Intensive cultivation of a calanoid copepod for live food in fish culture

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Project No. 1996/398

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OBJECTIVES:

1. Determine the most effective procedures for increasing the scale of intensive production of *Gladioferens imparipes* to enable reliable supply of animals with minimum labour and maximum automation.
2. Assess the relative nutritional value of *G. imparipes* and other live food animals as food for various species of marine fish larvae through survival and growth trials of fish on different diets. This is to involve fish which are currently cultivated and other species for which eggs or larvae can be obtained.
3. Determine the diet of copepods which will provide a biochemical profile in the copepods which best serves the nutritional requirements of larval fish.
4. Undertake a cost/benefit analysis of cultivated copepods in fish aquaculture.
5. Produce a detailed manual of procedures for intensive cultivation of *G. imparipes* for distribution by sale to commercial hatcheries within Australia.

NON-TECHNICAL SUMMARY

OUTCOMES ACHIEVED

Intensive copepod culture techniques developed during this project will further efforts by Australian hatcheries towards commercial-scale culture of difficult marine fish species, particularly WA dhufish. Successful culture of these species will benefit both commercial culture facilities and the broader Australian economy. The importance of this project has been recognised by the Aquaculture Development Unit (Fremantle, Western Australia), who has established intensive copepod cultures for use in further research into culturing new species. The manual of procedures for copepod culture has been requested by numerous commercial facilities and private persons, including Ocean West (Exmouth, WA) and WA Ocean Park (Shark Bay, WA)

Provision of suitable food for the early larvae of fish is one of the difficulties which currently restricts the development of marine fish culture. In many estuaries and coastal marine environments, where fish breed, the food item that is most likely to be encountered by a first feeding fish is a nauplius larvae of a planktonic copepod. Copepod nauplii are recognised as food by many fish, they elicit a feeding response, they are the appropriate size and they may provide nutritional benefits if they have been feeding on phytoplankton. In the natural environment the chances of survival for individual fish larvae are extremely small. High attrition occurs as fish larvae fail to

encounter suitable food when their yolk reserves are depleted or they are eaten if they are encountered by a larger predator.

In aquaculture it should be possible to provide conditions whereby juvenile fish have greater prospects of surviving the first few critical days than occurs in nature. Timely provision of suitable food and relief from predators should allow high rates of survival.

Current aquaculture practises provide adequately for the critical needs of some fish species with well developed technology for the production of rotifers and brine shrimp as live food. For some other species of fish these are not suitable as food and research laboratories in various parts of the world have worked towards the effective cultivation of marine copepods for use as an alternative food in aquaculture.

The aim of this study was to develop a system for the cultivation of a calanoid copepod which occurs in estuaries in the southwest of Western Australia. This copepod, *Gladioferens imparipes*, seemed suitable for use in marine fish aquaculture; it can tolerate a wide salinity range, including sea water, has planktonic nauplius larvae and is sufficiently robust to grow well in cultivation. The objectives of the study were to develop a scale of culture which would be realistic for use in aquaculture, develop a system to automate routine procedures in the copepod culture to minimise labour and to investigate the effectiveness of using copepod nauplii from intensive cultures to enhance the survival, health and growth of fish larvae. A final objective was to provide a manual of operations to assist people to develop, maintain and use cultures of *G. imparipes* for use in aquaculture. This manual is available as a separate appendix to this report.

Development of copepod cultures involved increasing the scale of cultures from units of 60 L, which had previously been effective, to units of 500 L and 1,000 L. A culture unit of 500 L, with recirculating water and automatic control of the daily operations for collecting nauplii and replenishing water quality continued producing ~ 450,000 nauplii per day for 409 days. This exceeds the production statistics published for other calanoid copepods. However, this rate of nauplius production is less than predicted from the potential reproductive performance of individual *G. imparipes* copepods held in optimum conditions. Further research should identify the cause and remedy the under-production of the recirculating culture.

Where sea water is readily available high water quality can be sustained in copepod cultures by flow-through rather than recirculation. Early indications are that nauplius production rates will improve under this regime.

The fatty acid profiles of algae used as food for copepods and the fatty acid profile of nauplii which had grown with those algae as food both indicated that laboratory grown copepods will provide HUFA which are known to be essential in the diet of larval fish.

