic pathways involved in converting 18-carbon-chain fatty acids into their 20- and 22-carbon-chain derivatives. Most freshwater fish and higher vertebrates are capable of following these pathways to completion but, generally speaking, marine fish cannot because of the absence or extremely reduced activity of the Δ5-desaturase enzyme.[1] In theory, if eicosapentaenoic acid (EPA, 20:5n-3) is present in the diet, the fish should be able to synthesize docosahexaenoic acid (DHA, 22:6n-3). In practice, this process occurs too slowly to supply the demand for DHA, which is particularly high during the larval and juvenile stages.[2,3] This means that for marine fish, both DHA and EPA must be provided, preformed, in the diet.

The essentiality of these two polyunsaturated fatty acids (PUFAs) has been established for a long time.[4-7] Less widely accepted, however, is that by the same argument of absent/inadequate Δ5-desaturase activity, arachidonic acid (ARA, 20:4n-6) must also be provided, preformed, in the diet. Compelling evidence for the dietary essentiality of ARA for marine fish has been provided.[8,9]

Several reviews have been published concerning the functions of essential fatty acids (EFAs) in marine larvae.[3,10,11] Briefly, DHA has important structural and functional roles in biological membranes, being especially abundant in fish. As in all vertebrates, DHA is further concentrated in neural tissues like the eye retina and the brain, and is therefore crucial during early development when there is a huge demand for DHA during the rapid growth and development of the eyes, brain and neural network. ARA is the main precursor of eicosanoids, whilst EPA modulates eicosanoid production by competing for the same enzyme systems that convert ARA to eicosanoids.

Since competitive metabolic interactions occur between the EFAs, it is important to consider their relative proportions to each other as well as their absolute

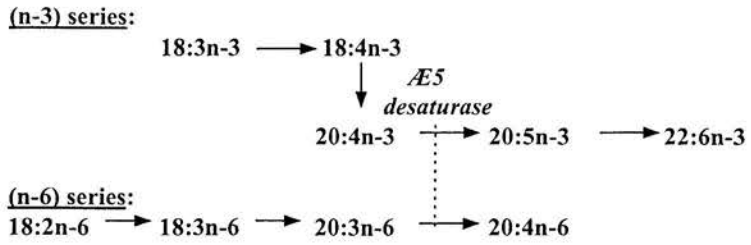

Figure 1. Conversion of C18 polyunsaturated fatty acids to their C20 and C22 derivatives (modified from Sargent[1]).

Table 1. EFA levels (weight% total fatty acids) in wild marine fish eggs, larvae, copepods and *Artemia* (data from Klunsøyr et al.,[12] Bell[13] and McEvoy et al. [14]).

Fatty acid	Cod Eggs	Cod Larvae	*Tisbe* (copepod)	*Artemia* nauplii
ARA	1.7	1.8	1.7	1.2
EPA	14.8	15.0	11.2	4.3
DHA	29.3	30.2	24.7	0.0
DHA:EPA	2.0	2.0	2.2	0.0
EPA:ARA	8.7	8.3	6.6	3.6

amounts when estimating how much of each EFA should be provided in larval fish diets.[3] For any particular species of fish, we should aim to match the EFA levels which occur in the wild eggs, larvae and natural prey of that species, since natural selection should have ensured that these are close to optimal. Taking cod as an example, Table 1 gives the EFA profiles of wild cod eggs and yolk-sac larvae.

The high levels of DHA, yielding a DHA:EPA ratio of approximately 2, is a common feature in many fish eggs and larvae. Despite its biological importance, ARA is not a particularly abundant fatty acid in nature and the 2% ARA present in wild cod eggs and larvae represents a generous provision. When the larvae initiate exogenous feeding, their natural food, copepods, contain a high level of DHA similar to that in wild eggs. Copepod DHA:EPA ratios and ARA levels are also comparable to those found in wild cod eggs. Although this is a simplification, because the FA profiles of copepods vary between species, developmental stage and diet,[15] the principle that "Nature knows best" is a good basis on which to estimate larval fish dietary requirements.

By contrast, when the EFA content of EG-grade *Artemia* nauplii is compared to that of copepods (Table 1), the former are clearly deficient in both the n-3 EFAs whilst their %ARA is 33% lower than the copepod species shown (*Tisbe*). So, although *Artemia* are convenient to use and it is possible to generate huge biomasses of them within 24 h, it is essential that their EFA content be raised substantially prior to their use as live prey for marine fish larvae.

Furthermore, there is now considerable interest in the quantities and quality of polar lipids in larval diets.[14,16,17] Copepods generally have a higher proportion of polar lipids than *Artemia*, the latter being rich in triglycerides (TAG).[18] So, not only are copepods a rich source of EFAs, the EFAs are generally present as polar lipids, which are believed to be advantageous in terms of their uptake and assimilation by fish larvae.[16,17,19,20] Hence, ideally, when enriching live prey we should aim not only to increase their EFA content but to do so in polar lipid form.

Table 2 is a brief review of some of the inanimate enrichment diets and presentation methods presently available. One of the most common enrichment practices at the moment is to allow live prey to feed on micellar emulsions of marine oils. These can be either TAG-rich natural fish oils or PUFA-concentrated products which can be rich in ethyl esters, free fatty acids or TAGs. The oils can be emulsified with detergents such as Tween 80 or 85, or with various lecithins which also act as natural antioxidants.[14] Obviously the degree of EFA enrichment will depend upon the EFA content in the oils and lecithins used. Most commercially available natural marine fish oils have relatively low DHA:EPA ratios, making it difficult to induce the desirable DHA:EPA ratio of 2:1 in *Artemia* enriched with these oils; a notable exception being tuna orbital oil (TOO), which has a DHA:EPA ratio of 4:1.[14, 38] When this oil is emulsified with 12% herring roe lecithin and used as an *Artemia* enrichment diet, a DHA:EPA ratio of 1.8 and a DHA content of 14% are generated in the subsequent nauplii.[14] Similarly, the efficiency of EFA enrichment will depend upon the nature and properties of the encapsulated oil when using microcapsules.

Liposomes have been successfully used to feed *Artemia* nauplii,[29,31] including n-3 PUFA liposomes from phosphatidylcholine (PC) extracted from herring roe.[14] However, liposomes made from pure herring roe PC proved very unstable, and it was necessary to add stabilizing cholesterol and dipalmitoyl PC (a synthetic, completely satu-

Table 2. Review of enrichment diets and techniques

Diet	Description
Micellar emulsions	Marine oils emulsified with detergents or lecithins[14,21-23]
Microcapsules	Oil diet contained in nylon-protein/all protein capsules[24-26] or gelatin-acacia capsules[27-30]
Liposomes	Microscopic phospholipid bilayer vesicles[14,29,31]
Silages	Crumbed silage of fish neural tissue[32]
Spray-dried algae/ protists	Dried *Schizochytrium* sp.[33-35] Dried polar lipid extract of *Crypthecodinium* sp.[36,37]

rated PC). Obviously, doing so diluted the n-3 PUFA content of the liposomes and considerably reduced their efficiency in raising n-3 HUFA levels in *Artemia* nauplii. However these liposomes successfully increased the nauplii's polar lipid content both in real and relative terms.[14]

The next technique listed in Table 2 is the use of a neural tissue silage[32] using brain and retinal tissue from fish head byproducts of commercial fisheries. The product is a particulate, crumbed diet which was used to enrich both *Artemia* and rotifers. The authors expected the silages to be rich in polar lipids as well as DHA. This was true of silages made from cod and whiting brains and eyes but, interestingly, their tuna eye silage consisted almost completely of TAG. This tuna eye silage was particularly successful in increasing DHA levels, especially in rotifers, whilst cod brain/eye silages seemed promising as diets for *Artemia* nauplii.

The use of spray-dried algae/protists seem to have great potential in terms of EFA enrichment: they are non-greasy and, as whole cell products, have the added advantage of delivering a more complete range of nutrients to the live prey, compared to oil emulsions.[33-35] A recent development is the availability of polar lipid-rich extracts with which authors have obtained 17% DHA in enriched *Artemia* and high DHA:EPA ratios.[36,37]

With all these diets, the basic enrichment theory is the same — we hope the live prey will feed upon and effectively bioencapsulate the EFA-rich diet, thus passing it to the larval fish which are too small to successfully feed on formulated diets at this stage. However, in practice, EFA enrichment is not so simple, and there are several problems that need to be considered.

The first of these problems is the autoxidation of PUFAs in the enrichment diets prior to bioencapsulation. In attempting to enrich our live prey with EFAs, we are by definition dealing with highly PUFAs and the presence of oxidation agents caused by enriching live prey in vigorously aerated, warm seawater. This means the PUFAs in the diets will be vulnerable and could generate potentially toxic oxidation products.

Workers at the University of Stirling tested TOO and cod liver oil enrichment emulsions (both emulsified with 3% Tween 80) which had feeding *Artemia* present at a concentration of 200 000 nauplii /L, against control enrichment media containing no *Artemia*, only illuminated, aerated seawater/oil emulsion at 27°C.[39] N-3 PUFA levels remained high in the control media, but dropped drastically towards the end of the 24-h enrichment period in the *Artemia* media. This reduction in emulsion PUFA content was particularly drastic in TOO emulsions which had the highest initial PUFA levels. Both of the experimental oil emulsions in this study were unprotected against peroxidation except for any natural antioxidants that were already present in the oils.

Emulsions can be protected against peroxidation with lecithins, and a dose-dependant antioxidant effect of soya PC has been demonstrated when this is added as an emulsifier to TOO.[39] Other natural antioxidants like α-tocopherol and ascorbic acid are often added to oils for protection against peroxidation, but they are usually added in the form of oil-soluble α-tocopherol acetate and ascorbyl palmitate, which are only really effective after digestion in the gut, not in the enrichment emulsions beforehand.[3] Other alternatives are ethoxyquin and butylated hydroxyanisole, which are both synthetic. Another simple solution is to shorten enrichment time to allow a safety margin before the risk of peroxidation increases significantly.[39]

There is little information available about peroxidation times in other types of enrichment diets. Intuitively, one would expect peroxidation problems to be reduced in diets in which the PUFAs have a barrier between themselves and the oxidation agents. For example, microcapsules with their protein coat and spray-dried algal cells which will have a cell wall albeit probably lysed by the drying process. The dried algal cells may well be further protected by their natural antioxidant systems, including pigments which should still be present within the cells.

A second inherent problem in the enrichment process is the metabolic conversion of lipids within enriched *Artemia* nauplii. Included in this are lipid class conversions and, perhaps most troublesome, the ret-

Table 3. Polar lipid and DHA in enrichment diets and subsequent *Artemia* nauplii.

	12% herring roe/ 88% TOO	Super Selco™	Liposomes (herring roe PC)
Polar lipid in diet (weight %)	12.9	3.5	77.0 (38.5 DPPC)
Polar lipid in enriched nauplii (mg PL/g DBW)	32.5[a]	34.5[a]	40.1[b]
DHA in diet (weight %)	24.2[a]	27.9[b]	15.2[c]
DHA in total lipid of enriched nauplii (weight %)	14.1[a]	6.3[b]	2.0[c]
Polar lipid-DHA in enriched nauplii (weight %)	2.5[a]	0.6[b]	1.5[c]

Different letters within rows indicates statistical differences between treatments ($P<0.05$)

roconversion of DHA to EPA. Several authors have pointed out that it is very difficult to increase the absolute amount of polar lipid in *Artemia* nauplii.[41-43] To illustrate this point, Table 3 shows data from a study in which *Artemia* nauplii were fed different enrichment diets with markedly different PL contents.[14]

The herring roe/TOO emulsion contained over 3.5 times more polar lipid than the Super Selco™ (INVE, Belgium), yet the amounts of polar lipid in the nauplii fed each were not significantly different. Although significant polar lipid enrichment occurred in nauplii fed the liposomes, this may have represented solidified indigestible DPPC in the guts of the nauplii, since 27°C is below the phase transition temperature of DPPC.[14] The addition of DPPC in order to stabilize the liposomes markedly reduced the DHA content of the liposomes to 15%. This is reflected in the DHA content in the total lipids extracted from enriched nauplii: only 2%. However, the polar lipid-DHA content is higher than might be expected from the total lipid-DHA figure. The polar lipid-DHA levels of 1.5% and 2.5% in nauplii fed, respectively, liposomes and 12% herring roe PL/88%TOO compared with the 0.6% polar lipid-DHA in Super Selco-fed nauplii, implies that the amount of polar lipid-DHA in the nauplii can be influenced by the level of polar lipid-DHA in the enrichment diet. Furthermore, we have preliminary data demonstrating that employment of 20% fish roe PL/80%TOO emulsions yield enriched nauplii containing 5% polar lipid-DHA, i.e, approximately twice as much as in nauplii fed the 12% lecithin emulsion. So, although it is difficult to alter the amount of polar lipid in the nauplii, it is possible to alter the proportion of DHA in polar lipid form.

Conclusive evidence for lipid class conversions as well as retroconversion of DHA to EPA in the guts of *Artemia* comes from a radiotracer study recently carried out at the University of Stirling in collaboration with Dr. Juan Carlos Navarro from the Instituto de Acuicultura in Castellon, Spain.[44] Several C^{14}-labelled fatty acids were used as substrates to investigate the metabolism of fatty acids and lipids in *Artemia* nauplii, including universally labelled DHA made in-house at Stirling by Drs. R.J. Henderson and M.V. Bell. Since all 22 carbon atoms in the DHA were radiolabelled, any chain-shortened products from its catabolism would also be labelled and therefore traceable. All the labelled fatty acids were converted to ethyl esters and used to spike Super Selco™ enrichment emulsions. In replicated trials carried out in Spain, nauplii were enriched for 24 h using the spiked emulsions. The enriched nauplii were then removed from the emulsions, a sample was taken from each, and the remaining nauplii were put into clean seawater for a further 24-h starvation period. Immediately after the 24-h enrichment period, most of the radioactivity was recovered as TAGs in the enriched nauplii, not ethyl esters, and 20% DHA had retroconverted to EPA. After a further 24-h starvation, the amount of radioactivity in naupliar polar lipids increased whilst the amount in TAGs decreased, indicating mobilization and utilization of stored lipids for growth. Since 44% of the naupliar DHA had been retroconverted to EPA at the end of this second 24-h period, it is not surprising that DHA:EPA ratios in enriched *Artemia* nauplii are considerably lower than those found in the original enrichment diets.[39]

Retroconversion of DHA contributes to the problem of "disenrichment" of live prey prior to their consumption by the fish larvae, i.e., the loss of the EFA with which the prey had been enriched. As part of a wider turbot and halibut feeding program, we recently followed EFA levels after enrichment with ARA-rich and EPA-rich emulsions, starving the nauplii for 12 h and 24 h at 3 different incubation temperatures.[45] The starvation period generally affected the EFA profile more than temperature, although loss of EFAs was generally more rapid at higher temperatures. Naupliar DHA levels decreased by more than 50% over 24 h: a similar rate as measured in the radiolabelled study previously described. On a more positive note, a study by Evjemo et al.[46] compared the rate of disenrichment in two species of *Artemia: A. franciscana* and a Chinese species, *A. sinica*. They found that loss of DHA was much slower in *A. sinica*, with 84% DHA remaining in the enriched nauplii after 72 h storage at 22°C.

Thus, in the future, it may be possible to carry out careful species selection for the desirable characteristic of DHA retention in *Artemia* nauplii destined for marine hatchery use. For now, it is possible to reduce disenrichment by cold storage of enriched nauplii and carefully adjusting prey densities in larval fish tanks to ensure nauplii are eaten within 12 h and starving nauplii do not remain in the tanks for long periods. If logistically possible, it would certainly be advantageous to perform two staggered enrichments each day, to generate freshly enriched nauplii for both morning and evening larval feeds.

