The Use of Calanoid Copepods in Semi-Intensive, Tropical Marine Fish Larviculture

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Abstract

The benefits of using copepods in aquaculture include: their superior nutritional value, high digestibility, movement patterns which trigger a strong feeding response in fish larvae, small size and simply the fact that they are part of the natural food chain for fish in the wild. Despite these significant advantages the use of copepods in aquaculture remains limited, mainly because of the inability to culture them cost effectively and at high density.

Over a period of ten years we have been able to develop a reliable culture method for the calanoid copepods *Acartia* sp. and *Parvocalanus crassirostris* and to successfully use these to culture a variety of tropical reef fish species both in Australia and in Hawaii. Some of the fish species, such as the golden snapper, *Lutjanus johnii* (Bloch) and the peacock hind, *Cephalopholis argus* (Bloch and Schneider) appear to require copepods to successfully negotiate past first feeding. Others such as the flame angelfish *Centropyge loricula* (Günther) seem to need copepods for their whole larval cycle of over 70 days. All fish species we have cultured using copepods as a supplement to other live prey have shown increased growth and survival.

The use of copepods in aquaculture is unlikely to compete cost effectively with techniques such as semi-automated, intensive larviculture systems for fish species that are easily cultured on rotifers, but for some high valued reef fish species at least, the use of copepods is highly desirable if not essential.

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1. Introduction

Interest in the use of copepods in aquaculture has grown since the 1980's. Over the past few years there have been several review articles published and numerous conferences, conference sessions and workshops dedicated to discussions of copepod culture and the important role that copepods can play as feeds for marine fish larviculture (Bell *et al.,* 1997; Støttrup 2000; Kleppel & Hazzard 2002; Lee *et al.,* 2005). The three main copepod orders, Cyclopoida, Calenoida and Harpacticoida have each been investigated for their suitability as feeds for larval and juvenile fish (Marcus 2005). While each copepod order has its advantages and disadvantages, it is generally agreed that the benefits of using copepods for larviculture include:

i. Their superior nutritional value in comparison to rotifers and *Artemia.* When fed an adequate mixed diet of microalgae, copepods are excellent sources of the Highly Unsaturated Fatty Acids (HUFA's); Docosahexaenoic Acid (DHA), Eicosapentaenoic Acid (EPA) and Arachidonic Acid (ARA) (Watanabe *et al.,* 1983; Støttrup and Jensen 1990). While it is true that rotifers and *Artemia* can be 'boosted' to also contain high levels of HUFA's, copepods contain most of their HUFA content in the structural or polar lipid fraction (Bell *et al.,* 2003). On the other hand *Artemia* contain most of their HUFA in the storage lipids (triacylglycerols) (Nanton & Castell 1999). The higher proportion of HUFA's in the polar lipid fraction means the lipids are more biologically available to the larvae that feed on them (McKinnon *et al.,* 2003). The HUFA's are also likely to be present in the correct ratios to enhance survival and growth of fish larvae (Watanabe *et a.,l* 1983; Nanton & Castell 1998; McKinnon *et al.,* 2003).
ii. Copepod nauplii may be more easily and completely digested than either rotifers or *Artemia* (Pederson 1984; personal observation, 1995). This is especially true for harpacticoid and calanoid species but from our experience may not necessarily be the case for cyclopoid species.
iii. Copepods are natural sources of the antioxidant, astaxanthin and Vitamins C and E. (Van der Meeren 2003). The powerful antioxidants found in copepods can protect the HUFA'a against peroxidation and are also considered beneficial to the health of fish larvae (McKinnon *et al* 2003).
iv. The movement of copepods and their nauplii triggers the feeding responses in fish larvae. The 'jerking' swimming action of most copepod nauplii and adults is believed to be an important stimulus for initiating feeding by fish larvae (Buskey 2005; Marcus 2005).
v. Use of copepods in larval fish diets has been associated with a decrease in fish malpigmentation and deformity rates (Bell *et* al 1997; Bell 1998; Støttrup 2000). Hamre, *et al.,* (2005) associated use of copepods with a significant improvement of both eye migration and pigmentation in Atlantic halibut, *Hippoglossus hippoglossus* L.).
vi. Many copepods have small sizes (<100 μm) for at least one or more developmental stages that make them suitable as first feeds for small larvae and/ or larvae that have a small mouth gape. For example *Parvocalanus crassirostris,* a tropical calanoid copepod, has a width of less than 100 μm up to stage III copepodid or just over 4 days of age (McKinnon *et al* 2003).

