

# Tiny Arctic Crustacean Improves Larval and Broodstock Diets

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## Far in the Arctic North

thrives a unique decapod microcrustacean that has potential to improve the larval and broodstock nutrition of a wide variety of aquaculture species. The organism is analogous to *Artemia* in some respects, except that its content of highly unsaturated fatty acids and pigments is considerably richer. Also, its length measures about 800  $\mu$ , as compared to 420-530  $\mu$  for newly hatched *Artemia* nauplii.

## Arctic Lake

The culture lake is located in northern Canada. It is a pristine Arctic salina measuring about 400 km<sup>2</sup> in area. The unique physical and chemical properties of the lake allow unimpeded culture of the organism on a continuing annual basis.

The depth of the salina is no greater than 2 m, which allows good penetration of sunlight. The chemistry of the lake is extreme. The salinity can be over 80 ppt, while the ionic strength of the water is excessive. Typical levels of sulphate ion are very high, thus causing hydrolysis and a very acidic pH of about 3.0.

The inhospitable climate and water chemistry of this ecosystem exclude most traditional predators. No fish, birds, or reptiles inhabit the area, and most microorganisms cannot survive within the lake.

## Eggs and Harvest

The zooplankter that grows in this cold, salty, and acidic water is a blood-orange crustacean that owes its coloration to a high level of astaxanthin pigment (the same pigment that colors shrimp, lobster, and salmon). Adults lay two types of eggs: summertime eggs, which rapidly hatch, and wintertime eggs that are capable of entering diapause and surviving for many years prior to hatching.

These diapause eggs may or may not become activated by the late-spring conditions of strong sunlight and high water temperature. In fact, it appears that eggs from various years may hatch during any summer, thus providing an elaborate mixing of the gene pool and evolution of the species to meet natural hardships.

## Production

Typically, fishing for adults commences in early June. The harvested organisms are drained and immediately flash-frozen into 750-g blocks. A small amount of the antioxidant Ethoxyquin® is added prior to freezing to retard oxidation of the valuable nutrients. The harvested product is being commercialized under the trade name Cyclop-eeze®.

The frozen blocks are vacuum-sealed to prevent oxidation. Freeze-dried product is derived from the frozen wafers by the process of lyophilization, which allows removal of up to 80% of the water from the organisms at the modest temperature of 21° C. The process of freeze-drying preserves the content and activity of enzymes, pigments, and long-chain, highly unsaturated fatty acids (HUFA).

Oil is extracted from the freeze-dried material using an organic solvent extraction system under vacuum.



*Cyclop-eeze® are harvested from a shallow, salty lake in northern Canada.*



*Several frozen and freeze-dried products are made from the processed 800- $\mu$  organisms.*

**Table 1. Comparison of nutrients in Cyclop-eeze and *Artemia nauplii*.**

PARAMETERS	CYCLOP-EEZE	ARTEMIA NAUPLII*
Protein	62%	71%
Lipid	36%	17%
Ash	3%	5%
Carbohydrate	3%	3%
Astaxanthene	3,000 to 7,500 ppm	31 ppm
Canthaxanthene	15 ppm	102 ppm
18:3n3 (Linoleic Acid)	10.45%	1.3%
20:5n3 (EPA)	11.74%	0.25
22:6n3 (DHA)	11.09%	2.7%
Enzyme Activity (Superoxide Dismutase)	30,000	22,500
Average Body Length	800 um	500 um

\*Equilibrium between free and esterified forms depends on environmental factors

Note: Cyclop-eeze Sample: Argent #70280  
Artemia Sample: Argent #95-p Argemtemia Platinum Label

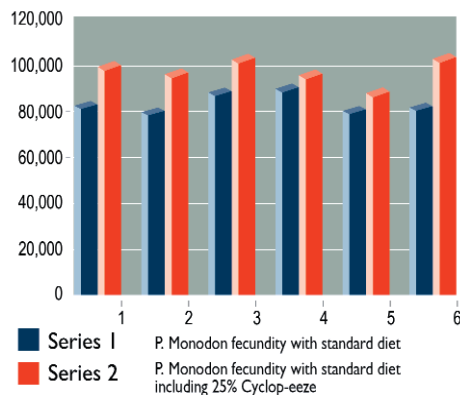
The processing technology renders the complete lipid, pigment, and lipophilic content into viscous oil. This oil can be used directly for feed inclusion, or it can be emulsified with a surfactant to make a feed booster for rotifers, *Artemia nauplii*, and the like.

