A Review on the Status and Progress in Rearing Copepods for Marine Larviculture. Advantages and Disadvantages. Among Calanoid, Harpacticoid and Cyclopoid Copepods

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Abstract

Copepod species of the genera Calanoida, Harpacticoida and Cyclopoida are used in aquaculture as food for marine larval fish. The calanoids are the most well-known species; most are easily identified as adults and therefore relatively easy to isolate from the wild. They require phytoplankton as food, and many calanoids are broadcasters shedding their eggs individually. The eggs sink to the bottom, which, when siphoned, removes both eggs and other debris. This is usually sufficient to maintain tank hygiene. Many calanoids cannot be kept at high densities and require therefore large volumes for their culture. Harpacticoids are generally benthic grazers, can be maintained at very high densities and therefore in much smaller volumes. They require surface area, which can be provided by placing structures within a tank, although this may complicate the method for cleaning tanks and maintaining tank hygiene. Harpacticoids do not require phytoplankton and can be fed inert feeds. Both harpacticoids and cyclopoids have egg sacs and different methods have been developed for harvesting the nauplii from the culture tanks. The harpacticoids seem particularly difficult to separate from the debris, whereas this seems easier to achieve with cyclopoids nauplii. The cyclopoids, like the harpacticoids, can be maintained in high densities and seem also relatively easy to culture.

Pond cultures using fertilisers are also used to produce copepods for rearing fish larvae. The fish are either reared within the same system or in separate units. Generally, all 3 genera are represented in the zooplankton blooms. The disadvantages of pond cultures include the risk of parasite transfer, no control over species occurrence and abundance, and in temperate climates this type of culture is seasonally restricted.

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1.0. Introduction

The search for the ideal copepod for marine fish larvae that can be cultured intensively is ongoing. In general, copepods are nutritionally suitable for marine fish larvae (Sargent *et al.,* 1997; Støttrup 2000; McKinnon *et al.,* 2003) and constitute a large percentage of the diet in the natural environment (Hunter 1981; Munk & Nielsen 1994; Pepin & Penney 1997). When compared to rotifers and *Artemia* nauplii, the traditional live feeds provided to marine fish larvae, copepods can improve larval growth and survival and the ratio of normally pigmented juveniles when fed either alone or as a supplement (Kraul 1983; McEvoy *et al.,* 1998; Nanton & Castell 1999). Thus, the ability to culture these organisms at a scale adequate for marine larviculture would present a major step forward for the production of many marine species that require a nutritionally better-suited diet than that provided by the traditional live prey.

Although more than 11,500 species of copepods have been classified (Humes 1994), the number of species that are cultured at larger scales relevant for rearing fish larvae are very few and fall within three of the ten orders of copepods, the Calanoida, Harpacticoida and Cyclopoida. The calanoid species are most abundant in the pelagic environment in coastal waters and have therefore received the most attention by researchers (Mauchline 1998). Species belonging to the *Acartia* and *Calanus* genera are most widely studied but those belonging to *Temora*, *Paracalanus, Pseudocalanus* or *Centropages* are also well known (Mauchline 1998). In aquaculture, species belonging to the genera *Acartia*, *Centropages* and *Eurytemora* are in most widespread use in mono- or mixed cultures (Støttrup 2003). Among the primarily epibenthic harpacticoid copepods, species belonging to the genera *Euterpina*, *Tigriopus* and *Tisbe* have been among the preferred candidates for aquaculture (Støttrup 2003). Very few cyclopoid species have been reared in the laboratory. *Oithona* spp. and *Apocyclops* spp. appear to be the best candidates suitable for multi-generation cultures and ideal as food for marine fish larvae (Støttrup 2003).

This review focuses on the advantages and disadvantages of calanoids, harpacticoids or cyclopoids in marine mass culture. Since the nutritional merits of these copepods are well documented (Sargent *et al*.1997; McEvoy *et al*. 1998; Støttrup 2000; 2003; McKinnon *et al.,* 2003), this review targets aspects of the culture of the different copepod species that render them more or less suitable for mass production.

2.0. The advantages and disadvantages of utilising calanoids in marine aquaculture

Calanoid species such as *Acartia* spp., *Eurytemora* spp and *Centropages* spp. have been studied for mass culture purposes. More recently paracalanid copepods belonging to the genus *Parvocalanus* have been reported to be well suited for intensive culture as well as suitable live prey for marine fish larvae (McKinnon *et al*. 2003; Shields *et al.,* 2005).

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2.1. Collecting new cultures

Because of their abundance in coastal habitats, calanoids are easy to collect and to establish in culture. They can be collected with plankton nets to selectively sieve nauplii (80-250µm), copepodite stages (80-350 µm) or primarily adult stages (250-600 µm) (Støttrup 2003). Another possibility is to use light traps to attract copepods during the night. These copepods can then be pumped or air-lifted to a floating cage with appropriate mesh size or to a land-based tank if close enough to shore (eg. Yamashita & Arakawa 1974 cited in Uye 2005). Toledo *et al.,* (2005) also exploited the strong phototactic response in *Acartia tsuensis* to harvest these from fertilised ponds. Since it is generally easier to identify the adult stage, it may be advisable to start a culture by collecting adults. The starter culture should consist of a minimum of 150 adults of both sexes. The recommended minimum tank size for pelagic copepods is 100 L with a low ratio of bottom area to height (Støttrup *et al.,* 1986; Støttrup 2003) (Fig. 1). Starting with a small number of copepods, however, the initial volume of seawater should be low since copepods need a minimum concentration of suitably-sized phytoplankton to obtain maximum filtration rates to yield maximum growth rates (Berggreen *et al.,* 1988; Støttrup & Jensen 1990; Payne & Rippingale 2000c). Low concentrations of copepod would result in excess phytoplankton in the tanks. The excess phytoplankton would sediment out and, if the bottom is not cleaned daily, this build up in sediment may seriously compromise the culture. Thus it may be advisable to start up cultures in small flasks and, as the culture expands, transfer it to 20 L- tanks and then subsequently to larger tanks with initially small seawater volumes, increasing the volume as the culture grows. See for example the description on the isolation and start-up procedures used for *Parvocalanus* sp. by Shields *et al*. (2005).

