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Introduction to Marine Copepod Culture for Live Feeds Production

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Copepods are the largest and most diversified group of crustaceans. They are estimated to include over 24,000 species, 2,400 genera, and 210 families, and, considering their small size, a great number of species probably remain to be discovered. They may be the most numerous multicellular animals on the earth, outnumbering even the insects.

Copepod habitats include fresh to hypersaline waters, marine and subterranean cave systems, moist leaf litter of forests, aquatic sediments, the deepest ocean trenches and hydrothermal vents, as well as the open ocean. Many copepod species are free-living, but possibly a greater number are commensal or parasitic on almost every phylum of aquatic animal. The usual length of adult copepods is 0.04 to 0.08 inch (1 to 2 mm), although free-living species range from 0.08 to 0.20 inch (0.2 to 15 mm) and some of the peculiar parasitic forms can reach as much as 12 inches (300 mm) in length.

The three most abundant free-living copepod Orders, and the ones used in aquaculture, are Calanoida, Cyclopoida, and Harpacticoida. The Calanoida and Cyclopoida are primarily planktonic, whereas the Harpacticoida are primarily benthic. Copepods are particulate feeders and consumers of a vast array of phytoplankton and microzooplankton. Some also ingest protozoa, organic particulate matter, and other metazoan zooplankton including copepods. The great abundance of copepods in the oceans makes them a primary food source for first-feeding, small-mouthed larvae of marine fish, which consume the small naupliar stages (50–100 µm).

Many copepods have two swimming modes. One is a slow and steady motion created by the movement of their mouthparts. The second involves a succession of rapid "jumps" propelled by appendages on the prosome. This "jumping" motion can be an important stimulus to feeding in larval fish.

Most copepods reproduce sexually. During mating, the male copepod grips the female with his first pair of antennae, which may be modified for this purpose. Some calanoids release eggs singly, whereas other calanoids, cyclopoids, and harpacticoids typically carry eggs in sacs. Some calanoids may form resting (subitaneous or diapause) eggs that delay hatching after release, and may offer the potential for storage as a ready source of nauplii in fish hatcheries. The larval phase consists of 6 naupliar stages and 5 copepodid stages before the adult stage is reached. This can require a week or up to a year, depending on the species and environmental conditions.

Culture techniques

There are three general modes of production for copepods and their nauplii as live feeds for larviculture applications. These range from extensive and semi-intensive production to intensive production. While these represent general categories, there are myriad production methods that can incorporate elements of any of these approaches.

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Extensive production

Early uses of copepods in fish culture included the harvest of wild copepods from the ocean using plankton nets and subsequent transfer to the fish hatchery. The diversity of copepods in the wild and their patchy distribution resulted in considerable unpredictability of these harvests. The need for a reliable copepod source led researchers to adapt traditional "open pond" fish culture techniques for copepod production. Essentially, the technique consists of pumping ambient seawater into a pond and adding additional organic and/or inorganic fertilizers to induce a phytoplankton bloom that will support the growth of endogenous zooplankton. This method inevitably produces a mixed population of planktonic invertebrates, only some of which are copepods. Some protocols involve further processing of the bloom to concentrate the zooplankters and select those in the appropriate size-range for transfer to hatchery tanks. Most frequently, fish eggs or larvae are stocked into a fertilized pond at the peak of the zooplankton population. While this method has been employed in the culture of a variety of fishes to varying degrees of success, it shares some fundamental shortcomings with the oceanic harvest using plankton nets. The species composition of the bloom and the timing of the bloom to provide the appropriate mix of size fractions cannot be reliably manipulated, and are dependent on abiotic factors as well as the particular ambient phytoplankton and zooplankton communities occurring in the pond when it is seeded. Moreover, the cyclic nature of plankton blooms limits the ability of extensive systems to produce copepods of the appropriate size fraction at sufficiently high densities for prolonged periods. As a result, extensive production systems must be quite large and/or include multiple units to compensate for the low production per unit volume and the fluctuation of yields. Additionally, exposure to other biota and a large volume of ambient seawater increases the risk of transferring pathogens to the hatchery.