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There is also agreement on the negative aspects of using copepods as commercial larval fish feeds. These include an apparent inability to be cultured in high densities. While rotifers are routinely cultured in numbers exceeding 2,000 per mL and *Artemia* can also be hatched and cultured at high density, copepod cultures rarely exceed densities of 2 per mL for adults and 10 per mL for nauplii (Støttrup *et al.,* 1986; Mckinnon *et al.,* 2003). Harpacticoid copepods may reach densities of more than 100 per mL (Fleeger 2005) but their epibenthic nature means they may not be available to fish larvae as food (McKinnon *et al.,* 2003; Shields *et al.,* 2005). This places a major restriction on the practicality, reliability and cost-effectiveness of using copepods for commercial fish larviculture.

Other problems with copepods include seasonal variation of their abundance in the wild and the possibility of the introduction of unwanted organisms, such as barnacles, comb jellies (ctenophores) and parasitic copepods, into fish cultures if wild zooplankton is directly fed to the fish larvae (Benetti *et al.,* 2001; Helland *et al.,* 2003).

In Darwin, we commenced investigating the application of copepods to larval fish rearing in 1993 when we were faced with difficulties in culturing the tropical Lutjanid, golden snapper, *Lutjanus johnii* (Bloch). Early attempts at larval rearing using either the normal strain rotifer, *Brachionus plicatilis*, or a small strain, local rotifer, *Syncaeta* sp, as first feed were characterised by total mortality of the larvae within five days from the commencement of feeding. At the time we observed that rotifers were rarely ingested, and if they were, then were often being excreted intact by the larvae, indicating that ingested rotifers were not being digested. Starved larvae exhibited similar survival times to those fed rotifers.

Information from Thailand provided further evidence that rotifers were not suitable as a first feed for Lutjanid larvae and that early stage nauplii of the calanoid copepod genus *Acartia* were more suitable because of their smaller size and high digestibility (Doi & Singhagraiwan, 1993; T. Singhagraiwan, pers. comm.).

Subsequent research at the Darwin Aquaculture Centre resulted in the development of a method for the semi-intensive culture of *L. johnii* juveniles. The method relied on the use of nauplii of a locally available copepod of the *Acartia* genus as a first feed for the larvae. Once the fish larvae had copepods included in their diet, their survival quickly moved from zero to over 30% after metamorphosis.

Between 1996 and 1998 the semi-intensive method for snapper production was further improved by the development of a reliable method for the culture of the copepods in the hatchery. This effectively removed the reliance on the unreliable wild copepod stocks and reduced the risk of the introduction of undesirable organisms into the larval fish tanks.

Since 2002 further refinement to the method has including extending the culture technique to another species of calanoid, *Parvocalanus crassirostris,* and successfully using these copepods in Hawaii to produce a range of fish species including, the flame angelfish, *Centropyge loricula* (Günther); crimson jobfish, *Pristipomoides filamentosus* (Valenciennes); almaco jack, *Seriola rivoliana* (Valenciennes) and the peacock hind, *Cephalopholis* argus (Bloch and Schneider).

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2. Methods

We initially based our culture method for golden snapper on the method developed in Thailand by Doi & Singhagraiwan (1993) for the culture of mangrove red snapper, *Lutjanus argentimaculatus* (Forsskål). The Thai method involved the collection of adult *Acartia* sp. copepods from a large semi-tidal, earthen pond and then using these copepods in prepared culture tanks to create a 'bloom' of nauplii which was then fed to the fish larvae. The copepods were first stocked into 7,000 L outdoor tanks that had been previously fertilised to encourage algae growth. When nauplii numbers increased, usually after two days, the entire contents of the copepod tanks were transferred to the larval cultures. Although the method was usually successful, survival rates obtained for the fish were often variable and the whole operation was at risk of failure if there was any unforeseen reduction in the copepod population in the semi-tidal pond.

In the mid 1990's at the Darwin Aquaculture Centre we began producing *L. johnii* fingerlings using a method similar to that developed in Thailand. Like the Thai method, we initially relied on a wild population of *Acartia* sp. from a nearby salt water lake. However, a seasonal decline in the *Acartia* sp. population due to monsoonal rains necessitated the development of techniques to maintain stock cultures of *Acartia* sp. in the hatchery and we also required the ability to scale up stock cultures to provide sufficient copepods for the commercial production of *L. johnii* fingerlings.