Fig. 1. Picture of *A. tonsa* culture tanks used at a cod hatchery on Bornholm. The culture was started from cold-stored non-diapause eggs provided by Dr. Myron Peck from a culture in Hamburg University, and belongs to the same stock originally isolated in 1981 by Støttrup *et al*. (1986). Photo: J. Støttrup.

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2.2. Food requirements

Pelagic copepods must be fed planktonic algal species. The minimum algal concentrations required to achieve maximum growth rates depend on the size of algal cells provided to cultures. Thus a density or around 10^5 cells ml^{-1} is sufficient when using small algae < 5 µm such as *Isochrysis galbana* or *Nannochloropsis oculata*, around 10^4 cells ml^{-1} when using larger algae such as *Rhodomonas salina*, and 10^3 cells ml^{-1} using even larger algae, >12 µm such as *Ditylum brightwelli, Thalassiosira weissflogii* or *Crypthecodinium* sp. (Støttrup 2003; Muller-Fuega, *et al.,* 2003). The need for planktonic algal species as food for copepods may be perceived as a disadvantage because continuous algal cultures have not been introduced in most aquaculture units. Most still rely on batch cultures using glass flasks and bags or transparent cylindrical tanks for their algal production (Muller-Fuega *et al*. 2003) (Fig. 2). Not all algae are equally nutritious to the copepods and the fatty acid distribution in the algae may affect their growth and production, as well as their fatty acid profile (Støttrup & Jensen 1990; Jónasdóttir 1994; Payne & Rippingale 2000a; b). Dietary content of amino acids may also be important for copepod fecundity (Kleppel *et al.,* 1998) although lipids seem to have a stronger influence (Jónasdóttir 1994).

Fig. 2. Picture of algal cultures flasks and bags. *Rhodomonas* cultured in flasks
(a) and plastic bags
(b) at a cod hatchery on Bornholm are used to feed the copepods *A. tonsa* for the RESTOCK project. Photo: J. Støttrup.

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2.3. Densities in culture

Calanoids are primarily suspension feeders and typically require large volumes of water for this life-style. In culture, maximum densities reached are 100-2000 L^{-1} although densities of 3000^{-1} were obtained for cultures of *Eurytemora affinis* (Turk *et al.,* 1982; Støttrup *et al.,* 1986; Chesney 1989; Støttrup 2003).

In starting cultures, high copepod densities are obtained over the first few days and then these generally decline and stabilize at a lower level. Toledo *et al*. (2005) observed a rapid increase from 60 to 1,800 copepodites and adults L^{-1} in 1,000 L tanks when a mixture of microalgae and yeast was used as food. The culture peaked 3-5 days after the inoculation and rapidly decreased thereafter.

Paracalanid species of the genus *Parvocalanus* can attain high population densities of 30 ml^{-1} and were higher than recorded for another warm-water calanoid species (5 ml^{-1} for a tropical *Acartia* sp.) (Shields *et al*. 2005; Schipp *et al.,* 1999) and these densities are much higher than those achieved for temperate species such as *A. tonsa* (Støttrup 2000).

2.4. Production and harvest of eggs

The adult calanoids produce eggs over an extended period, depending on the species. In many calanoid species the eggs are spawned freely into the water column. Calanoid species with freely- (broadcast-) spawned eggs are preferable for culture purposes as the eggs sink and can easily be harvested by siphoning the bottom of the culture tank. Various *Acartia* species produce on average 11-50 eggs $female^{-1}$ day^{-1} totalling over 1200 eggs from one single fertilisation (Mauchline 1998). Fig. 3 depicts a female *A. tonsa* with an attached spermatophore.

Fig. 3. Picture of a female *A. tonsa*, with a spermatophore attached near its genital aperture and an egg about to be produced within the genital somite. Photo: S.R. Sørensen.

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An egg collecting devise was tested whereby the adults were retained in smaller tanks within larger tanks (Toledo *et al*. 2005). A filter at the bottom of the adult retainer tank allowed the eggs and algae to sink through to the larger tank. From here, both eggs and algae were airlifted back into the retainer tank, but passing first through a smaller mesh filter which retained the eggs but allowed the algae to pass. This system was relatively efficient in collecting eggs from *A. tsuensis* and the highest number of eggs collected was just under 2 eggs individual^{-1} (copepodite + adult). The authors suggest further improvements such as stocking with primarily adults (eg. using a larger mesh size to separate the adults) and possibly increasing the feeding (or adding yeast). The egg production and hatching rate might be further improved by improving the quality of the algal diet provided. Toledo *et al*. (2005) use a combination of *Tetraselmis* sp., *Chaetoceros* sp., and *Nannochloropsis* sp. All these algae have low or no content of docosahexaenoic acid (DHA, 22:6n-3) and high content of eicosapentaenoic acid (EPA, 20:5n-3) (Muller-Fuega *et al*. 2003). Calanoids do not have the enzyme required for the bioconversion of EPA to DHA (Sargent & Hendersen 1986) and there is evidence that they may need these fatty acids for growth and reproduction. Replacing one or two of the algal species with another with high DHA levels would improve the nutritional value of the diet and possibly productivity.

Fecundity improved when adult *Parvocalanus* sp. were fed a mixture of microalgae, *Chaetoceros* sp. and *Isochrysis* sp., compared to monoalgal diets (Shields *et al*. 2005) although an even higher egg production (31 eggs female^{-1} day^{-1}) was obtained from *P. crassirostris* females fed the dinoflagellate *Heterocapsa niei* (McKinnon *et al*. 2003). System productivity was high for these paracalanid species with daily nauplii production of 3750 L^{-1} for *Parvocalanus* sp. in 400 L cultures (Shields *et al*. 2005). This was a higher production than that obtained for a tropical *Acartia* species (444 L^{-1} in a 1000 L batch culture) and 878 L^{-1} for *G. imparipes* in 500 L batch cultures (Schipp *et al*. 1999; Payne & Rippingale 2000c).

