ENRICHMENT OF *ARTEMIA* FOR USE IN FRESHWATER ORNAMENTAL FISH PRODUCTION

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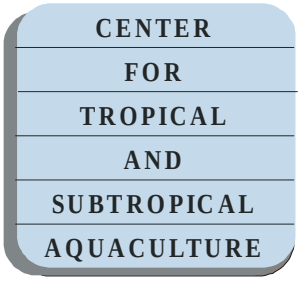


TABLE OF CONTENTS

INTRODUCTION

The dietary requirements of all aquaculture species can be categorized by five different classes of compounds: 1) proteins, 2) lipids, 3) carbohydrates, 4) vitamins, and 5) minerals. It is not the intent of the authors to provide an exhaustive review of each nutrient mentioned but rather to focus on the importance of the lipids, in particular the fatty acids, in aquaculture. A comprehensive introductory text for review of all of the components of an artificial diet was written by Tacon (1990). The purpose of this manual is to describe what fatty acids are, their nutritional importance, and ways they can be used to enrich live feeds. Two live feeds which are ubiquitous in the culture of aquatic organisms, the rotifer *Brachionus sp.* and the nauplii of the brine shrimp *Artemia*, are the main vehicles by which the nutritional components are delivered to the fish larvae. This manual focuses on the use of and enrichment of *Artemia*. The rotifer is a topic to be covered under a separate manual and the authors refer the reader to the publications by Snell and Hoff (1988), Fulks and Main (1991) and Tamaru et al. (1993a) for more information regarding the use of rotifers in hatchery operations.

LIPIDS

Lipids are substances found in both plants and animals. They are relatively insoluble in water yet soluble in organic solvents such as ether, chloroform, and toluene. Lipids fall into two basic categories (glycerol-based and nonglycerol-based) according to the presence of the alcohol, glycerol. In the discussions to follow, the focus will be only on the glycerol-based lipids, which make up simple fats and oils.

The lipids are important sources of metabolic energy (adenosine triphosphate or ATP) and are the most energy-rich of all classes of nutrients. In particular, the free fatty acids which are derived from fats and oils are the major source of fuel for metabolism in fish muscle (Tacon 1990). Lipids are also essential components of cell membranes, serve as carriers for fat-soluble vitamins, and are the source of essential fatty acids. Fatty acids are necessary for maintaining cell membrane integrity, lipid transport, and are the building blocks for many hormones.

Composition

Fats and oils occur naturally in foodstuffs and in the fat deposits of most animals in the form of triglycerides and phospholipids. Triglycerides are esters of fatty acids and the alcohol, glycerol. The chemical composition of a triglyceride is presented in Fig. 1. The simplest fat would have the same fatty acid for the three locations on the glycerol molecule at which it can bond. A discussion on the chemical structure of fatty acids (e.g., R1-COOH, R2-COOH, R3-COOH) is presented in the following section. Few naturally occurring fats or oils exist as a single triglyceride and most fats are mixed triglycerides, as the fatty acids are different.

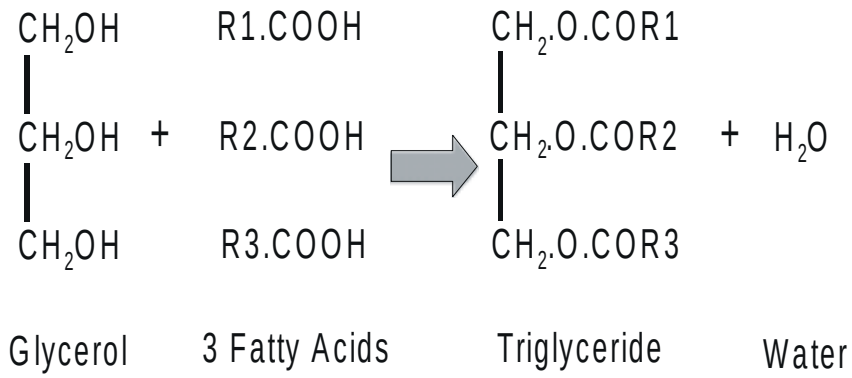


Figure 1. Chemical composition of a triglyceride.