Semi-intensive production

Semi-intensive production of marine copepods offers many advantages when compared with extensive production, including increased biosecurity, the ability to target a chosen copepod species and life stage, and increased production efficiency. Semi-intensive production is usually conducted indoors in climate-controlled facilities where temperature and photoperiod can be held within optimal ranges. Depending upon the species chosen for cultivation, a series of tanks may be used for grow-out of nauplii until sexual maturity. A separate set of "production" or "harvest" tanks may be used in conjunction with maturation tanks. A "stock population" tank may be maintained for stocking of grow-out tanks.

Production tanks are configured to collect and concentrate the eggs or nauplii. Depending on species, collections are generally conducted daily or on an as-needed basis to ensure a consistent supply of nauplii for ongoing larviculture operations. Whereas most semi-intensive copepod production is in a static batch culture environment, some production protocols have explored the incorporation of culture water recirculation with water treatment options such as solids removal, biofiltration, and ultraviolet sterilization. Sub-optimal water quality has the potential to negatively impact nauplii production and culture longevity, however optimal conditions are species-specific and further research is needed to define appropriate parameters.

Feeding of semi-intensive copepod systems differs considerably from extensive practices. Live microalgae must be cultured onsite or purchased because many copepod species do not thrive on algal concentrates. Production of clean microalgae, free of non-target protozoan and metazoan contamination is crucial, as these organisms can quickly outcompete the copepod species and result in a culture crash. While monoalgal diets can be used to culture numerous species of copepods, mixtures of two or three different algal species are commonly used to ensure that essential dietary requirements are met and for optimal production and stability of the culture. *Tisochrysis lutea, Chaetoceros muelleri,* and *Tetraselmis chuii* are three commonly used microalgae for the culture of numerous calanoid and cyclopoid copepod species. Research has demonstrated that copepod nutrition can influence parameters such as naupliar production, development time, and fatty acid composition. Since copepods lack the ability to synthesize fatty acids, the option exists to manipulate their nutritional profile through diet; thereby optimizing their nutritional profile as a feed for individual cultured species. It should be noted that the production of live microalgae represents a significant production cost, and reduction or elimination of the use of live microalgae would help decrease the cost of semi-intensive copepod production.

Intensive production

Intensive production further exploits the benefits associated with semi-intensive production of marine copepods. As with semi-intensive production, culture is conducted indoors, with comprehensive environmental controls. The main difference is the replacement of live algae as a copepod feed with inert diets such as algal concentrates. It should be noted that alternatives to live algae would only be viable for certain copepod species. While the elimination

of live algae reduces overall labor inputs for the hatchery and increases biosecurity, it is not without its drawbacks. Through the elimination of live algae, the beneficial aspects of live algae such as conversion of carbon dioxide (CO_2) to oxygen and microbiome stabilization are lost. Consequently, intensive production requires additional water quality monitoring and control. Also similar to semi-intensive production, most intensive copepod production cultures currently are conducted in static batch cultures for upwards of 30 days or more. However, given the increased water quality monitoring and control needed for microbiome stabilization, recent investigations are looking further into RAS applications as well as probiotics towards improved culture environment management and control.

Feeding of intensive copepod systems differs considerably from both extensive and semi-intensive practices. Current practices utilize dosing of copepod culture vessels with algal concentrate feeds; with dosing regimen dependent upon copepod densities and required nauplii production. These algal concentrates can be utilized as single-species feeds, blended with other single-species feeds, or purchased as multi-species feeds. For production of the copepod *Apocyclops panamensis,* a typical production diet is a blend of five algal species represented in the Reed Mariculture product Rotigrow Plus®. Investigations into optimizing feeding practices using algal concentrates for copepod broodstock maintenance as well as nauplii production are being pursued.