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Influence of Dietary Levels of Eicosapentaenoic and Arachidonic Acids on the Pigmentation Success of Turbot (*Scophthalmus maximus* L.) and Halibut (*Hippoglossus hippoglossus* L.)

L. A. McEvoy, A. Estevez, J. G. Bell, R. J. Shields, B. Gara and J. R. Sargent

Turbot and halibut larvae were fed until metamorphosis on live prey enriched with high, medium and low levels of either eicosapentaenoic acid (EPA, 20:5n-3) or arachidonic acid (ARA, 20:4n-6) in order to investigate the role of these fatty acids in skin pigmentation success in flatfish. The 6 test enrichments comprised blends of specialty triglyceride oils emulsified in seawater with soya phosphatidylcholine (PC) added at 12% (w:w). A previously employed enrichment comprising 12% soya PC:88% tuna orbital oil (TOO) was included as a control diet. Ascorbyl palmitate and α-tocopherol acetate were added to all diets at concentrations of 100 mg/kg oil. Turbot larvae in triplicate 60-L containers (750 larvae/tank) were fed from day 3 to 14 post-hatch on rotifers enriched for 6 h with the previously described emulsions followed by a diet of enriched *Artemia* nauplii from day 11 to 45. Similarly, 18-day post-first-feeding halibut larvae in triplicate 100-L tanks (40 larvae/tank) were fed for 43 days on *Artemia* nauplii enriched for 12 h on one of the 7 diets. Following metamorphosis, all surviving fry were counted, measured and weighed, and the incidence of normal pigmentation in each tank was assessed. Pooled brain samples were taken from each tank for fatty acid analysis. Brain fatty acid profiles reflected the dietary treatments. There was a significant positive correlation between high levels of ARA in turbot brain and an increased incidence of malpigmentation ($r=0.945$, $P<0.000$), and a converse relationship between high levels of EPA and decreased numbers of malpigmented fry ($r=0.610$, $P<0.010$). Levels of EPA and ARA in halibut brains showed similar trends with respect to malpigmentation, but the correlations were less statistically significant ($r=0.560$, $P<0.010$ and $r=0.389$, $P<0.100$ for ARA and EPA, respectively). Both species showed a significant, positive correlation between the incidence of normal pigmentation and brain EPA:ARA ratios ($r=0.871$, $P<0.001$ in turbot, and $r=0.547$, $P<0.05$ in halibut), with the greatest number of normally pigmented fry occurring when their brain EPA:ARA ratio was raised above 4:1.

Introduction

Malpigmentation continues to pose a problem in marine flatfish cultivation, and tends to reduce the market price.[1] Pigmentation is thought to be under both neural and neuroendocrinological control[2] and thus may be influenced by dietary essential fatty acid levels, since docosahexaenoic acid (DHA, 22:6n-3) has important structural and functional roles in cell membranes and is important for normal neural development. In addition, ARA and EPA are precursors of bioactive eicosanoids which are involved in synaptic transmission and intra- and inter-cell signaling.[3-5] When considering essential fatty acid requirements in fish, it is important to remember that competitive metabolic interactions exist between these fatty acids, making it necessary to consider their relative proportions to each other as well as their absolute amounts in the diet.[5] Reitan et al.[6] obtained a positive correlation between DHA:EPA ratios in 12- and 22-day-old turbot larvae and their subsequent pigmentation rates at 27 days. However, to date no one has investigated the effects of varying dietary EPA:ARA ratios on pigmentation success.

This paper describes a preliminary investigation into the subsequent pigmentation success of turbot

Table 1. Oils utilized for *Artemia* enrichments in 1997 turbot and halibut feeding trials

Diet	Type of oil	Supplier
EPA 10	50% TOO[a] +50% Marinol[b]	[a] Croda Universal Ltd., UK
EPA 20	Marinol[b]	[b] Marine Oil Refiners, South Africa
EPA 30	EPA 30 Triglyceride	Croda Universal Ltd., UK
ARA 3	97% TOO +3% ARASCO™	Martek Bioscience Corp., USA
ARA 7	85% TOO +15% ARASCO™	
ARA 15	65% TOO +35% ARASCO™	

ARASCO™ contains 40% ARA in the form of mixed triglycerides manufactured and purified from *Mortiella alpina.*
[a] TOO = Tuna orbital oil
[b] Marinol is a blend of sardine and anchovy oils.

(*Scophthalmus maximus* L.) and halibut (*Hippoglossus hippoglossus* L.) fed with live prey enriched with varying levels of eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (ARA, 20:4n-6).

Materials and Methods

Tuna orbital oil (TOO) and a selection of specialty triglyceride oils were blended to generate 6 experimental oils containing high, medium and low levels of either EPA or ARA (Table 1). A control enrichment oil of 100% TOO was also incorporated in the trial. All oils were emulsified using 12% (w:w) soya PC (Epikuron, Lucas Meyer, Germany), and ascorbyl palmitate and α-tocopherol acetate were each added at a rate of 100 mg/kg oil. These 7 emulsions were used to enrich live prey in feeding trials with larval halibut and turbot.

Halibut trials were conducted at the Seafish Aquaculture Marine Hatchery at Ardtoe, Scotland. Triplicate 100-L black, static tanks were used for each dietary treatment and stocked randomly with a single batch of 18-day post-first-feeding (PFF) larvae (40 larvae/tank). Temperature was maintained at 12°C and illumination was provided by dimmable tungsten floodlights (initially 50 lux at the water surface; gradually increasing to 500 lux by 35 days PFF). Every second day, 20% of the tank water was exchanged and 2 L of *Nannochloropsis* were added. The larvae were fed twice daily with *Artemia* nauplii enriched (12 h @ 27°C) with one of the 7 experimental emulsions. Residual prey levels were monitored and feeding levels adjusted accordingly.

The turbot feeding trial was conducted at the Centro de Experimentacion en Acuicultura, Riveira, La Coruña, Spain. Each treatment was triplicated in 60-L mesh-bottomed (150 μm) containers randomly suspended in a 6000-L raceway. Temperature was maintained at 18°C (± 2°C) and individual airlifts provided aeration to each of the containers. A photoperiod of 12L:12D was employed throughout the trial. Each container was stocked with 750 larvae and these were initially fed with enriched rotifers (6h @ 20°C) from day 4 posthatch (PH) (3 rotifers/mL) to day 13 PH (16 rotifers/mL), and with enriched *Artemia* nauplii (12 h @ 27°C) from day 11 PH (0.1 nauplii/mL) to day 45 PH (10 nauplii/mL). Every 2 to 3 days during the rotifer feeding stage, one third of the raceway water was changed and 20 to 30 L concentrated *Isochrysis galbana* and *Nannochloropsis gaditana* were added. Five low-concentration, wide-spectrum antibiotic baths were administered on days 6, 8, 10, 12 and 16. The raceway was converted to an open system with UV-treated filtered seawater from day 16 onward.

The halibut trial ran for 42 days and the turbot trial for 45 days. The fish were then anaesthetized with MS-222 (Sandoz), weighed, measured, and their pigmentation success assessed. Brain samples were taken for fatty acid analysis by capillary gas-liquid chromatography.[7]

Results and Discussion

Table 2 shows the ranges of EPA and ARA levels in live prey enriched with the experimental emulsions. The EPA:ARA ratios were successfully manipulated in the live prey to generate a wide range of values. Ideally, DHA levels should have been maintained at a constant level. However, this proved very difficult to achieve and the DHA contents of the live prey enriched with the EPA emulsions contained significantly less DHA than the ARA emulsions (Table 2). Nonetheless, these high EPA emulsions generated the highest rates of normal pigmentation and no significant correlation between DHA:EPA ratio and normal pigmentation was found in either species

Table 2. Ranges of EPA and ARA levels in rotifers and *Artemia* fed experimental enrichment emulsions

Emulsions	
% ARA 20:4(n-6)	0.9 - 15.1
% EPA 20:5(n-3)	4.1 - 68.4
EPA:ARA	0.3 - 38.0
Rotifers	
% ARA 20:4(n-6)	0.8 - 8.4
% EPA 20:5(n-3)	2.8 - 58.7
EPA:ARA	0.3 - 41.0
Artemia	
% ARA 20:4(n-6)	1.0 - 7.9
% EPA 20:5(n-3)	5.5 - 33.2
EPA:ARA	0.7 - 33.0
% DHA in *Artemia* fed EPA diets	3.0 - 4.6
%DHA in *Artemia* fed ARA diets	6.0 - 9.9
DHA:EPA in *Artemia* fed EPA diets	0.1 - 0.3
DHA:EPA in *Artemia* fed ARA diets	1.3

Growth and survival was high in all treatments and neither species showed any significant differences in these parameters between treatments, within species. Despite the generally conservative nature of brain tissue, brain fatty acid profiles from the various treatments closely reflected the dietary levels of EPA and ARA.

When the incidence of normal pigmentation was correlated with percentage ARA and EPA levels in turbot brain total lipid, there was a significant correlation for both fatty acids (Equation 1, ARA; Equation 2, EPA).

(1) $y = -17.394x + 125.736$, $r = 0.945$, $P < 0.000$

(2) $y = 226.546 \log x - 155.738$, $r = 0.610$, $P < 0.010$

The best pigmentation rates therefore occurred with low levels of ARA and high levels of EPA. Halibut pigmentation versus brain percentage ARA and EPA levels showed similar trends, but only the ARA correlation was statistically significant (Equation 3, ARA; Equation 4, EPA).

(3) $y = -36.052 \log x + 30.498$, $r = 0.560$, $P < 0.010$

(4) $y = 42.810 \log x - 24.735$, $r = 0.389$, $P < 0.100$

Considerably lower levels of normal pigmentation were obtained in the halibut trial compared to the turbot trial. The high EPA treatment, which generated the highest mean number of correctly pigmented fry, yielded 28% correctly pigmented halibut as opposed to 86% normal turbot. When the incidence of normal pigmentation was correlated with EPA:ARA ratios in the brains of both turbot and halibut, there was a statistically significant correlation for both species (Equation 5, turbot; Equation 6, halibut).

(5) $y = 108.258 \log x + 13.691$, $r = 0.871$, $P < 0.001$

(6) $y = 25.983 \log x + 1.509$, $r = 0.547$, $P < 0.05$

EPA:ARA ratios above 4:1 yielded the best pigmentation results and 100% malpigmentation occurred at ratios below 1:1.

Figure 1 illustrates the interactions between ARA and EPA. Briefly, increasing dietary ARA tends to increase eicosanoid efficacy, whereas increasing dietary EPA should reduce eicosanoid efficacy. The apparent significance of the EPA:ARA ratio in the tissues of turbot and halibut implies that eicosanoids may be involved in the pigmentation process. Since the balance of EPA and ARA in cells and tissues can influence an individual's ability to respond to stress, this finding corroborates the general belief amongst mariculturists that stressful environmental conditions such as overcrowding, unsuitable illumination, etc., can increase the incidence of malpigmented fry. Naess and Lie[1] determined the period during which pigmentation patterns are determined in halibut, the so-called "pigmentation window", to occur before larvae reach 2.5 mm myotome length (approximately 16 mm standard length).

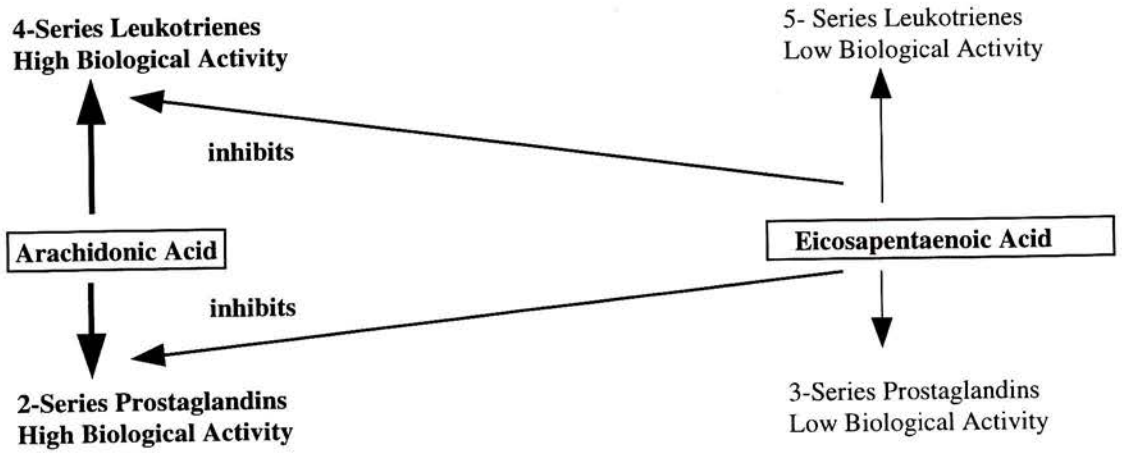

Figure 1. Interactions between arachidonic acid and eicosapentaenoic acid in the production of eicosanoids. Redrawn from Sargent (1995) with permission.[5]

The findings presented here may imply that increased stress, particularly during this critical "pigmentation window" period, may also increase the incidence of malpigmentation. It is interesting to note that in the halibut trial, the larvae were moved into their experimental tanks close to this critical period, presumably resulting in increased stress levels at this time. This may explain the depressed levels of normal pigmentation seen in all the halibut treatments compared with those in the turbot trial.

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Evaluation of Commercial Enrichment Media for Enhancing Nutritional Value of *Artemia* for Larval Halibut (*Hippoglossus hippoglossus*) Culture

T. Blair, F. Powell, P. Brooking and J. Castell

To improve the enrichment protocol for producing *Artemia* nauplii to feed larval halibut (*Hippoglossus hippoglossus*) 3 enrichment media (a high protein microdiet mixed 50:50 with cod liver oil, DHA Selco and Algamac-2000) were fed at concentrations of 1 or 2 g/million nauplii for 6-, 12-, or 24-h exposure periods. There were significant effects of media, concentration and time on total lipid content and fatty acid composition of *Artemia* nauplii lipids. DHA Selco and Algamac-2000 each increased nauplii DHA (22:6n-3) to over 7% of the total fatty acids. Algamac-2000 transferred the highest DHA/EPA ratio to the nauplii (2.9 after 12-h exposure to 2 g/million nauplii). The microdiet-cod liver oil emulsion had the least effect on nauplii lipids, appeared to be poorly assimilated and is not recommended for *Artemia* enrichment. A second trial with 2, 3, and 4 g/million nauplii concentrations of DHA Selco and Algamac-2000 was conducted. Concentration of the enrichment media had little effect. However, length of exposure and type of media had significant influences. DHA Selco enrichment provided higher total lipid content, while the highest DHA/EPA ratios (2.5-3.3) resulted from 24-h exposure to Algamac-2000. Furthermore, Algamac-2000 provides the advantage of producing cleaner, less oily nauplii and, being a complete algal meal, probably supplies a more complete nutrient package including proteins, amino acids, vitamins, minerals and essential fatty acids.

Introduction

In 1996, Maritime Mariculture Inc. (MMI) began a pilot commercial halibut culture project in cooperation with Dr. K. G. Waiwood (Department of Fisheries and Oceans, St. Andrews, NB). Initially, wild captured zooplankton were used as food for halibut larvae, and larvae were successfully reared through metamorphosis and weaned onto commercial microdiets. Shortages of zooplankton in the Bay of Fundy in late spring and early summer put later larval batches in danger of starvation. Unenriched and poorly enriched *Artemia* nauplii were substituted for wild zooplankton and reasonable numbers of juvenile halibut were produced. These nauplii-fed fish suffered from a higher incidence of albinism and malpigmentation, were smaller, and were slower to develop than the zooplankton-fed fish.

