Live Food for Marine Fish Larvae

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The choice of a particular fish species for aquaculture starts a cascade of subsequent choices of live feeds for a fish hatchery. Once a fish species is chosen, the first larval feed (zooplankton such as copepods, rotifers or *Artemia*) may be chosen, and each of these zooplankters has different algae requirements for optimum performance. The first feed is usually determined by larval mouth size. For this discussion, the choices are large, medium and small mouth fish larvae. The fish chosen as examples here are species raised by the author, but many other species fall into similar categories of size and nutritional needs.

Choosing a first feed for marine fish larvae

Some fish larvae have simple requirements for live feed, especially larvae with large mouths. A hatchery that only produces mahimahi (*Coryphaena hippurus*) or flying fish (ie., *Hirundichthys* spp.) would not need algae production facilities, and thus would not require much space for live feeds. For these species of fish, small strain *Artemia* may be used as a first larval feed until the larva is large enough to ingest enriched (ie, 2nd instar) *Artemia*. At that stage, most marine fish perform much better if their *Artemia* is enriched with a source of highly unsaturated fatty acids (HUFAs), such as DHA Selco. This method works for short term feeding because these species (and probably most species) store enough of the critical fatty acids in their eggs to allow larvae to double their weight before depleting their reserves (Kraul *et al.*,,, 1992). Although *Artemia* works for these species, these fish may also be raised with rotifers or copepods, and *Artemia* may not be the cheapest choice even if it is the simplest. There are examples (ie, some flat fishes) where larval survival is adequate without enriching *Artemia*.

Most marine fish larvae require prey smaller than *Artemia*, and these prey require more complicated culture facilities. Different live feeds have different needs. Some zooplankton (ie, rotifers) eat almost any species of algae or even processed feeds, but other zooplankton are very specialized and will only grow well on certain species of algae. This principle also applies to algae. Certain algal species can be grown at very high densities with cheap nutrients, but other algal species require intensive care and more costly nutrients.

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Examples of fish that require medium sized feed for first feeding larvae are grey mullet (Mugil cephalus), Pacific threadfin (*Polydactylus sexfilis*, called moi in Hawaii), jacks *(Seriola dorsalis*, *S. rivoliana*, *Caranx ignoblis*), and tuna (Katsuwonis pelamis in this example). As with larger larvae, un-enriched rotifers may be used for first feeding, but should be enriched with a HUFA source as soon as possible so that larval HUFA reserves are not depleted. First feeding success for larvae with smaller mouths (ie, jacks) is sometimes improved by stimulating rotifer egg production in the larval tank, and this bypasses the opportunity to enrich those rotifers. Rotifers should be enriched and flushed daily as soon as possible. Jacks and tuna have higher HUFA requirements than mullet or threadfin, but all marine fish do better with proper feed enrichment. Although these "medium mouth" fish can feed well on rotifers, their survival and strength can be significantly improved by using copepods, especially at first feeding (Kraul S., 1983, Kraul, *et al.,,,* 1993). Seriola rivoliana, for example can achieve double the yield (20% *vs* 10%) at fingerling size when they are started on copepods. Higher survival at first feeding in this case is mainly due to feed size. The economic value of using copepods depends on the particular situation at each hatchery: copepod culture requires more labor than rotifer culture, and 10% survival can be more than adequate for many situations. Tuna have very high nutritional requirements, and this author has only raised them on copepods, even though they can eat rotifers.

Small mouthed fish larvae may require feeds smaller than juvenile rotifers. Examples from the author's experience include peacock grouper (*Cephalopholis argus*), flame angelfish (*Centropyge loricula*), and snappers (*Pristipomoides filamentous*). This particular snapper will ingest rotifers at first feeding, but does not survive well unless fed copepod nauplii. Peacock grouper is a hardy larva and grows large enough to ingest rotifers within a few days feeding. Centropyge angelfishes represent the smallest mouth size, and require copepod nauplii for at least ten days. Even smaller feed items, such as the ciliate Euplotes sp. can be cultured and are associated with successful culture of some fish larvae. Research at Pacific Planktonics indicates that this ciliate may be too small to contribute to larval fish growth under our conditions.

