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Cultured microalgae as aquaculture feeds

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Abstract. Approximately 90% of the 14.5 million metric tons of aquaculture-produced animals in 1993 were reared using phytoplankton as a feed source during one or more stages. Worldwide aquaculture production is growing at 7.2% per annum, with trends toward intensification and greater control over total nutritional input. Algal production for feeds is divided into intensive monoculture for larval stages of bivalves, shrimp, and certain fish species, and extensive culture for growout of bivalves, carp, and shrimp. Favored genera of microalgae for larval feeds include *Chaetoceros, Thalassiosira, Tetraselmis, Isochrysis,* and *Nannochloropsis.* These organisms are fed directly and/or indirectly to the cultured larval organism. Indirect means of providing the algae are through artemia, rotifers, and *Daphnia,* which are, in turn, fed to the target larval organisms. At a calculated cost of $50/kg dry algae equivalent, there is significant pressure to replace algae with prepared diets. The largest algal production (100,000 liters/day) facilities support bivalve larval and nursery cultures. Bivalve growout technology usually depends on natural sources of phytoplankton-rich water. Natural algal production in shrimp ponds supplies up to 50% of the nutrition of these animals during growout. The major challenge facing algologists is reducing production costs while maintaining reliability.

The production of algae for feed is a critical component in the culture of bivalves, shrimp, and most fish. Martek Corporation of Columbia, Maryland has estimated the value of microalgae cultured by aquaculturists worldwide to be US$34 million/yr in support of the bivalve and shrimp industry. Table 1 provides a breakdown of aquaculture production by major animal groups for 1993, as reported to FAO. This table accounts for 90% of the total fish and shellfish production defined by FAO. Those species not included in Table 1 either do not have a larval cycle included in their culture, or too little is know of the actual culture conditions by the authors. Of the 14.66 million metric tons total defined in Table 1, better than one half came from traditional bivalve (which includes weight of shell) and carp species, both of which rely heavily on natural phytoplankton productivity for food throughout their culture cycle. Eighty-five percent of all aquaculture production comes from eastern Asia (Akiyama 1992). In Taiwan alone, over 5 billion bivalve (*Meretrix lusoria*), 5.85 billion shrimp (*Penaeus* spp.), and 152 million fish larvae were produced commercially in 1990 with the help of algae and other live feed organisms (Liao et al. 1991).

The aquaculture industry worldwide has experienced an annual growth rate of 8.7% over the 1979 to 1989 period (Akiyama 1992). Over the six-year period preceding that presented in Table 1, culture of those species represented therein increased by 7.2% annually. While constraints on growth, such as disease, pollution, government regulation, and competition for optimal culture areas restrict the industry, there is ample reason to believe the industry will continue to grow at a similar pace for several more years. In 1993, total aquaculture production accounted for approximately 22% of the total world harvest of fish and shellfish for direct human consumption.

The production of algae is considered secondary to the production of the target aquaculture animal. With the possible exception of the large-scale bivalve hatcheries, algal culture is a ''side room'' activity. Algal densities are measured in terms of cells per milliliter to match units with larval stocking densities. Output is measured as liters per day rather than grams of dry weight per square meter per day. There is generally less emphasis on maximizing growth rate. Of greater importance is having a reliable supply of algal culture that can be transferred to the animal production units when needed.

The following sections provide an insight into the algal production requirements and production methods for each of the groups of animals defined in Table 1. We hesitate to estimate the total annual algal production undertaken by the aquaculture industry. Instead, we have focused on the production of zooplankers because: (1) these are more easily quantified, (2) zooplankton usually require algal production

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Table 1. Major animal aquaculture species listed relative to larval feeding regimen.[a]

Algal feed throughout	Mix: algae/ zooplankton	Only zooplankton	Prepared diet
Oysters 1,019,451	Marine shrimp 675,465	White bream 218,921	Salmon 391,245
Scallops 975,578	Milkfish 354,515	Sea bream 89,470	Trout & char 326,013
Mussels 1,046,474	Chinese carp 4,685,120	Sea bass 32,746	Tilapia 550,600
Clams 901,374	Common carp 1,240,551	Yellowtail 141,799	Catfish 337,968
Miscellaneous[b] 0.18×10^6	Miscellaneous[c] 1.59×10^6		Miscellaneous[d] 7,500
Total: 4.12×10^6	8.93×10^6		1.61×10^6