The chemical structure of a phospholipid is presented in Fig. 2. The R1 portion is a fatty acid, usually a saturated fatty acid. The R2 portion is also a fatty acid, often a highly unsaturated fatty acid (HUFA) such as arachadonate or docosahexaenoate, and the X portion is typically serine, choline, or ethanolamine. The most variable components of fat or oil are the fatty acids that attach to it thus greatly affecting the physical and chemical properties.

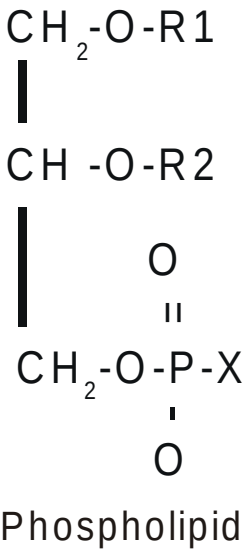


Figure 2. A typical phospholipid.

Fatty Acids

At least 40 different fatty acids are known to occur naturally in plants and animals. They all can be represented by the chemical formula:

$$CH_3 (CH_2)_n COOH$$

Where: n = 0 in acetic acid
n = 1 in propionic acid
n = 2 in butyric acid
n = up to 24 where n is usually an even number

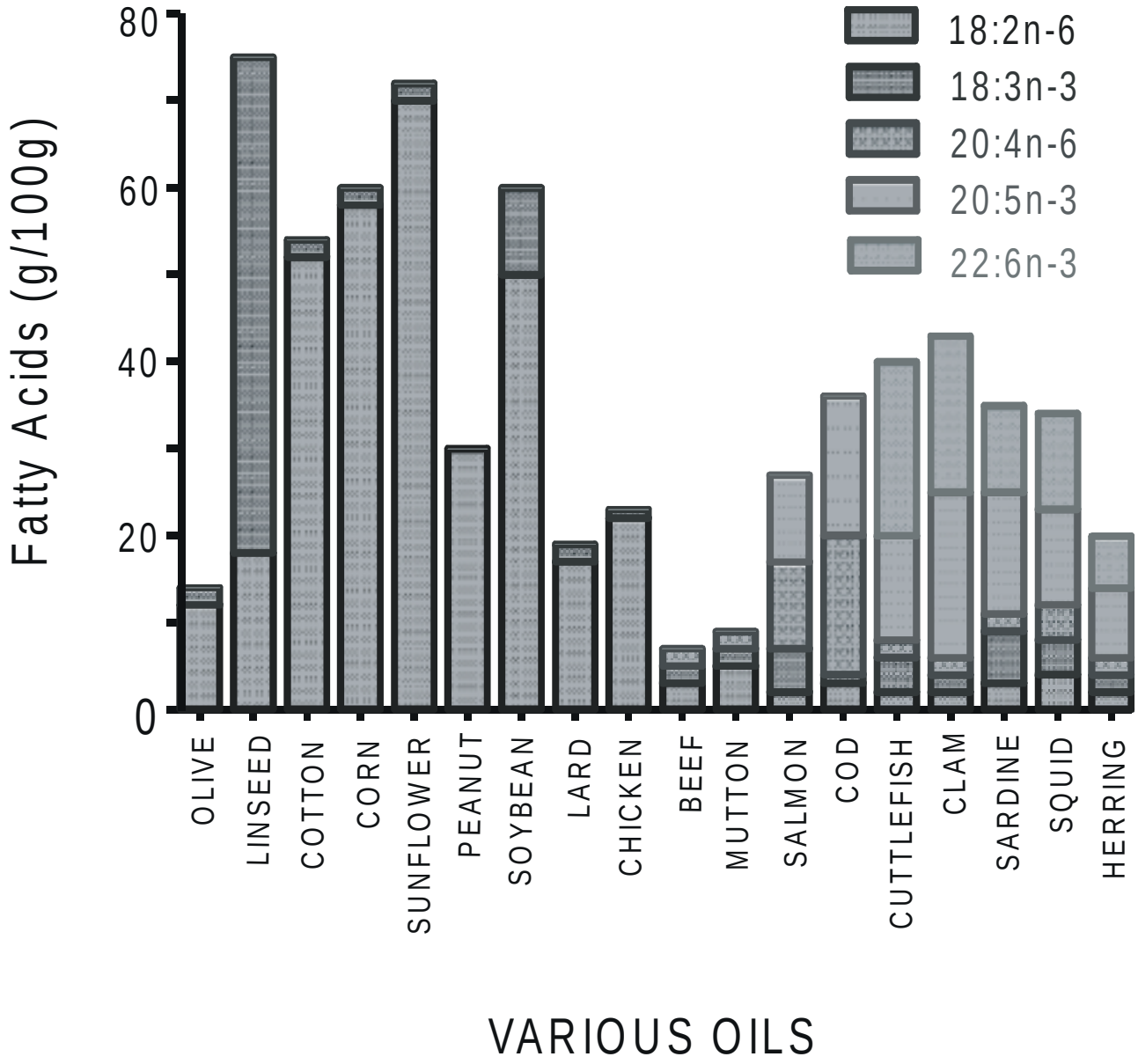
Most naturally occurring fatty acids contain a single COOH (carboxyl group) and a straight unbranched carbon (C) chain which may contain no double bonds (saturated fatty acid) or one double bond (mono-unsaturated fatty acid) or more than one double bond (polyunsaturated fatty acid or PUFA). The degree of unsaturation (i.e., number of double bonds) greatly influences the physical properties of the fat or oil. In general, unsaturated fatty acids are more chemically reactive and have lower melting points than the corresponding saturated fatty acid (i.e., carbon chain of the same length). Examples of saturated, monounsaturated, and polyunsaturated fatty acids of the same carbon chain length are:

FATTY ACID	STRUCTURE	ABBREVIATION
Stearic Acid	$CH_3(CH_2)_{16}COOH$	18:0
Oleic Acid	$CH_3(CH_2)_7\ CH{=}CH(CH_2)_7COOH$	18:1n-9
Linolenic Acid	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	18:3n-3

Because of the cumbersome chemical formulae, abbreviations have been adopted to denote the fatty acids. In the examples given above, 18 represents the number of carbon atoms in the chain, the number before the n (e.g., 3n) represents the number of double bonds and the number after the hyphen represents the location or carbon atom of the first double bond when counting from the methyl group (i.e., CH_3) portion of the fatty acid or from left to right in the examples given above. On the basis of the classification given, the polyunsaturated fatty acids (PUFAs) are divided into three main families; oleic (n-9), linoleic (n-6), and linolenic (n-3). The family names of the PUFAs are derived from the shortest chain member of the group.

In general, animals are incapable of synthesizing fatty acids belonging to the n-6 (linoleic) and n-3 (linolenic) series. It is presumed that only plants are capable of synthesizing these families of fatty acids. Animals must therefore have a diet containing these fatty acids in a ready-to-use form referred to as essential fatty acids (EFAs). A breakdown of the various n-3 and n-6 essential fatty acids found in the raw materials used to formulate feeds for both land and aquatic animals is presented in Fig. 3.

It was once believed that the EFAs required by land animals are of the n-6 series and that aquatic organisms require the fatty acids of the n-3 series. The fatty acids found in the various fats and oils used to formulate various feeds for either type of organism reflect that major difference. However, it is now believed that both terrestrial and aquatic organisms require fatty acids in both the n-3 and the n-6 series. Understanding the differences in the



requirements amongst the different animal groups is one of the more notable advances made in aquaculture that has led to the development of various enrichment media for boosting the appropriate fatty acid content in the diets of cultured aquatic organisms.

Figure 4. *Artemia* nauplii, and L- and S-type rotifers.

LIVE FEEDS

A major task in devising a protocol for the artificial propagation of a fish species is the development of a feeding regimen for the larvae. Live feeds are a convenient and often essential food source for the larvae of some cultured species, especially those without a fully developed digestive system. In such cases, live food organisms provide digestive enzymes that breakdown the food ingested by larvae and can be described as naturally encapsulated bags of nutrients. Two major concerns among aquaculturists are providing organisms appropriate to the size of the larvae at the first feeding stage and then supplying the large numbers of feed organisms necessary to maintain the larvae. two live feeds, the rotifer Brachionus sp. and the nauplii of the brine shrimp Artemia (see Fig. 4) satisfy both the dimensional and numerical requirements (Lubzens et al. 1989; Sorgeloos et al. 1991). The discovery that the nutritional quality of both these food organisms can be manipulated to meet the particular nutritional requirements of larvae has resulted in major strides in the hatchery production of fish. Enrichment or “boosting” of the fatty acids of both food organisms has become incorporated into the larval-rearing protocols for many fish species (Sorgeloos et al. 1991).