Application techniques in larviculture

Red snapper

Although attempted since the 1970s, successful culture of red snapper (*Lutjanus campechanus*) was not achieved until the late 1990s when larvae were reared in ponds with mixed, bloomed zooplankton. Using those results, Ogle et al. (2005) designed an extensive copepod production system in which ambient estuarine water was pumped through a coarse filter into approximately 18,500-gallon (70,000-L) holding tanks and bloomed. After blooming, approximately 40 percent of the water was sieved to the desired size fraction to provide zooplankton for feeding red snapper larvae. Subsequently, tanks were re-filled with estuarine water and bloomed for the next harvest. Multiple tanks were bloomed and harvested in a rotating succession to provide a steady supply of zooplankton. Calanoid species, *Acartia tonsa* (Figs. 1 and 2) in particular, dominated the harvest. During two seasons in which production was precisely quantified, the system produced up to 6.5×10^6 copepods per tank per day with an average daily production of approximately 190 nauplii per gallon (50 nauplii/L). Over the five years during which this system was used, copepod production supported an average of about 150,000 red snapper larvae per year, which resulted in an average of less than 10,000 (3,574 to 28,545) post-larval fish per year, about 6.8 percent (2.1 to 14.3 percent) survival (Ogle et al. 2005). Although this extensive system occasionally resulted in high (>10 percent) survival rates through the larval phase, inter- and intra-batch variability was high (0 to 34 percent survival) and unpredictable. The inconsistency in copepod yields, limited production capacity, and recurring disease problems in the snapper larvae, linked to exposure to ambient water, all spurred transition toward a closed-system intensive culture method. By 2013, a closed, batch culture system for continuous, intensive production of *Acartia tonsa* (Figs. 1 and 2) had been developed. The system consists of a series of 12 pairs of grow-out tanks (250 gal or 900 L) that are used to sequentially stock six egg production tanks (500 gal or 1900 L), which provide eggs that are hatched to produce nauplii for both feeding fish larvae and restocking the growout tanks. By seeding tanks sequentially on a rotating schedule, almost continuous production is maintained. Operation of the system between 2013 and 2015 yielded an average of 22 million eggs per day with an average hatch rate of 49 percent. This translates into approximately 3,650 nauplii per gallon/day (965 nauplii/L/day), a near 20-fold improvement over the extensive system. The system produces sufficient nauplii to feed batches of up to 120,000 red snapper larvae. While survival rates of red snapper larvae are still low (rarely exceeding 5 to 10 percent), this intensive copepod production system has

Figure 1: *Acartia tonsa* adult female with eggs.

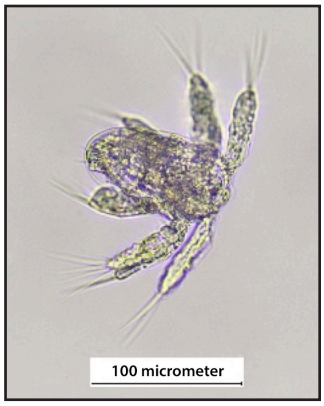

Figure 2: *Acartia tonsa* nauplius.

improved the consistency of red snapper larviculture through greater control of the quantity and size of nauplii that can be harvested on a given day to feed larvae, and has led to improved biosecurity.

Marine ornamental wrasses (*Halichoeres melanurus*)

Traditional live feeds such as rotifers (*Brachionus* spp.) and *Artemia* have been ineffective as an initial diet for ornamental labrid species including the Melanurus wrasse, *Halichoeres melanurus,* a species desirable within the marine ornamental trade. The calanoid copepod *Parvocalanus crassirostris,* in particular, has emerged as a viable live feed for the production of numerous species of importance to the marine aquaculture industry. Research by Groover (2018) has demonstrated that *P. crassirostris* can be an effective component of larval nutrition for the Melanurus wrasse. Feed densities of ≥5 nauplii per milliliter resulted in increased ingestion rates and allowed this species to be reliably cultured through its larval phase numerous times. Feeding regimes developed during this research offered copepod nauplii daily over ~30 days of culture. While this protocol proved to be effective, further optimization that reduces the feeding duration and quantity of copepod nauplii is sought to reduce production costs and increase the economic viability of production. Copepods used in these trials were produced in the semi-intensive production systems described above and fed live microalgae.