[a] Worldwide 1993 harvest is given in metric tons (FAO 1995).
[b] Includes abalone and other freshwater and marine mollusks.
[c] Includes Indian carps and other cyprinids, mullet, perch and perch-pike, sturgeon, flatfish, *Macrobrachium*, and other freshwater shrimp.
[d] Includes cod and grouper.

as well, and (3) the period of time (nonbivalve) larvae exist exclusively on algae is minor compared to the period spent feeding on zooplankters.

As evident in Table 1, Martek's focus on the bivalve and shrimp sectors is certainly justified. For similar reasons, the following sections describe algal production by these two industries. Production of algae for rearing zooplankton, for inclusion into growout feeds, and in growout culture itself is also touched upon.

Bivalve hatcheries

The culture of bivalves is among the oldest of the aquaculture industries. Growout culture ranges from providing attractive settling sites in highly productive, near-shore environments for naturally produced spat to placing hatchery-reared, settled spat in rafts into such sites. In either case, growout depends on naturally occurring bacteria and phytoplankton for food. Algal culture occurs only in hatcheries producing eyed larvae or spat. It is estimated that worldwide, 50% of the bivalves harvested came from hatchery seed.

Bivalve hatcheries rely on a broad range of algal species. These include *Thalassiosira pseudonana, Skeletonema* sp., *Chaetoceros* spp., *Chlorella minutissima, Isochrysis galbana, Pavlova* spp., *Phaeodactylum tricornutum, Nitzschia* sp., *Gomphonema* sp., and *Tetraselmis subcordiformis*. Any given hatchery may use a number of species.

Coast Oyster Company of Quilcene, Washington is the world's largest oyster-growing company, producing over 30 billion eyed larvae per year (Donaldson 1991). Algae are cultured to feed broodstock, larvae, and seed. Broodstock are fed algae up to two months prior to spawning. While this period is long, the number of broodstock animals is relatively small, consuming 5% of the total algal production. Broodstock feeding focuses on maintenance rather than nutrition/reproduction. Feeding larvae in the hatchery lasts up to 21 days and consumes 42% of the total algal output. Larval nutrition is important, determining the species of algae Coast Oyster has selected. *Skeletonema* (clonal designation ARC-3) and *Chaetoceros calcitrans* (CCAL) are used for larvae up to 150 μm and *Thalassiosira pseudonana* (UW3H) is used for larger larvae, broodstock, and juveniles. At Coast Oyster, settled spat are also sold, meaning that seed oysters are fed as well, consuming 53% of the algal output.

Table 2. Units devoted to microalgal culture at Coast Oyster Company.[a]

Vessel	Units inoculated per day	Units in system
1-liter flasks	12	24
20-liter Carboys	9	18
3000-liter tanks	4–5	18 available
20,000-liter tanks	6–7	20 available

[a] Daily algal harvests are as high as 100,000 liters/day at 3×10^6 cells/ml (Donaldson 1991).

All algae at Coast Oyster Company are grown in a batch system designed to maximize yield. Guillard's F/2 culture medium is used with 10% CO_2 injected into the aeration lines for 1 min of every 45 min. The primary concern is to maintain axenic cultures in log-phase growth. Table 2 provides an inventory of tanks and their frequency of use in supporting algal production. Transfers are effected at 6×10^6 cells/liter. Lights for the larger tanks are high intensity discharge (HID) light fixtures suspended 1–1.5 m above the tanks. During production season (March through October), harvests are targeted at 100,000 liters of algae per day at a culture density of 3×10^6 cells/ml. Assuming total annual production of algae at 20×10^6 liters, this would translate into 1500 eyed larvae/liter or 2×10^6 algal cells per eyed larva. Algal production accounts for 18% of total hatchery expenditures. The estimated cost of algal production at Coast Oyster Co. is $50/kg dry weight equivalent.

