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Production of live prey for marine fish larvae

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Abstract — *Tropical marine fish larvae vary in their requirements for live planktonic food. Selection of live prey species for culture depends on larval size and larval tolerance of water quality. This report describes some of the cultured prey species, and their uses and limits as effective food for fish larvae. Methods are presented for the culture of phytoplankton, rotifers, copepods, and other live feeds. Difficulties in rearing certain species of marine fish are compared to their dietary requirements.*

Feeding methods are more complex for larvae from smaller eggs, more pristine environments, and pelagic versus demersal embryonic environments. Copepods are the natural diet of almost all first feeding marine fish larvae, and can be cultured in unlimited quantities. The nutritional profile of a cultured copepod, ***Euterpina acutifrons*** *surpasses all other live larval foods. The smallest and most sensitive reef fish larvae tested will consume nauplii of* ***E. acutifrons****, but survival is poor due to a toxicity effect.*

Dependable phytoplankton culture is the key to successful larval rearing. Sterilization, inoculation, nutrient enrichment, species selection, and facility design are discussed.

Rotifer production depends on temperature and algal species. About 2.5 % of larval rearing volume is sufficient rotifer production volume for densely stocked, fast-growing fish larvae. Algal enrichment is the safest method when rotifers are used for marine fish larvae, and yields two-fold growth of rotifers to 100-150 rotifers/ml.

Copepods are cultured to densities of 20-50 copepodites/ml (0.1 g/l.), with population growth rates of 15 %/day. Algal species and water quality maintenance determine copepod growth rate and harvestable biomass.

INTRODUCTION

Marine fish larvae are more difficult to raise than anadromous fishes or invertebrates. Much of this difficulty can be attributed to complex requirements through a relatively long hatchery phase (see Figure 1). Species become more difficult to raise when their eggs are smaller or less numerous, or when artificial spawn induction is required. Length of time in the hatchery phase, and number of food species required increases the chances for making fatal errors or introducing catastrophic disease, and larval tolerance to organic pollutants makes a big difference in the amount of care needed to achieve high yields.