An efficient method was developed which involved feeding 1,000 L cultures of copepods once per day with a mixed ration of three species of algae and, after eight days, screening these cultures to harvest adult copepods and late-stage copepodids. The regular screening also reduced contamination of the cultures by rotifers and other undesirable zooplankton.

The cultures were scaled up by either stocking the adult copepods and copepodids into larger culture tanks (5,000 l) or directly into larval rearing tanks (20,000 or 40,000 l). In the larval rearing tanks the adult copepods continued to produce nauplii which were consumed directly by the fish larvae. This production method eliminated the need to handle the easily damaged copepod nauplii and simplified larval rearing.

Description of the larval rearing method for L. johnii.

On D0 (Day 0, the day the larvae hatched) large fibreglass tanks (20,000 or 40,000 l) were filled with sand-filtered, chlorine disinfected, sea water and inoculated with microalgae *Rhodomonas* sp. and *Isochrysis* sp. to a total algal cell density of between 5-10 x 10^5 cells per mL. Also on D0, adult *Acartia* sp. were stocked into the tanks at a density of 30 to 60/ l. First feeding fish larvae were stocked into the tanks at a density of 1-2/ L on D2. The plankton bloom was monitored daily and extra algae or zooplankton added as necessary. After feeding on *Acartia* nauplii for 3 to 4 days the larvae were able to consume and digest rotifers. Water exchange commenced from D10, commencing with a 10% daily exchange, increasing to 100% by D30. *Artemia* nauplii were added to the tank at 0.5/ L from D12 to D30.

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Weaning commenced after day D25 and lasted for 3 to 7 days. The fish were transferred to nursery tanks just after metamorphosis (D35-D40). The larval rearing regime is summarised in Figure 1. and a photograph of the 40,000 litre culture tanks is presented in Figure 2.

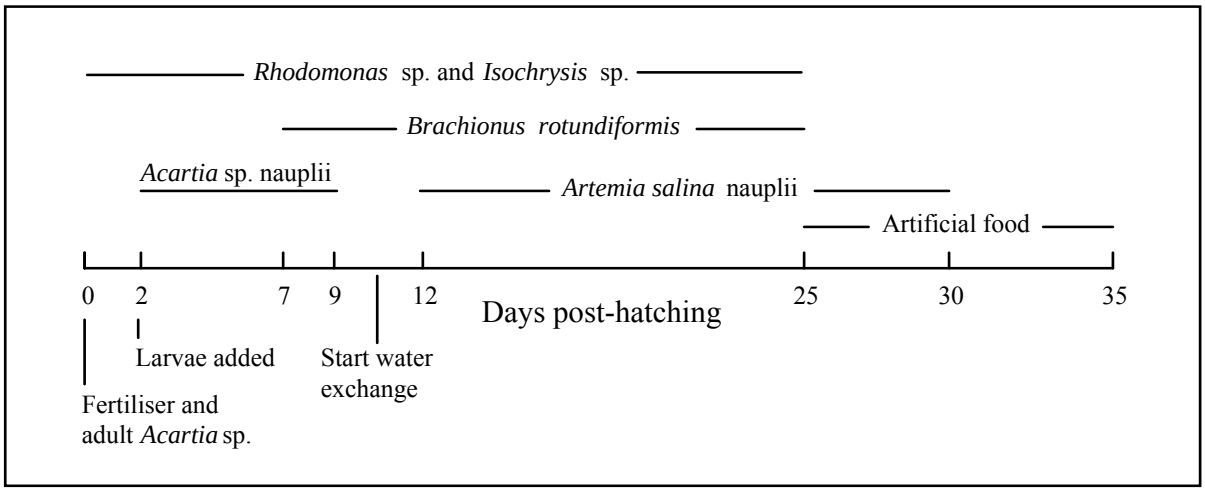

Figure 1. Larval rearing regime in 20 and 40 m^3 tanks for *Lutjanus johnii* from first feeding larvae, through to metamorphosis and weaning. Slight variations of this protocol have been successfully trialled on a number of other tropical reef fish species.