ARTEMIA

The brine shrimp is a relatively primitive form of crustacean of the class Brachiopoda (so named for the gills on their legs). The lack of a true carapace places them in the suborder Anostraca, and further in the family Artemiidae. There is still disagreement as to the number of species listed in the genus *Artemia*. Brine shrimp are naturally found in lakes or pools having extremely high salinities. The fact that these bodies of water regularly dry out and then fill again is thought to be one of the selective pressures that charted the mode of reproduction (i.e., cyst production) characteristic of the *Artemia* currently exploited in the aquaculture industry. While the adult form is primarily used as a live, frozen, or freeze-dried food in the aquarium trade, the nauplius stage is used exclusively in fish hatchery operations.

Adult stages of the brine shrimp exhibit both oviparity and ovoviviparity. With ovoviviparity, both fertilized and parthenogenic eggs can undergo full development within the adult until the free swimming nauplius stage. Oviparity in *Artemia* is characterized by fertilized eggs becoming arrested at the blastula stage of development. These are encased in a complex shell that is impermeable to most substances. Production of cysts is initiated by changes in environmental conditions such as an increase in salinity. The cysts are shed from the body and desiccated osmotically or dried on the shores of pools or lakes. The desiccation process is thought to be necessary for further development after the cysts are rehydrated.

It was recognized long ago that freshly hatched *Artemia* nauplii are a high value feed for fish larvae and fry. Because of the size of the nauplius stage, *Artemia* also represent the only practical food source for the early stages of many fish and crustacean larvae (Bardach 1972), but using them as a larval food is not foolproof. For example, the dry weight and caloric content of nauplii can decrease as much as 25% within 24 h after hatching when kept at 25 C. This means the nauplii must be fed to the larvae as soon as possible after hatching or be stored at low temperatures to decrease their rate of metabolism. Another drawback is that they have been found to be nutritionally deficient, especially in the long chain polyunsaturated fatty acids (PUFAs). The later shortcoming, however, can be corrected by enrichment of the *Artemia* nauplii.

Hatching of *Artemia*

Commercial harveting of *Artemia* cysts is done in three major areas, San Francisco Bay, California and the Great Salt Lake, Utah, in the United tates, and the salt lakes of Saskatchewan in Canada. However, 80% of the cysts that are sold commercially originate from the Great Salt Lake (Sorgeloos et al. 1991). A list of distributors can be found in Appendix 1. Because of considerable variability in the hatching percentage of cysts from different origins and even in separate batches from the same strain (Sorgeloos et al. 1986), the hatching percentage should be tested periodically. This can be done by incubating and hatching a specific amount of cysts (1 g contains approximately 250,000 cysts). The hatching percentage can be calculated by estimating the total number of nauplii and dividing by the number of cysts incubated.

The cysts are merchandised in vacuum-packed cans that must be refrigerated after opening. They are sold in a dormant stage of development and are viable for years if stored dry and and in their original containers. In nature, *Artemia* cysts await environmental cues such as rain and then sunlight to emerge from dormancy. Hatching of *Artemia* is a relatively simple process if certain parameters are followed. A constant water temperature (25 to 28 C), salinity (15 to 35 o/oo), pH 8.0, saturated DO levels accomplished by vigorous aeration, and strong illumination (2,000 lux) are essential to initiate the hatching response (Sorgeloos et al. 1986). A method for hatching of *Artemia* cysts described below is based on an amount intended to supply a medium-size hatchery with two 3000-L larval rearing tanks containing 30,000 larvae each that require a feeding of 60 x 10^6 nauplii/day. For individual purposes the amount may need to be increased or decreased.

Large-Scale Hatching

Materials:

two 100-L fiberglass cylinders with funnel shaped bottom and drain
50-L glass aquarium
black plastic sheet
12" length of glass tubing (1/8" diameter)
10' of airline tubing (1/8" diameter)
flashlight
20-L plastic buckets marked with liter graduations
aeration source and airstones
top loading balance (10 kg capacity)
submersible heaters
collection net (100 m)
100-L plastic tub
tygon tubing 1" diameter
sea water supply
500-L graduated cylinder
1-ml glass pipette
8x ocular magnifier
lights (100 watt)

Step 1. *Incubation of Cysts*: Fill fiberglass cylinders with sea water and aerate vigorously. Place heaters in cylinders and set temperature at 28 C. Measure amount (grams) of *Artemia* cysts required and add to the cylinders. (This is assuming that the hatching percentage of the *Artemia* cysts is already known.) An alternative to measuring cysts by weight is to measure them by volume by converting the number of grams of cysts to the number of ml of cysts. Continue vigorous aeration and illuminate with lights hung above the cylinders. Incubate overnight.