Several researchers have indicated that enriching *Artemia* with 22:6n-3 before feeding to larval marine flatfish significantly enhanced larval vision at low light intensities,[1] improved neural development,[2] and reduced pigmentation problems.[3-5] The objective of our trials was to evaluate the effectiveness of various enrichment media, enrichment concentrations and exposure times on the lipid content and fatty acid composition of *Artemia* nauplii.

Materials and Methods

In trial 1, *Artemia* nauplii were hatched over 24 h in 24 ppt seawater at 28°C. Nauplii, stocked at 100 000/L in 250-L conical tanks, were enriched with the following media in triplicate: (1) Algamac-2000, (2) DHA Selco, and (3) a microdiet:cod liver oil (CLO) emulsion (50:50 w/w). Enrichments were tested at 1 and 2 g/million nauplii over 6, 12 and 24 h except for Algamac-2000, which was not tested at 2 g and 24-h exposure. The *Artemia* nauplii were exposed to fresh seawater containing the enrichment media at the test concentrations for 6 h. Nauplii were then washed on a 150-µm screen with filtered seawater. Samples (about 1.5 g) were removed, rinsed with distilled water, and stored in plastic vials at -80°C until analyzed. Remaining nauplii were again exposed to fresh seawater with media added at the same concentrations. After a further 6 h, the washing and sample collection procedure was repeated. Remaining nauplii were returned for further 12-h enrichment and final samples were taken.

Enrichment media and *Artemia* samples were freeze-dried. Lipids were extracted and weighed using a $CHCl_3$:MeOH:0.88%KCl(H_2O) (8:4:3)[6] phase separation technique. Fatty acid methyl esters (FAMEs) were prepared by adding 2 mL 7%$BF_{3(MeOH)}$ and 0.5 mL toluene to 1 mg lipid and heating to 50°C for 16 h. After cooling, 5 mL H_2O were added. The FAMEs were extracted in 6 mL hexane and concentrated to 50 µL. FAMEs were applied over a 1-cm streak on a silica gel G thin layer chromatography plate. The plate was developed in hexane:diethyl ether:acetic acid (90:10:1). The methyl ester band (sprayed with 2'-4'-dichlorofluorescene and viewed under UV light) was scraped into a test tube and extracted with 5 mL $CHCl_3$:MeOH (2:1). Silica was removed by filtering through glass wool, the solvent evaporated and the methyl esters dissolved in 0.2 mL hexane for analysis by gas liquid chromatography (GLC).

FAMEs were analyzed using a Varian 3400 GLC equipped with a Supelco Omegawax™ 320 capillary column and temperature programmed from 160 to 240°C at a rate of 3.5°C/min and held at 240°C for 12.25 min (total run time 35 min). Retention times and integrated peak areas were determined using the Varian Star integration package and peaks were identified by comparisons with known FAMEs in the Supelco Omega Test standard or from previously identified peaks in a menhaden oil FAME sample.

Table 1. Fatty acid composition (mean% ± SD)* of lipids in enrichment media and *Artemia* nauplii used in trial 1.

Media			n	% Lipid***	20:4n-6**	20:5n-3**	22:6n-3**	DHA/EPA
Microdiet			1	15.60	0.5	8.6	6.9	0.79
Cod Liver Oil			1	99.10	0.4	7.1	16.4	2.30
DHA Selco			1	82.19	1.1	6.0	19.8	3.31
Algamac-2000			1	16.23	6.6	1.1	28.4	26.30
Artemia	**Concentration (g/million nauplii)**	**Time (h)**	**n**	**% Lipid**	**20:4n-6**	**20:5n-3**	**22:6n-3**	**DHA/EPA**
Microdiet: CLO	1	6	2	16.01	0.4	6.4	1.0	0.2
DHA Selco	1	6	2	15.72	0.6	8.1	2.6	0.2
Algamac-2000	1	6	2	14.64	0.4	1.1	0.8	0.8
Microdiet: CLO	2	6	2	15.17	0.4	2.8	0.8	0.3
DHA Selco	2	6	2	16.51	0.6	8.6	3.3	0.4
Algamac-2000	2	6	2	15.80	0.5	1.5	2.6	1.8
Microdiet: CLO	1	12	3	17.59 ± 1.22^{ab}	0.4 ± 0.0^{a}	2.6 ± 0.2^{abc}	1.8 ± 0.2^{a}	0.7 ± 0.0^{a}
DHA Selco	1	12	3	16.68 ± 0.46^{ab}	0.7 ± 0.0^{cd}	3.0 ± 0.2^{bd}	4.3 ± 0.4^{bc}	1.4 ± 0.2^{ab}
Algamac-2000	1	12	3	15.47 ± 3.34^{ab}	0.6 ± 0.1^{bc}	1.3 ± 0.1^{a}	2.0 ± 0.5^{a}	1.5 ± 0.3^{ab}
Microdiet: CLO	2	12	3	15.44 ± 0.07^{ab}	0.5 ± 0.0^{ac}	2.4 ± 0.1^{abc}	1.7 ± 0.2^{a}	0.7 ± 0.1^{a}
DHA Selco	2	12	3	20.09 ± 1.62^{ab}	0.8 ± 0.0^{cd}	4.1 ± 0.1^{de}	5.6 ± 0.1^{cd}	1.4 ± 0.0^{ab}
Algamac-2000	2	12	3	15.81 ± 0.62^{ab}	0.8 ± 0.0^{de}	1.8 ± 0.2^{ab}	5.3 ± 1.2^{cd}	2.9 ± 0.8^{c}
Microdiet: CLO	1	24	3	16.86 ± 0.46^{ab}	0.5 ± 0.0^{ab}	4.5 ± 0.3^{ef}	2.6 ± 0.2^{ab}	0.6 ± 0.1^{a}
DHA Selco	1	24	3	15.23 ± 2.51^{ab}	0.9 ± 0.0^{de}	4.5 ± 0.6^{ef}	5.1 ± 0.2^{cd}	1.1 ± 0.2^{a}
Algamac-2000	1	24	3	17.70 ± 3.80^{ab}	1.0 ± 0.1^{e}	3.1 ± 0.5^{cd}	6.9 ± 0.9^{de}	2.3 ± 0.1^{bc}
Microdiet: CLO	2	24	3	15.08 ± 1.47^{a}	0.5 ± 0.0^{ab}	4.5 ± 0.3^{de}	2.6 ± 0.2^{ab}	0.7 ± 0.2^{a}
DHA Selco	2	24	3	20.58 ± 0.28^{b}	0.9 ± 0.0^{de}	5.4 ± 0.7^{f}	7.5 ± 0.4^{e}	1.4 ± 0.1^{a}
Algamac-2000	2	24	0	–	–	–	–	–

*Different letters in any column indicate significant difference at $P<0.01$.
**Fatty acids as % of total lipid.
***Total lipid as % of dry tissue weight.

The concentration of each identified fatty acid was expressed as a precentage of the total lipid recorded in that sample. GLM ANOVA with Tukey's HSD test with significance set at $P<0.01$ was used to test for differences in percent composition of each of the fatty acids among the samples. Statistical calculations were performed using SYSTAT version 7.0 for Windows.[7]

Upon completion of trial 1, a second trial was conducted following the same protocol. In trial 2, an unenriched control group was used, along with 2 media (DHA Selco and Algamac-2000) at concentrations of 2, 3 and 4 g/million nauplii, and exposure times of 0, 12 and 24 h. Samples were collected and analyzed as described in trial 1.

Results and Discussion

The enrichment media analyses are presented in Table 1. Algamac-2000 had the highest DHA (28.4%) and highest DHA/EPA ratio (26.3). The fatty acid composition of Algamac-2000 was similar to values in the literature,[8] except that our analysis indicated a lipid level of 16.23% instead of the reported 32%. This difference might be due to variations between batches or laboratory techniques. Algamac-2000 is a relatively new product used for enriching rotifers and *Artemia,*[8] or as a feed supplement for molluscs.[9] DHA Selco had the second highest DHA/EPA ratio (3.31). Feeding larval Japanese flounder (*Paralichthys olivaceus*) and European turbot *(Scophthalmus maximus)* with DHA Selco-enriched *Artemia* reduced malpigmentation in the metamorphosed juveniles[3] compared with fish fed unenriched nauplii. This effect was attributed to the high DHA level (19.8%) in this product. The microdiet lipid was lowest in DHA (6.9%) and had the lowest DHA/EPA ratio (0.79). This product was only 15.6% lipid; therefore, the ratios in the 50:50 mixture with cod liver oil (CLO) were offset by the 16.4% DHA and 2.30 DHA/EPA ratio in CLO fatty acids

The results of trial 1 are presented in Table 1. The 6-h treatments were sampled in duplicate and were not used in statistical comparisons. DHA Selco at 2 g/million for 24 h effected the greatest increase in total lipid content (20.58%) while Microdiet:CLO at 2 g/million for 24 h was least effective (15.08%). DHA Selco and Algamac-2000 were about equally effective in increasing 22:6n-3 (5.1% and 6.9% respectively after 1 g for 24 h). The best DHA/EPA ratios resulted from Algamac-2000 treatments (2.9 after 2 g for 12 h and 2.3 after 1 g for 24 h). The microdiet-CLO mixture had the lowest DHA/EPA ratios (no greater than 0.7 for any treatment). It appeared that CLO was poorly assimilated by the nauplii.

Table 2. Fatty acid composition (mean% ± SD)* of lipids in *Artemia* nauplii used in trial 2.

Artemia	Conc****	Time (h)	n	% Lipid***	20:4n-6**	20:5n-3**	22:6n-3**	DHA/EPA
Unfed	0	0	3	14.0 ± 0.5^{ab}	0.6 ± 0.1^{a}	0.0 ± 0.0^{a}	0.5 ± 0.2^{a}	0.3 ± 0.1^{a}
Unfed	0	12	3	9.9 ± 0.5^{a}	1.1 ± 0.0^{ab}	0.0 ± 0.0^{ab}	0.9 ± 0.3^{ac}	0.3 ± 0.1^{a}
DHA Selco	2	12	3	16.9 ± 1.0^{bc}	1.3 ± 0.1^{bc}	0.5 ± 0.0^{cf}	8.1 ± 0.6^{bde}	1.3 ± 0.1^{bc}
Algamac-2000	2	12	3	15.7 ± 0.4^{bc}	1.7 ± 0.1^{cde}	0.1 ± 0.0^{ace}	9.1 ± 1.2^{def}	1.9 ± 0.2^{cd}
DHA Selco	3	12	3	18.3 ± 0.1^{bcd}	1.0 ± 0.1^{ab}	0.4 ± 0.1^{acd}	6.9 ± 3.1^{bcd}	1.7 ± 0.7^{c}
Algamac-2000	3	12	3	14.7 ± 0.2^{bf}	1.4 ± 0.0^{bc}	0.1 ± 0.0^{ac}	6.9 ± 1.2^{bcd}	1.8 ± 0.0^{cd}
DHA Selco	4	12	3	19.8 ± 1.2^{cde}	1.5 ± 0.2^{bc}	0.6 ± 0.1^{bf}	12.3 ± 1.6^{deg}	1.6 ± 0.1^{c}
Algamac-2000	4	12	3	14.9 ± 0.8^{bf}	1.6 ± 0.1^{cde}	0.1 ± 0.0^{ac}	6.1 ± 1.2^{abf}	1.6 ± 0.1^{c}
Unfed	0	24	3	10.1 ± 0.3^{a}	1.3 ± 0.2^{bd}	0.2 ± 0.1^{ac}	2.9 ± 2.2^{ab}	0.7 ± 0.4^{ab}
DHA Selco	2	24	3	18.9 ± 0.9^{cdf}	1.8 ± 0.3^{cef}	0.7 ± 0.1^{ef}	11.1 ± 1.7^{defg}	1.6 ± 0.1^{c}
Algamac-2000	2	24	3	17.7 ± 0.9^{bd}	2.1 ± 0.2^{ef}	0.2 ± 0.0^{bce}	16.0 ± 3.2^{g}	3.3 ± 0.1^{e}
DHA Selco	3	24	3	21.4 ± 2.7^{de}	1.6 ± 0.1^{cd}	0.7 ± 0.0^{f}	13.0 ± 1.3^{eg}	1.5 ± 0.1^{c}
Algamac-2000	3	24	3	14.7 ± 0.5^{bf}	2.2 ± 0.0^{f}	0.2 ± 0.0^{cf}	15.3 ± 1.8^{g}	2.6 ± 0.3^{de}
DHA Selco	4	24	3	23.5 ± 2.4^{e}	1.6 ± 0.3^{cde}	0.6 ± 0.1^{def}	12.8 ± 1.2^{deg}	1.8 ± 0.2^{cd}
Algamac-2000	4	24	3	15.2 ± 1.6^{bf}	1.8 ± 0.1^{cdf}	0.1 ± 0.0^{ace}	10.6 ± 1.4^{dfg}	2.5 ± 0.1^{de}

*Different letters in any column indicate significant difference at $P<0.01$.
**Fatty acids as % of total lipid.
***Total lipid as % of dry tissue weight.
****Enrichment media concentration as g/million nauplii.

The results of trial 1 might have been compromised by the use of 2 different cans of *Artemia* cysts. Zero time samples to compare the original lipid compositions of the nauplii were not taken. The 1 g, 6-h microdiet-CLO replicas and the 1 and 2 g, 6-h DHA Selco had 6.4 to 8.6% 20:5n-3, while the replicas of the other 3 groups of 6-h sample treatments had much lower 20:5n-3 levels. These appear more related to the original nauplii lipid composition than to the enrichment media. Thus the levels of 20:5n-3 in the subsequent treatments may be unreliable for comparisons.

Trial 2 was initiated to reevaluate DHA Selco and Algamac-2000 at 2, 3 and 4 g/million nauplii for 12 and 24 h (Table 2). In this trial, differences in concentration of either media had no significant effect on the fatty acid and lipid composition of the *Artemia*. Differences in media and exposure time had the greater influence. Although DHA Selco produced the highest lipid content of the treatments (23.5% and 21.4% for 4 and 3 g at 24 h), it appeared that Algamac-2000 produced higher DHA/EPA ratios (3.3, 2.6 and 2.5 for 2, 3 and 4 g Algamac-2000/million after 24-h exposure). The *Artemia* maintained consistently low 20:5n-3 levels while levels of 22:6n-3 increased with enrichment. These DHA/EPA ratios are all higher than the 2.0 minimum believed necessary for marine fish larval development. Furthermore, even at 2 g for 24 h Algamac-2000 gave the best results for 22:6n-3 enrichment (16.0%) and for 20:4n-6 (2.1%). For hatchery operators Algamac-2000 held an additional advantage, since unlike the other treatments, it does not produce an oily scum in the enrichment tanks.

We gratefully acknowledge Mr. Lealand Lei, Aquafauna Bio-Marine, Hawthorne, CA for generously supplying samples of the Algamac-2000 product for the enrichment trials.

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Intensive Hygienic *Artemia* Production

Tania De Wolf, Marleen Dehasque, and Peter Coutteau

New trends in aquaculture require more intensive and hygienic techniques of live food production. A "disinfecting continuously" (DC) concept for *Artemia* hatching and enrichment was developed to control microbial populations during *Artemia* production. Bacterial blooming was prevented during the hatching of DC cysts, resulting in bacterial levels in the hatching medium below 10^3 CFU/mL (total counts on marine agar) compared to levels of 10^7 CFU/mL in the control treatment. The counts for *Vibrio* spp., potential pathogens for the larval stages of fish and shrimp, were reduced from 10^6 to 10^2 CFU/mL. After enrichment with a DC enrichment emulsion, the *Artemia* nauplii had 10 000 times fewer bacteria than those obtained by a non-disinfected standard enrichment. The lower biological oxygen demand and the improved water quality due to the application of the DC concept allowed the development of an innovative product for the combined hatching and enrichment of *Artemia* using decapsulated cysts. This "all-in-one" concept allows enriching at double densities, reduces the requirements for tank volume and labor, and results in enriched *Artemia* with a nutritional value similar to those obtained through standard enrichment methods.