Copepod culture

Copepods offer several advantages and disadvantages when compared to other hatchery feeds. The main differences are fatty acid values, sizes of nauplii and adults, and ease of culture. Copepods are able to synthesize HUFAs from algae, so enrichment is not needed. This is good for large ponds where uneaten live feeds cannot be easily removed. In this way, labor is reduced because once the copepods are stocked in a larval fish tank, they may not need to be restocked. Copepods have a large size range from 1^{st} nauplii to adult copepodite, and are a good size for the entire hatchery phase for some fish. The disadvantage with copepods is their relatively slow growth rate and low culture density compared to rotifers. To use copepods successfully, more space must be alloted and more live algal cultures must be available.

Several copepod species can now be cultured at useful densities. The author has cultured *Euterpina acutifrons* at densities of 100/ml, and the paracalanids *Parvocalanus crassirostris* and *Bestiolina similis* at densities of 20 to 90/ml (Figure 1). Euterpina is a harpacticoid, and is thus more tolerant of handling and high density culture. Some fish larvae do not feed well on Euterpina as a first feed (Kraul, S. 1989), but all marine fish larvae tested will do well on Euterpina as a second feed. In literature reports (McKinnon *et al.*,,, 2003), Paracalanids are often cultured at densities of 1 to 20/ml, so they are more difficult to provide in large numbers to fish larvae. Analysis of fatty acid contents of these three copepod species shows some differences, but all of them have adequate HUFA content to promote good larval fish survival.

Recent research at Pacific Planktonics has resulted in significant gains in production of the calanoid copepod, *Bestiolina similis*. In 2004, the author worked at another company using *Parvocalanus crassirostris*, a species that is also being used by The Oceanic Institute (OI) in Hawaii. OI reports (Shields and Laidley, 2003) obtaining copepod densities of 1 to 4 copepodites per milliliter (ml) and 5 to 25 nauplii per ml, with average harvests of 4 nauplii per ml per day. The other hatchery uses very large culture tanks but only obtains densities of 2 to 3 copepods per milliliter, with significant labor costs. McKinnon *et al.*,,, (2002) reported a maximum density of 8 *B.similis* copepods per milliliter in their cultures. We routinely obtain greater than 20 copepodites plus 20 to 50 nauplii per milliliter in our normal cultures, and have obtained as many as 91 nauplii per ml in a small tank.

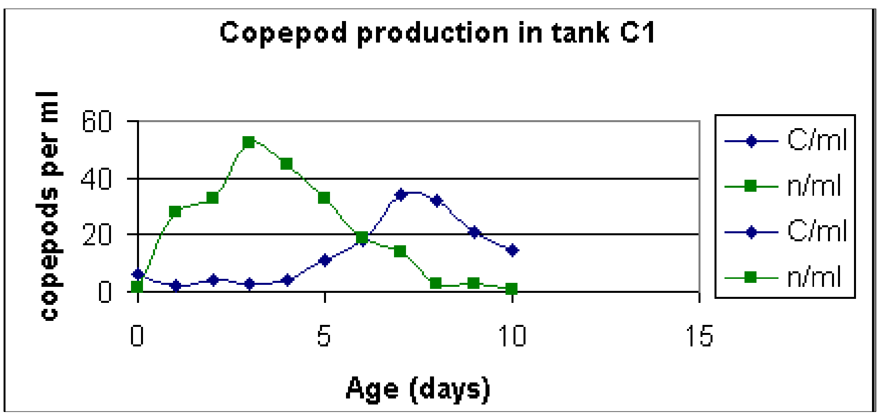

Figure 1: Typical culture densities of *Bestiolina similis* at Pacific Planktonics