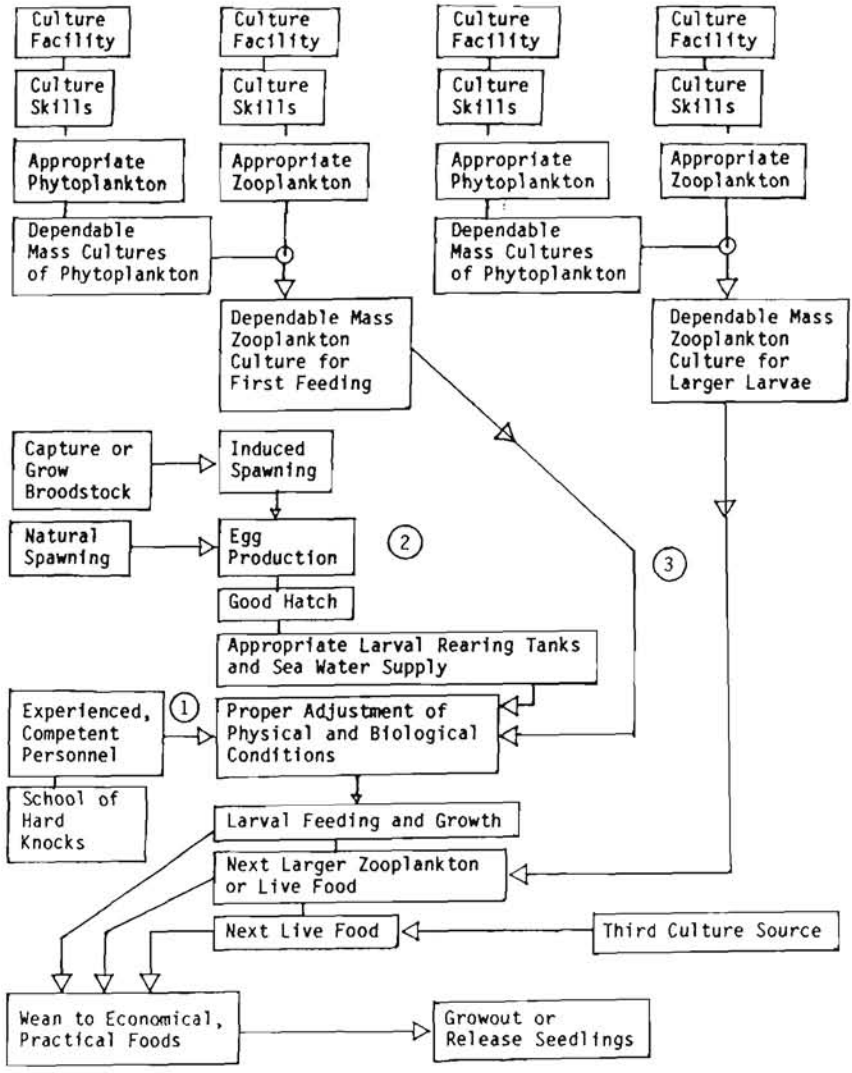

Fig. 1. — Necessary components of successful marine fish seedling production.

OUR EXPERIENCES WITH VARIOUS LARVAE

Because of its large inventory of tropical marine fishes, the Waikiki Aquarium frequently has the opportunity to attempt to rear many types of larvae. Some of our attempts succeed, and some fail. Those larvae that survive our manipulations fall into one of the following descriptions : eggs larger than 0.9 mm; larvae hatch from demersal nest, ready to feed; larvae found naturally in stressful environments (i.e., tidepools, mangrove swamps, nutrient-rich waters, etc.). Some larvae survive first feeding

without problems but seem to suffer from nutritional inadequacy after 2-3 weeks (eg. reef shrimps.). Most larvae of tropical reef fishes hatch from pelagic eggs in offshore waters, are less than 750μ diameter, and fail to survive first feeding in our hatchery. These larvae do not ingest rotifers, *Brachionus plicatilis*, probably because of size. The dinoflagellate, *Gymnodinium spendens*, has been used successfully for anchovy larvae (Lasker et *al.*, 1970). Our preliminary tests with this small (20 × 30 × 40 μ) flagellate show ingestion by *Scorpaenid larvae* (1.3 mm TL), but poor survival. Further tests may be more successful. Although the smallest of these larvae consume nauplii of our cultured copepod, *Euterpina acutifrons*, they usually become weak and cease feeding within 1 day. This particular problem is common to larger larvae from pristine environments, namely the mahimahi, *Coryphaena hippurus*. Thus we are using mahimahi as a model to develop methods of using cultured plankton for feeding tropical marine fish larvae.

There are other approaches to first feeding of delicate larvae : Victor Oiestad (Kvenseth and Oiestad, 1984) promotes natural zooplankton blooms in large natural and artificial impoundments, then stocks hatched larvae, with good survival. Similarly, redfish larvae are stocked in Texas ponds, and Rabbitfish larvae are stocked in mangrove ponds. Stocking extensive « natural » environments is successful, but difficult to control and analyze. We are hopeful that the study of plankton cultures, using mahimahi as a model, will help us understand the nutritional and environmental requirements of larvae, so that we can improve hatchery yields.

MAHIMAHI AS A MODEL

Mahimahi spawn without artificial inducement, and their larvae are larger (4.5-5.0 mm at hatch) and grow faster than most tropical marine fishes. However, their need for several different live diets, and their sensitivity to pollutants (they normally spend their larval phase in pristine open ocean water) call for stringent technology. Therefore, mahimahi are a sensitive model for testing live feeds, and they have the advantage of being available from natural spawns year round.

Successful hatchery culture of the mahimahi *Coryphaena hippurus* depends on meeting relatively fastidious dietary and environmental requirements. Yolk sac larvae require upwelling sufficient to counteract downward migration. First feeding larvae prefer copepodites (electivity index, e = + 0.52) and nauplii (e = + 0.20) of the cultured copepod *Euterpina acutifrons*, but survive significantly better on the rotifer *Brachionus plicatilis* (e = + 0.15). Thus, for first feeding larvae, food preference (Figure 2) is not a valid indicator of optimal diet. Some first feeding mahimahi larvae ingest newly hatched brine shrimp *Artemia franciscana* (e = - 0.25), but larval survival is poor if brine shrimp are used as a sole diet.