By siphoning the tank bottom daily, the eggs are harvested and transferred to separate tanks to hatch over 24-48 hours to provide newly-hatched nauplii. By siphoning the bottom sediment through a filter with an appropriate mesh size for the species egg-size, several benefits are achieved:

- Tank hygiene. The bottom is cleaned daily removing sedimented algae, faeces and other debris collecting together with ciliates and other protozoans.
- "Clean" copepod eggs. The eggs are separated from the debris by filtering through an appropriate mesh size; eg. 45 μm were used to collect *A. tonsa* eggs (Støttrup *et al*. 1986).
- Egg separation. The eggs are separated from the other developmental stages and can be put into separate containers for hatching to produce newly-hatched nauplii. This also reduces potential cannibalism from the adults since calanoids are known to cannibalize nauplii (Cutts 2002).
- Size-targeting. Keeping the eggs produced on one day in a separate hatching tank allows the possibility to target specific sizes of nauplii or copepodites for feeding fish larvae. Many species have isochronal development, such as the calanoid *A. tonsa* and it is easy to predict the time needed to reach a particular stage of development when maintained at a stable temperature. Mixing batches of eggs from different days into the same hatching tank is therefore not preferable.

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• Storing eggs. It is possible to harvest the eggs, concentrate them and store them under anoxic conditions under refrigeration (4ºC) for later use (Støttrup et al., 1999; stored up to 12 weeks,). This practise has been routinely used in the laboratory at the Danish Institute for Fisheries Research to stockpile larger numbers of copepods eggs, or to take a pause in the culture during the holiday season. The eggs could then be set to hatch in the normal manner either to start up a new culture or to provide a large number of similar-sized individuals to feed fish larvae than normally produced daily in the culture system. Since these eggs are non-diapause eggs, their viability decreases with storage time; thus the storage time is limited (weeks to a few months) and the duration of viable storage may vary with species or storage conditions. In *A. tsuensis* egg viability was ensured after a week of storage under refrigeration by disinfecting the eggs prior to storage (Toledo *et al*. 2005). Peck and Holste (2006) quantified the decrease in hatching of *A. tonsa* eggs with increasing anoxic storage time at 4°C through six months (85% fresh hatch and a linear decrease of roughly 4% every 20 days).

Although calanoids with freely-spawned eggs may be preferable for mass rearing, a culture method was developed for the calanoid *Gladioferens imparipes*, whose females carry a brood of 25-45 embryos (Payne & Rippingale 2000a, b). An automated double 500L system using recycled water and daily collection of nauplii was developed (Payne & Rippingale 2000b) but the production observed in this system fell well below the potential and further work was required to improve the technology and increase the efficiency of harvesting the nauplii from the system.

The nauplii hatch from the eggs and undergo six molts (naupliar stages NI to NVI) before molting into copepodites. The copepodids also go through six developmental stages, the final (CVI) being the adult stage. This prolonged development which may require as little as 6-7 days (eg. *Pseudodiaptomus annandalei*; Su et al., 2005), but is often 12-14 days or longer, renders their culture more problematic when compared to, for example, the rotifer culture. Rotifer development may take as little as half a day but may also be as long as just over 24 h, depending on culture conditions (Lubzens & Zmora 2003). Thus rotifer cultures can be harvested daily. On the other hand, the prolonged development has its advantages. The eggs can be harvested and separated from the remaining developmental stages and hatched or grown to provide the developmental stage (size) of prey that best matches that required for the feeding fish larvae as described above.

2.5. Dormant eggs

Dormancy at some point during the life cycle is a characteristic of many copepods and dormancy during the eggs stage is characteristic for many coastal and estuarine calanoid species (Grice & Marcus 1981). To date 44 calanoid species have been identified with a resting egg stage categorised on the basis of observations of nauplii hatching from sediment samples collected on the seabed (Mauchline 1998). Thus, it is unsure whether all these species have diapause eggs or whether the eggs are quiescent nondiapause eggs or some other intermediate egg type (Marcus 2005). *Acartia* species such as *A. tonsa*, and *A. clausi*, *Eurytemora affinis*, *Centropages hamatus* all have diapause eggs (Marcus 2005) and possibly also *Temora longicornis*, whose eggs were

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found in densities exceeding 10^4 eggs^{-2} in outdoor ponds used to raise cod *Gadus morhua* L. larvae (Næss 1996). The distinction between diapause and non-diapause eggs is important because whereas diapause eggs remain viable after storage (eg. exposed to sulphide or anoxia) non-diapause eggs suffer reductions in hatching after storage and their viability is reduced with prolonged storage (Marcus 2005). On the other hand, non-diapause eggs can rapidly hatch (hours to days depending upon the species and temperature) whereas diapause eggs can only hatch after an obligatory "resting" phase. However, diapause and non-diapause eggs may be indistinguishable form each other using conventional microscopic techniques (Lindley 1990). Diapause eggs are also more resistant to disinfection. Naess & Bergh (1994) compared hatching and subsequent nauplii survival after exposing eggs to 3 different disinfectants conventionally used in hatcheries.

The advantages of dormant eggs are multiple. Lavens & Sorgeloos (1996) suggested using copepod resting eggs as an inoculum to initiate copepod cultures. Marcus (2005) further suggested their use for short- or more importantly long-term storage to ensure stable production of newly hatched nauplii for feeding marine fish larvae. Another advantage also pointed out by her was the reduction of contaminant risks due to the fact that resting eggs of several taxa were "resistant to surface disinfection agents commonly used in aquaculture". The use of dormant eggs also serves as a stock in case of population crashes that may occur during cultivation as pointed out by Marcus (2005). Thus dormant eggs could as noted by Marcus (2005) "facilitate the availability of copepod nauplii for aquaculture".