Marine ornamental neon gobies (*Elacatinus oceanops*)

The neon goby, *Elacatinus oceanops,* is popular in the marine ornamental trade because of its colorful lines, amenability to cohabitate with many other fishes, schooling tendencies, and its relative ease of culture utilizing traditional finfish larviculture feeds and methods. Protocols incorporating rotifers as a first live feed, subsequently transitioning to *Artemia,* and then cofeeding and subsequent complete weaning onto dry diets, can typically be achieved in about 25 to 30 days with an average survival from newly hatched sac fry to weaned fingerlings of about 40 percent. As is being recently discovered in the larviculture of many finfish species around the world, even species with good larviculture results without the use of copepod nauplii can benefit from their inclusion in the larval diet, specifically around the first feeding stage. The neon goby is one such example. Early investigations into the augmentation of the standard larviculture protocols (days 1 to 12 post hatch) with *Apocyclops panamensis* (Figs. 3 and 4) nauplii, ranging from 1 to 3 nauplii per milliliter, fed four times per 24-hour period, have consistently doubled survivals from newly hatched sac fry through to weaned fingerlings from 40 percent to over 80 percent. Similar results have been reported with the yellowtail clownfish (*Amphiprion clarkii*) in which survivals through day eleven, post-hatch, increased from around 40 percent to over 80 percent with the inclusion of calanoid copepod (*Centropages typicus*) nauplii during early larval feeding stages.

Figure 3: *Apocyclops panamensis* adult female with eggs.

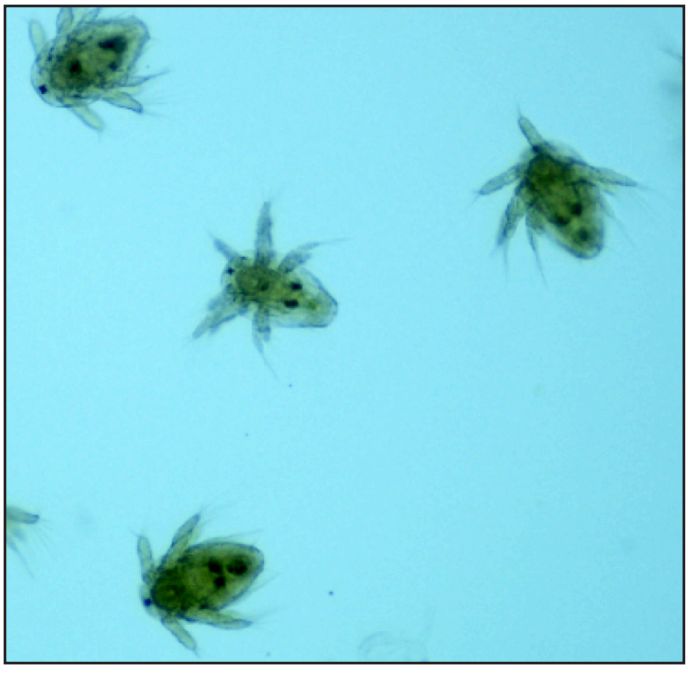

Figure 4: *Apocyclops panamensis* nauplii.

Summary

With the ever-expanding demands for aquaculture to increase production of current and emerging fish species, as well as for improved efficiencies, pressure for innovations in hatchery operations will only increase. The marine copepod is emerging as a key component of finfish larviculture through strain selection, optimized culture techniques (Fig. 5), development of large-scale production systems, and integration into larviculture production protocols. While currently more challenging to produce than traditional live feeds such as rotifers and *Artemia,* copepods are quickly rising in importance as a necessary component of live feeds regimes for marine fish. Continued research, along with experience gained during expanded use of copepods in aquaculture, will undoubtedly enable the US aquaculture industry to diversify the number of fish species that can be cultured commercially.

Figure 5: Example of semi-intensive/intensive production system.

Suggested readings

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