Food preference and optimum diet composition change as larvae grow. From the seventh to 20th post-hatch day, larval survival and growth rates are significantly higher when larvae are fed cultured copepods rather

than rotifers or brine shrimp. Fry occasionally begin accepting nonliving foods by day 20. From first feeding through day 20, larvae gain 20 % body weight per day with a food conversion efficiency of 0.3 at ambient Hawaii temperatures (23 to 26°C) (Kraul et *al.*, 1989).

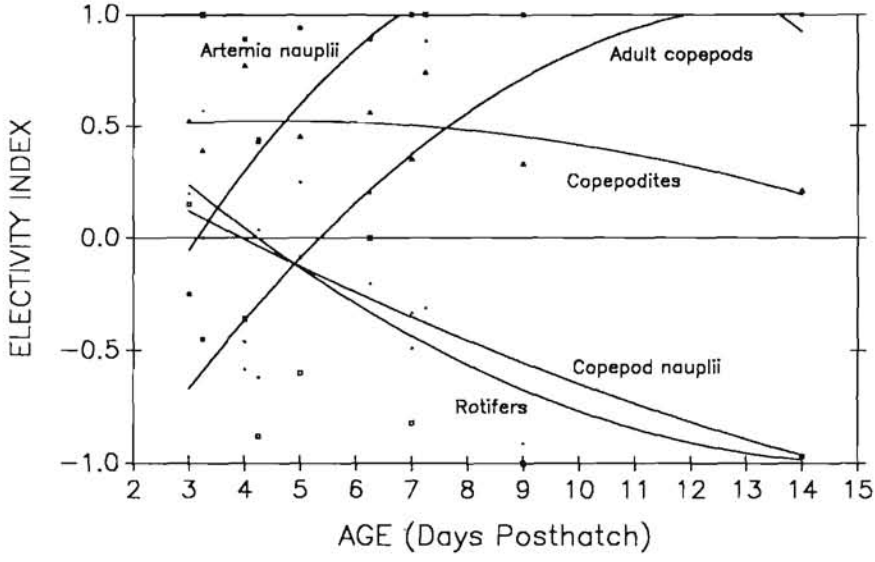

Fig. 2. — Food preference of larval Mahimahi *(Coryphaena hippurus).*

Mahimahi larvae appeared to select food based on size and some other visual quality : (optical reflectivity, motion). When rearing water became supersaturated with oxygen, newly feeding larvae ingested gas bubbles of appropriate size until their guts were full of gas. This phenomenon has also been observed in mullet (Kraul, 1983) and other fishes. Copepods were selected over rotifers of equal width. Movement and light refraction differed in these two prey species. Nevertheless, larvae consumed any appropriately sized live food items when they were sufficiently abundant.

Lower survival on copepods during the first week was not a nutritional problem since larval growth rates were not significantly different on any of the diets offered. Survival of starved larvae was greater than survival of copepod-fed larvae by day 4 (Figure 3). Carnivorous attacks on the larvae by *E. acutifrons* were not observed. The first feeding larvae's deleterious reaction to copepods may have been caused by a toxin or microorganism associated with copepod culture medium. Older larvae were able to tolerate copepod « poisoning », perhaps through the development of a functional immune system, and derived better nutrition from copepods than from brine shrimp. Improved larval survival on a copepod diet has been demonstrated for mullet (Kraul, 1983).

There is a tank size effect at first feeding. Growth was comparable in large (4,000 l.) and small (200 l.) tanks through day 5 but was signifi-

cantly greater in the large tank by day 9 (7.92 +/− 0.39 mm total length for large tank larvae with all foods versus 6.48 +/− 0.34 mm for copepod-fed and 6.64 +/− 0.30 mm for rotifer-fed larvae in small tanks).