Step 2. *Separation of Shells and Nauplii*: After *Artemia* cysts have hatched (at this stage the *Artemia* are called nauplii) turn off aeration and remove heater and airstone. Wait approximately 5 minutes for the empty casings to float to the surface of the water. Support collecting net securely in 100-L plastic tub and fill tub with sea water. Separate the cyst casings from the hatched *Artemia* by draining the contents of the cylinder into the collecting net. Be sure to close the drain before the suspended cyst casings can flow through the net. *NOTE: DO NOT OVER-FLOW THE NET.*

Step 3. *Collection of Nauplii*: Fill an empty aquarium with sea water leaving enough room to accommodate the collected nauplii. Remove the collecting net from the plastic tub and rinse collected nauplii with sea water. Then place contents of the collecting net into the aquarium but do not aerate. Cover the aquarium with a black plastic sheet leaving an uncovered space near the bottom and use a flashlight to illuminate the uncovered area. Wait approximately 5 minutes and siphon nauplii from the bottom of the aquarium using the glass tubing connected with a piece of airline tubing as a siphon hose. Let the nauplii drain into a 20-L plastic bucket. *CAUTION: DO NOT LET NAUPLII COLLECT ON THE BOTTOM OF THE AQUARIUM FOR LONGER THAN 15 MINUTES OR THEY WILL SUFFOCATE*.

Step 4. *Quantifying Nauplii Harvested*: Add sea water to bring the volume of the siphoned nauplii in the plastic bucket to the 15 liter mark and aerate vigorously to mix. Fill graduate cylinder with sea water to the 500 ml mark. Remove 2 ml of sea water with the pipette and replace with 2 ml of sea water containing nauplii from the plastic bucket. Swirl the contents of the graduate cylinder, use the pipette to remove 1 ml aliquot with the pipette, and count the number of nauplii in the pipette using the 8x magnifier. Repeat this process five times and calculate the average number of nauplii per ml. Multiply this value by 250 to obtain the estimate of the number of nauplii/ml in the plastic bucket. Multiply this number by 15,000 ml to obtain the total number of nauplii available. The amount of nauplii required for feeding or for enrichment can be removed volumetrically.

Small-Scale Hatching

The following method has been simplified for those needing only small amounts of *Artemia* nauplii.

Materials:

two 2-L cylinders with funnel shaped bottom (plastic soda containers with the bottoms removed work well)
12" glass tubing (1/8 " diameter)
5' airline tubing (1/8' diameter)
flashlight
aeration source and airstones
collection net (100 m)
sea water supply (fresh water + 35 grams of sea salt per liter of fresh water)

Step 1. *Incubation of Cysts*: Fill one cylinder with 1 L of sea water and aerate vigorously. Measure amount of *Artemia* cysts required and add to the cylinders (3 grams of cysts per liter is a good rule of thumb). An alternative to measuring cysts by weight is to measure them by volume (1 level teaspoon of cysts 5 grams of cysts). Illuminate and incubate for approximately 24 h with vigorous aeration.

Step 2. *Separation of Cysts and Nauplii*: After *Artemia* have hatched (at this stage the *Artemia* are called nauplii), stop the aeration and remove the airstone. Wait approximately 5 minutes for the empty casings to float to the surface of the water. Darken the room and use a flashlight to illuminate the bottom of the cylinder to attract the nauplii.

Step 3. *Collection of Nauplii*: After approximately 5 minutes most of the nauplii will have clustered at the bottom of the cylinder. Remove the nauplii using the piece of glass tubing connected with the airline tubing as a siphon hose. Let the nauplii drain into a collection net. The number of nauplii needed for feeding larvae will dictate how the procedure may be scaled up or down.