Introduction

Artemia nauplii, extensively used as live food in early marine larval rearing, are heavily contaminated with bacteria that bloom during the hatching process. As this bacterial flora consists mainly of *Vibrio* spp., *Artemia* nauplii can be considered as a possible carrier of pathogenic bacteria that can cause disease outbreaks during larval rearing of other species. Verdonck et al.[1] found a distinct correlation between the bacterial strains in the larval intestines and the ones in the administered live food, supporting the hypothesis of bacterial transfer from live diets to the predator larvae. Furthermore, the intensification of production techniques in marine hatcheries has increased the sensitivity of the larval stages to stress, which in turn increases the chance for disease outbreaks. In this perspective, the DC products were developed. DC is an application that prevents and provides a continuous suppression of the growth of bacteria, ciliates, and fungi during hatching and enrichment of *Artemia*. The aim of this study was to produce "cleaner" *Artemia* nauplii, with low bacterial numbers and consistent quality. In this paper, the effects of application of DC products on the bacterial loads in *Artemia* production are presented.

Materials and Methods

To evaluate the effect of the DC concept on the bacterial load and nutritional value of ready-to-feed *Artemia* nauplii, the following treatments were compared:

1. Standard hatching and enrichment,
2. Standard hatching and DC enrichment,
3. DC hatching and DC enrichment,
4. "All-in-one" hatching and enrichment.

In treatments 1, 2, and 4, premium GSL *Artemia* cysts (Inve Aquaculture NV, Belgium) were used, while in treatment 3, DC *Artemia* cysts were used.

Hatching and enrichment were performed in 1-MT cylindroconical tanks equipped with open tube aeration and airstones that maintained dissolved oxygen at a minimum level of 4 ppm. Prior to incubation of the cysts, the diluted sea water (30 ppt) was sand-filtered and disinfected with 10 ppm of active chlorine, and subsequently neutralized with 10 ppm sodium thiosulphate to remove excess active chlorine. In all treatments, 2 g of cysts were incubated/L of seawater.

For treatments 1, 2, and 3, standard hatching was performed (24 h at 28°C, strong illumination) using non-decapsulated cysts. In treatments 1 and 2, no disinfection was used during the hatching, while in treatment 3, DC cysts were hatched. After hatching, the nauplii were harvested, rinsed, and transferred into the enrichment tanks at a density of about 300 nauplii/mL.

Treatment 1 was subsequently enriched with DHA Selco (Inve Aquaculture NV, Belgium), adding a first dose at T24 (24 h after incubation, 300 ppm) and a second at T36 (300 ppm). Treatments 2 and 3 were enriched using the same procedure, with DC DHA Selco.

In treatment 4, decapsulated cysts were used and incubated at 2 g dry weight equivalent/L. At the time of incubation, 200 ppm A1 DHA Selco was added. After hatching (24 h), a second dose of A1 DHA Selco was

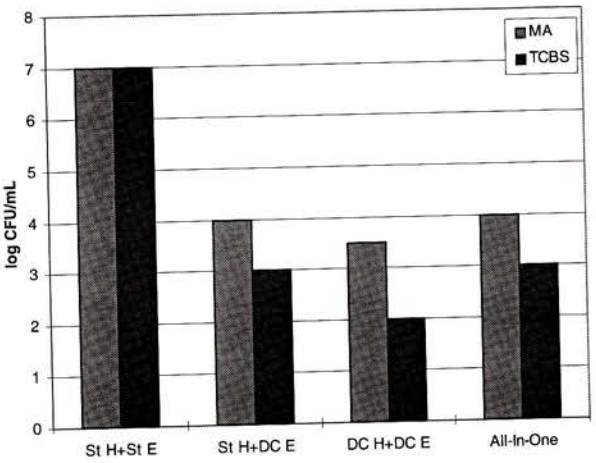

Figure 1. Bacterial load of the enrichment medium 48 h after time of incubation (St = standard, H = hatching, E = enrichment).

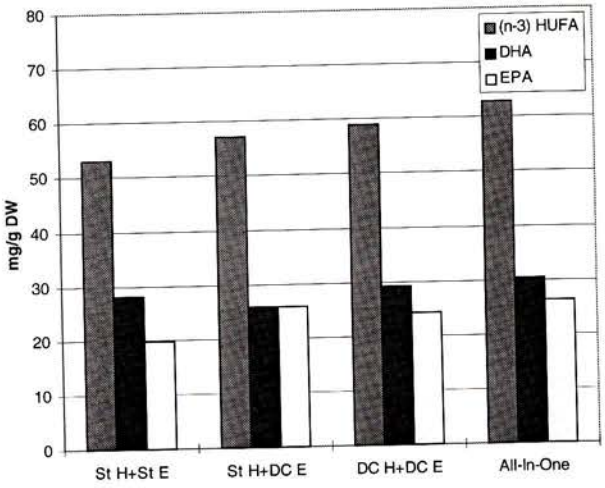

Figure 2. HUFA values of enriched *Artemia* 48 h after incubation (St = standard, H = hatching, E = enrichment).

added (300 ppm) in the same tank (without intermediate harvest and washing), and a third dose of 300 ppm was added 36 h after hatching.

Forty-eight hours after incubation of the cysts, samples of the enriched nauplii were taken for fatty acid methyl ester (FAME) analyses (preparation by direct transesterification[2])and of the enrichment medium to control the bacterial load. A dilution series was made in sterile saline solution and 0.1 mL of the appropriate dilution was put on marine agar (MA) plates for total count and on TCBS (thiosulphate citrate bile sucrose) plates for *Vibrio* counts. The plates were incubated for 24 h at 25°C and colonies were counted. Numbers are expressed as log CFU/mL enrichment medium.

Results and Discussion

Bacterial Load

The bacterial load after enrichment was reduced dramatically by using a DC enrichment emulsion (treatment 1 vs. treatment 2) (Fig. 1). Total marine bacteria and *Vibrio* counts only reached 10^4 CFU/mL and 10^3 CFU/mL respectively, compared to 10^7 CFU/mL for total count as well as *Vibrio* count in the control treatment. This means a reduction of potential larval pathogens by a factor of 10 000.

Applying DC during the hatching process reduced the bacterial load again 10 times in comparison with the application during enrichment only (treatment 3 vs. treatment 2).

The "all-in-one" treatment gave similar results as the DC enrichment even though density during enrichment was doubled compared to the control, and no transfer to a new clean-water tank was done before starting the enrichment (treatment 4).

Enrichment Values

Enrichment levels were slightly higher when the DC concept was used (treatments 2 and 3 compared to treatment 1) (Fig. 2). When the enrichment was done in the hatching tank ("all-in-one" concept), high enrichment levels were maintained, even at double naupliar density. Bacterial respiration is lower using the DC concept, leaving more dissolved oxygen available for the nauplii. This allows enrichment of the nauplii at higher densities even without intermediate harvest.

In conclusion, it was shown that hatching and enrichment in the same tank using A1 ("all-in-one") produced healthy, clean, well-enriched nauplii. This technique saves labor (no intermediate harvest, only 1 tank to clean instead of 2), lowers space and operating costs, and significantly reduces the risk of transferring pathogenic bacteria to the larval rearing tank.

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The Use of *Artemia* in Feeding Larval Wolffish (*Anarhichas lupus* L.)

C. I. Hendry and L. C. Halfyard

The necessity of providing enriched live feed (i.e., *Artemia* nauplii) to larval Atlantic wolffish (*Anarhichas lupus* L.) during the first-feeding stage was investigated. In various other marine fish, the presence of live feed promotes the instinctive predatory response. It is also thought that live feed supplies digestive enzymes that the fish larvae are unable to synthesize. Newly hatched wolffish were raised to 40 days post-hatch in raceways supplied with recirculating seawater maintained at 8°C. Dry feed and *Artemia* nauplii were supplied from feeders controlled by automatic timers. All measured growth parameters (wet weight, body length, specific growth rate) were significantly higher in wolffish fed diets supplemented with unenriched *Artemia* than those fed on dry feed alone. The highest growth parameters were in fish larvae fed diets supplemented with enriched *Artemia*. Survival between different treatments followed the same trend as the growth parameters. Providing larval wolffish with dry feed supplemented with enriched *Artemia* is therefore necessary to obtain the best growth performance and survival.

Introduction

The Atlantic wolffish (*Anarhichas lupus*) is an excellent potential candidate for aquaculture due to the prime quality of its flesh.[1] There is no commercial fishery in the Canadian Atlantic area,[2] therefore direct market competition would be negligible. To successfully culture this species, it is necessary to develop an understanding of its biology.

Artemia and dry diets, usually enriched with essential fatty acids (EFAs), are used routinely in marine larviculture. However, there are conflicting reports on the importance of *Artemia* and live food to some species. It has been suggested that *Artemia* are not necessary for the production of first-feeding wolffish, since survival can be above 80% on dry diet alone.[3]

Terrestrial animals require n-6 (linoleic series) highly unsaturated fatty acids (HUFAs) as EFAs for their normal growth, while marine fish require n-3 (linolenic series) HUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It has been suggested that the different requirement in fish is due to the lower body temperature. In marine fish, DHA is generally more important for growth and development than EPA.

The aims of this study were to evaluate the necessity of providing live *Artemia* to developing larval wolffish and the effect of enriching *Artemia* with an essential fatty acid (i.e., DHA).

Materials and Methods

A wolffish egg mass was collected at Bauline, NF, and incubated at 6°C in an upwelling Heath tray system. Upon hatching, the larvae were randomly distributed to 9 raceways at a density of 50 fish/L. Conditions in the raceways were: 1.26 L/min flow of recirculating seawater, 8°C water temperature, 206-244 lux light intensity and LD 18:6 photoperiod. Both the *Artemia* and dry diets were provided with automatic feeders (Fig. 1). Cleaning and removal of mortalities was done daily and water quality was monitored weekly.

There were three treatments: (1) dry diet only (D), (2) dry diet supplemented with unenriched *Artemia* (UA+D), and (3) dry diet supplemented with *Artemia* enriched with DHA Selco (EA+D). Each treatment had 3 replicates, and the feeding trial continued to 40 days post-hatch (dph). Fish were fed 9 times during the 18-hour photoperiod and the *Artemia* (1000/L) and the dry diet (in excess) were introduced at the same time. At each of the 3 sampling times (days 0, 20, and 40), body length and wet weight were recorded from 20 fish per tank. Mortalities were also recorded. Specific growth rates were obtained from the measurements of wet weight using the following formula taken from Strand et al.[3]

$$SGR = \left(e^{\frac{(\ln wt_2 - \ln wt_1)}{(t_2 - t_1)}} - 1\right) \times 100$$

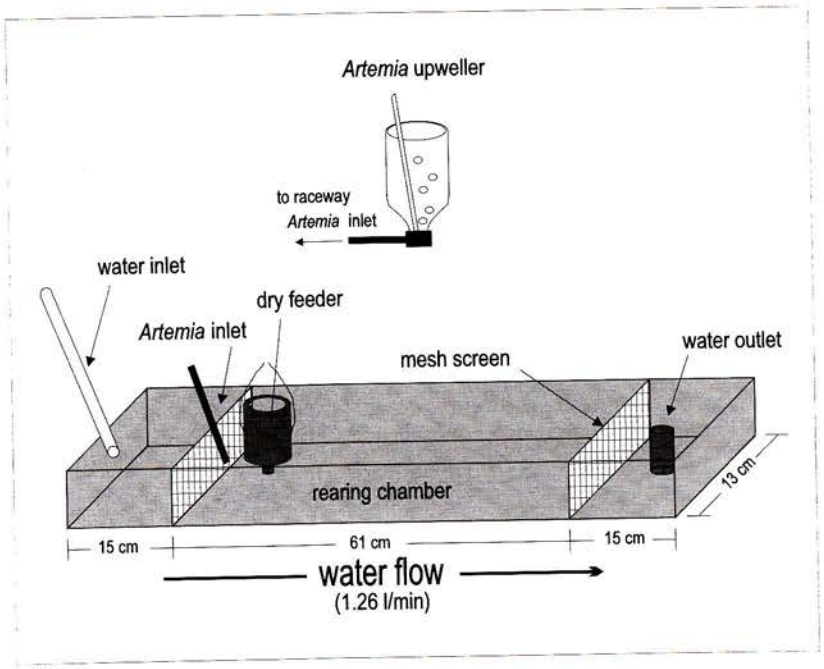

Figure 1. Schematic representation of raceway system used to raise larval wolffish in this experiment.

Growth parameters and survival were analyzed for significant differences between replicates and treatments ($P<0.05$) using one-way analyses of variance.

Results and Discussion

Water quality parameters remained relatively constant during the experiment and were within acceptable limits for temperature, salinity, dissolved oxygen, ammonia, and pH.

Results from replicates within each treatment (except 1B) were not significantly different and were pooled together. Group 1B had growth characteristics larger than all the other groups and was therefore considered to be an outlier and was not used in the statistical analyses. It is, however, interesting that this was the only experimental group reared on the outer edge of the culture system. It is therefore possible that this group of fish reacted to the constant human traffic passing by the side of the system by moving to the surface and perhaps obtaining more food per fish.

It is evident from Figure 2 that wet weights and lengths increased more in larvae fed the diet supplemented with unenriched *Artemia* than in larvae fed an *Artemia*-deficient diet ($P<0.05$). This suggests that *Artemia* are required for the successful rearing of wolffish larvae. The results support other studies showing that total replacement of live prey is still not possible in the culture of marine fish and better growth results are obtained when formulated diets are used in combination with live prey from first feeding.[7]

However, it is still uncertain why *Artemia* is essential to the diet of marine larval fish. It has been suggested that this live form of food stimulates the instinctive predatory response of the larvae, which is important given the limited availability of yolk sac reserves in the larvae. Another suggestion is that living prey contain digestive enzymes (proteases) which support and accelerate predatory digestive processes.[5] A final explanation is that perhaps the *Artemia* supply some needed nutrition to the wolffish larvae.

The wet weights and lengths of larvae fed the DHA-enriched *Artemia*-supplemented diets were signifcantly higher than those fed *Artemia* without enrichment ($P<0.05$). This suggests that it is not only necessary to add live prey (*Artemia*) to the dry diets of marine fish larvae, but that the zooplankton must also be enriched with HUFAs (i.e., DHA). This is not surprising since DHA is required by most marine larval fish as an EFA.[6]

The specific growth rates (a function of wet weight over time) were also larger in the unenriched treatment than in larvae receiving the *Artemia*-deficient diet ($P<0.05$), and was highest in those fed the enriched *Artemia* diet ($P<0.05$). It is also interesting to note that these SGR values were higher than those seen in other studies.[3,7,8] However, the dry diets used in the various studies have been different, which may suggest the commercial dry diet or enrichment used in

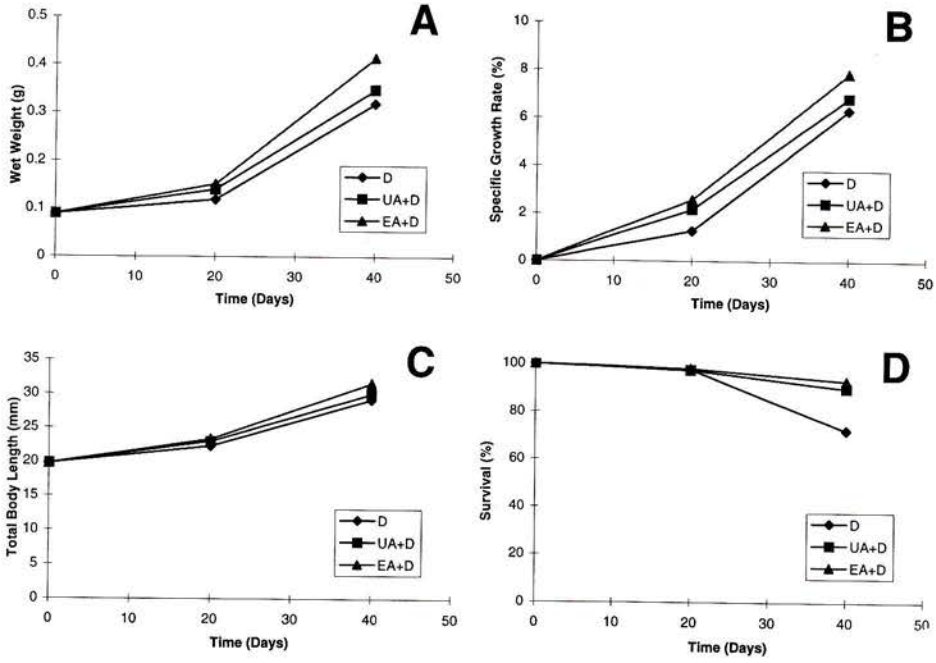

Figure 2. Differences in growth parameters ((A) wet weight, (B) specific growth rate, (C) total body length) and (D) survival between dietary treatments over the 40-day experimental duration.

this experiment had better nutritional qualities. It has even been shown that there can be significant differences in growth performances of different egg masses. Perhaps the egg mass used in this experiment had superior growth attributes.