Tab. 1. — Daily feeding rations for 20000 Mahimahi larvae. Based on starting weight of 0.7 mg, growing 25 %/day, FCE = 0.3

		Number of food items × 1 million			
DAY	GRAMS OF FOOD	≠ R(× 10^6)	≠ C(× 10^6)	BS(g) cysts to start for	≠ H(× 10^5)
2	1.1	6			
3	1.3	7.3			
4	1.6	9.1		3d BS :	
5	2.0	11.4			
6	2.4	14.2	1.0		
7	3.1	17.8*	1.3*	3*	
8	3.8	22.3	1.6	4	
9	4.8	27.8	2.0	5	
10	6.0	34.8	2.4	6	
11	7.5		3.1	7	
12	9.3		3.8	9	
13	12		4.8	11	
14	15		6.0	14	
15	18		7.5	18	
16	23		9.3	22	
17	28		11.6	28	
18	36		14.6	35	.4
19	44		18.2	44	.5
20	56		22.7	55	.6
21	69		28.4	68	.8
22	87		35.5	85	1.0
23	108			107	1.2
24	136			133	1.5
25	169			167	1.9
26	212			208	2.4
27	265			260	3.0
28	331			325	3.7
29	414			406	4.7
30	517			508	5.8

* Quantity in each column fulfills the entire food ration. In practice, one food type is decreased while the next type is introduced, and the food ration is provided by the sum of the two types.

Nutrition has been shown to be a factor with the types of food reported here. Watanabe et *al.* (1978a, b) found that *Tigriopus* sp. copepods had a high proportion of essential fatty acids regardless of their medium, whereas rotifers (and *Artemia*) were not as nutritious unless their culture medium was optimum. Enrichment of rotifers and brine shrimp with essential fatty acids is currently being studied, using the nutritional profiles of copepods as a standard.

Heavy mortality during the transition to nonliving foods has been observed in other species (Bromley and Howell, 1983), and stressful energy expenditures during the metamorphic transitions in these and other species (Corbin, 1977) may explain the importance of diet in improving survival. Our current studies show that the use of newly hatched mahimahi larvae as a food (starting at day 18, when PLs are over 15 mm TL) greatly

improves postlarval survival through weaning. Once PLs are weaned onto squid (with a vitamin supplement), they suffer few mortalities. Development of a commercially practical diet is in progress.

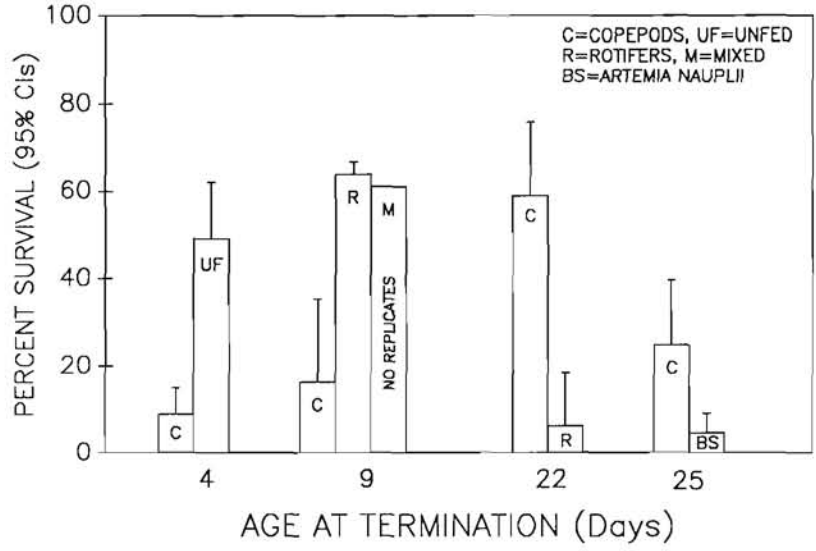

Fig. 3. — Effect of diet on larval mahimahi survival.

Summary of Mahimahi as a Model

Rotifers, copepods, and brine shrimp were accepted in varying degrees as a first food by larval mahimahi. Best survival was obtained by feeding rotifers days 2 to 8, copepods day 6 to 21, brine shrimp day 10 to 25, and newly hatched mahimahi larvae after day 18. Mahimahi larvae increased their weight at least 20 % per day through day 21, and had a food conversion efficiency of 0.27 to 0.33. Total live plankton consumption through 21 days was 1,000 rotifers plus 1,333 copepodities plus 2,500 brine shrimp nauplii per larva. From day 18 through 40, each PL will consume about 8,000 newly hatched mahimahi larvae.