The conditions required for the production of diapause eggs, mainly photoperiod and temperature, are well-known and documented (Marcus & Murray 2001; Marcus 2005). Often the diapause eggs must be stored for some time under specific conditions before they can be induced to hatch. For example diapause eggs of *Labidocera aestiva* must be stored at 5 ºC for 10-30 days before they can be induced to hatch at 19 ºC (Marcus 1987). Those of *Centropages hamatus* require 4 months at 29 ºC before they can be induced to hatch at temperatures below 20 ºC (Marcus 2005). After storage, diapause eggs generally require a longer incubation time before hatching compared to non-diapause eggs of the same species and at the same incubation temperature (Marcus 2005). Apparently it may be possible to shorten the incubation time following storage by exposing the eggs to different temperature or to certain chemical conditions (Marcus 2005). Thus it seems there is much bio-technological work needed to develop a large-scale system to produce sufficient numbers of diapause eggs at reasonable costs and to examine the conditions required to reduce the time to hatching after storage.

2.6. Size and availability as food for fish larvae

The width and length of newly-hatched nauplii vary among calanoid species. The prey width is considered the most important metric for the ability of the fish larva to ingest the prey since the prey are ingested whole and the mouth size (gape size) of the fish would limit what it could capture (Chesney 2005). The widths of nauplii of different species vary considerably and a two-fold difference is apparent between Acartia sp, for example 30 µm (Chesney 2005) versus 65 µm (Schipp *et al*.1999). The diversity of size spectra in copepods not only among species but also

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within species provided by the numerous developmental stages is considered an advantage in aquaculture.

The paracalanid species of the genus *Parvocalanus* also have small newly-hatched nauplii. The NI stage of *P. crassirostris* was measured at ~38 µm in width (McKinnon *et al*. 2003) and slightly larger for another species; 41µm in width (Shields *et al*. 2005). Both these species were found ideal for larvae of the red snapper *Lutjanus campechanus* (Poey, 1860). *A. tonsa* nauplii were successfully fed to Baltic cod (*Gadus morhua* L.) larvae in an ongoing project in Denmark (Fig. 4).

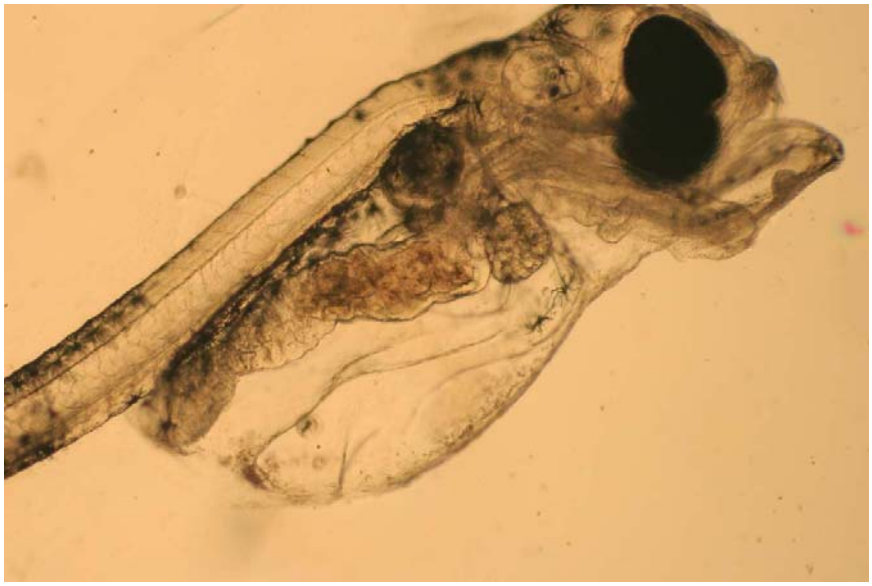

Fig. 4. Picture of a Baltic cod larvae with *A. tonsa* nauplii in its stomach (above) and a closer look at the stomach contents (below). Photo: S.R. Sørensen.

The calanoids are largely planktonic species and are thus in the water column at all times. The encounter rate depends not only on the concentrations of both prey and predator in culture tanks, but is also influenced by turbulence within the tank, illumination and prey contrast. The first feeding fish larvae may be relatively ineffective feeders but rapidly gain competence and capture success approaches 100% within a few days (Chesney 2005). The non-feeding first stages of the copepod nauplii may be difficult to perceive because they are nearly transparent (Fig. 5a). The older feeding nauplii become more visible to the fish larvae because of the algal pigment residues in the naupliar stomachs (Fig. 5b). Thus it may be advantageous to feed fish copepods that have fed on phytoplankton.

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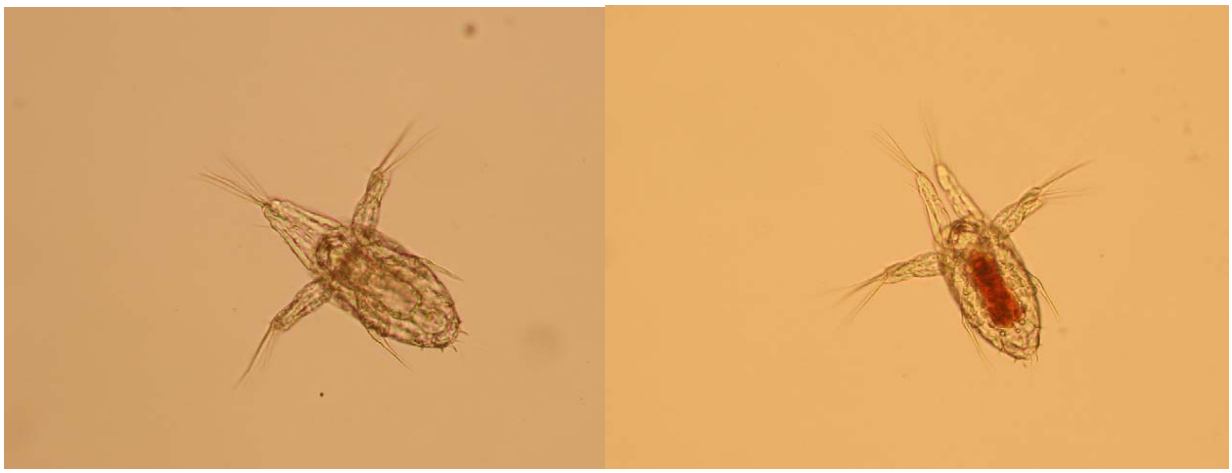

Fig.5. Picture of an *A. tonsa* nauplii without food in its stomach (5a) and one with food (*Rhodomonas* sp.) in its stomach (5b). Photo: S.R. Sørensen.