Algae choices

Choice of zooplankton species may determine the algal species that needs to be raised. Using *Artemia* allows one to use any algal species as a green water background, or to avoid using algae completely. Choosing rotifers as a feed allows the use of almost any algae because modern enrichment media (ie, Algamac 2000) is the main provider of nutritional value in the rotifer when it is eaten by fish larvae. Thus, the best choice for algal species when using rotifers is an alga that is easy to raise at high density, and that does not foul the larval rearing tank. For instance, *Tetraselmis chuii* grows at high density ourdoors using cheap nutrients. This algal species is good for harpacticoid copepods and rotifers and as green water. *Chaetoceros gracilis*

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also grows at high density outdoors using cheap nutrients. Chaetoceros is ok for copepods and green (brown) water. *Isochrysis galbana*, Tahitian strain (T.Iso) grows at high density ourdoors with costly nutrients, can give very high yield in paracalanoids and is easier to culture than *Rhodomonas*. *Rhodomonas* spp. usually grows to lower densities (by weight) and is harder to grow, but provides very good nutritional value for copepods (ie, has very high HUFA content). *Dinoflagellates* provide the best nutrition for copepods, and yield the highest copepod growth rates, but are very difficult to grow. *Nannochloropsis* is one of the easiest algae to grow, uses cheap nutrients, is not a good feed for the copepods mentioned here, but is good for rotifers and as green water.

Algae species makes a big difference in yield of copepods, and our results differ from others. McKinnon, *et al.*,,, (2003) found about equal egg production in Bestiolina using *Isochrysis* and *Tetraselmis*. We did not compare egg production in phase 1, but found much better performance using *Isochrysis galbana* (T-Iso) than we did with *Tetraselmis chuii*. The *Tetraselmis* we were using settles to the bottom easily when not aerated sufficiently, and probably suffocates copepod eggs prior to hatch. The results are dramatic, and we do not use *Tetraselmis anymore*. We cannot aerate *Bestiolina* cultures enough to keep *Tetraselmis* suspended without harming the copepods.

Comparing copepods

Figure 2 shows fatty acid values for some of the copepod species that the project director has worked with. The values vary with copepod species and the algae they were fed. *Bestiolina similis* and *Parvocalanus crassirostris* (ParvoA) were fed *Isochrysis galbana*. ParvoB was fed *Rhodomonas*. *Euterpina acutifrons* was fed *Tetraselmis chuii*. When used as a feed for marine fish larvae that have a high demand for omega-3 HUFAs, all of the copepods performed well, and did not seem to be deficient in nutrients. In fact, copepods are one standard for measuring the performance of feeds that must be enriched, such as rotifers and *Artemia*. Feeds higher in omega-3 fatty acids increase stress resistance in fish larvae (Kraul, *et al.*,,,, 1993).

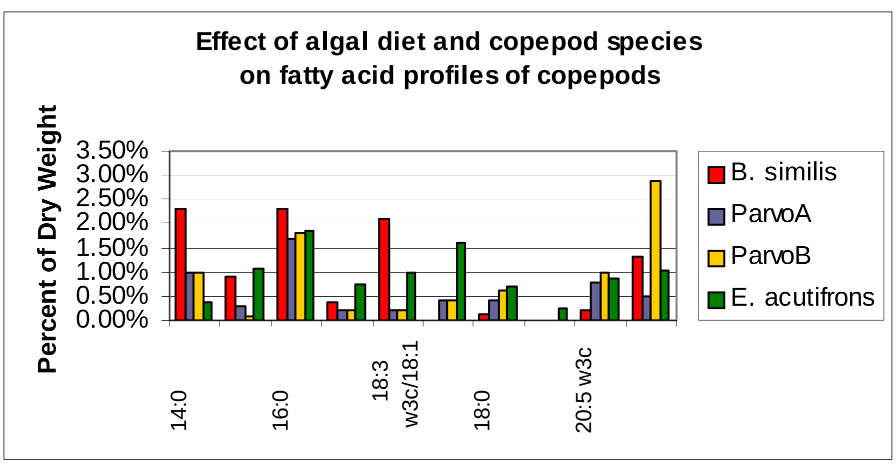

Figure 2: Comparison of fatty acids in 3 species of cultured copepods and 3 species of algae.