PLANKTON CULTURE METHODS

A. Algae culture

Microscopic, single-celled algae (phytoplankton) is cultured at the Waikiki Aquarium for use as a food for filter feeders such as the *Tridacna* clam and various corals, and for feeding microinvertebrates such as brine shrimp *(Artemia)*, rotifers, and copepods. Microinvertebrates are used for feeding larval and juvenile fishes and corals. We culture several families of phytoplankton; the techniques for their culture are almost identical.

Some of the species we use are *Tetraselmis chuii, Chaetoceros gracilis,* and *Gymnodinium splendens.* To keep track of which species is in which container, each species is given a code of the first two letters of the genus name, i.e. TE, CH, GY.

Procedures

1) Clean the containers. Bottles should be acid cleaned and flushed. Large vats should be scrubbed lightly and rinsed out. The object here is to remove most of the surface-bound organic matter so that the chlorine sterilization (next step) is more effective. Be sure to fill acid cleaned bottles completely with tap water to remove acid vapors.
2) Fill containers with seawater, and add 1.0 ml of Clorox (5.25 % sodium hypochlorite) for every litre of seawater. Let the containers sit unaerated away from strong light overnight. If water is needed sooner, add 5 ml of Clorox per litre and let stand at least 2 hours. The object here is to maintain a chlorine residual of > 10 ppm overnight. Residual chlorine is affected by organic load, so more will be needed in dirtier water. A swimming pool test kit is adequate for measuring residual. Strong light and aeration will disperse chlorine and reduce its strength.
3) Neutralize the chlorinated seawater by adding 1.0 ml of sodium thiosulfate solution (1N, 250 g Na2S2O3.5H2O per litre of distilled water) for every 4.0 ml of Clorox that was added earlier. Neutralization is complete when water is mixed (i.e. turn on air). Airstones are not recommended for algae cultures because they take too long to clean. A plastic pipette or piece of lead on an airhose will do. If airstones are used, soak them in full-strength Clorox between batches and rinse them thoroughly before use.
4) Add nutrients to the sterile seawater. Our « F/2 » nutrient stocks (Guillard, 1975) are made up at 500 times final concentration (Appendix B). Thus, 2 ml of nutrient should be added to each litre of sterile seawater. Nutrients are kept in a plastic bottle in the refrigerator. Normally, 2 l. bottles get 4 ml of F/2; 100 l « buckets » get 200 ml of F/2, etc.
5) Add algae inoculant. It is best to check microscopically and record cell counts and health occasionally. You can usually tell by color if a 2 l culture is healthy and dense enough to use as inoculant for 100, 200, or 600 l cultures. Note the date, inoculant source, inoculant quantity, and algal species on a piece of tape (or directly on glass bottles) whenever a new culture is started. This procedure will help you to determine how well a culture is doing, and is good for inventory control.

B. Rotifers

Much literature is available on the culture of *Brachionus plicatilis* (Theilacker and Mc Master, 1971; Snell and Carrillo, 1984). At Waikiki Aquarium, rotifers reproduce parthenogenically about 100 % per day,

feeding exclusively on *Tetraselmis chuii* (TE). At densities above 100 R/ml, we replenish 50 % of rotifer water with undiluted TE daily, to maintain 100 % rotifer growth.

The keys to using rotifers successfully are : use only heathy phytoplankton of good nutritional value; clean the rotifer container frequently, especially before using the rotifers as a larval food; keep the harvesting screen (we use 60ñ nytex) submerged to avoid irreversible drying; monitor rotifer density and change water before density decreases.

C. Copepods

The harpacticoid copepod, *Euterpina acutifrons*, is a nearly ideal food for larval marine fishes due to its size, trophic ecology, nutritional value, culturability, and (most importantly) acceptability by pelagic marine fish larvae. There are few literature references to successful copepod culture. Suggested readings include Theilacker and Kimball (1984); and Zurlini et *al.* (1978). Culture techniques are easy, using the following suggestions.