2.7. Conclusions for calanoid cultures.

Although much experience has been gained in culturing different calanoid species, there is still a need for more progress. The focus should be on achieving stable cultures i.e. finding the optimal density for maximum production. This includes the optimal feeding regime for cultures, the optimal quality (size and nutrition), and how to ensure the food is available for the copepods to maximise production. Finally tank hygiene needs to be maintained and eggs or nauplii harvested. A method that includes all these features in the most efficient manner, requiring a minimum of labour and ensuring stability would be a great step forward towards the introduction of calanoid cultures in mariculture.

Non-diapause eggs of calanoids can be collected and stored for later use. Although the storage time is limited, these stored eggs are useful as a supplement during low periods of eggs production or during rearing when larger quantities of nauplii are required than afforded by the production system on a daily basis. Work towards improving the quality of the cold-stored non-diapause eggs may help to increase the benefits of storing non-diapause eggs.

The bio-technical development for the production and storage of diapause eggs would also be a major step forward for use of calanoids in marine larviculture.

To ensure easily detected prey, especially for first-feeding larvae, feeding stages of nauplii should be provided to the fish larvae.

3.0. The advantages and disadvantages of harpacticoid copepods

Species of the genera *Tisbe*, *Tigriopus* and *Euterpina* have been primarily targeted for mass cultures in aquaculture. The approaches have been diverse ranging from intensive batch systems

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(Støttrup & Norsker 1997, extensive pond cultures (Fukusho 1980) to mesh baskets suspended in larger, larval fish tanks (Kahan *et al*., 1982).

3.1. Ease of production

Most harpacticoid species are epibenthic organisms, grazing organic debris from stable substrates. They have a high tolerance to a wide range of environmental conditions (Uhlig 1984). Harpacticoids are also very productive and can attain high population densities in culture. As such they are ideal candidates for culture and it is only their relatively small size (adults are typically around 1 mm in body length) and their use of epibenthic habitats that make them unfavourable as live prey for marine fish larvae. Exceptions include the pelagic harpacticoid *Euterpina acutifrons,* which was used successfully to rear pelagic larvae of mahi mahi *Coryphaena hippurus* (L.) (Kraul *et al.,* 1992), and *Tisbe holothuriae* nauplii, which swim freely during the early stages and are suitable for the very early feeding stages of some marine fish larvae e.g. turbot larvae (Støttrup & Norsker 1997).

3.2. Densities in culture

Harpacticoid densities in culture can exceed 40,000 individuals L^{-1} and, because of their benthic lifestyle, can be mass-cultured in relatively small units (Støttrup 2003). Although less fecund than broadcast spawning calanoids (Kiørboe & Sabatini 1995), they have a high reproductive capacity (Uhlig 1984; Pinto et al., 2001) and relatively short life cycles (7-29 days for different *Tisbe* species; 12-21 days for different *Tigriopus* species, depending on species and rearing temperature). *N. lacustris* has a generation time of 10-12 days (Rhodes 2003). Since most harpacticoids can survive and grow within large ranges of environmental conditions, harpacticoids can be maintained at high temperatures (>20 ºC) to obtain fast-growing cultures. They are relatively tolerant to decreased water quality due to either a build up of tank detritus or nitrogenous wastes products such as ammonia (Støttrup & Norsker 1997; Cutts 2002) and can be maintained in dense cultures without aeration as long as oxygen is not depleted or severely reduced.

3.3. Production and harvest of nauplii

Because most harpacticoid females bear their eggs in egg-sacs, it is not possible to separate the eggs from the main cultures. Cultures techniques need to be developed that effectively harvest the newly-hatched nauplii. A continuous system was developed by Støttrup & Norsker (1979) for *T. holothuriae* producing on average ½ million nauplii and copepodites daily over a 1½ month period. This system utilised the observed lifestyle/traits of the nauplii and copepodite stages of *T. holothuriae* (Fig.6) to separate these from each other. The first naupliar stages swim freely in the water column but are not as strong swimmers as the adults. Therefore prior to the automated harvest, a light would switch on driving the copepodites and adults to swim away from the light source towards the bottom of the tank. The flow would then be reversed so the water was drawn from the surface instead of the bottom harvesting the nauplii from the water column. This would provide a naupliar sample without any debris. However, the harvest was not entirely effective

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and many nauplii remained in the tank and grew in the system. Evidence suggested that this culture was food limited and that productivity could have been increased without compromising the culture stability. Another continuous mass culture was developed by Sun & Fleeger (1995) for *Amphiascoides atopus* in a relatively small basal surface area (4 m²) with a large volume of 1440 L. about ½ million individuals were harvested daily form this system during a 5 month period.