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Nutritional values and enhancement of larval feeds

Profiles of the major fatty acids contained in our larval feeds is presented in Table 1 below, and in Figure 3. Lipids were assayed by Microbial ID, Inc. of Newark, Delaware. Proteins were assayed by Eurofins Scientific, Inc., in their Des Moines Iowa facility. All feeds were concentrated on plastic screen mesh and blotted "dry" from under the screen. This does not represent true dry weight, but with further air drying at Microbial ID, the fatty acid values seem comparable to our previous results. Protein assays (Figure 4) were performed on feed blotted without further drying, and are thus lower in percent protein than true dry weights, but adequate for comparing relative values among our feeds. There are considerable differences among the feeds. *Bestiolina similis* had the highest protein and lipid contents of all the small feeds, but had less total fat than *Artemia* (brine shrimp) enriched with DC-DHA Selco. The ciliate we can culture in volume, *Euplotes sp*., showed a surprisingly good level of protein and lipid, and was able to incorporate enrichment products. Ciliates did not incorporate enrichment products as well as standard feeds like rotifers and *Artemia*, but the levels attained would seem to offer sufficient nutrition to support larval fish health and growth.

Enrichment procedures were based on our methods of enriching other larval feeds. Protein Selco Plus (PSP) was rinsed through an 11um screen and added to 10 liters of concentrated ciliates. This allowed us to rinse out the excess PSP after enrichment prior to the assay. During larval fish testing, the first feeding of ciliates each day was either un-enriched, or only enriched 2 hours. The manufacturer suggests 8 hours of enrichment for rotifers, but we felt it was impractical to enrich at midnight until we could assess preliminary data. The assayed ciliates were enriched 4 hours. The last daily ciliate feeding was enriched 8 hours. Chart 2 shows that ciliates were able to incorporate modest amounts of protein from PSP. Some fatty acids, especially the omega 3's 20:5 (EPA) and 22:6 (DHA) were boosted significantly by PSP.

Ciliates and rotifers (assayed as a control) were enriched 2 hours with 300ppm Algamac 2000, our standard dose. Ciliates did not incorporate quite as much lipid as rotifers, but still boosted their nutritional values to a level nearly comparable to rotifers. During a 2 hour enrichment, one might not expect ciliate tissues to metabolize the enrichment, so an assay reflects the nutritional values of the enrichment medium. Our experience with rotifers as a feed for other larval fishes indicates that this 2 hour enrichment greatly increases the health and growth rate of marine fish larvae.

Enrichment with DC-DHA Selco showed very different incorporation values for ciliates and *Artemia*, but still showed considerable enrichment of ciliate omega 3 fatty acids. Overall, we conclude that ciliates do not seem totally without nutritional value, and ought to support larval fish survival, even if only for a few days beyond normal starvation time. Enriching ciliates with any of the tested products is effective and boosts protein and lipid values to a level of at least 50% of standard feeds. Successful enrichment of ciliates did not improve their value as larval fish feed at our farm because the fish larvae did not ingest enough ciliates to fill their guts. Control groups fed copepod nauplii always survived better than ciliate fed fish larvae.