1) Do not use an algae that gets too slimy and settles heavily. You will want to aerate sufficiently to suspend the algae, but not so much that you interupt sexual coupling. Slimy surfaces will trap nauplii. *Chaetoceros gracilis* ($4 \times 4 \times 5\ \mu$) works well. You may get faster growth and a higher fecundity if a dinoflagellate or other flagellated green phytoplankter is present. Normal growth rates at the Waikiki Aquarium are 10-15 % per day (up to 100 fold increase in 8 days), with harvest densities of 20 to 50 adults copepods per ml.
2) Partial shading helps, if cultures are outdoors. Keep the cultures in a growth phase, and change them over to a clean container every few weeks.
3) Inoculate with 1-10 % of your harvest. I like to keep the density above 1 per ml so I can count them with a 1 ml. pipette, but they will grow fast at densities of 1 per litre. Algal densities of 5×10^4 to 2×10^5 cells per ml will give good growth rates. You can approximate these densities as visibilities of 7 to 10 cm.
4) Do not let rotifers enter the system. They will usually outproduce the copepods. It is difficult to keep these two zooplankters separate with screens because their sizes overlap. If you do get a rotifer takeover, isolate 10-100 gravid female copepods and start over in 2 to 40 l of new medium (check them in a microscope to make sure there are no rotifers).
5) There are 6 naupliar stages, and 6 copepodite stages, including the adult. Size is $50 \times 50 \times 70\ \mu$ (N1) to $150 \times 175 \times 700\ \mu$ (C6). I use a 37 μ screen to harvest N1 & N2, and a 100 μ screen for copepodites. Generation time is about 8 to 11 days under best conditions, at temperatures of 24-26°C. If you stock your rearing container with all sizes of copepods, new nauplii will be produced by the adults to replace those consumed by the fish larvae.

D. Brine shrimp

For our purposes, *Artemia* spp. are not cultured, but merely hatched, fed, and used as nauplii. Other review at this workshop discuss brine shrimp in detail.

E. Other plankton

If postlarval marine fishes will not accept nonliving foods after metamorphosis, they can sometimes be fed grown out brine shrimp. However, we find that brine shrimp are nutritionally deficient for mahimahi postlarvae. It is possible that new enrichment technology will allow the use of juvenile and adult Artemia as a food for postlarval fishes. At the Aquarium, we use newly hatched mahimahi larvae as a food for postlarval mahimahi when they are larger than 15 m TL. Hatchling mahimahi are not cultured, but merely hatched, rinsed, treated with Prefuran, and added to the rearing tank as a live food. Growth and survival are excellent using hatchlings, and we almost always have enough for mass cultures.

Other larger plankton is generally difficult to culture, or of low yield. Cladocerans (Takami et *al.* 1978) and calanoid copepods may be used on a limited scale.

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APPENDIX I
FOOD REQUIREMENTS FOR REARING 20,000 MAHI-MAHI LARVAE IN 4000 LITERS

A. CULTURE CAPACITIES

1. *ROTIFERS*

MINIMUM DENSITY & QUANTITY = > 2/ml and > 200R/larva

a) 2/ml × 4 × 10^6 ml = 8 million rotifers = 400 R/larva
b) 200 R/larva × 20000 larva = 4 million rotifers

CULTURE & BACKUP :

a) 8 million = 40 R/ml × 200 litres = feed for day 2.
b) 20 million = 100R/ml × 200l = suggested minimum preparation.
c) Tank ≠ 1 = 100 R/ml × 150 l = 15 million on day 2.
   Tank ≠ 2 = 50 R/ml × 150 l = 7.5 million on day 2.
d) 1 million R = 0.18 g (our wet weights).

2. *COPEPODS*

a) 1 million copepodites (> 100 μ screen size) = > 2.3 g.
b) Day 6 feed = 1 million C = 50 l × 20 C/ml.
c) Use Table 1 after day 6, using 3 l weight samples, rather than ≠ C/ml.

3. *HATCHLING (H) mahimahi = 0.9 mg*

On day 18, 36 g of food can be provided with 40000 newly hatched mahimahi larvae.