### Nutritional Composition

This microcrustacean offers several nutrients of importance in aquaculture diets, especially in the crucial stages of larval rearing and sexual maturation (Table 1). Many fish and crustacean species require substantial levels of HUFA, particularly eicosapentanoic acid and docosahexanoic acid, during larval development. *Artemia* are typically deficient in these nutrients. Inadequate levels of HUFA can result in mortality, slow growth, deformities, poor stress resistance, and improper pigmentation.

Perhaps one of the most dramatic aspects of this product is its extraordinarily high content of a natural red pigment called astaxanthin. Levels of 3,000-7,500 ppm are typical of the freeze-dried product. This pigment not only imparts vivid coloration in shrimp, ornamental fish, and salmon, but is also thought to improve the viability of eggs when offered in broodstock diets.

Crustaceans utilize free amino acids to osmoregulate; consequently, organisms raised in hypersaline environ-



**Figure 1. Fecundity of *P. monodon* eggs augmented with Cyclop-eeze.**

ments tend to be very rich in free amino acid content. This rich content of free amino acids, coupled with high levels of betaine, create a strong feeding stimulus for most predators. This reduces losses through feed waste and leaching of nutrients, and improves feed efficiency.

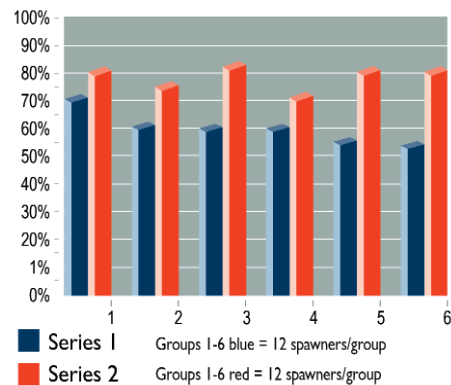
The use of freeze-drying technology also preserves the content of digestive enzymes in Cyclop-eeze. In early-larval forms with rudimentary digestive systems, enzymes within prey organisms are thought to be important in improving bio-availability of nutrients.

Finally, an immune-stimulating effect has been noted with this product. Studies have documented enhanced survival in bacterial challenge tests and hatchery production systems.

### Aquaculture Applications

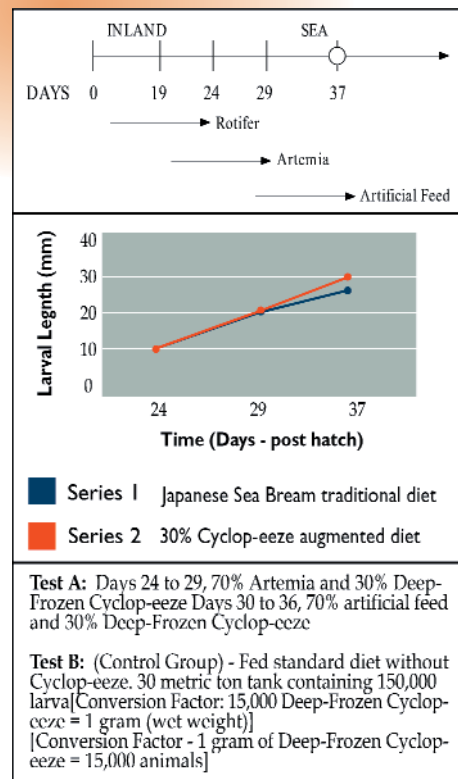
A number of trials have been conducted using Cyclop-eeze as a feed ingredient. In trials with *P. monodon* larviculture, initial feeding of 400-µ freeze-dried particles began during late mysis/early PL<sub>1</sub>. From PL<sub>2</sub> onward, deep-frozen product was offered. The results showed an increase in survival to PL<sub>29</sub> from 18% to 40%. Coloration was also enhanced, and growth rates were comparable to pure *Artemia* feeding.

In trials with *P. monodon* broodstock, performance of spawners receiving a traditional diet of fresh squid, clams, mussels, and commercial brood diet was compared with 25% by weight replacement with deep-frozen Cyclop-eeze. The addition of Cyclop-eeze significantly improved fecundity and hatchability (Figures 1 and 2).