Providing *G. imparipes* copepod nauplii in the diet of some species of fish was shown to be beneficial. For West Australian seahorses (*Hippocampus angustus*) , snapper (*Pargus auratus*), and West Australian dhufish (*Glaucosoma hebraicum*), animals

with copepod nauplii in the diet had higher survival and faster growth than controls with only rotifers in their diet.

Intensive aquaculture is warranted for fish with high market value. The cost of reliable copepod nauplius production to provide a beneficial supplement to the diet of first feeding fish larvae is not excessive. Indeed, there is growing evidence that for species such as the groupers, provision of copepod nauplii is the only commercially viable option.

KEYWORDS: **Calanoid, copepod, culture, fish larvae, first food.**

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The bulk of this work was conducted at Curtin University of Technology on the premises of the Department of Environmental Biology. The assistance and interest of staff and students alike at this school is gratefully acknowledged. In particular, we would like to thank both technicians who where directly involved with the project, John Corbett and Diane Webb.

Studies on large-scale copepod cultures were conducted at the Aquaculture Development Unit in Fremantle, Western Australia. For this, we would particularly like to thank Greg Jenkins and Ken Frankish for their generous co-operation. The assistance of all the ADU staff, particularly Gavin Partridge, Craig Poller and Bruce Ginbey, is also acknowledged.

BACKGROUND

There is a high demand world-wide for seafood and other table fish and traditional approaches to fish harvest from the sea are not able to meet this demand. The development of commercially viable aquaculture is widely acknowledged as important. There is potential for cultivated fish to become a significant proportion of the total market for staple food, for luxury food and for aquariums.

Successful cultivation through many generations has only been achieved for a few species of fish which are of economic importance. It is an important objective for aquaculture research to increase the number of species which can be reliably bred and thereby provide new market resources without risking the survival of wild populations or natural ecosystems.

Most species of marine fish produce large numbers of larvae which, in nature, have very high rates of mortality at the critical stage after yolk reserves are depleted and the fish commence exogenous feeding. In aquaculture it should be possible to reduce this high mortality both by timely provision of adequate and appropriate food and by removing the risk from predators. The serious difficulty comes in identifying and providing suitable food for this critical first feeding stage of the fish larvae.

The importance of **live** food for fish larvae at the critical first feeding stage is well known. In the natural environment of the sea, the potential food items most likely to be encountered by fish larvae are the nauplius stages of calanoid copepods. During evolution, larval fish of many species must have developed effective predation strategies to recognise and capture nauplii and some fish may have developed partial dependence on them.

Marine calanoid copepods which feed primarily on phytoplankton reflect the biochemical composition of their diet in their body tissues and storage compounds. Hence the fatty acids such as EPA and DHA, which are essential in the diet of marine fish larvae (and other vertebrates), are provided in nature through a food chain leading from phytoplankton through herbivorous copepods to fish.

Herbivorous calanoid copepods are particularly suitable as food for fish. The size range (100μm nauplii to 1,000μm adult) fits the gape of many larval fish, copepod nauplii elicit a strong feeding response from many fish larvae and copepods have naturally high levels of essential fatty acids and amino acids.

There is field and laboratory evidence that calanoid copepods are the natural, and preferentially selected, food of many larval fish. Despite research effort, intensive cultivation has only been achieved for very few species of calanoid copepods and reliable supply for aquaculture has proved difficult.

The most widely used food items for larval fish in aquaculture are the brine shrimp *Artemia* and the rotifer *Brachionus plicatilis*. Although successful as food for many fish, especially after enrichment with HUFA, these organisms are not ideal in all circumstances or for all fish species.

Culture systems for live food organisms are varied, reflecting the diverse requirements of different organisms and different ideas on how best to culture them. Rotifers are generally maintained as semi-continuous batch cultures, requiring frequent removal of rotifers and renewal of water to maintain the health of the cultures. This is very labour intensive. Most copepod culture systems also have high labour requirements (Støttrup et al. (1986)), and this has led to interest in developing automated techniques, and the system developed by Støttrup & Norsker (1997) represents the benchmark for automated copepod culture systems. While this system has many interesting features, it was developed to suit a particular harpacticoid copepod species and is not at all suitable for use with calanoid copepods.