The survival of the wolffish larvae in this experiment was highest in the enriched *Artemia* diet ($P<0.05$). However, the results appear contrary to Strand et al.,[3] who obtained survival rates above 80% without any live feed. The mean survival for larvae on the dry diet after 40 days was 72.3%. Survival rates remained stable through to 100 days post hatch.[9]

Conclusions

This experiment explored the possibility of excluding supplementation of dry feed with live *Artemia*, or even eliminating the need to enrich the *Artemia* with expensive, highly concentrated fatty acids (i.e., DHA). Unfortunately, neither constituent could be eliminated without observing significant decreases in growth and survival.

The authors would like to thank Mr. Ray Fitzgerald for his help in raising and maintaining the wolffish larvae.

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Enzyme Activity as a Tool for Assessing the Cultured Condition of Rotifers and Fish Larvae — A Preliminary Study

Adriana B. De Araujo, Wenresti G. Gallardo, Terry W. Snell and Atsushi Hagiwara

Neonates of *Brachionus plicatilis* hatched from cysts were exposed to a concentration series of free ammonia (control to 9.8 ppm) and increasing viscosity (relative viscosity against filtered seawater = 1 to 1.169); the latter was regulated by the addition of methyl cellulose. Activities of glucosidase and esterase in rotifer guts decreased with increasing free ammonia and viscosity, respectively. There was a significant correlation of enzyme activity with rotifer life span and fecundity at 25°C on a diet of *Nannochloropsis oculata*. This same technique was applied to *Paralichthys olivaceus* fish larvae reared in six 80-L tanks for 20 days post-hatching and fed a diet of *B. plicatilis* at three densities (0.5, 5, and 10 individuals/mL). Esterase and phospholipase activity of fish larvae were quantified by fluorometry or image analysis. Significant correlation was observed between esterase activity and notochord length ($P<0.05$). Low esterase and phospholipase fluorescence intensities were observed at low survival and growth rates.

Introduction

In order to assess the status of rotifer mass cultures, several techniques have been developed. Egg ratio, ingestion rate, and swimming speed can be good indices of population health of cultured rotifers.[1] The viscosity of rotifer culture media is negatively correlated with rotifer population growth rate.[2]

In ecotoxicology, in vivo enzyme activity has been used in freshwater cladocerans[3] and rotifers[4] to estimate chronic toxicity. The activity of certain rotifer enzymes can be determined in vivo or in vitro with substrates that have been used in ecotoxicology. These substrates are non-fluorescent, but are cleaved by endogenous enzymes to yield fluorescent products. The technique requires a short period of substrate incubation to quantify enzyme activity and can be included into the daily routine in finfish hatcheries. In order to develop this technique, laboratory research is necessary to clarify how rotifer enzyme activity is changed by known environmental stressors, such as free ammonia, food shortage, and contamination of protozoa and bacteria.

This paper reports the results of the effect of free ammonia and viscosity stressors on in vivo esterase and phospholipase activity of rotifers and the correlation of esterase activity with growth and survival of fish larvae.

Materials and Methods

Rotifers

For the rotifer experiments, we used neonates hatched from resting eggs that were previously mass produced[5] and stored in cans.[6] Resting eggs were incubated[7] after disinfection,[8] and the hatched neonates were used as test animals to assess[9,10] the response to the stressors unionized ammonia and change of culture viscosity. Unionized ammonia was added to seawater at seven concentrations between 0 and 9.8 ppm following a previously described protocol.[11] Relative viscosity of experimental seawater was regulated at five levels between 1 and 1.169 by the addition of methyl cellulose.[2] Temperature and salinity were constant at 24°C and 22 ppt.

Newly hatched neonates were individually cultured in a 100-µL solution to obtain lifespan and fecundity data. The culture medium contained 7×10^6 cells/mL *Nannochloropsis oculata* and was replaced daily. For enzyme activity measurements, 100 unfed newly hatched neonates were inoculated into 1-mL test solution and incubated for 2 hours. Rotifers then were

exposed to fluorogenic substrates (Molecular Probes Co.) for the enzymes esterase, phospholipase, or glucosidase for 15 minutes. These substrates are non-fluorescent but are cleaved by endogenous enzymes to yield fluorescent products (fluorescein). Following the protocol described previously,[12] fluorescence measurements were made using a fluorometer (Turner, TD-700) with fluorescein excitation and emission filters.

Table 1. Correlation between enzyme activity and rotifer population growth.

Enzyme	Ammonia	Viscosity
Esterase	n.s.	*
Glucosidase	**	**
Phospholipase	n.s.	n.s.

* $P<0.05$; ** $P<0.01$

Fish Larvae

To investigate whether this technique is also useful for fish larvae, Japanese flounder larvae *(Paralichthys olivaceus)* were reared in 100-L (water volume = 80 L) polycarbonate tanks. Newly hatched larvae were stocked into each tank at 30 larvae/L and reared at 3 food densities (10, 5, and 0.5 rotifers/mL) with 2 replicates each. Temperature was constant at 17°C and salinity at 35 ppt. Other details of this rearing method were the same as previously described.[13,14]

It is not clear whether rotifer enzymes are immediately decomposed after ingestion by *P. olivaceus* larvae, or kept active in the larval gut. To avoid such problems, enzyme activity in *P. olivaceus* larvae was measured daily in the morning before feeding. Larval esterase and phospholipase activities were measured in vivo by image analysis[4] with the same substrates used in the rotifer experiment. To visualize enzyme activity, five larvae were incubated in the dark at 24°C for 30 minutes. Images were captured with a CCD video camera (Sony, XC-77) attached to a compound microscope (40 to 100x magnification). The intensity of the localized fluorescence was quantified using an NIH Image. Twenty larvae were taken from each rearing tank to determine notochord length daily. On day 20, percent survival was determined.

Linear regression was performed to determine if there was a significant relationship between larval growth and survival rates and in vivo enzyme activity. Multiple comparison (PLSD test) was then performed to identify the differences between treatments.[15]

Results and Discussion

Rotifers

As has been reported by several authors,[2,11,16] the fecundity and lifespan of individually cultured rotifers decreased as the unionized ammonia concentration and viscosity of the medium increased. Glucosidase fluorescence was well correlated with fecundity and lifespan for the rotifers exposed to various concentration of unionized ammonia and also with lifespan for the rotifers exposed to higher

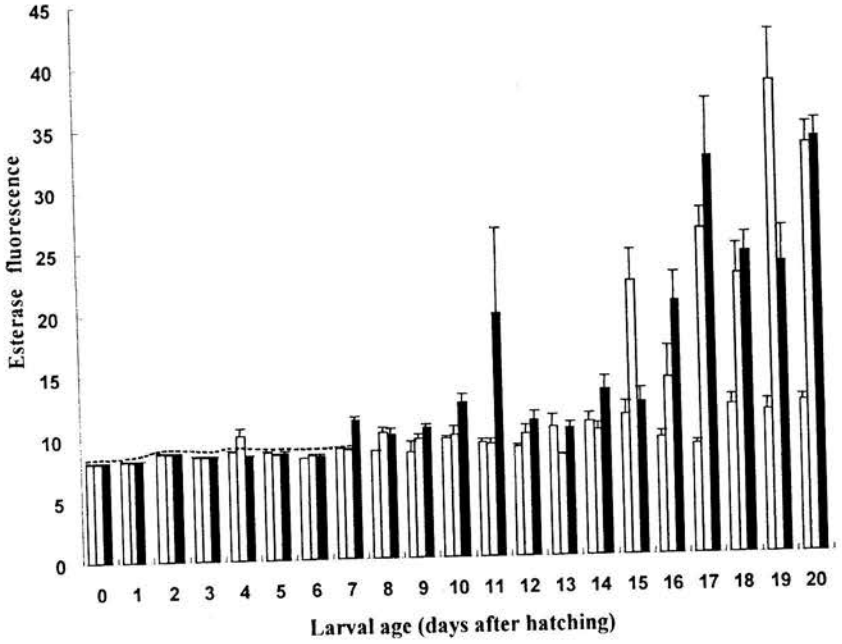

Figure 1. Esterase activity of *Paralichthys olivaceus* larvae fed at a rate of 0.5, 5, and 10 rotifers/ML (white, gray, and black columns, respectively). Vertical bars are SD of three replicates. Mouth opening was on day 3. The dotted line shows enzyme activity of larvae under starved conditions.

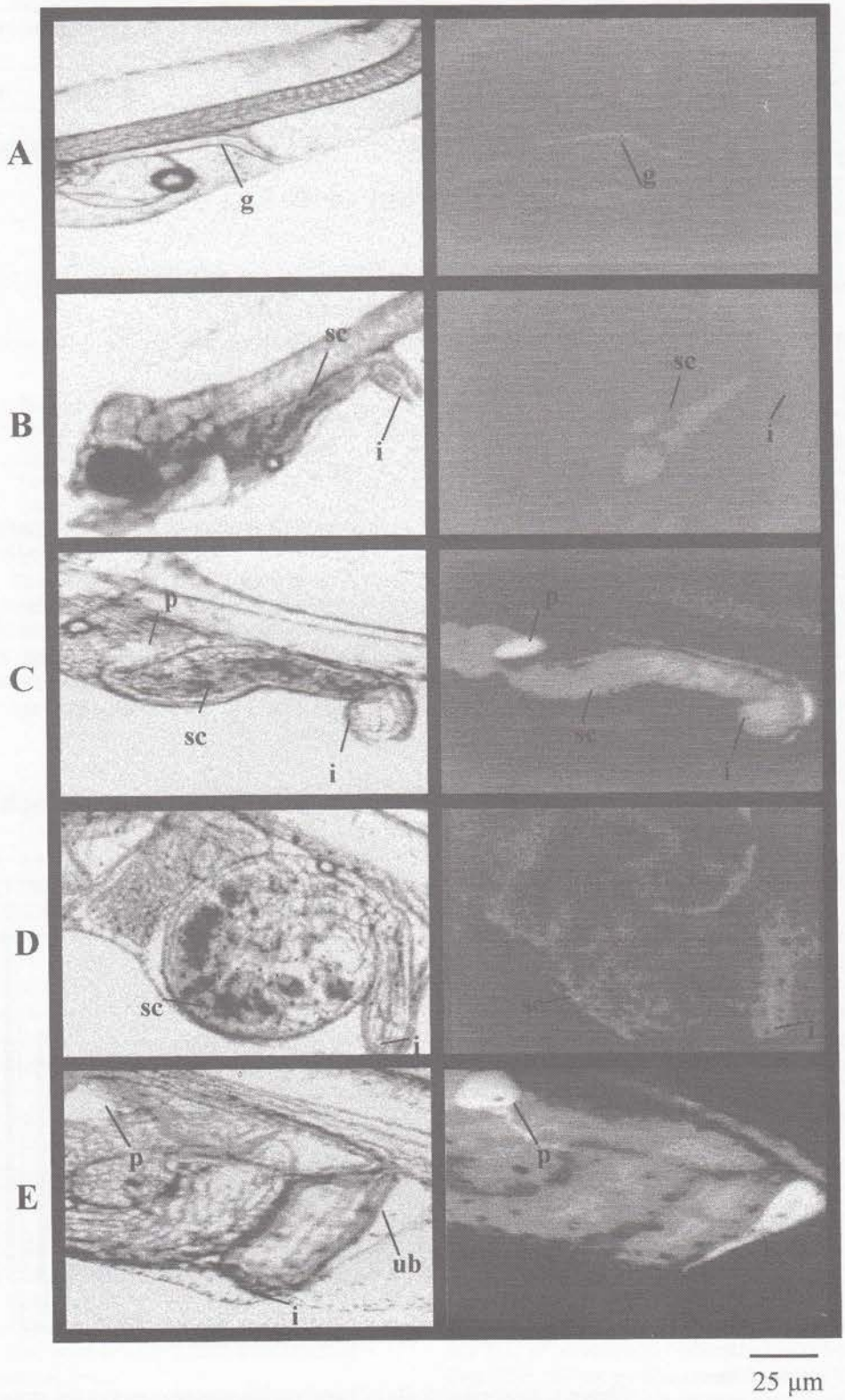

Figure 2. Compound (left) and fluorescent (right) microscopic images of *Paralichthys olivaceus* larva exposed to esterase substrate (cFDAam). Photos A-E (right) show higher esterase activity in larva on days 1, 5, 10, 15 and 20 posthatch, respectively (g – gut, i – intestine, p – pancreas, sc – stomach, ub – urinary bladder).

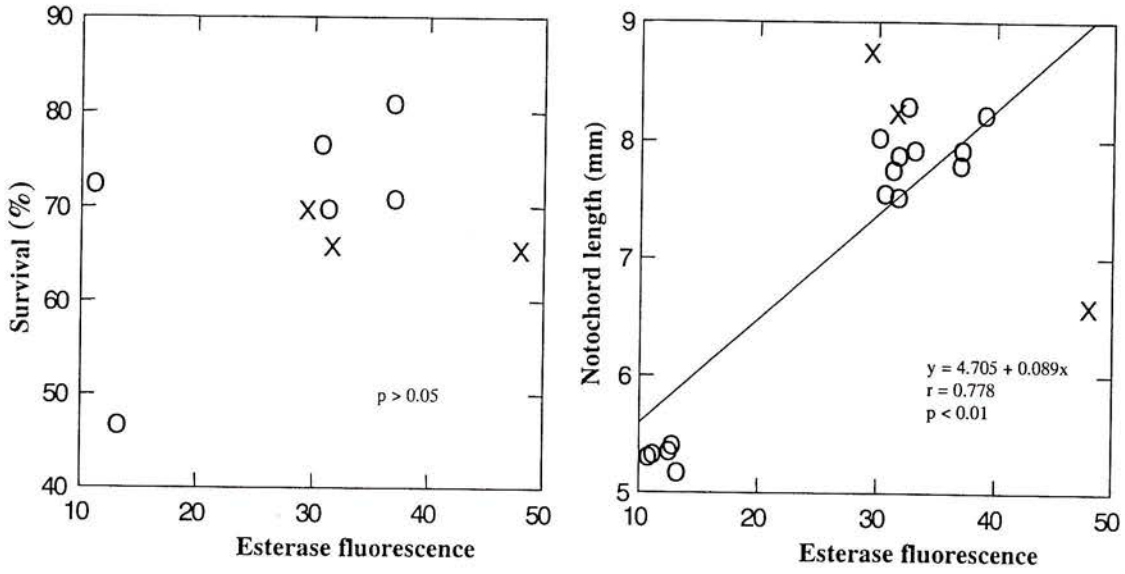

Figure 3. Relationship between esterase activity and survival (left) and between esterase activity and larval growth (right) of *Paralichthys olivaceus* larva. O and X indicate that data were obtained from larval rearing trials from different spawnings.

viscosity (Table 1). High correlations were also obtained for esterase versus rotifer fecundity and lifespan under various viscosities. These indicate that glucosidase and esterase activity measurements of rotifers can be a useful tool to assess physiological condition of rotifers exposed to environmental stress (Table 1).