The algal culture scaling-up steps of a typical commercial algal production unit are described in Duerr et al (1997). Sato (1991) provides an extensive description of each of the steps of large-scale hatchery production of algae at The Oceanic Institute. Figure 1 and 2 are photographs of final algal growout tanks in hatcheries showing two means of lighting common to the industry: HID lamps from above in Fig. 1, and fluorescent lamps from the side in Fig. 2. Some hatcheries prefer restricting final growout volumes to smaller units than those illustrated in Fig. 1 and 2. For example, the National Marine Fisheries Service hatchery in Milford, Connecticut, maintains axenic culture throughout, ending with 15-liter units that can achieve 100×10^6 cells/ml. They have estimated productivity rates equal to those of heterotrophically grown algae (Wickfors personal communication).

Algal and/or zooplankton culture for fish and shrimp

As listed in Table 1, the provision of algal food to fish and shrimp larvae includes direct, indirect, and no addition (given prepared diet instead), depending upon the species cultured. After the yolk-feeding stage, larval fish and shrimp listed in the two central columns enter a critical period in their transition to aggressive feeding on natural productivity or prepared diets. Continued growth can occur only if sufficient amounts of properly sized algae and/or zooplankton are available in the larval culture vessel; the mouth size of the larvae determines what size food particle

Fig. 1. A 5000-liter fiberglass tank with a high intensity discharge light suspended above the tank. The top is normally uncovered and aeration is provided.

Fig. 2. A 2000-liter polyethylene bag tank with a bank of fluorescent lamps provided across the back side. Each bag is sealed except for aeration inlets and outlets. Photo from State of Washington, Fish and Game Hatchery Clam Hatchery, Quilcene, WA.

Fig. 3. Rotifer (*Brachionus plicatilis*) with an egg cluster attached to the posterior end. Cilia line the oral cavity; feed and lipid droplets can be seen in the gut. Adults are 150 μm in length. The culture of the rotifer depends primarily on live algal material for food.

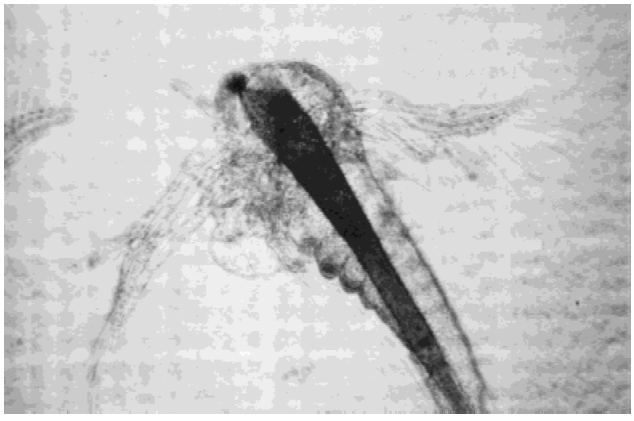

Fig. 4. Newly hatched brine shrimp (*Artemia salina*) nauplii (220 μm length) may be used as a food source for shrimp and fish larvae. When larger artemia (up to 1 cm in length) are required, live algae serves as their primary food source. The dark area is a nutrient emulsion-packed gut.

it will receive. Use of live feeds in larval fish and shrimp culture is a well-established procedure. As such, a number of algal and zooplankton strains of the correct size and nutritional content have been identified for each of the major aquaculture species. Species-specific hatchery procedures that define concentrations of algae needed as the larvae grow, as well as the concentrations and sizes of zooplankton to add once the algal particles are too small, have been developed. At the point in development that the larvae can ingest food particles as large as 100 μm, hatcheries will typically introduce a zooplankton food source to the larval culture vessel.