Research at Curtin University, W.A., extending over 25 years, provided information on the ecology and general biology *Gladioferens imparipes*, an estuarine calanoid copepod. This work showed the animals to have many attributes making them amenable to intensive cultivation and acceptable as a dietary item for some species of marine fish. Small laboratory cultures of *G. imparipes* were maintained to provide animals for research and for ecotoxicology testing. Success in using the copepods as food in growth trials of larval pipefish (*Stigmatopora argus*) prompted further interest in use of the copepods in aquaculture and development of culture systems to a scale appropriate for commercial aquaculture. The present report describes the research in this direction which was supported by FRDC from June 1996 - June 1999.

NEED

1. There is need to maximise the efficiency of marine fish aquaculture by optimising survival, health and growth at the early larval stage of those fish species which are currently reared in captivity. *Artemia* and rotifers are commonly used to feed larval fish but for some fish the nauplius larvae of copepods may be a better food. With reliable copepod cultivation having been demonstrated at a small scale it was important to evaluate the nutritional benefits which could accrue to fish from providing copepods as the first food, and to evaluate the economic feasibility of copepod production at the scale required for commercial aquaculture.

   If economical copepod production could be achieved, Australian aquaculture industries could achieve substantial increase in productivity through using these animals as alternatives for or supplements to the usual food for larval fish.

2. There is benefit in extending the number of marine or estuarine fish which can be economically reared as table fish. For some fish species (some of which were identified at World Aquaculture '99), neither *Artemia* nor rotifers are useful as first food items. Identification and provision of suitable food for early larval stages is critical to further developing the range of species of fish in cultivation. There is need to evaluate copepod nauplii from intensive cultivation as first food for fish which until now have not been successfully reared in captivity.

3. There is benefit in extending the range of marine fish species, especially from tropical waters, which can be successfully reared to supply the lucrative trade in fish for aquarium display. Present reliance on wild capture of these fish involves environmental risks which should be reduced. As more restrictions are imposed on wild collection of these fish the value of 'guilt free' tank bred fish is likely to increase. The need to evaluate copepod nauplii as food for early larvae of some marine ornamental fish was reiterated at the 1999 meeting of the World Aquacultue Society in Sydney where a number of presentations stressed to economic value of these fish. Interest in marine ornamentals is also indicated by the conference entitled Marine Ornamentals '99 held in Hawaii.

4. Many biological resources from the sea are of research interest and potential economic value. Reliable provision of a natural food in the form of copepod nauplii will enable successful laboratory maintenance of more species of these animals, permitting more detailed study and evaluation of their scientific and economic value.

OBJECTIVES

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5. To produce a detailed manual of procedures for intensive cultivation of *G. imparipes* for distribution by sale to commercial hatcheries within Australia.

METHODS

Development of copepod cultures

Recirculating 500 L culture

Prior to commencement of the present project 60 L batch cultures of copepods were successfully operating with manual control of the procedure for nauplius collection. The same principles were used in developing a 500L culture with Programmable Logic Control (PLC) used to control the daily collection of nauplii and recirculation of water through a biological filter and protein skimmer (Figure 1). The PLC operated from the 240 volt electrical grid but controlled all the electrical components by 12 volt circuits.

Seawater for all cultures was obtained from Fremantle harbour. Water was aged for at least 30 days, chlorinated / dechlorinated and diluted to S 27 (75% of seawater) with deionised water before use. Temperature was maintained at 23±1°C, and dissolved oxygen, pH, ammonia and nitrite levels were monitored regularly.

Algae for use as food for copepods was routinely cultured in 20 L Nalgene ® carboys using Guillard's F/2 medium and CO_2 supplemented aeration. *Isochrysis galbana*, the usual food, was taken from cultures with >7 million cells.ml^{-1} and added daily to copepod cultures to 100,000 cells.ml^{-1}.

Flow-through 1,000 L culture

A 1,000 L copepod culture was developed following the same principles as used in the smaller culture but with continuous slow flow of sea water and manual operation of the nauplius collection process (Figure 2).