Figure 2. The two 40 m^3 tanks used for semi-intensive larval rearing of tropical marine finfish at the Darwin Aquaculture Centre. A. Tanks ready to be filled with chlorine-disinfected sea water. Aeration lines are suspended in the tanks; B. Tank under operation, half covered with 90% shade-cloth to control light levels. Feeder tanks containing extra zooplankton can be seen in the background.

Description of the copepod culture method.

Stock culture tanks of 1,000 L volume were filled with 1 µm bag-filtered, chlorine-disinfected sea water. Adult copepods were added at an initial density of approximately 50 per litre and fed daily with a ration of *Rhodomonas* sp. and *Isochrysis* sp. microalgae to a total algal cell density of 5-10 x 10^4 cells per mL. Originally, *Tetraselmis* sp. algae was also used but this was later determined to not be necessary.

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The tanks were operated without water exchange and were provided with gentle aeration via a 4 mm air tube set at a flow rate of around 500 mL per minute and fitted with a 25 mm air stone.

After 8 days of culture, a time period which we determined gave the maximum yield of adult and late-stage copepodids, the tanks were harvested into a 100 L collecting tub fitted with a 90 µm mesh screen in the centre. The collected copepods were further rinsed for another 20-30 minutes before being counted and either used to start another culture or added directly to larval fish tanks.

A more complete description of the copepod culture method was published several years ago (Schipp, Bosmans & Marshall 1999). Improvements and/or changes since that time include:

i. Reducing the number of algal species used to two.
ii. Developing the ability to gently collect nauplii and to use these for directly feeding small scale larval cultures.
iii. Using algae grown by a continuous culture method to further boost the productivity of the copepods
iv. Successfully adapting the method for another species of calanoid, *Parvocalanus crassirostris.* A nine day culture period was determined to be the most effective for this species.
v. Upscaling the copepod culture method to even larger tanks > 40,000 litres, to enable the daily harvest of between 20 to 40 million adult and late stage copepodids.

3. Results and discussion

Our research has focused on the use of calanoid copepods specifically because of the desirable characteristics of this order. The calanoid copepods that we have investigated from the genera of *Acartia* and *Parvocalanus* are both reasonably productive (in copepod terms) broadcast spawners producing in excess of 30 eggs per female per day (McKinnon *et al., 2003*. They also have small to very small nauplii which remain at a suitable prey size for small mouthed reef fish larvae for two to four days and are commonly available locally in our tropical marine environment.

Another desirable trait of the calanoid copepods is that all stages are planktonic. By comparison, harpacticoid copepods despite often being able to be cultured in higher densities than the calanoids, are mainly epibenthic and therefore not readily available to foraging fish larvae. Harpacticoid copepods have an advantage in that they can elongate 18 chain fatty acids and therefore create their own HUFA's (Nanton & Castell 1998). Even though calanoid copepods cannot do this and rely on the HUFA source in their diet, a mixed algal ration of species containing appropriate HUFA levels is sufficient to overcome this as a problem.

The one species of cyclopoid copepod we examined as a candidate for snapper culture, (*Apocyclops dengizicus*), although relatively easy to culture using a fresh fish meal based diet, was apparently unable to be digested by the larvae and so its use was discontinued. Another potential reason for not using cyclopoids is that some species have been observed to predate directly on fish larvae (Frimpong & Lochmann 2005).

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Many studies have shown that diet is very important in copepod culture and can be directly linked to reproductive performance and nutritional content of the copepods (Lacoste, Poulet, Cueff, Kattner, Ianora & Laabir 2001.; Støttrup & Jensen 1990). Our research supports this concept. We have investigated a variety of algal pastes and formulated micro diets as copepod feeds but failed to find any that was a suitable replacement to live algae. The quality of the live algae is also important and when copepods were fed with algae produced from a continuous culture system the performance and reliability of the copepods improved. The reason for this is probably that continuous algal culture systems produce algae that is not only 'cleaner' than batch cultures but that the algae is always maintained in the fast growing 'log phase' and is therefore better nutritionally.

In 2003, McKinnon *et al* published a paper suggesting that the calanoid copepods *P. crassirostris* and *Bestiolina similis* may be a better alternative to *Acartia* sp. for aquaculture given the smaller size, less cannibalistic nature and apparent higher vulnerability to predation of these two species. At about the same time as this paper was published we had the opportunity to examine *P. crassirostris* and *B. similis* in culture and concluded that, for us at least, *P. crassirostris* was easier to maintain in culture than *B. similis* and could be cultured at higher densities than previously obtained for *Acartia* sp. (Schipp *et al.,* 1999) (see Fig. 3) although not as high as those reported for *Parvocalanus* sp. by Shields *et al.,* (2005). The most important result we obtained was that, when copepods were included in their diet, the larvae of every fish species we cultured grew and survived better.