Fish Larvae

Percent hatching of the fertilized fish eggs was 98%. Esterase and phospholipase activities were detected in *P. olivaceus* larvae from day 0. Even for unfed larvae, these activities were seen until their death (Fig. 1). Esterase activity was specifically localized in the stomach, pancreas and urinary bladder. Localization of esterase activity is shown for larvae on day 5, 10, 15 and 20 posthatch (Fig. 2). Esterase activity was higher as larval length increased. A significant correlation ($P<0.01$; $r = 0.78$) was obtained between the esterase activity and notochord length (Fig. 3). No significant relationship was observed between larval enzyme activity and percent survival.

The increase in fluorescence with larval age indicates that an increasing amount of enzyme is produced by the larvae as they grow older. The observed fluorescence which started in the intestines and appeared later in the stomach indicates that development and production of enzyme starts in the hindgut and then proceeds to the foregut. As found in studies on *Theragra chalcogramma*, the increase in digestive enzyme activities after the transition period could be associated with the morphological changes of the digestive organs.[17] Survival did not show a significant correlation with esterase and phospholipase activity. But whenever these fluorescence intensities were low, survival and growth were also low. The results of these experiments suggest that it is difficult to assess the health of fish larvae at different stages and sizes. More data are required to determine whether the physiological condition of larvae can be determined from enzyme activity data. Monitoring of larval behavior involved in swimming and ingestion could be an another tool to reach this objective.[18,19]

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Evaluation of Several Commercial Enrichment Media for Enhancing the Nutritional Value of Rotifers Fed to Winter Flounder (*Pleuronectes americanus*)

T. Blair, J. Batt, R. Melanson, S. Kirk, and J. Castell

The influence of several commercial enrichment diets on the composition of rotifers (*Brachionus plicatilis*) and the subsequent effects on the survival and lipid composition of larval winter flounder (*Pleuronectes americanus*) fed the enriched rotifers were studied. The survival of the fish larvae appeared to be directly related to the levels of docosahexaenoic acid (DHA) in the enriched rotifers. In addition to the effect of dietary treatment, there appeared to be individual tank influences on larval culture and survival success. These were reflected by differences in total larval lipid content within and among treatments. The lipid content of the larvae perhaps indicates general feeding success and might be used as an early warning index of larval nutrition status.

Introduction

One difficulty in the culture of cool-water marine finfish is overcoming the poor survival from the first-feeding stage to metamorphosis. Larvae have been successfully reared through these stages using diets consisting only of rotifers or rotifers combined with with green water (*Isochrysis galbana*).[1] Though rotifers may be reared on a diet of baker's yeast, they tend to be deficient in the essential fatty acids (EFA) docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) that are critical for survival and development of marine fish larvae.[2,3]

It was reported in the early 1980s that rotifers fed yeast could be nutritionally enriched with DHA and EPA by exposing them to a mixture of fish oils and "omega-yeast".[4-6] Since then a number of commercial "enrichment" media have been marketed for enhancing the nutritional value of rotifers. The effectiveness of three of these commercial media to improve the nutritional value of rotifers was recently evaluated.[7] The objectives of our study were: 1) to confirm this work and include several additional commercial enrichment media, and 2) to evaluate the nutritional value of these enriched rotifers when fed to larval winter flounder (*Pleuronectes americanus*).

Materials and Methods

Stock rotifers (*Brachionus plicatilis*) fed on Microfeast L-10 were enriched for 7 to 14 h in inverted 2-L soft drink bottles which had the bottoms removed. Triplicate rotifer enrichments were done with each enrichment medium: 1) Super Selco, 2) Dry Selco, 3) High DHA Selco, 4) Microfeast L-10, 5) Microfeast L-10E, 6) Microfeast MB-30 and 7) Microfeast MB-32. Rotifers were harvested onto an 80-µm screen and washed to remove excess food. Rotifer samples (1.5 g) were sealed in vials and stored at -80°C until analyzed.

Newly hatched flounder larvae were reared in 118-L medium-density, polyethylene containers with 30° conical bottoms. Feeding commenced when the larvae were 2 days old with 100 000 rotifers added twice a day for 5 days. The quantity of feed provided was then increased to 500 000 rotifers twice a day. Larval culture continued until metamorphosis began (the point when more than 6 larvae had the left eye move across the body midline).

Enrichment media, rotifer and larval samples were freeze-dried. Lipids were extracted and weighed using a $CHCl_3$:MeOH:0.88%$KCl_{(H_2O)}$ (8:4:3)[8] phase separation technique. Fatty acid methyl esters (FAMEs) were prepared by adding 2 mL 7% $BF_{3(MeOH)}$ and 0.5 mL toluene to 1 mg lipid and heating to 50°C for 16 h. Then 5 mL H_2O were added. The FAMEs were extracted in 6 mL hexane and concentrated to 50 µL. FAMEs were applied over a 1-cm streak on a silica gel G thin layer chromatography (TLC) plate. The plate was developed in hexane:diethylether:acetic acid (90:10:1). The methyl ester band (visualized by spraying with 2′-4′-dichlorofluorescene and viewing under UV light) was scraped into a test tube and extracted with 5 mL $CHCl_3$:MeOH (2:1). Silica was removed by filtering through glass wool, evaporating the solvent and dissolving the methyl esters in 0.2 mL hexane for analysis by gas liquid chromatography (GLC).

FAMEs were analyzed using a Varian 3400 GLC equipped with a Supelco Omegawax™ 320 capillary

column and temperature programmed from 160° to 240°C at a rate of 3.5°C/min and held at 240°C for 12.25 min (total run time 35 min). Retention times and integrated peak areas were determined using the Varian Star integration package and peaks were identified by comparisons with known fatty acid methyl esters in the Supelco Omega Test standard or from previously identified peaks in a menhaden oil FAME sample.

The concentration of each identified fatty acid was expressed as a percentage of the total lipid recorded in that sample. GLM ANOVA with Tukey's HSD test with significance set at $P < 0.01$ was used to test for differences in percent composition of each of the fatty acids among the samples. Statistical calculations were performed using SYSTAT (v. 7.0) for Windows.[9]

Results and Discussion

The major EFAs of the enrichment media are reported in Table 1. The content of DHA (22:6n-3) ranged from 3.1% in Dry Selco lipid to 39.1% in High DHA Selco. The EPA (20:5n-3) ranged from 5.1% of the Dry Selco lipid to 27.0% in Super Selco. The arachidonic acid (ARA; 20:4n-6), which has been shown to be essential in juvenile turbot diets[10] and is probably essential in the diet of other larval marine fish, was very low in all enrichment media ranging from 0.4% in High DHA Selco to only 1.7% in Super Selco.

The effects of the enrichment products on the total lipid and major EFA composition of rotifers are presented in Table 1. There were considerable differences in the rotifer lipid content with Microfeast L-10E giving lipid of 13.0% wet tissue weight and Microfeast L-10 fed rotifers having only 4.6% lipid. There was little dietary effect on the content of ARA, but there were significant effects on the DHA and EPA levels. The Dry Selco treatment produced the lowest rotifer levels of DHA (4.4%), while High DHA Selco resulted in the highest level in the rotifers (11.7%). The EPA was lowest in rotifers enriched with Dry

Table 1. Fatty acid composition (mean% SD)* of lipids in enrichment media, rotifers and winter flounder larvae.

Diet	Selco			Microfeast			
	Super	Dry	High DHA	L-10	L-10E	MB-30	MB-32
n	1	1	1	1	-	1	-
ARA**	1.7	0.7	0.4	0.8	-	1.6	-
EPA**	27	5.1	8.9	19.4	-	25.9	-
DHA**	22.8	3.1	39.1	15.2	-	19.1	-
DHA/EPA	0.84	0.61	4.39	0.78	-	0.74	-
Rotifers							
n	3	3	3	3	1	3	-
% Lipid***	7.1 ± 2.1	6.1 ± 1.0	6.3 ± 1.1	4.6 ± 3.6	13.0	6.2 ± 1.4	-
ARA	1.2 ± 0.0	1.1 ± 0.2	1.0 ± 0.0	1.2 ± 0.1	1.2	1.0 ± 0.0	-
EPA	14.0 ± 1.6^{a}	7.0 ± 0.9^{b}	10.6 ± 0.4^{c}	7.6 ± 0.2^{b}	11.0	10.1 ± 1.0^{bc}	-
DHA	10.2 ± 1.2^{a}	4.4 ± 0.5^{b}	11.7 ± 1.0^{a}	4.6 ± 0.1^{b}	9.8	6.4 ± 0.4^{b}	-
DHA/EPA	0.73 ± 0.01^{a}	0.63 ± 0.07^{a}	1.10 ± 0.13^{b}	0.60 ± 0.01^{a}	0.89	0.64 ± 0.02^{a}	-
Flounder Larvae							
n	3	2	3	2	3	2	3
% Lipid	4.6 ± 3.1	3.6 ± 0.7	5.2 ± 1.7	3.6 ± 0.3	4.8 ± 4.0	4.5 ± 1.9	3.7 ± 0.8
ARA	1.7 ± 0.1	1.7 ± 0.2	1.3 ± 0.2	1.5 ± 0.2	1.2 ± 0.4	1.6 ± 0.1	1.5 ± 0.1
EPA	8.5 ± 1.7	7.7 ± 0.5	5.9 ± 0.7	6.6 ± 1.3	8.7 ± 2.9	9.3 ± 0.7	9.0 ± 1.0
DHA	11.6 ± 1.8	9.2 ± 0.6	11.8 ± 2.9	7.6 ± 1.8	8.8 ± 1.8	12.2 ± 1.5	12.4 ± 1.6
DHA/EPA	1.38 ± 0.18^{a}	1.19 ± 0.00^{a}	1.97 ± 0.28^{b}	1.16 ± 0.04^{a}	1.03 ± 0.14^{a}	1.31 ± 0.07^{a}	1.37 ± 0.02^{ab}
% Survival	38.0 ± 13.5	28.4 ± 25.6	42.4 ± 26.4	30.4 ± 33.5	45.1 ± 10.4	36.7 ± 31.4	54.1 ± 7.4

*Different letters indicate significant difference at $P<0.01$.

**Fatty acids as % of total lipid.

***Total lipid as % wet tissue weight.

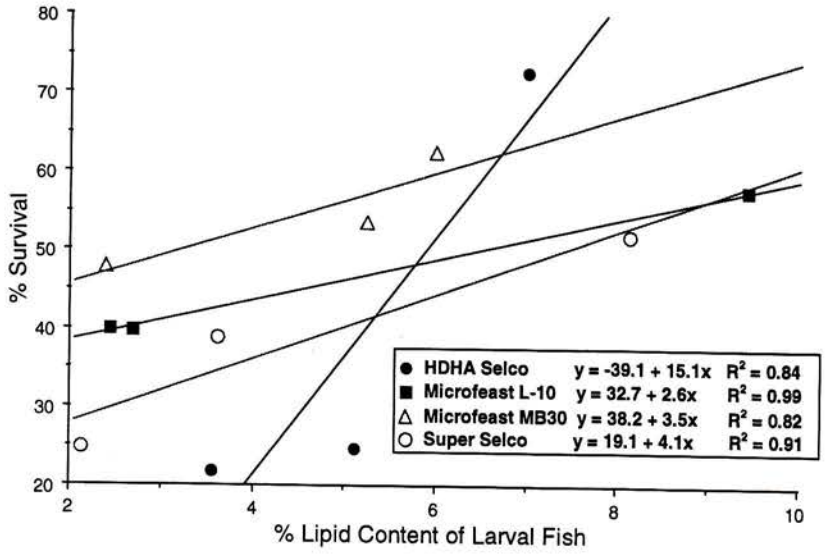

Figure 1. Relationship between total lipid content and survival of larval winter flounder.

Selco (7.0%) and highest in those treated with Super Selco (14.0%). Although the High DHA Selco did result in a DHA/EPA ratio of 1.10, none of the enrichment treatments resulted in a DHA/EPA of greater than 2, which has been suggested as the desired ratio.

The total lipid and fatty acid compositions of larval winter flounder fed rotifers grown on various commercial enrichment media are presented in Table 1. There were much smaller differences in DHA and EPA levels in the fish larvae than there were in rotifers fed the various commercial enrichment diets. Microfeast L-10, which resulted in low DHA levels in rotifers, also produced low DHA levels in larvae. Similarly, High DHA Selco resulted in high DHA levels in both the rotifers and the fish fed the rotifers. However, while the DHA levels in rotifer lipids ranged from 4.4 to 11.7%, the levels in larval lipids ranged from 7.6 to 12.4%. The ratio of DHA/EPA in the fish larval lipids followed the trends set by rotifer lipids, but the ratios in larval lipids were consistently higher than those of the rotifers. This could result from either consumption of the algae used to "green" the water or lipid supplied to the larvae via the yolk.

The mean percentage survival for treatments are reported in Table 1 and the relationship between survival and total lipid is shown in Figure 1. Groups fed rotifers with high levels of DHA (High DHA Selco, Super Selco and Microfeast L-10E, MB-30 and MB-32) had the highest average survival and only MB-30 suffered an individual tank crash. The highest larval DHA level and highest survival were obtained in fish fed rotifers enriched with MB-32. Unfortunately, lipid and fatty acid composition of samples of the media and enriched rotifers were not analyzed. However, there were large variations within treatments in survival. There were also differences within treatments in the total lipid content in larvae. Though three replicates per treatment were not enough to develop a reliable correlation between survival and lipid content, the trends suggest that monitoring total lipid content in individual culture tanks might indicate relative feeding success and larval condition.

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Preliminary Trials Using a Harpacticoid Copepod, *Tisbe* sp., as a Diet for Larval Haddock and American Plaice

Dominic A. Nanton and John D. Castell

Preliminary feeding trials were conducted to compare growth and survival of marine fish larvae (American plaice, *Hippoglossoides platessoides*, and haddock, *Melanogrammus aeglefinus*) fed rotifers (*Brachionus plicatilis*) or harpacticoid copepods (*Tisbe* sp.). The plaice and haddock larvae were observed feeding on the copepods and larvae fed *Tisbe* grew significantly better ($P < 0.10$) than larvae fed rotifers. However, haddock larvae fed *Tisbe* did not survive as well from 0 to 18 days post-hatch as those fed rotifers.

Introduction

The culture of most cold-water marine fish species requires the provision of live prey for a variable period beginning with the onset of larval feeding. When marine fish larvae are offered copepods and other zooplankton such as rotifers *(Brachionus plicatilis*), they tend to select the copepods.[1] Harpacticoid copepod diets have been used for the larviculture of many marine fish species, including saury,[2] mahimahi,[3] and black sea bream.[4]

The advantages of using harpacticoid copepods as live food in marine fish culture are their ability to be mass cultured and high essential fatty acid (EFA) composition (e.g. 20:5n-3 and 22:6n-3) which is independent of the long-chain EFA composition of the diet.[5,6] A further advantage is that any harpacticoids which are not eaten immediately maintain their nutritional value by eating detritus and the biofilm that rapidly develops on the tank (both of which can cause problems in the rearing of marine fish larvae[6]). The disadvantage of using harpacticoids for marine fish culture is that they do not remain suspended in the water column.