Effect of culture conditions on *G. imparipes*

Survival at low temperature

Copepods of the same age class were grown to maturity at 25°C in water of S 27 and fed *Isochrysis galbana* daily to excess. When most of the animals were mature and reproducing, nauplii were separated from the adults by screening through 149 μm mesh. Nauplii and adults were separately placed in clean seawater (S 27) and fed excess *I. galbana* for 2 hours. After feeding, both groups of copepods were again placed in clean water and distributed evenly between 12 2 L containers, 6 containers each for nauplii and adult copepods. Three containers with nauplii and 3 with adult copepods were randomly selected and held in darkness in each of two domestic refrigerators set at 4±1.5°C and 8±1.5°C.The positions of the containers with the refrigerator was rotated daily to minimise the effect of spatial temperature differences.

Copepods from each container were sampled at 3 day intervals by removing 3 consecutive 40 ml volumes while thoroughly vigorous mixing the contents of each container. Each 120 ml sub-sample was then warmed to room temperature before a small quantity of *I. galbana* was added. After 24 hours, the number of living and dead copepods in each sub-sample was determined. Living nauplii and adult copepods sub-

sampled on days 21 and 42 were placed in clean water. These animals were fed daily until live nauplii of the next generation were observed.

Effect of food type on survival and growth

Nauplii of an approximately uniform age class were collected from copepod cultures maintained at 22°C and S 27 by screening culture water through 100 μm mesh. They were then transferred to filtered (1.2 μm) seawater (S 27) to a density of 1 nauplius. ml^{-1}. Nauplii were then randomly allocated to each of 36 150 ml plastic bowls such that each bowl contained approximately 70 nauplii. The water volume in each bowl was adjusted to 100ml. Two groups of 18 bowls each were floated in separate water-baths maintained in darkness at 20°C and 25°C. Five treatment diets were each randomly allocated to three bowls in both groups, with the remaining three left unfed. Diets consisted of the algae *Isochrysis galbana*, *Chaetoceros gracilis*, *Dunaliella tertiolecta* and *Nannochloropsis oculata* and fresh bakers yeast (*Saccharomyces cerevisiae*). Algal cultures were originally obtained from the CSIRO Marine Laboratories in Hobart, Tasmania, and maintained in logarithmic growth phase using Guillard's f/2 medium in non-axenic conditions.

Nauplii were fed daily with equivalent concentrations by volume of the treatment diets. The mean cell volumes of each food organism was determined using an electronic particle counter (Coulter ®). Prior to feeding each day, the turbidity of each algal culture was measured using a Hach Kit Drel/5 and this reading used in a predetermined correlation equation to calculate cell density. The accuracy of this equation was monitored regularly. Suspensions of fresh yeast in deionised water were stored at 4°C and renewed every two days. A haemocytometer was used to determine cell densities of each new suspension. Cell volume and density were then used to calculate a quantity of food such that treatments received 1 ppm food by volume each day up to day 4, increasing to 2 ppm for the duration of the trials. These quantities ensured excess food in all treatments. Every third day, at least 90% of the water volume in each bowl was replaced with clean water.

Copepod Survival

Copepod survival on each diet was assessed each day until egg production commenced. Estimates of copepod survival were made for each container, a score given according to the approximate percentage survival:

Score	Survival
3	>90%
2	50-90%
1	10-49%
0	<10%

Containers with a score of zero were removed from the trial. Adult copepods were counted at the end of the trial. Results were analysed using oneway ANOVAs and Tukey's W procedure.

Maturation

Female copepods were judged to be mature when carrying their first clutch of embryos. Newly mature females were removed from containers daily, counted and kept in clean water with other females from the same treatment. Daily collection of females continued until all were mature. The number of adult males remaining was recorded and all males from the three replicates of each treatment pooled.

Cumulative proportions of mature females reared on treatment diets were logit transformed and regressed against time. The time taken for 50% of the females to reach maturity on each diet was predicted from these regressions and compared using oneway ANOVAs and Tukey's W procedure. Regression gradients, indicating the rate of maturation, were compared using dummy variables.

Nauplius Production and Female Growth

Adult male and female copepods from each diet treatment were mixed and evenly distributed between six containers and fed with the same experimental diets for a further four days. Three males and three embryo-bearing females from each diet were then transferred to a clean container. Survival of the adult copepods was monitored daily. After exactly 96 hours the adult copepods and newly produced nauplii were preserved in formalin containing Rose Bengal stain. Nauplii were later concentrated by gentle centrifuging, pipetted onto a slide and counted under a compound microscope. Oneway ANOVA and Tukey's W procedure were used to compare the prosome lengths of females and the total number of nauplii produced by copepods which had grown to maturity on the different diets.