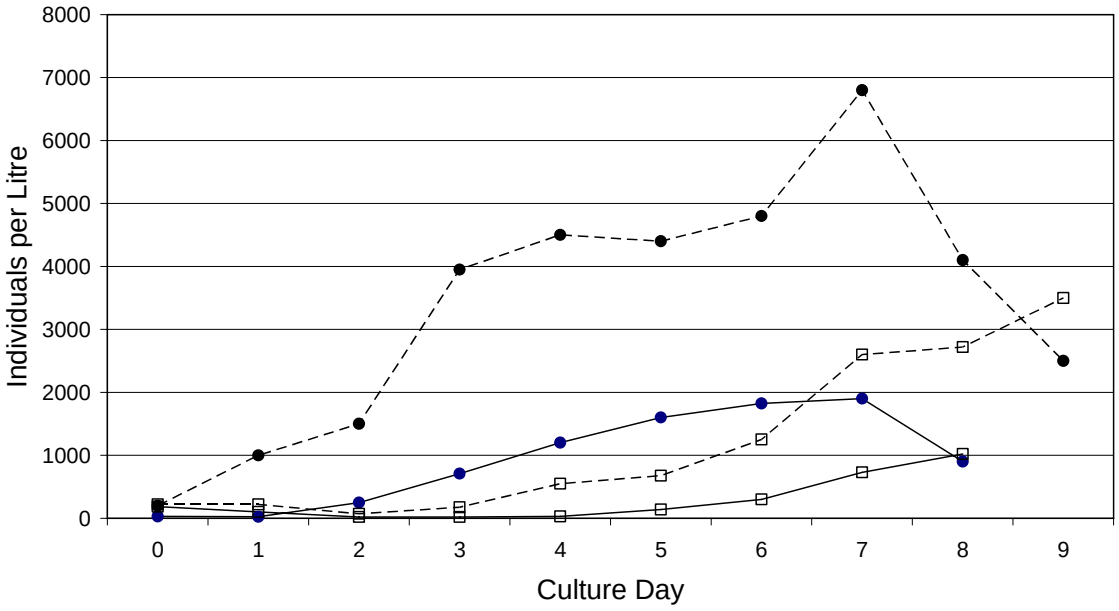

Figure 3. Average densities of *Acartia* sp. copepods (solid line) cultured in Darwin in 1999 compared to *Parvocalanus crassirostris* (dashed line) cultured in 1000 litre tanks in Hawaii in 2002. ● = nauplii; □ = adults + copepodids. An 8-day culture cycle was used for *Acartia* sp. and *P. crassirostris* had a 9-day cycle. Day 0 = the day the culture tanks were started.

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From our experience both *P. crassirostris* and *Acartia* sp. have proved to be suitable as first feeds for a wide variety of fish species. The fish that we have cultured can be divided into two broad groups. Those whose early survival is strongly linked to feeding on copepod nauplii and who do not survive well, if at all, if copepods are not used. This group includes, *Lutjanus johnii*, *Centropyge loricula* (Fig. 4), *Pristipomoides filamentosus* and *Cephalopholis argus.* The other group is those fish that do not need copepods as first feed and who will do quite well on rotifers or *Artemia* but when copepods are used as a supplement, their growth is significantly enhanced. This group includes, barramundi, *Lates calcarifer* (Bloch); almaco jack, *Seriola rivoliana*; giant trevally, *Caranx ignobilis* (Forsskål); and the common dolphinfish, *Coryphaena hippurus* (Linnaeus).