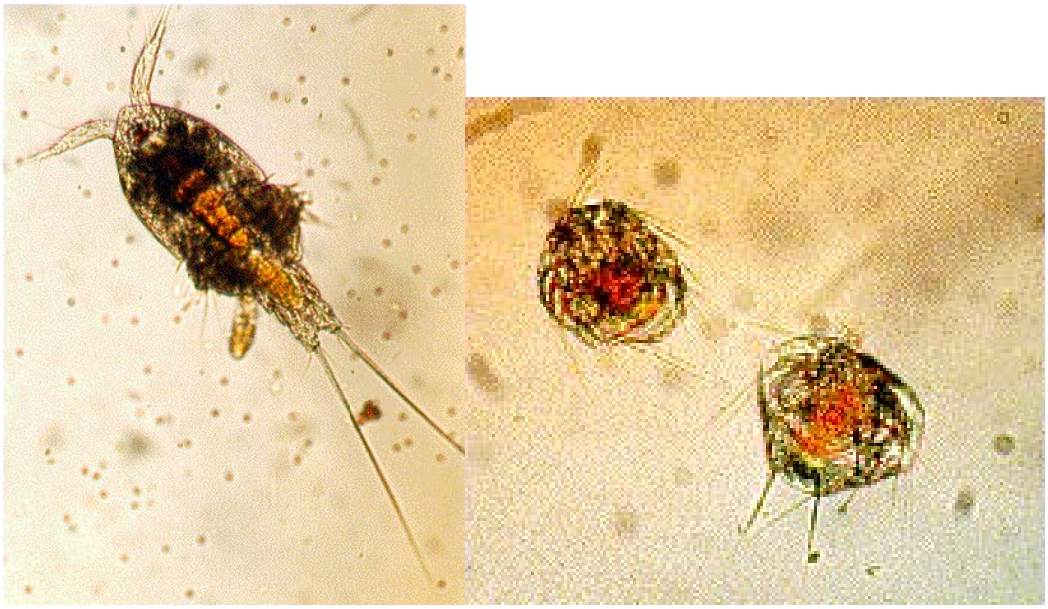

Fig. 6. Picture of an adult *Tisbe holothuriae* (6a) and its nauplii (6b). Photo: J. Støttrup.

Practically any type or shape of tank can be used to culture a harpacticoid like *T. holothuriae*, but the highest densities are obtained when the surface area to volume ratio is high. In the continuous system described by Støttrup & Norsker (1997), small plastic balls were introduced to provide a substrate and increase the surface area to volume ratio within the tank.

In batch cultures of *T. holothuriae*, it was possible for Støttrup & Norsker (1997) to obtain between 300,000 and 500,000 nauplii day^{-1} from a flat tray culture (40 x 60 cm; 3 L). This corresponded to a daily volume output of 100,000 nauplii L^{-1}. The whole culture had to be filtered daily, but the adults were very tolerant to this handling and continued to produce nauplii for the entire two-month monitoring period. Several researchers have encountered problems separating the nauplii from the culture debris. Rhodes (2003) found that the nauplii of *N. lacustris* would cling to the bottom and aggregate with faecal and food debris detritus. Many nauplii are photonegative and thus tend to collect and aggregate within this debris. To separate nauplii or adults from debris, *T. holothuria* was concentrated in a 500 ml or 1000 ml cylinder. The cylinder was placed on a tripod with a light source from below. After about 15 minutes the top 90% of the volume was poured into a new container providing relatively debris-free nauplii or copepodites. This was especially effective for the copepodites probably because these are stronger swimmers (Norsker & Støttrup, unpublished observations).

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3.4. Food requirements

Harpacticoid species can feed on a microalgal diet, but they are not dependent on a constant supply of suspended feed. Most harpacticoids feed by scraping organic matter off surfaces and it is possible to culture these organisms in small volumes with a large surface area (Støttrup & Norsker 1997). They can be fed a variety of inert feeds including formulated feeds normally used in aquaculture for fish. This type of feed should be administered with great care to avoid hygiene problems developing in the culture tanks. Food quality is, however, important for development and fecundity and the diet offered will therefore influence the performance of the culture.

Marine fish larvae require feeds with high levels of essential fatty acids such as DHA, EPA and arachidonic acid (ARA, 20:4n-6). The copepods produced should therefore contain high levels of these fatty acids. In calanoids these essential long-chain unsaturated fatty acids must be present within the microalgae diet as they lack the necessary enzyme to convert EPA to DHA and may lack the chain-desaturation and -elongation enzymes necessary for the conversion of linolenic acid (18:3n-3) to EPA (Sargent & Hendersen 1986; Fraser et al., 1989). The copepods may indeed require these essential fatty acids for their own population growth. On the other hand, the harpacticoid *T. holothuriae*, apparently has the necessary Δ-5 and Δ-6 desaturase and elongase enzymes necessary for bioconversion of shorter chain n-3 fatty acids to EPA and DHA (Norsker & Støttrup 1994; Nanton & Castell 1998). This species had high levels of essential fatty acids regardless of the levels in the diet. Rhodes & Boyd (2005) examined the effect of using formulated feed compared to an algal diet on the growth and lipid composition of the harpacticoid *N. lacustris*. This species can apparently also elongate fatty acids chains and utilise dietary (18:3n-3) and linoleic acid (18:2n-6) to biosynthesise marine fish essential fatty acids such as DHA, EPA and ARA. Apparently, these epibenthic harpacticoids have evolved differently to the planktonic calanoids and no longer depend on dietary input of HUFA to maintain normal growth and reproductive capacity.

3.5. Size and availability as food for fish larvae.

The smallest nauplii are those of *T. holothuriae* and *N. lacustris* (≈70-80 μm) (Dahms & Bergmans 1988; Fleeger 2005). Due to their relatively small size, the nauplii of *T. holothuriae* are appropriate for the larvae of only a few marine fish species (i.e., those that have very small mouths) or as a starter or rotifer supplement diet for species such as turbot *Psetta maxima* L. Newly hatched nauplii of a *Tisbe* sp. collected near Halifax, Canada measured 90 μm in length (Nanton & Castell 1998) and are therefore almost the same size as newly-hatched *A. tonsa* nauplii.

For the fish larvae to feed on these nauplii they also need to be able to encounter them or perceive their presence. The harpacticoid *Euterpina acutifrons* is pelagic and some harpacticoid species have planktonic, immature stages (Neunes & Pongolini 1965). The early naupliar stages of *Coullana* (*Scottolana) canadensis* and *Tisbe* spp. are planktonic (Hicks & Coull 1983) and *T. holothuriae* nauplii were observed swimming in the water column (Støttrup & Norsker 1997).