A preliminary, small-scale study was performed with American plaice (*Hippoglossoides platessoides*) and haddock (*Melanogrammus aeglefinus*) larvae fed a diet of either rotifers (*B. plicatilis*) or the harpacticoid copepod *Tisbe* sp. The main objective was to determine if the fish larvae would grow and survive on a diet of *Tisb.* Based on nutritional value alone, the larvae would be expected to show increased growth and survival because of the high *EFA* value of *Tisbe* compared with rotifers. However, this benefit might not be realized because *Tisbe* sink to the bottom of the tanks and are less available than the rotifers to the fish larvae swimming throughout the water column.

Materials and Methods

Eggs from one naturally-ripe American plaice were stripped and dry fertilized with the sperm from an adult male. The fertilized eggs were transferred to floating trays that had 200-µm mesh bottoms and were held in a flow-through seawater system at 2°C. Hatching occurred approximately 14 days after fertilization. Before the larvae were placed in culture containers, initial lengths were measured using vernier calipers The containers used to culture the fish larvae consisted of 15-cm (6-in) diameter white PVC pipe 15 cm in height with a 40-µm mesh screen inserted 5 cm from the bottom (total volume 1.9 L). The containers were placed in tanks provided with flow-through seawater at 2.0°C (range 1.2 to 2.6°C). The larvae were cultured in green water with approximately 250 mL (ca. 5 million cells/mL) of the alga *Isochrysis galbana* (clone T-iso) added daily. To allow for optimal aeration, a stream of air was directed over the surface of the tanks. Two arches (2 x 3 cm) were cut from the bottom of each container to improve water flow and exchange through the mesh bottom. One container holding 20 plaice larvae was fed with the *Tisbe* diet and four containers, each with 50 plaice larvae, were fed the rotifers. The imbalance in the numbers of fish larvae in the containers was due to an insufficient number of copepods being available to feed a large number of fish larvae. The main objective, however, was to determine whether the fish larvae would grow and survive when fed a diet of *Tisbe*.

The rotifers were fed to the plaice larvae daily at an initial rate of approximately 10 animals/mL. The density of *Tisbe* in the larval culture containers was approximately 0.9 copepods/mL in the > 200-µm size fraction (mainly adults) and 3.5 copepods/mL in the < 200-µm size fraction (copepodites and nauplii).

Table 1. Survival and growth measurements of American plaice (*Hippoglossoides platessoides*) larvae reared at 2°C and fed harpacticoid copepods (*Tisbe* sp.) or rotifers (*Brachionus plicatilis*). The copepods were fed baker's yeast and the rotifers were fed Microfeast® L-10 larval diet.

	Copepod-fed [a]		Rotifer-fed [b]		
Time (days)	Length (mm)	% Survival (n=20)	Length (mm)	% Survival Median	Range
0	4.21 ± 0.31	100	4.35 ± 0.38	100	100-100
6	6.32 ± 0.43*	–	5.56 ± 0.56	–	–
12	–	65	–	24	68-10
15	–	25	–	12	48-6
23	–	0	7.61 ± 0.72	4	14-0

[a] Survival in plaice larvae in a container with an initial population of 20 larvae
[b] Survival in plaice larvae held in 4 containers each with an initial population of 50 larvae
* Significant differences at $P < 0.05$

Fertilized haddock eggs, obtained from Dr. K.G. Waiwood (Biological Station, Department of Fisheries and Ocean, St. Andrews, NB) were placed in floating trays and treated similarly to those of the plaice. Newly-hatched larvae were measured, separated into two culture containers, and reared in the same conditions as those used for the plaice larvae. There were 30 larvae in each container and they were fed either the rotifers or copepods. The concentration of *Tisbe* was approximately 0.7 copepods/mL in the >200-µm size fraction (mainly adults) and 2.7 copepod/mL in the < 200-µm size fraction (copepodites and nauplii). The rotifers were fed at a concentration of approximately 10 animals/mL.

The cultured harpacticoid copepods were fed a diet of baker's yeast in excess (ca. 1 mg dry weight of diet/L of seawater) and maintained at a temperature of 20 ± 1°C in MacDonald Plexiglas hatching jars provided with constant gentle aeration. The rotifers were cultured at 20°C and fed 3 g of Microfeast L-10® larval diet daily (3 million rotifers in 100 L of 20-ppt seawater).

Results and Discussion

The plaice larvae that were fed copepods (*Tisbe*) had significantly higher growth ($P < 0.05$) than those fed rotifers, when assessed after 6 days (Table 1). This result may be partially due to the fact that the *Tisbe*-fed larvae were reared at a lower density than those fed rotifers. At 12 days post-hatch, survival of the *Tisbe*-fed plaice larvae (65%) was similar to that of the rotifer-fed larvae (68%). However, survival of the plaice larvae that were fed rotifers was variable (Table 1) and survival in both groups declined precipitously 2 to 3 weeks after hatching. This mortality could have been due to a number of factors: 1) deteriorating water quality, particularly in the tanks receiving *Tisbe* as food (the copepods could not be completely separated from the detritus in the culture container before they were fed to the fish larvae); 2) inappropriate containers used to culture fish larvae (containers with dark walls and conical bottoms would have been preferable); and 3) rotifers may have been too small as live food for the 2-week-old plaice larvae. Even so, the larvae were observed to feed on *Tisbe*, and relatively good growth and survival of the larvae was obtained for the first two weeks after hatching even though the culture conditions were less than optimal.

The haddock larvae fed the copepods demonstrated better growth but poorer survival compared to larvae fed rotifers (Table 2). Unlike the culture conditions for the plaice larvae, haddock larvae fed the two diets were reared at the same density, suggesting that the better growth was due more to diet than to the effect of stocking density. Feeding behavior of the haddock on the *Tisbe* larvae was also observed. The largest decline in survival was during the first 2 weeks post-hatch for the *Tisbe*-fed larvae and after approximately 2.5 weeks for the rotifer-fed larvae. The reasons are probably similar to those proposed for the plaice larvae.

One first-feeding haddock larva fed *Tisbe* for 17 days after hatch was thin-sectioned longitudinally so that the gut contents could be examined. This larva contained a partially digested adult or later-

Table 2. Survival and growth measurements of haddock (*Melanogrammus aeglefinus*) larvae reared at 2°C and fed harpacticoid copepods (*Tisbe* sp.) or rotifers (*Brachionus plicatilis*). The copepods were fed baker's yeast and the rotifers were fed Microfeast® L-10 larval diet.

Time (days)	Copepod-fed		Rotifer-fed	
	Length (mm)	Survival (%) (n=20)	Length (mm)	Survival (%) (n=30)
0	3.47 ± 0.41	100	3.47 ± 0.41	100
8.5	5.36 ± 0.76*	57	4.57 ± 0.15	93
18.5	5.89 ± 0.61**	23	5.00 ± 0.41	57
21.5	–	10	–	10
25.5	–	3	–	7

* Significant differences at $P < 0.10$
** Significant differences at $P < 0.05$

stage copepodite (length 200 µm) in the stomach lumen, a smaller early copepodite (length 100-200 µm) in the intestinal lumen, and a third well-digested copepod in the most posterior section of the intestinal lumen.

These preliminary larval feeding trials demonstrated that both haddock and plaice larvae will readily ingest the harpacticoid copepod *Tisbe* and will grow on this diet for the first two weeks after hatching. The culture conditions for these fish larvae were not optimal and work needs to be done on a larger scale with better culture methods to determine whether rotifers or *Tisbe* are superior as a live food diet. The copepods spend most of their time on the walls and bottom of the culture container, so are not as accessible to the fish larvae as the rotifers which swim in the water column. This also may be a reason for the decline in survival that occurred approximately two weeks after hatching.

In summary, the more rapid growth of the marine fish larvae fed *Tisbe* compared with those fed rotifers could be due to either (or both) the increased EFA value of the copepod or the lower larval density in the copepod-fed tanks. The low survival of the copepod-fed haddock larvae could be due to the inaccessibility of the live food to the larvae. A floating tray system similar to the one designed by Kahan et al.[7] could be used to make *Tisbe* more accessible to the fish larvae. Because of their detritivorous benthic nature, *Tisbe* may better serve as a tank cleaner and an EFA-rich live food supplement to rotifers and brine shrimp, rather than as the sole live food diet.[6] Further studies comparing growth and survival of fish larvae on a live food diet with or without *Tisbe* should be conducted to determine the value of *Tisbe* as a live food supplement.

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The Production and Use of Copepods in Larval Rearing of Halibut, Turbot and Cod

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This paper summarizes some of the results from a 2-year Concerted Action project financed by the European Commission. The aim of the project was to review the literature on the use of live feed and the rearing techniques for cod, *Gadus morhua*, halibut, *Hippoglossus hippoglossus*, and turbot, *Scophthalmus maximus*. Ongoing research was also coordinated to provide further knowledge in key areas identified during the project. Analytical work was carried out to supplement present knowledge in the field of larval nutrition. Different rearing strategies are used for the three species of fish larvae examined: rotifers and *Artemia* nauplii are commonly used as live prey for turbot, whereas for cod and halibut culture, copepod-based rearing techniques have gradually been replaced by more traditional techniques based on the use of *Artemia* nauplii alone or with rotifers as a starter diet. The shift to more traditional live prey has been accompanied by an increased incidence of malpigmented or deformed juveniles. Copepods are unquestionably nutritionally better than rotifers and *Artemia*, but because of the difficulties in obtaining sufficient numbers of copepods at the right size and at the right time, rotifers and *Artemia* remain the preferred species for larviculture. A compromise adopted by many hatcheries is to provide copepods for a short duration during the larval phase, a strategy that has been shown to be particularly important for obtaining high survival rates and normally-pigmented juvenile halibut. In extensive turbot culture systems using copepods as live feed, *Artemia* are still used when the copepod supply is decreasing, as they provide an important energy source in the form of triacylglycerols.

Introduction

In 1995, a 2-year Concerted Action project funded by the European Commission was initiated. The project reviewed the literature on the rearing of cod, *Gadus morhua*, halibut, *Hippoglossus hippoglossus*, and turbot, *Scophthalmus maximus*, with particular reference to the use of live food. An economic evaluation of halibut production and its prospects was also included in the project,[1] and ongoing research on larval rearing and copepod culture was co-ordinated to provided new information in key areas identified in the review.

The larviculture of most marine fish species depends on the provision of live prey during the larval stage. Successful mass culture techniques for rotifers and the easy acquisition and hatching of *Artemia* nauplii have resulted in their widespread use and the rapid development of fish rearing technologies. *Artemia* and rotifers provide a wide size range of prey that are applicable for most marine fish species. However, they are inherently nutritionally inadequate, particularly in essential fatty acids such as eicosapentaneoic acid (20:5n-3, EPA), docosahexaneoic acid (22:6n-3, DHA) and arachidonic acid (20:4n-6, ARA). These fatty acids are important for the normal development of several marine species and recently there was been interest in the significance of the dietary ratio of DHA to EPA. Although significant nutritional improvements have been gained from using enrichment emulsions for culturing rotifers and *Artemia* nauplii, rearing success with species such as halibut has, until recently, been limited.

Materials and Methods

The project was comprised of several literature reviews and the coordination of ongoing work. The production and use of live prey (rotifers, *Artemia* and copepods) was reviewed as well as their use in marine fish larviculture.[2,3] Further, the methods for rearing cod, halibut and turbot were reviewed[4,5] and several experiments, using a variety of methods, were carried out on these species during the course of the project.[6-13] Finally, the biochemical and nutritional analytical procedures used in larviculture were coordinated, sampling from different sources were planned, and several experiments were conducted to provide novel information in this field.[14-16]

Results and Discussion

In terms of larval survival and growth, halibut, cod, and turbot rearing systems depending on naturally-produced prey organisms in extensive rearing systems have in most studies proven to be superior to strategies using cultured prey organisms in intensive systems.[17-20] It has not been determined whether this is a direct result of the diet or of the lower larval densities and general biotic and abiotic conditions in the extensive systems.

When traditional live foods fail to fulfill the nutritional requirements of fish larvae in intensive culture systems, copepods are often used as an alternative or supplementary live food. Attempts have been made to develop intensive cultures for several species of calanoids and harpacticoids such as *Acartia* sp., *Paracalanus* sp., *Eurytemora* sp., *Tigriopus japonicus* and *Tisbe* sp., and of the cyclopoid copepod *Oithona* spp.[21-24] However, the the large-scale laboratory culture of copepods is still in the developmental stage and this is the major constraint to the use of copepods in commercial hatcheries.

Several attempts have been made to culture copepods in outdoor tanks on a large scale. The calanoid *Acartia tsuensis* has been cultured successfully in 24-m^3 outdoor tanks and fertilized sea water with sustainable exploitation rates of 27% per day.[25] Between 1 and 5 kg of the harpacticoid *Tigriopus japonicus* have been harvested at regular intervals from co-cultures with rotifers in outdoor 200-tonne concrete tanks for a period of about 3 months.[26] These zooplankton were seeded to extensive cultures of *Chlorella minutissima*, and baker's yeast and enriched yeast (w-Yeast) were added at regular intervals. The maximum copepod density registered in this system was 22 048/liter. In Denmark, success in rearing of turbot juveniles on a commercial scale is dependent on outdoor cultures of copepods and other natural zooplankton. The zooplankton are collected from the fjord where the hatcheries are situated and added to plastic-lined earthen ponds or to outdoor tanks (as in the case of the Maximus A/S turbot hatchery). In the latter system, the copepods are grown in an outdoor tank and used to supplement the declining copepod populations in the larval rearing tanks. In this outdoor culture, a predominance of 4 calanoid copepod species were registered,[27] but other species of calanoids may be present and benthic harpacticoids are usually in abundance in such systems. But even in outdoor systems the cultures may periodically be dominated by one planktonic species, as seen in the outdoor rearing systems used for cod in Denmark.[28]

Cod and halibut are also reared in outdoor units in Norway using natural zooplankton. These systems, ranging from the utilization of natural lagoons to plastic bags within lagoons have been described in detail.[4] The species of zooplankton reported as dominant by these authors were mainly copepods. The spring bloom of copepod nauplii in the lagoon systems are largely attributed to the hatching of resting eggs. When bags are used for larval rearing, they are filled with filtered seawater and the copepods are harvested through filters that concentrate a specific size range. Initially, copepod nauplii are used for the first-feeding fish larvae. They are concentrated using 80 μm and 250 μm filters. The larger copepod sizes are provided by replacing the 250 μm filter with a 350 or a 600 μm filter.[4]

More recently, marine copepods have been reared in outdoor tanks ranging from 1.6 to 250 m^3 in size.[29] The main species was the calanoid *Eurytemora affinis*, but *Centropages hamatus* and *Acartia* spp. were also found in the culture. The naupliar peaks ranged from 200 to 400 individuals/liter and the copepodite and adult peaks were 25 to 50 individuals/liter. These trials showed the potential for initiating copepod cultures inoculated with sediments containing resting eggs. The copepod diet, both in size and HUFA content, was shown to be important for the success of the culture of calanoids. It was estimated that large mesocosm tanks with a 10% daily exploitation rate could supply the energetic demands of 26 to 102 halibut larvae /m^3 culture volume for a 2-month period under local Norwegian weather conditions.[29] Higher production values were estimated for the more intensive cultures, although the period of culture may be much shorter.

The commercial viability of these semi-extensive systems has been demonstrated by Maximus A/S, a turbot hatchery in Denmark. This company has further refined the extensive rearing method described by Paulsen and Andersen.[30] This reliable method resulted in the productions of about half a million turbot juveniles annually beginning within 3 years of its start in 1990. These relatively small mesocosms are strictly monitored daily to ensure a steady supply of algae and copepods within the rearing tanks. A separate tank

used only for copepod cultures is used to supplement the copepod supply if this becomes necessary. As the turbot larvae grow, *Artemia* nauplii are added to the system, although this is usually not necessary during the first week of feeding.[4,31] Because the majority (99%)[31] of the fish produced in this system are normally pigmented (in Europe it is relatively difficult to sell turbot juveniles which are not well pigmented), there is a low percentage of discards of the valuable juveniles from the system. The critical moment for these culture systems is the timing of the zooplankton production and the addition of the fish larvae.[32] It appears important to have a good and growing supply of algae in order for the copepod broodstock to provide a constant supply of nauplii for the first-feeding fish larvae.