The zooplankters most commonly used are rotifers (*Brachionus plicatilis*) (Fig. 3) and brine shrimp or artemia (*Artemia salina*) (Fig. 4). To a much lesser extent, cladocerans (*Moina macrocorpa*, *Daphnia* spp.) and copepods (*Euterpina acutifrons*, *Tigriopus japonicus*) are used. These organisms range in size from 100-μm diameter (newly hatched rotifers) to 1–2 mm (adult artemia and copepods). For zooplankton to grow and reproduce in the hatchery, algal food is necessary. It is provided to the newly hatched rotifers or artemia (nauplii), until these zooplankters reach the desired size. Then, just prior to harvesting, the zooplankton may be given a boost of algae or a formulated emulsion to pack the gut of the zooplankter. This increases its nutritional value to the target culture species feeding on it. Finfish hatcheries producing algae (to feed rotifers or artemia) typically use the following species of algae: *Nannochloris oculata*, *Nannochloropsis oculata*, *Chlorella* sp., *Chlamydomonas* sp., *Tetraselmis tetrathele*, and *T. chuii*.

Hatcheries prefer to use artemia whenever possible because eggs (cysts) are readily purchaed as a dry, dormant-phase product. Upon immersion in seawater, the cysts hatch (nauplius stage) and can be ingested immediately, if the target fish or shrimp larvae are the correct size. Copepods are considered the most nutritious zooplankter, but they are very difficult to culture.

Animals in Table 1, column 2 (receiving a mix of algae and zooplankton) have the smallest mouths at the time of

Table 3. Estimated 1993 production levels of major, hatchery-reared, phyto- and zooplankton-consuming aquaculture species.

Culture animals	Animals harvested ($\times 10^6$)	Fry stocked ($\times 10^6$)	Larvae stocked ($\times 10^6$)	LarvaZooDays ($\times 10^9$)[a]
Marine shrimp: *Penaeus* spp.	19,782.9	35,922.7	94,421.1	976.57
Common carp *Cyprinus carpio*	2,707.5	4,426.7	14,211.1	58.35
Freshwater shrimp: *Macrobrachium* spp.	316.6	959.4	1,298.5	25.18
Sea bream/sea bass: *Sparus aurata; Pagrus major; Dicentrarchus labrax; Lates calcarifer*	71.7	217.3	904.1	16.89
Milk fish *Chanos chanos*	118.2	147.7	369.3	5.43
Chinese carp: silver, grass, bighead, mud, and crucian	50.9	56.5	134.8	1.44

[a] LarvaZooDays = fry stocked + (larvae stocked − fry stocked)/2 × (days fed zooplankton). The halved figure assumes mortality occurs linearly during the zooplankton feeding period. The calculation also assumes 100% survival during nursery.

first feeding of those listed in the subsequent columns. Some of these culture animals are capable of ingesting food particles as small as 5 μm in diameter; none are incapable of eating zooplankton at this stage. Fish and shrimp larvae are aggressive and strike at the closest prey organisms they can find. Only properly sized food particles that the animals can efficiently consume are therefore provided in the hatchery. As the larvae grow, the size of the food particle is increased.

Table 3 provides an estimate of the hatchery-dependent algal and zooplankton feeding needs for those groups of species with the largest hatchery demands for algal culture. Larval production levels for each species were calculated from the 1993 harvest, taking into account the fraction of the total harvest derived from hatcheries, the mean size of the animal at harvest, and the survival rates during the growout and hatchery phases, on a country-by-country production basis. This resulted in the estimated number of hatchery-reared fry stocked into growout ponds and larvae stocked into the hatchery, respectively. Hatchery methods in each country culturing the particular animal were used to calculate the weighted mean number of days the larvae were fed zooplankton and the mean number of larvae fed during their hatchery experience. These calculations resulted in the ''LarvaZooDays'' figures. In the case of the common carp, cultured algae may be used in both hatchery-dependant larval production as well as extensive larval rearing ponds stocked with larvae.

Fish that are high in total production (Table 1), but that have no algal production in support of their larval rearing include the white bream and yellowtail. White bream are cultured from captured wild fry, and yellowtail from captured wild larvae added to cages placed in bays and fed natural zooplankton attracted by lights. The ''miscellaneous'' species of fish and shrimp from the middle columns also depend on considerable algal/zooplankton production for their larval-rearing. While mullet production is relatively small, because the larvae feed on algae and zooplankton for 45 days, their LarvaZooDay requirement is 2.5×10^9, exceeding that of all Chinese carp species (Table 3). Similarly, sturgeon have a 90-day requirement for live feeds and an estimated LarvaZooDay requirement of 0.5×10^9.