4. *LARVAL WEIGHT GAIN ANALYSIS*

a) Rotifers/first feeding : Assume larva growth = 0.7 mg × 20000 larvae × 25 % weight gain/day × 0.3
   FCE = 1.05 g food on day 2 = 5.8 million Rotifers. Use table 1 for subsequent days.
b) Copepods : Use weights, not numbers, unless scale is not available.

APPENDIX II
ALGAL NUTRIENT (F/2) PREPARATION

Algal nutrients are prepared by adding 4 major chemicals, 5 trace elements, 3 vitamins, and 1 optional chemical (silicate : we already have plenty in our water) to de-ionized or distilled water. This « F/2 » stock is stored in the refrigerator and added to the sterilized seawater at 2 ml per litre to give the final concentrations listed below.

ELEMENT (AW)	FINAL CONC.	SOURCE (FW)	AMOUNT OF SOURCE PER 3.5L STOCK
N (14)	12 mg/l	NaNO3 (85)	128 g
P (31)	1.2 mg/l	KH2PO4 (136)	9.2 g
EDTA (292)	4.3 mg/l	Na2EDTA.2H2O (372)	9.7 g
Fe (56)	0.65 mg/l	FeCl3 (162)	3.4 g
Trace elements	(see below)		1.8 ml @ x 5
Vitamins	(see below)		17.5 ml

TRACE ELEMENTS are easier to measure as dilute stock solutions. A single pipette can be used to add each of these to the F/2 stock. Each of these 100 ml bottles is enough for about 100000 l of algae culture. These bottles do not have to be refrigerated. Keep each trace element in a separate 100 ml. bottle.

ELEMENT (AW)	FINAL CONC.	SOURCE (FW)	AMOUNT OF SOURCE PER 100 ML STOCK
Cu (64)	2.5 ug/l	CuCl2.2H2O (170)	0.66 g
Zn (65)	5.0 ug/l	ZnCl2 (136)	1.05 g
Mn (55)	50.0 ug/l	MnCl2.4H2O (198)	18.00 g
Co (59)	5.0 ug/l	Co(NO3)2.6H2O (291)	2.47 g
Mo (96)	3.0 ug/l	Na2MoO4.2H2O (242)	0.76 g

VITAMINS B12 and biotin should be kept frozen, if possible, at 100 mg per 50 ml distilled water. To prepare vitamin stocks, add 16 mg (8 ml) of thawed, stirred vitamins to 300 ml distilled water. Also add 6.6 g Thiamine HCL (vitamin B1). Store this solution in the refrigerator and use 17.5 ml per 3.5 l. F/2 stock.

Final concentrations using these doses are :

B1 = 2.2 × 10-4 g/l;
B12 = 5.4 × 10-7 g/l;
Biotin = 5.4 × 10-7 g/l

SILICATES may be added if needed to diatom cultures at a final concentration of 4.3 mg/l. 17.4 g of Na2SiO3. 9 H 2O in 400 ml of distilled water can be used as a source (1 ml/l) of enrichment for small cultures. For large cultures, it is easier to weigh out 4.35 g of silicate per 100 L of culture. Hawaiian waters have abundant silicate, so it is not added.

BORON in the form of boric acid is sometimes used in µg/l quantities for seawater sources which lack boron. Hawaiian waters are not limited by Boron, so it is not added.

SUBSTITUTIONS :

You can compute the grams (g) of any source mineral needed with this formula :

$$\frac{\text{(final conc.) (500 ×) (FW) (3.5 l)}}{\text{(AW) (moles of element per mole of source)}} = \text{g of mineral added to 3.5 litres of F/2 stock}$$

i.e., for N : $\frac{(12\ \text{mg/l})\ (500)\ (85\ \text{g/mole})\ (3.5\ \text{L})}{(14\ \text{g/mole})\ (1\ \text{mole/mole})\ (1000\ \text{mg/g})}$

= 128 g of NaNO3

Substitutions can also be made by the following proportion :

Amount of new source

$$= \frac{\text{(FW new source) (amount of old source)}}{\text{(FW old source)}}$$

as long as the new element has the same number of moles of the important element as the old source.

SOME SUBSTITUTIONS :

CuSO4 (FW = 160)	0.62 g/100 ml stock
CoCl2.6H2O (FW = 238)	2.02 g/100 ml stock