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On the other hand, nauplii of *N. lacustris* do not swim (Fleeger 2005) and are, therefore, unsuitable prey items for fish larvae whereas the copepodite/adult stages do swim and are similar in size to rotifers. These post-naupliar stages could thus be used in mariculture to replace or supplement rotifers. Supplementing a rotifer diet with *T. holothuriae* nauplii increased feeding in turbot larvae (Støttrup & Norsker 1997). Apart from their presence in the water column, prey swimming behaviour and prey contrast are also considered important factors affecting larval fish feeding (Buskey 2005). Providing *feeding* nauplii to fish larvae enhances prey contrast and this together with their apparent low predator escape responses (Buskey 2005) makes these species ideal live prey for first-feeding fish larvae.

3.6. Conclusions for harpacticoid cultures

Harpacticoids can be cultured in high densities and are apparently easier to culture than calanoids. Many species, however, have epibenthic life-stages, a life history trait that reduces the range in prey sizes that can be utilised by pelagic fish larvae. Their nutritional value is similar to that of calanoids, but they are not dependent on planktonic food particles or on the provision of fatty acids that are essential to marine fish species, since some species are able to chain elongate and desaturate shorter-chain fatty acids.

Progress is needed in the biotechnical development. It should be possible to develop semi-automated systems for feeding and harvesting nauplii that would minimises labour and make culturing harpacticoids more efficient.

4.0. The advantages and disadvantages of cyclopoid copepods

A few species of cyclopoids have been cultured in the laboratory mainly belonging to the genus *Apocyclops*. Adult copepods of *Apocyclops panamensis* were harvested from a brackish pond (10-27 ‰) using a sump pump to pump the pond water into three 125 L tanks fitted with 100 µm mesh sized bags (Lipman 2001; Fig. 7). The water was transferred to the laboratory and filtered through different filters. The adults were harvested from the 150 µm to 350 µm mesh size fraction. *A. panamensis* has been reared in intensive and extensive systems to feed the larvae of red snapper *Lutjanus campechanus.* Being omnivorous, they can be fed phytoplankton, yeast or other feeds. In most cases the species were isolated from coastal brackish waters, adapted to laboratory conditions and subsequently cultured. In pond cultures, calanoid species generally develop and dominate but very often cyclopoids from the genus *Oithona* also develop along with a few harpacticoid species (Gaudy 1978; van det Meeren & Naas 1997; Toledo et al., 1999).
Some cyclopoids species have very short development times; 4-5 days to maturation for *Apocyclops royi* (Su *et al*. 2005), which make them very ideal for mass culture.

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Fig. 7. Photo of trap for *Apocyclops panamensis*. Photo by kind permission, E.E. Lipman.

4.1. Densities in culture

Cyclopoid copepods of the genus *Apocyclops* can be cultured at densities similar to harpacticoids. In intensive systems, Shirgur (1989) produced *Apocyclops dengizicus* at densities averaging 16,000 L^{-1}. Cheng et al. (2001, in Phelps et al., 2005) were able to achieve densities up to 33000 nauplii L^{-1}. *A. borneoensis* was cultured in large 15 m^3 systems obtaining densities of 2300 adults L^{-1} and a total density of 4400 individuals L^{-1} (James & Al-Khars 1986). Not surprisingly, the optimal adult stocking density of *A. panamensis* within intensive batch cultures was found to be half the maximum stocking density of 5120 adults L^{-1} (Phelps *et a*l. 2005). At a stocking density of 2560 adults, the average production of 8.2 nauplii and copepodids L^{-1} was almost twice that at the highest adult stocking density.

4.2. Production and harvest of nauplii

Cyclopoids, like harpacticoids, have their eggs contained within paired egg sacs, which remain attached to the female genital segment until they hatch (Huys & Boxshall 1991; Fig. 8). Lipman (2001) achieved a significantly higher production of *A. panamensis* compared to *A. tonsa* during a 14 day culture. Also *A. panamensis* survived better in these cultures than *A. tonsa*. Mean production of *A. panamensis* was 773 nauplii and copepodites L^{-1} as compared to 325 *A. tonsa* nauplii and copepodites L^{-1} (Lipman 2001). In intensive batch systems (40L) stocked with an adult density of 5,120 *A. panamensis* adults L^{-1} the production of nauplii and copepodids harvested after 4 days was 17,873 L^{-1}(Phelps *et al*. 2005). This was equivalent to 4.7 nauplii

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female^{-1}. The highest production per female (8.1 to 8.5 nauplii female^{-1}) was, however, obtained at the lower stocking densities of 320- 2560 adults L^{-1}. It was therefore recommended to stock the culture with 2560 adults L^{-1} to obtain high yields and high productivity. Thus in continuous systems for this species, it may be preferable to maintain adult densities at around 2000 L^{-1} and sustain the culture for longer periods. Higher yields and higher numbers of nauplii adult^{-1} were obtained from domesticated individuals of the same species (Phelps *et al*. 2005). The number of offspring produced daily per female varied with temperature in culture of *A. royi* fed *Chaetoceros muelleri* (Su *et al*.2005). The lowest (8 nauplii^{-1} female^{-1} day^{-1}) was at 25ºC and higher rates (12 to14 nauplii female^{-1} day^{-1}) were produced at 30ºC and 35ºC respectively. The highest cumulative number of offspring per female was 222 at 30ºC.

Fig. 8. Picture of an *Apocyclops panamensis* female bearing egg sacs. Photo by kind permission, E.E. Lipman.

As is the case with harpacticoids, problems arise due to the need to harvest nauplii from adult cultures, and no viable solutions have been developed to date. Filtration and subsequent separation from the debris is the order of the day, but the latter may be more easily accomplished with cyclopoids since they are planktonic. The problem of contaminant build-up would also need to be overcome especially if cyclopoids are fed inert feeds.

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4.3. Food requirements.

Cyclopoids are omnivorous and can be fed a mixture of feeds, but most researchers feed phytoplankton or a mixture of phytoplankton and yeast to these copepods (Phelps *et al*. 2005).