ENRICHMENT PROCEDURE

Although widely used as a live feed for a variety of species under culture, the nauplii of most strains of *Artemia* are lacking in certain nutritional elements required by some species of fish, in particular the long chain omega-3 polyunsaturated fatty acids or PUFAs (Sorgeloos et al. 1991). The cysts from China test positve for DHA, however, they are extremely difficult to obtain and very expensive. Most strains also have a low energy to protein ratio. These

deficiencies, however, can be remedied by an “enrichment” process in which the *Artemia* nauplii are fed a source rich in PUFAs to ultimately improve their nutritional composition.

Artemia nauplii can be enriched with homemade or commercial preparations. The commercial products described in this manual are used for comparison purposes only and the mention of them is not an endorsement. The ornamental fish farmer must weigh costs as well as convenience in deciding on a particular method. Homemade preparations are usually employed in large-scale hatcheries where the number of fry produced range between 100×10^3 and 1.0×10^6 per hatchery cycle. The process for enriching *Artemia* nauplii from scratch using menhaden oil is presented below. It should be noted that other oils such as cod liver oil, squid oil, and sardine oil can be substituted for menhaden oil, depending on cost and availability.

Materials:

hot plate magnetic stirrer
blender
polysorbate emulsifier**
tap water
250-ml graduate cylinder
two 250-ml bottles with caps
10-ml pipette
menhaden oil**
thermometer
500-ml glass beaker
refrigerator
** = see Appendix 1 for source

Step 1. *Heating of Menhaden Oil*: Place 200 ml of menhaden oil (Zapata Haynie Corporation, Medville, Virginia, USA) into glass beaker and heat on hot plate to 50 C.

Step 2. *Mixing of Ingredients*: Pour heated menhaden oil into blender and add 200 ml of hot (40 to 50 C) tap water and mix.

Step 3. While mixing, add 10 ml of polysorbate emulsifier (Gulf Pacific Industries, Auckland, New Zealand) and continue mixing until fluid turns into a creamy white solution.

Step 4. *Storage of Enrichment Medium*: Pour mixture into 250-ml bottles and cap. Label bottle with date and refrigerate. This solution is useable for 2 or 3 weeks when kept refrigerated. When the medium begins to sour or turn rancid, it should be replaced with a fresh batch.

Step 5. *Enriching Nauplii*: The emulsion should be used at a concentration of 0.25 ml per liter of sea water or 250 ppm. Aerate the mixture for at least 10 minutes to ensure good mixing before adding *Artemia*. Incubate the nauplii with the enrichment medium at a density of 300 - 400 nauplii/ml for a minimum of 6 h before feeding to fish larvae. A common practice is to enrich the nauplii overnight and feed the fish larvae the following morning. *CAUTION: ENRICHING NAUPLII OVERNIGHT WILL RESULT IN LARGER INDIVIDUAL NAUPLII THAN NEWLY HATCHED NAUPLII*.

Step 6. *Collection of Enriched Nauplii*: Pour enriched nauplii into collecting net and rinse thoroughly with sea water. Place nauplii into a 20-L bucket and add sea water to the 15 liter mark. Calculate the number of nauplii/ml as described previously and feed to fish larvae by removing the appropriate volume from the bucket.

Larger quantities of enriched nauplii can be produced but require storage at 5 C at a density of 5,000/ml with appropriate aeration (Sorgeloos et al. 1991). Nauplii stored in this way maintain their enrichment for up to five days.

For enrichment of smaller quantities of *Artemia*, commercial preparations such as Selco, Algamac-2000, Sander's Rich, or Microfeast may be more cost-effective and convenient as the emulsification process is already done. To reconstitute the enrichment medium carry out Steps 5 and 6 but use the recommended concentrations printed on the label. Several of these commercial preparations have been investigated and the fatty acid profiles of newly hatched nauplii compared against nauplii that have been enriched using the process described above (Table 1).

Table 1. Fatty acid profiles of Artemia nauplii using various enrichment media. Values are reported in mg/100 mg dry weight.