Figure 4. A selection of flame angelfish, *Centropyge loricula*, juveniles approximately 100 days old that had been grown in a semi-intensive production system using the calanoid copepod *Parvocalanus crassirostris.*

For the fish that appear to require copepods in their diet it is interesting to note that the copepods are not necessarily have to be in very high numbers in the larval rearing tanks. Traditional fish larval culture works on the premise that live feeds such as rotifers need to be present in high density (usually somewhere between 10 and 20 per mL) to increase encounter frequency between fish larvae and prey to ensure high larval survival. One of the recognised problems with copepods is that it is usually close to impossible to maintain these high densities for copepod nauplii in culture (Støttrup *et al., 1*986). Many copepods, particularly *Acartia* sp., can be cannibalistic (McKinnon *et al* 2003) which can be a limiting factor for culturing them at high densities as can the apparent ability of the copepods to limit their reproductive capacity as their density increases (Peck & Holste 2006). So far densities in our *P. crassirostris* stock cultures have averaged about 7 nauplii and 3 adult/ late stage copepodids per mL after nine days of culture. These figures are comparable with those obtained by McKinnon *et al.,* (2003). Nauplii densities as high as 7.0/ mL have rarely been obtained in our fish culture tanks where the range is more commonly 0.1 to 2.0 / mL. Despite rarely being able to obtain high nauplii densities, from our experience, this is not necessarily a limiting factor in semi-intensive culture where stocking rates of fish larvae are in the region of 2-4 per litre. Most fish larvae appear to be very capable of successfully finding and

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consuming copepod nauplii even if the nauplii are present in only low numbers. It is well recognised that larvae will preferentially feed on copepods, even amongst a bloom of rotifers (Kuhlmann, Quantz & Witt 1981; Van der Meeren 1991; Ali, Mohn Salleh & Siti Noraziah 1998). In many of our fish cultures when nauplii numbers were as low as 0.5 per mL and rotifers more than 10 per mL, nauplii were the dominant prey item in the stomach contents of first feeding larvae.

The question about whether or not it is cost effective to culture and use copepods for aquaculture is difficult to answer. Certainly for the fish species listed above for which copepod nauplii are almost considered essential for survival past first feeding, then copepods may be cost effective – providing the market price of these fish justifies the extra expense. For the other fish species that 'do better' when copepods are used in addition to traditional live feeds then the use of copepods would be dictated by their cost of production and this would need to be examined on a case by case basis. It certainly does not appear practical to try and use copepods as a complete alternative to rotifers and *Artemia* but the way we have used them, to 'kick start' the feeding process for reef fish larvae, is perhaps the best approach. A number of other authors have also recognised that complete rotifer/ *Artemia* replacement is not practical but that the targeted application of copepod nauplii to first feeding larvae can be extremely beneficial (Bell *et* al 1997; Støttrup & Norsker 1997; Støttrup 2000; Shields *et al.,* 2005). The use of resting stages or dormant eggs of calanoid copepods has also been suggested as a means of increasing the functionality of using copepods in marine systems (Marcus 2005). We have tried on several occasions to induce resting production in tropical calanoids and so far it has not been possible and there are no references in the literature to suggest that resting egg production is practised by tropical calanoids.

In Darwin, we now employ a 'semi-automatic' intensive, recirculating larval rearing system for *L. calcarifer*. We are able to produce 600,000 weaned fingerlings (15 mm body length) from two, 6,000 litre tanks after a 28 day culture cycle. Rotifers are cultured in high density of over 1500 per mL and pumped automatically into the larval tanks to maintain a feeding density of 20 rotifers per mL. The system also utilises one of the new generation micro-diets, Gemma Micro™ from Nutreco, as a co-feed from day 8 post hatch which results in the fish being fully weaned by day 18. *Artemia* use is almost insignificant at less than 3 x 440 g cans per million fish produced. It is hard to see a copepod based rearing regime being able to compete with such a system.

4. Concluding comments

Our experience over ten years with copepod culture has been interesting. We have been able to develop a reliable culture method for two calanoid copepod species that has enabled us to keep captive cultures going in the hatchery for many months at a time. We have successfully used these copepods to produce a wide range of tropical fish species and, for a few of these species at least, achieved some of the best survival rates obtained so far in captivity.

At present the fish program at the Darwin Aquaculture Centre is focussed entirely on the commercial production of barramundi and not on reef fish or copepod production. This has been at the request of local industry but this situation may change in the near future as established farms look to diversify their production. Once again high value reef fish and copepod research would be back on our agenda. It is difficult to estimate how much more efficient we could make

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copepod production. The natural limitations on the productivity of the tropical calanoid copepods we have studied would most likely mean that any future gains we make would be in the areas of technology, for example automating part or all of the copepod production process, rather than further investigations into copepod biology.

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