4.4. Size and availability to fish larvae.

A. panamensis, *A. dengizicus* and *A. royi* are all ideal candidates for aquaculture (Phelps *et al*. 2005). The sizes of the nauplii of *A. royi* range from 110 to 265 μm and the adults are about 1 mm in length (Chang & Lei 1993 in Su *et al*. 2005). This species is used to feed larvae of grouper *Epinephelus coioides* (Hamilton, 1822) in a private hatchery in Taiwan (Su *et al*. 1997). A further species, *Apocyclops borneoensis* was used to replace *Artemia* for rearing *Acanthopagrus cuvieri* (Day, 1875) (James & Al-Kars 1986).

4.5. Conclusions for cyclopoids cultures

Cyclopoids are apparently easier to culture than calanoids and can be maintained at higher densities in culture. They can be fed a variety of foods, although most have used phytoplankton in intensive systems.

The major disadvantage is the inability to harvest eggs as with broadcasting calanoid species. In the culture of *A. tonsa* (Støttrup *et al.,* 1986) the processes of egg harvest and cleaning of tank bottom are combined. In the rearing systems for harpacticoid, cyclopoid and some calanoid species (i.e. those species that bear their eggs until hatching) the harvest and tank cleaning processes are separate and often result in more labour-intensive systems.
Once collected, the nauplii of cyclopoids are easier to separate from debris than harpacticoid nauplii and the potential for an automated (batch or continuous) harvest of the nauplii is expected to be higher.

5. Pond cultures

Pond culture using fertilisers has been employed by different researchers for a variety of fish species. Pond cultures can yield average densities of 10- 300 individuals L^{-1} of a mixture of copepod species that includes members of all 3 genera discussed in this review (Svåsand et al., 1998). For example, Colura et al., (1987) obtained 374 nauplii L^{-1} of *Acartia* sp. and *Oithona* sp., and Phelps *et al*. (2005) produced >1000 nauplii L^{-1} of *A. panamensis* . Pond cultures are still in use in commercial systems in Denmark where they are started each year with diapause eggs that hatch from the sediment. The diapause eggs were most likely produced towards the end of the previous year's production (Engell-Sørensen et al., 2004). The method is relatively simple as described for example in Engell-Sørensen *et al*., (2004) and Phelps *et al*., (2005), although production is limited to the summer months in temperate climates. Most often the fish are reared in these systems, and one pond can be reserved for copepod production for adding to the other rearing ponds in cases when it becomes necessary during fish larval rearing (van der Meeren & Naas 1997; Støttrup 2000; Engell-Sørensen *et al*., 2004).

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Apart from the limited season in temperate climates, other disadvantages of pond systems include the risk of parasite transmission or the accidental introduction of larvae of predatory species. Most often, the species developing cannot be regulated and, in many systems, the dominant species alternates between species. In systems in temperate climates it is not unusual to observe an initial bloom of rotifers during start up, followed thereafter by blooms of copepods and decreased rotifer abundance (Støttrup 2003).

6.0. General comments for copepod cultures

Photoperiod is important for all copepods and affects egg production (Støttrup 2003; Peck & Holste 2006). Thus an appropriate photoperiod should be a requisite for all copepod cultures. Most species tolerate gentle aeration and supplying gentle aeration is especially beneficial for planktonic species that require phytoplankton as this helps keep the phytoplankton in suspension.

Nauplii can be stored in cool temperatures. For example Payne & Rippingale (2000d) stored nauplii of *Gladiferens imparipes* in static seawater at 8 ºC for up to 12 days with excellent survival. In the laboratory newly-hatched nauplii of *A. tonsa* were stored at 6-7ºC at densities of 30 to 100 ml^{-1}. Survival was highly variable as shown in Fig. 9 (own unpublished data). On average around 80% survived to day 5 and >60% survived to day 8. There was no difference in survival between those fed *Rhodomonas* sp. during cold storage and those that were not fed. More work is needed to examine the ideal conditions for stockpiling nauplii in cold storage and to examine the changes in the nutritional value of nauplii that are cold stored for a number of days with or without algae.

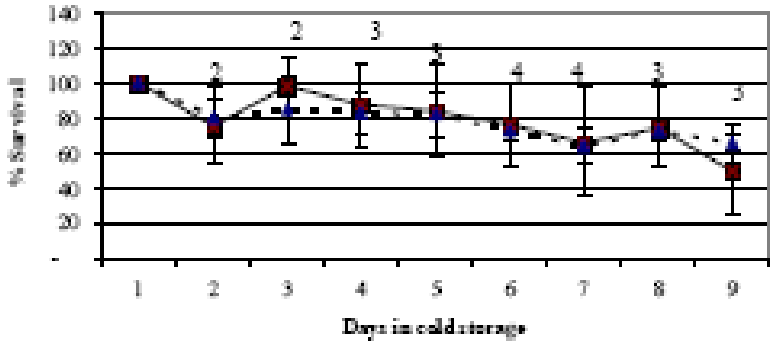

Fig. 9. Survival of newly-hatched nauplii kept in cold storage in 200 ml beakers at densities from 30-100 ml^{-1} with (▲) and without (■) the addition of *Rhodomonas* sp. Four replicates were monitored and each was sampled daily and counted in triplicate. Not all the replicates in the unfed group were counted each day. The numbers in the fig show the number of replicates counted for this group.

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The egg or naupliar harvest does not need to be 100% efficient in copepod cultures. The unharvested eggs and/or nauplii seed the culture tanks and help to maintain longer-term stable production within a system. Allowing some of the eggs and nauplii to remain within tanks eliminates the need for a stock culture of adults to replenish the culture at intervals.

Acknowledgements

The author thanks Dr. Ana Puello and the other organisers of the VIII International Symposium on Aquaculture Nutrition for the invitation to submit this manuscript. I would also like to thank Dr. Myron Peck (University of Hamburg) for commenting on a draft of this manuscript.

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