Fatty Acid	Hatched *Artemia*	Menhaden Oil (250 ppm)	SELCO (300 ppm)	DHA SELCO (300 ppm)	MicroFeast L-10 (250 ppm)	ALGAMAT - 2000 (200 ppm)
14:0	0.08	0.36	0.14	0.11	0.12	0.14
16:0	0.92	1.77	1.85	1.77	1.58	1.45
16:1n-7	0.33	0.75	0.35	0.39	0.44	0.38
18:0	0.30	0.77	0.91	0.70	0.66	0.37
18:1n-9	1.25	2.38	1.77	2.64	2.17	1.84
18:2n-6	0.40	0.64	0.81	0.84	0.73	0.50
18:3n-3	1.96	2.57	4.97	3.30	3.80	2.76
18:4n-3	0.20	0.47	0.69	0.26	0.43	0.23
20:1n-9	0.03	0.12	0.08	0.17	0.07	0.04
20:4n-6	0.08	0.20	0.19	0.11	0.10	0.15
20:5n-3	0.44	1.02	0.59	1.83	0.82	0.68
22:1n-11	-	0.02	-	0.10	0.01	0.01
22:6n-3	-	0.50	0.25	2.33	0.16	0.11
Total Fatty Acid	5.97	11.55	12.60	14.50	11.10	8.66

- = not detectable

From Table 1 it can be seen that all of the enrichment procedures listed result in significant increases in total fatty acids compared to newly hatched *Artemia* nauplii alone. An increase in the total fatty acids equates to an increase in the caloric density of the feed (i.e., increase in energy content). The essential highly unsaturated fatty acids (HUFAs) 20:5n-3 or eicosapentaenoate (EPA) and 22:6n-3 or docosahexaenoate (DHA) are significantly higher in *Artemia* nauplii that have been enriched. The varying amounts of EPA and DHA reflect the differences in the amount of enrichment media used as well as quantitative and qualitative differences in the sources of these fatty acids (e.g., fish oil or algae) that make up the commercially prepared enrichment media.

The enrichment process is time dependent as depicted in Table 2. As the fatty acids are taken up by the *Artemia*, their fatty acid profiles change acording to the duration of the enrichment period. Newly hatched *Artemia* had 7.0 mg total fatty acids/100 mg dry weight *Artemia* with no detectable levels of DHA. After enrichment for 12 h the total fatty acids increased significantly to 10.3 mg/100 mg and the *Artemia* had significantly higher amounts of essential fatty acids in the n-3 and n-6 families. After 24 h of enrichment, significantly higher levels of essential fatty acids and total fatty acids were achieved. The results indicate that convenience and the length of enrichment process should also be considered when preparing *Artemia* nauplii as a food for the larvae of ornamental fish.

Table 2. Fatty acid profiles of *Artemia* nauplii using SELCO (300 ppm) enriched at 12 and 24 h, respectively. Values are reported in mg/100 mg dry weight.

Fatty Acid	Newly Hatched *Artemia*	SELCO (300 ppm) at 12 hours	SELCO (300 ppm) at 24 hours
14:00	0.07	0.10	0.14
16:00	0.89	1.24	1.85
16:1n-7	0.26	0.33	0.35
18:00	0.39	0.60	0.91
18:1n-9	1.27	1.83	1.77
18:2n-6	0.53	0.74	0.81
18:3n-3	2.93	4.44	4.97
18:4n-3	0.47	0.62	0.69
20:1n-9	0.04	0.06	0.08
20:4n-6	0.08	0.13	0.19
20:5n-3	0.05	0.12	0.59
22:1n-11	-	-	-
22:6n-3	-	0.05	0.25
Total	6.98	10.30	12.6

- = not detectable

Other live feeds

Commercial enrichment preparations have also been tested with other live feeds used in freshwater ornamental fish culture and have been found to result in similar results. Enrichment of freshwater rotifers and the cladoceran, Moina, have been investigated and the results are summarized in Table 3. Unenriched Moina and freshwater rotifers were grown with “green water” from a guppy tank. The enrichment media for Moina and the freshwater rotifers were Algamac 2000 (100 ppm) and Microfeast (100 ppm), respectively.

Table 3. Comparison of fatty acid profiles of unenriched and enriched freshwater food organisms. Values are reported in terms of mg/100 mg dry weight.