As estimated in Table 3, the single largest segment of the aquaculture industry relying on hatchery production of algae for larval feeding is the shrimp industry. Penaeid shrimps are all marine, primarily distributed in tropical and subtropical areas. Worldwide, their culture employs a variety of technologies. In 1990, there were approximately 4700 penaeid shrimp hatcheries in the world; 95% by number and 85% by output are in Asia (Rosenberry 1991). Shrimp hatcheries range in size from 50,000 to over 500,000 liters in rearing-tank capacity. The relative production efficiency ranges from approximately 500 shrimp postlarvae (PL)/liter/year in South and Central America to 100 PL/liter/year in Asia (Wilkenfeld 1992).

As shrimp larvae molt and metamorphose, feed requirements change; but algae are used throughout the larval stages. Algae are fed to shrimp beginning at the nauplius-5 (N-5) larval stage, and continue through the larval and postlarval (PL-10) rearing stages (Table 4). The most commonly cultured genera of algae, in order of their popularity, are *Chaetoceros, Tetraselmis, Skeletonema, Thalassiosira, Phaeodactylum,* and *Isochrysis.* Shrimp hatcheries commonly culture artemia nauplii to provide large feed particles. Table 4 provides a chronology of the live feeds present in the larval rearing tanks at The Oceanic Institute shrimp hatchery.

Algal culture in shrimp hatcheries commonly utilizes Guillard's F/2 medium and sterile cultures for all scale-up batches. Newly hatched larval shrimp are introduced into culture tanks and algae are added to the desired concentration. As described in Table 4, the larval rearing tanks are flushed at increasing rates as the larvae grow. The replacement medium restores the proper algal density. Similarly, artemia densities are maintained in the larval rearing tanks by transferring appropriately sized animals from separate artemia culture tanks. It is thought that in addition to nutritional considerations, the presence of algae contributes to conditioning the water and keeping ammonia levels low. Table 5 lists the number of larval rearing tanks and supporting algal and artemia culture tanks at The Oceanic Institute.

Zooplankton production

Scaling-up algal cultures for anticipated larval fish or shrimp needs can take 6–60 days, depending on the size of final culture vessel. The 25,000-liter outdoor tanks and 125,000-liter circulating algal raceways (CARs) at The Oceanic Insitute are exceptionally large in this regard (Fig. 5). Small batch culture systems for algae are the rule in all but experimental hatcheries. This reduces capital and labor

Table 4. The Oceanic Institute's feeding regimen of larval stages of marine shrimp.[a]

Stage	Substages	Duration[b]	Food	Daily water exchange[c]
Nauplius	N-1 to N-5; N-5 stage	36 h	Yolk sac; 1×10^5 algal cells/ml added to culture	None
Zoea or	Z-1	3–5 days	1.3×10^5 cells/ml;	50%
Protozoea	Z-2 & Z-3		1.5×10^5 cells/ml;	
	Z-3		2 artemia/ml added	80%
Mysis	M-1	3 days	1.2×10^5 cells/ml + 3 artemia nauplii/ml	80%
	M-2 & M-3		+ 5 artemia nauplii/ml	
Postlarval	PL-1 to PL-10	10 days	$0.8–1.0 \times 10^5$ cells/ml 8 artemia nauplii/ml	100%
Nursery	No further metamorphosis	1 month	Finely ground, prepared diets	Variable—outdoor

[a] From Wyban and Sweeney (1991).
[b] Assumes culture of *Penaeus vannamei* grown under ideal culture conditions. Other genera would experience other growth rates.
[c] For larvae stocked at 75–125 individuals/liter; nursery at 1/liter.

Table 5. Design specifications for The Oceanic Institute's larval shrimp rearing facility.[a]

	Specifications		
Equipment	Larval rearing	Algae culture	Artemia culture
Tanks			
Number	4	3	3
Capacity (liters)	1000	160	90
Diameter (cm)	120	44	44
Seawater			
Filters	10μ, 1μ, .5μ	1μ, 0.5μ, 0.1μ	1mm, 0.5mm
Temp. control	28°C	23°C	27°C
Lighting per tank	500-W halogen lamp	Four 100-W fluorescent tubes	One 100-W incandescent bulb

[a] From Wyban and Sweeney (1991).

costs and allows matching of algal batches with larval (batch) availability/quantity.