Cod and halibut have been reared on the copepod-based system, but more recently a shift towards the more traditional rotifer and *Artemia*-based methods has been evident. Concurrent with this trend is an increased incidence of abnormal juveniles. Experimental work indicates that fish species have different requirements. Turbot developed normal pigmentation when provided a mixture of copepods, but when the traditional diet was supplemented with monospecies of copepods, no correlation between the diet and incidence of normal pigmentation was found.[12,33] Halibut larvae reared on copepods (*Eurytemora velox*) developed superior pigmentation characteristics compared to those receiving enriched *Artemia*.[13] Furthermore, diet-related differences in rod:cone ratios were indicative of abnormal eye development among the *Artemia*-fed halibut. In a separate experiment, better pigmentation results were obtained when halibut larvae feeding on an *Artemia* diet were switched to copepods for 7 days before they had reached 16 mm in length.[11] After the 7-day period they were switched back to *Artemia* nauplii. Prey densities were increased from 1000 to 2000/L as the larvae grew. The copepods were harvested from an enclosed basin or from 250-m outdoor tanks used for copepod cultures.[29]

Biochemical analyses have confirmed copepods as a superior diet, suggesting that they should be the first choice for ensuring good growth, survival and development in marine fish larvae. However, the difficulties in obtaining sufficient quantities at the right time discourage their use. The risk of pathogen and parasite transmission via copepods can also be of concern in extensive production systems. It is apparent that the supply of copepods even for a limited period of time and in relatiively small quantities is crucial for the normal development of certain species such as halibut. Apart from essential nutrients, which only the copepods seem to be able to provide, enriched *Artemia* are also an important food source, providing the energy required for the rapidly-developing larvae in the form of triacylglycerols. In addition, vitamin C enriched *Artemia* may prove useful in stress prevention.

In intensive culture systems using manipulated broodstock which provide eggs year round, large-scale, year-round production cultures of zooplankton are required. Rotifers and brine shrimp are widely used and the culture techniques are well developed. However, the nutritional problems associated with the use of these prey have not been solved and there is a renewed interest in culturing copepods. While the extensive cultivation of copepods is biologically and economically feasible, and adaptable for commercial purposes, the development of intensive large-scale culture systems for calanoid or harpacticoid copepods has not been attempted or tested for economic viability.

Generally, planktonic filter-feeding species of copepods require large volumes of water and tolerate only low adult densities (<l00/L). Benthic grazers, such as many harpacticoids, may be cultured at high densities of several thousands per liter. This contrast in culture densities is demonstrated in the large-volume, labor-intensive method developed for *Acartia tonsa* (approximately 530 eggs/L culture volume)[22] and the small-volume method developed for *Tisbe holothuriae* (100 000 nauplii/L culture volume).[3] The benthic species may be produced more intensively by merely providing more surface area. In traditional culture tanks this can be achieved simply by filling the tanks with biofilter media. Planktonic filter-feeders often depend on a diet of planktonic algae, whereas benthic surface scrapers can feed on a variety of feeds, including inert food. This means that in culturing benthic surface scrapers, the requirements for algal cultures may be eliminated.

Production techniques for the harpacticoid *T. holothuriae* is similar to that for rotifer production in terms of productivity, labor-requirements, and stability of the system[3] and this species has been proposed as a substitute for rotifers in commercial systems. On a large scale, a rotifer tank can be transformed to a harpacticoid tank simply by filling up the tank with biofilter elements and thus increasing the surface area. Essentially the culture can be run the same as that for rotifer culture (batch cultures), although it is suggested that the tanks be emptied daily through two filters; a 180-µm superimposed in a 60- or 80-µm filter. The filtrate in the 180 µm mesh size could be returned to the refilled culture tank. Those harpacticoids retained by the 60 or 80 µm mesh size can be used for feeding or can be stored at < 5°C for up to 3 to 5 days before being used as food for fish larvae. Algae could be added to the culture tank to ensure a food supply for the copepods. A good rule of thumb is to apply 1 µg dry weight algae per adult in culture. Continuous culture of this species is also possible,[3] although the technique needs to be refined and tested in terms of

long-term stability and reliability. Kahan et al.[23] used floating baskets within the larval tanks to produce nauplii for the fish. The nauplii could swim through the bottom filter which retained the adult population. Such a system can also be used to supplement fish with a copepod diet.

It is often not realized there can be substantial populations of calanoid and harpacticoid copepods present in the filters of intensive hatcheries. Very few facilities have attempted to utilize these. The copepods can be collected and used for scaling up and as a dietary supplement for fish larvae. A simple approach would be to filter these copepods (for example between 100 and 250 μm) to reduce the presence of other plankton. An initial period of laboratory adaptation may be required before these organisms can be cultured more intensively. During the scaling-up period, overfeeding often causes the culture to collapse. A batch system is recommended for the initial period with a 7 to 10 day cycle and feeding with algae once every day or two. Not all copepod species are equally robust and after several batch cycles one species may dominate. If this species has the appropriate size and is readily consumed by the fish larvae, optimization of the culture system can then take place.

Recent results comparing the fatty acid content and distribution in different life stages of copepods with that in enriched *Artemia* showed that in terms of HUFA, the content of DHA, and the ratio of DHA to EPA, copepods are superior to enriched *Artemia* nauplii [34] primarily because of retroconversion of DHA to EPA by the *Artemia* nauplii.[35] This work only focused on fatty acids, and the content of arachidonic acid has also recently been recognized as being important. The copepods may contain other essential ingredients which have not yet been identified.

The challenge of devising reliable intensive production systems for copepods remains open for the next century. Until then, the convenience of purchasing and hatching of *Artemia* eggs will ensure their continued use and direct work towards solving the nutritional problems that arise from using *Artemia.*

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Calendar

• **Courses — Practical Aquaculture Technologies,** 19 – 30 July and 19 July – 6 August 1999, Harbor Branch Oceanographic Institution, Ft. Pierce, Florida. Week 1: recirculating systems (systems design & operation; removal, aeration and disinfection systems; flow requirements and carrying capacity; water quality parameters). Week 2 & 3: culture techniques for molluscs, crustaceans and finfish live feeds (microalgae, rotifers and artemia); site selection; health management; feeding protocols. Information: Dr. M. Davis-Hodgkins (tel 561 465-2400, fax 561 466-6590, e-mail acted@hboi.edu). This 2- or 3-week course costs US$1995 and $2995 (includes housing).

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• **US Trout Farmers Association Meeting and Trade Show,** 18 – 20 August 1999, Sheraton Inner Harbor Hotel, Baltimore. Program will highlight environmental concerns, such as effluents, and a workshop/short course will be held on fish production. Information: Pat Bethany (tel 304 728-2189, fax 304 728-2196, e-mail ustfa@intrepid.net).

• **American Fisheries Society Annual Meeting, Trade Show and Exhibition,** 29 August – 1 September 1999, Charlotte, North Carolina. Information: AFS 1999 Annual Meeting, Amy Fink. 5410 Grosvenor Lane, Suite 110, Bethesda, Maryland 20814 (tel 301 897-8616 (ext. 214), fax 301 897-8096).

• **ICES Symposium on the Environmental Effects of Mariculture,** 13 – 16 September 1999, St. Andrews, NB. Forum to share research results and enhance international cooperation and collaborative research on 1) the environmental effects of bivalve and fish farming in the coastal zone, and 2) the influence of local environmental factors on mariculture productivity. Information: Dr. D. Wildish, DFO, St. Andrews, NB EOG 2X0 (tel 506 529-5894, fax 506 529-5862, e-mail wildishd@mar.dfo-mpo. gc.ca).

• **Aliia HELEXPO**, 7th International Exhibition of Fisheries, Aquaculture and Relevant Equipment, 23 – 26 September 1999, Thessalonki, Greece. Information: HELEXPO, 154 Egnatia Str., 546 36 Thessaloniki, Greece (tel +30 31 291 111, fax +30 31 229 116).

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• **Marketing & Shipping Live Aquatic Products '99,** 14 – 17 November 1999. DoubleTree Hotel, Sea-Tac Airport, Seattle. Agenda: improved handling technologies, resource management, regulatory concerns, unwanted introductions of non-indigenous species, economics, and animal welfare issues. Information: JB Peters, 5815 NE Baker Hill Road, Bainbridge Island, WA 98110 USA (fax 360 394-3760, e-mail JohnBPeters@compuserve.com, website http://www.alaska.net).

• **Aquaculture Venezuela '99 and 2nd South American Aquaculture Congress,** 17 – 20 November 1999, Puerto La Cruz, Venezuela. Sponsored by the Latin American Chapter of the World Aquaculture Society. Contact: J. Cooksey, Conference Manager, 21710 7th Place West, Bothell, Washington, USA (fax 425 483-6319, e-mail worldaqua@aol.com).

• **Aquaculture America 2000**, 2 – 5 February 2000, New Orleans, Louisiana. Annual meetings of the US Chapter of the World Aquaculture Society, the American Tilapia Association, Striped Bass Growers Association, AFS Fish Culture Section, and the Louisiana Aquaculture Association. Sessions: freshwater crustacean, tilapia, red drum, marine shrimp, tropical fish, reptile, amphibian, salmonid, molluscan, and striped bass culture; water quality; aquaculture regulations; ploidy manipulaiton and sex reversal; recirculating systems; computers and aquaculture; nutritional requirements and diet formulation for shrimp and fish; and aquaculture as a teaching tool. Contact: John Cooksey, Conference Manager, 21710 7th Place West, Bothell, Washington, USA (telephone 425 485-6682, fax 425 483-6319, e-mail worldaqua@ aol.com). Deadline for abstracts: 31 July 1999.

• **International Conference on Risk Analysis in Aquatic Animal Health,** 8–10 February 2000, Paris, France. Sessions: the need for risk analysis; risk analysis methodology; areas of application to aquatic animal health including problems, research needs and environmental concerns, case histories and field studies; and recommendations and future prospects. Information: Dr. K. Sugiura, Office International des Epizooties, 12 Rue de Prony, 75107, Paris, France (tel 33 (0)1 44 15 18 88, fax 33 (0)1 42 76 09 87, website http://www.ole.int).

• **Conference on Aquaculture in the Third Millennium and Aquaculture and Seafood Fair 2000,** 21 – 25 February 2000, Bangkok, Thailand. Sessions: integrating aquaculture into rural and coastal development; aquaculture and poverty alleviation; involving stakeholders in policy making, planning and management; promoting sustainable aquaculture with economic incentives; building the information base for policy making; establishing legal, institutional and regulatory frameworks; aquaculture production systems; genetics, health management and disease control; nutrition and feeding; culture-based fisheries and enhancement; systems approach to aquaculture management. Exhibitions will be held on aquaculture nutrition and health, seafood and cold storage, and ornamental fish. Exhibition information: Ms Natprapa Yokputtaraksa, Production Management & Services Co., Ltd. (tel 66-2 862-113-4, fax 66-2 862-1132, e-mail pmsco@asiaaccess.net.th).

• **AQUA 2000,** 2 – 6 May 2000, Nice, France. Annual meetings of the World Aquaculture Society and the European Aquaculture Society. Information: John Cooksey, Conference Manager, 21710 7th Place West, Bothell, Washington, USA (tel 425 485-6682, fax 425 483-6319, e-mail worldaqua@ aol.com).

• **Annual Meeting of the Canadian Society of Zoologists,** 3 – 6 May 2000, St. Andrews, NB. Information: Dr. M. Burt, Huntsman Marine Science Centre, St. Andrews, NB (tel 506 529-1222, fax 506 529-1212, e-mail mburt@nbnet. nb.ca).

• **The 9th International Symposium on Nutrition and Feeding in Fish**, 21 – 25 May 2000, Miyazaki, Japan. Topics include: Challenges and strategies for aquafeed development in the 2000s, nutrient requirements and availability, nutrient metabolism and its control, alternative protein sources, fish health with reference to fish feed, larval and broodstock nutrition, and nutritional strategies and management of aquaculture waste. Information: Prof. T. Takeuchi, Tokyo University of Fisheries, Konan 4, Minato, Tokyo 108-8477 (tel +81-3-5463-0545, fax +81-3-5463-0553, e-mail take@tokyo-u-fish.ac.jp, website http://www. tokyo-u-fish.ac.jp/fish-nutrition).

• **Aquaculture Canada 2000,** 28 – 31 May 2000, Hotel Beausejour, Moncton, NB. 17th annual meeting of the Aquaculture Association of Canada. This millenial conference and exposition will cover a broad spectrum of aquaculture topics and will attract growers, scientists, administrators, educators and students. Information: Dr. Andrew Boghen, Dept. Biologie, Université de Moncton, Moncton, NB E1A 3E9 (tel 506 858-4321, fax 506 858-4541, e-mail boghena@umoncton.ca).

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Information:
Dr. Andrew Boghen
tel 506 858-4321, fax 506 858-454
e-mail boghena@umoncton.ca

• **Fishery 2000 Guangzhou,** The International Fishery Exhibition, 30 May – 1 June 2000, Chinese Export Commodities Fairground, Guangzhou, P.R. China. Exhibition of seafood, commercial fishing, fish farming and fish processing equipment and technology, seafood transportation systems, refrigeration equipment and technology, and seafood packaging. Information: Top Repute Co., Ltd., Room 2403, Fu Fai Commercial Centre, 27 Hillier Street, Sheung Wan, Hong Kong (tel 852 2851 8603, fax 852 2851 8637, e-mail topreput@hkabc. net).

• **3rd International Conference on Shellfish Safety,** 19– 24 June 2000, Southhampton College, Long Island University, NY. As with previous symposia in this series, presentations will be given dealing with shellfish biology and ecology, chemical and microbiological contamination and assessment, impacts of harmful and toxic algae, depuration technology, monitoring and management, aquaculture and harvesting sites, health and sanitation, and quality assurance programs and regulatory controls. Information: Dr. Sandra Shumway, Natural Science Division, Southampton College, 239 Montauk Highway, Southampton, NY 11968 USA (fax 516 287-8419, e-mail sshumway@southampton.liunet.edu).

• **Coastal Zone Canada 2000**, 17 – 22 September 2000, Reade and Convention Centre, Saint John, NB. Theme: Coastal Stewardship: Lessons Learned and the Paths Ahead. The conference will focus on 4 related subthemes: Aboriginal Practices, Community-based Actions, Coastal Health and Oceans Governance. Information: Coastal Zone Canada 2000 Secretariat, Department of Fisheries and Aquaculture, P.O. Box 6000, Fredericton, NB E3B 5H1 (tel 506 453-2253, fax 506 453-5210, e-mail czczcc2000@gov.nb.ca, website http://www.gov.nb.ca/dfa/czczcc2000.htm.

• **Third World Fisheries Congress,** 31 October - 3 November 2000, Beijing, P.R. China. Topics: Effect of sustainable fisheries on optmizing food composition and improving human health, scientific management, reasonable exploitation and protection of fisheries resources, fisheries technologies, effect of the fishery environment on sustainable development, healthy aquaculture and ecosystems, bio-technology and fisheries, aquatic products processing and comprehensive utilization, bio-diveristy and protection, fishery machinery and instruments, fishery economics, fishery policies and sustainable fisheries development, and application of information technology in fisheries. Conference Secretariat: China Society of Fisheries, Bldg 22, Maizidian Street, Chadyang District 100026, Beijing, P.R. China (tel 86 10 64194233, fax 86 10 64194231, email csfish@agri.gov.cn).