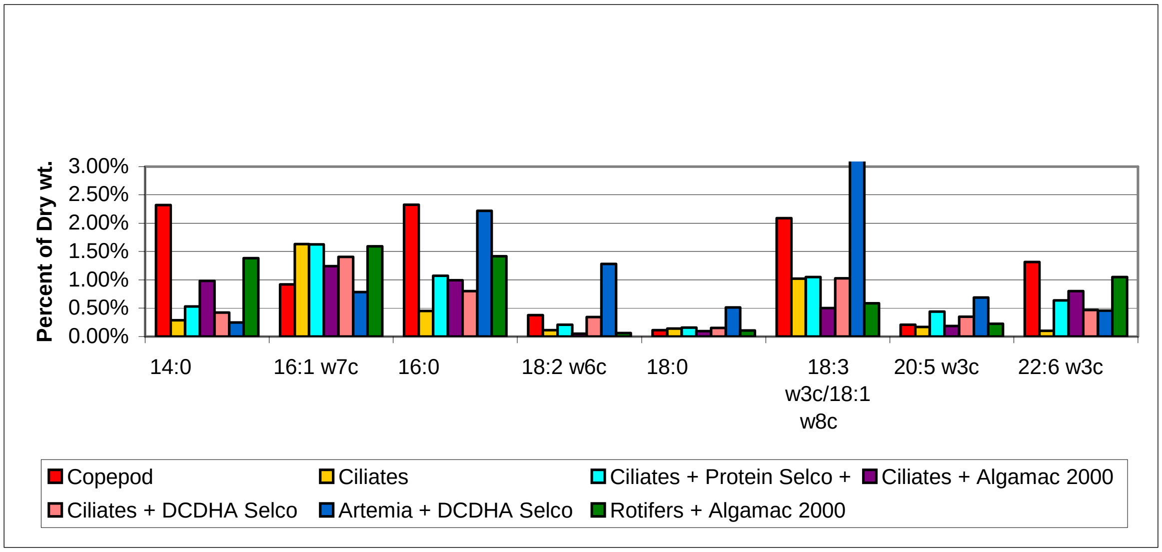

Figure 3: Major fatty acids in larval fish feeds at Pacific Planktonics

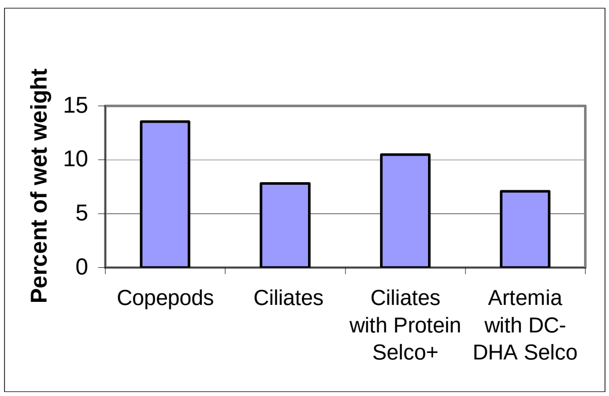

Figure 4: Relative protein content of feeds used at Pacific Planktonics

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Table 1: Profiles of major fatty acids in larval feeds used at Pacific Planktonics
Values are in percent of dry tissue weight. Total fatty acid includes values less than 1%, which are not shown here. See also Chart 1. Note that the 18:3 peak for *Artemia* is off the scale in Figure 3.

Fatty Acid	Copepods	Raw Ciliates	PSP Ciliates	Algamac 2000 Ciliates	DC-DHA Selco Ciliates	DC-DHA Selco *Artemia*	Algamac 2000 Rotifers
14:0	2.32%	0.29%	0.53%	0.98%	0.42%	0.25%	1.39%
16:1 w7c	0.92%	1.63%	1.63%	1.24%	1.40%	0.79%	1.59%
16:0	2.33%	0.45%	1.08%	1.00%	0.80%	2.22%	1.42%
18:2 w6c	0.38%	0.11%	0.21%	0.05%	0.35%	1.28%	0.06%
18:0	0.11%	0.14%	0.16%	0.10%	0.15%	0.51%	0.11%
18:3 w3c/18:1 w8c	2.09%	1.02%	1.05%	0.50%	1.03%	9.10%	0.59%
20:5 w3c	0.21%	0.17%	0.44%	0.19%	0.35%	0.69%	0.22%
22:6 w3c	1.32%	0.10%	0.64%	0.80%	0.47%	0.46%	1.05%
Total Fatty Acid	11.3%	5.60%	7.40%	6.30%	6.70%	19.00%	8.20%
(% of dry wt for all FAs)							

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