In tropical areas, where year-round fish and shrimp culture takes place, year-round live feed production systems are useful. Outdoor cultures of *Tetraselmis chuii* at a mean density of 1 million cells/ml have been maintained semi-continuously year-round in replicate CARs at The Oceanic Institute in support of rotifer production systems. In a smaller model CAR, F/2 culture medium and pulsed CO_2 addition resulted in similar densities and doubling rates (0.5X–0.7X/day) (Olaizola et al. 1991). Daily harvest of either of the outdoor culture systems consisted of transferring 30–50% of the algal culture to 1000-liter rotifer (*B. plicatilis*) culture vessel(s). Rotifers harvested the algae by eating them; rotifers were harvested by draining a required volume of the vessel through a screen and recovering the rotifers for addition to the larval fish or shrimp rearing tanks. The cost of algal/rotifer production by this system was \$650/rotifers x 10^9.

Nonlarval food uses of cultured algae

Dried algae are used in commercial larval, nursery, and growout diets of fish and shrimp. This is difficult to document because there is no international requirement to list ingredients in aquaculture feeds. Examining feed samples under a microscope can reveal the presence of algae. A number of studies have documented the nutritional excellence of dried algal products in shrimp and fish diets, as reviewed in Wood et al. 1991). Cost considerations, however, dictate that more than 1–2% inclusion levels of dry algae can be ruled out with respect to feeds for the major

Fig. 5. The 30,000-liter fiberglass tanks (foreground) and 125,000-liter circulating algal raceways (background) at The Oceanic Institute are used for large-scale algal production.

aquaculture species. High cost, specialty diets for ornamental fish, and Kuruma shrimp (*P. japonicus*) are throught to normally contain algae, primarily for their carotenoid content.

Several carp species, milkfish, and shrimp are known to derive nutrition from the natural phytoplankton production in growout ponds. In the case of milkfish, ponds will be prepared prior to stocking with fertilizers to promote the development of a blue–green algal mat called ''lab-lab'' in the Philippines. Once a mat is well developed, wild-caught milkfish fry are introduced, and they graze on this mat until it is completely consumed. Supplemental provision of dry feed increases slowly, as the animals grow and the algal mat disappears (Bardach et al. 1972). Shrimp derive 50–100% of their nutritional needs from natural productivity in growout ponds, depending on the stocking density (Anderson et al. 1987; Hunter et al. 1987). However very little, if any, effort is made by the fish or shrimp farmers to influence the natural productivity once the ponds are stocked, other than to regulate water turnover in the ponds. The ponds typically contain or multiple species of algae and will experience a succession of dominating species.

Trends

Live feeds continue to be expensive, relatively unreliable in quantity, and variable in quality. A related problem is that of bacterial contamination, causing difficulties in maintaining the algal culture and jeopardizing the target larval organisms. In spite of this, the current success of aquaculture hatcheries indicates that the problems can be overcome and that commercial hatcheries can operate economically. Even the smaller commercial hatcheries produce over 2 million liters of algae culture per year. To ensure that high quality algal material is available when needed, redundancy is usually built into production systems.

Heterotrophic algal culture is one option being studied to increase production and reliability. Cost estimates based on laboratory experiments are projected to be US$2–25/kg dry weight (Martek Corp., Columbia, MD). Limited success has been achieved in providing lyophilized algal material produced during periods when the hatcheries are otherwise inactive. While this increases reliability, it also increases cost (US$150–$250/kg dry weight). These products have not yet been demonstrated to be a complete substitute for live algal material.

In the near term, live feeds will continue to be the primary feedstock in aquaculture hatcheries. Advances in packing emulsions and formulated feeds are having an increasing impact in the hatchery. Many shrimp hatcheries are hybrids in that they provide both algae, packed artemia, and prepared diets. Eventually prepared diets may supersede live feeds, except perhaps artemia.

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