**Figure 2. Percentage hatchability of fertilized eggs.**

In trials with marine fish, dietary inclusion of frozen Cyclop-eeze improved growth rate of red sea bream and European sea bass fry (Figures 3 and 4). Both the extracted oil and frozen Cyclop-eeze were compared with a high-protein diet, cuttlefish meal, krill oil, and frozen krill for broodstock of Japanese red sea bream (Figure 5). The results were favorable in terms of increased buoyant eggs, reduced abnormal eggs, improved color, higher hatching rate, and greater overall production of fish seed.



**Figure 3. Comparison of Japanese red sea bream larval growth.**

**Test A:** Days 24 to 29, 70% Artemia and 30% Deep-Frozen Cyclop-eeze Days 30 to 36, 70% artificial feed and 30% Deep-Frozen Cyclop-eeze

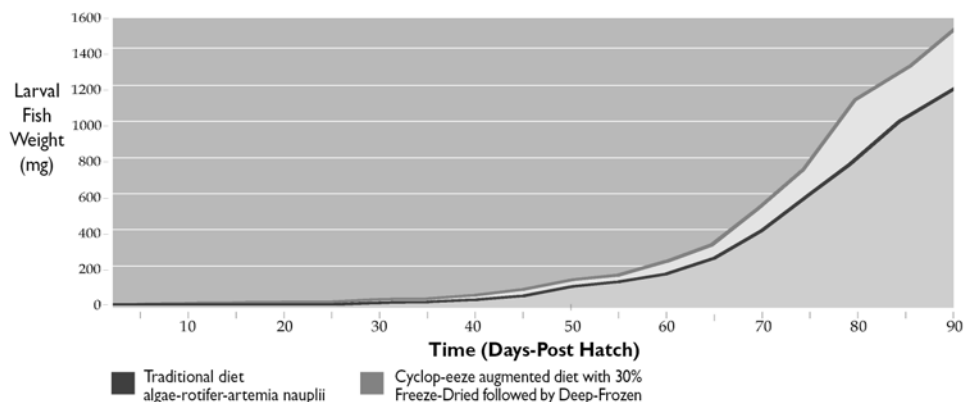
**Test B:** (Control Group) - Fed standard diet without Cyclop-eeze. 30 metric ton tank containing 150,000 larva [Conversion Factor: 15,000 Deep-Frozen Cyclop-eeze = 1 gram (wet weight)] [Conversion Factor - 1 gram of Deep-Frozen Cyclop-eeze = 15,000 animals]

## Recommended Feeding Rates

Dosage of Cyclop-eeze products is generally tapered, with maximum feeding regimens for larval stages and minimal augmentation during juvenile and adult stages. However, significant augmentation of broodstock diets is beneficial, typically increasing fecundity and enhancing hatchability and survival of larval species.

## Conclusion

A planktonic microcrustacean cultured in a saline and acidic Artic lake has interesting nutritional composition for use in aquaculture diets. It is rich in highly unsaturated fatty acids, astaxanthin, attractants such as free amino acids and betaine, digestive enzymes, and immune stimulants. Feeding trials with shrimp, marine fish, and salmonids have demonstrated improved growth rates of larvae and reproductive performance of broodstock.



**Figure 4.** Growth enhancement of European sea bass with Cyclop-eeze diet augmentation.

Developmental Stage	High* Protein	Fish meal + Cuttlefish meal	Krill Extract oil	Frozen Raw Krill	Cyclop-eeze Extract Oil	Cyclop-eeze Deep-Frozen
Avg. Eggs/Fish <sup>**</sup>	1.49 x 10 <sup>6</sup>	1.21 x 10 <sup>6</sup>	0.9 x 10 <sup>6</sup>	2.0 x 10 <sup>6</sup>	2.6 x 10 <sup>6</sup>	3.1 x 10 <sup>6</sup>
Buoyant Eggs* (%)	49.1	68.6	69.6	82.7	96%	98%
Abnormal Eggs <sup>**</sup> (%)	77.5	22.1	20.9	8.1	4%	2%
Av. Oil Globules	2.4	1.5	1.2	1.1	1	1
Color Index	17	27	39	49	93	92
Rate of Hatching (%)	83.1	93.7	67.5	90.3	98%	98%
Deformity (%)	14.8	4.7	8.4	2.0	2.0%	2.0%
Final productivity of fish seed from total eggs hatched	21.1	52.8	41.4	68.1	92%	93%

\* - buoyant eggs are those which float on the water surface and are alive      \* - in excess of 60% protein in an inert formulated diet  
 \*\* - abnormal eggs are those which do not float and probably nonhatching

**Figure 5.** Effects of broodstock diets of different composition on spawning and egg quality of Japanese red sea bream.