Fatty Acids	Unenriched Moina	Enriched Moina	Unenriched Freshwater Rotifers	Enriched Freshwater Rotifers
14:00	0.03	0.32	0.17	0.18
16:00	0.43	1.29	0.89	0.92
16:1n-7	1.36	0.86	0.05	0.24
18:00	0.22	0.26	0.21	0.27
18:1n-9	1.77	1.18	0.48	0.67
18:2n-6	0.11	0.08	1.12	1.54
18:3n-3	0.04	0.03	2.64	0.93
18:4n-3	0.01	0.03	0.17	0.00
20:1n-9	0.01	0.05	0.29	0.25
20:4n-6	0.16	0.21	0.05	0.08
20:5n-3	0.07	0.37	0.11	0.52
22:1n-11	-	-	-	-
22:6n-3	-	0.75	-	0.42
Total Fatty Acids	4.22	5.42	6.27	6.34

- = non detectable

The results clearly indicate that the enrichment procedures also elevate total and essential fatty acids of both of these live feeds used in freshwater ornamental fish culture. While the technologies for enriching live feeds used in the culture of marine food fishes have been employed at commercial scale for a long time, remarkably, the developed technology is not widespread in the freshwater ornamental fish industry. One reason for this is that all fishes do not require the long chain PUFAs as reported for some marine species (Tamaru et al. 1993b). The task to examine the effects of enriched *Artemia* on hatchery production of freshwater ornamental fishes is quite challenging as 1,500 species are reported to characterize the aquarium industry (Chapman et al. 1997). Some positive results, however, are being observed by freshwater ornamental fish producers in Hawaii who are utilizing enrichment procedures. Improved consistency and higher survival in larval discus, improved growth and survival of goldfish larvae, and increases in fecundity of angelfishes and guppies have been reported. All of these still need to be verified under laboratory conditions.

Conclusions

The enrichment processes for *Artemia* nauplii significantly elevate all of the fatty acids found in the nauplii. Most notable is the elevations of C20:5n-3 (EPA) as well as infusion of C22:6n-3 (DHA). Both of these have been implicated to be essential for larval growth and development in a number of fish species (Watanabe et al. 1983; Sorgeloos et al. 1991). It should be pointed out that investigations into the use of enriched *Artemia* often result in no obvious differences in survival and growth for a particular marine fish species (Ako et al. 1994; Dhert

et al. 1990; Kraul et al. 1993). However, there is a marked improvement in the ability of these fish larvae to tolerate being physically handled. The implications of these results are that the general health and well-being of the fish larvae have been significantly improved by the feeding of enriched *Artemia* nauplii. Including the enrichment of nauplii into the other established hatchery practices should insure that a higher quality seed will be produced from the hatchery.

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Appendix 1.

List of Distributors

Listing in this appendix does not constitute an endorsement of products or services but are recommendations for products or services that the listed manufactures, suppliers or organizations may provide.

Artemia Cysts

Argent Chemical	8702 152nd Avenue NE Redmond, WA 98052 USA Phone: 206-885-2112 Fax: 206-885-3777
Prime Artemia Inc.	1042 East Fort Union Blvd., Suite 400 Midvale, Utah, 84047 USA Phone: 801-978-9337 Fax: 801- 978-9303
San Francisco Bay	8239 Enterprise Drive Newark, California 94560 USA Phone: 510-792-7200 Fax: 510-792-5360
Sanders Brine Shrimp Company	3850 S. 540 W. Ogden, Utah 84405 USA Phone: 801-393-5027 Fax: 801-621-3825

Emulsifier

Gulf Pacific Laboratories	P.O. Box 40027 Glenfield Aukland, New Zealand Fax: 0064-9444-9264

Menhaden Oil

Zapata Haynie Corp.	P.O. Box 175 Reedville, VA 22539 USA Phone: (804)453-4211

Enrichment Media

SELCO	Artemia Systems Inc. Oeverstraat 7 9200 Dendermonde/Baasrode Belgium Phone: 32-52-331320 Fax: 32-52-341205
MICROFEAST	Provesta Corporation 15 Phillips Building Bartlesville, OK 74004 USA Phone: 918-661-5281 Fax: 918-662-2208
ALGAMAC-2000, ROTI-MAC	Aquafauna BioMarine Inc. P.O. Box 5 Hawthorne, California, 90250 USA Phone: 310-973-575 Fax: 310-676-9387
SANDERS RICH, DOCOSA GOLD	Sanders Brine Shrimp Co., L.C. 3850 S. 540 W. Ogden, Utah 84405 USA Phone: (801) 393-5027 Fax: (801) 621-3825