Effect of water quality on survival and growth

Copepod nauplii were collected from a 500 L culture and grown for 4 days on a diet of *Isochrysis galbana*. Approximately 130 nauplii (N5 and N6) were stocked into 20 130 ml bowls in a small volume of clean seawater (S 27). Nauplii in groups of four replicate bowls received treatment as below;

1. Clean seawater added, 90% exchange with clean water every 2 days.
2. Clean seawater added, 90% exchange with clean seawater every 6 days.
3. Clean seawater added, no water changes.
4. Water from 500 L culture reservoir tank added, 90% water change with reservoir water every 2 days.
5. Water from 500 L main culture tank added, 90% water change with culture tank water every 2 days.

All treatment water was at the same salinity (S 27) and temperature (23±0.5°C). Each treatment group was fed *I. galbana* according to the regime used in 500 L cultures. Dissolved oxygen, pH, ammonia, nitrite and nitrate were recorded in each treatment group at the start and finish of the trial.

Dead copepods were removed and counted daily until all animals were mature (approximately three weeks). Females were considered mature when carrying their first embryo sac. These were removed and counted daily until no females remained. Survival was compared using oneway ANOVAs and Tukey's W procedure. Cumulative proportions of mature females were logit transformed and regressed

against time. The time taken for 50% of the females to reach maturity in each treatment was predicted from these regressions and compared using oneway ANOVAs and Tukey's W procedure.

Gladioferens imparipes as food for fish

Size of copepods

Copepod nauplii were collected from a 500 L intensive culture and screened through 100 μm mesh to obtain nauplii of uniform size. These were stocked into three 2 L containers held in a water bath at 25±0.5°C and fed excess *Isochrysis galbana* daily. Ten copepods were removed from each container daily and their length measured with a microscope ocular graticule.

Larvae of snapper

The effect of including copepod nauplii in the diet of snapper (*Pagrus auratus*) was investigated using in a flow-through water system located at the Aquaculture Development Unit (ADU, Fremantle, Western Australia). Filtered seawater (5μm) was supplied to each of six 140 L cylindrical tanks and gently aerated. Temperature was maintained at 20 ± 1.5°C and light kept to a 10L:14D cycle. The microalga *Nannochloropsis oculata* was delivered to each container via peristaltic pump to maintain 1-3 million cells.ml^{-1} at all times. *Isochrysis galbana* was added once daily to each container to a density 50-100,000 cells.ml^{-1}. Filtered water was delivered to each tank and outlets were screened with 53 μm mesh. Daily water exchange occurred at the rate of 110% from when fish were added to the tanks to day 7 post-hatch, increasing to 150% from day 8 to 15 and 220% after day 16. Dissolved oxygen, pH and ammonia-nitrogen were measured in each container every fourth day.

Snapper larvae were obtained from naturally spawning second generation broodstock maintained at ADU. For each of two trials, two day post-hatch larvae were stocked into each tank at a density of 25. L^{-1}. Two diets were each randomly allocated to three replicate containers. Feeding commenced on day 4 post-hatch with live prey at the rate of 10 ml^{-1}, replenished three times daily. The length of 10 larvae from each container was recorded every two days in the first trial and every three days in the second. At the conclusion of each trial, all surviving fish were counted and swim bladder inflation recorded for 10 fish from each replicate. Larval length, survival and swim bladder inflation were compared using oneway ANOVAs.

For the first trial, the treatment diet consisted exclusively of copepod nauplii for the first six days of feeding followed by Super Selco® (SS) enriched rotifers. The control diet comprised SS rotifers throughout. Weaning onto SS enriched *Artemia* was conducted over a five day period when larvae had reached an average length of 7 mm. During this time *Artemia* rations were gradually increased and rotifer rations gradually decreased. Water exchange was increased to 250% during this period. The trial was terminated on the second day of the larvae being fed exclusively on *Artemia*.

For the second trial, the treatment group was fed a mixed diet consisting of 20% (by number) copepod nauplii and 80% SS rotifers. The control diet comprised SS rotifers

only. No weaning onto *Artemia* took place in this trial which was concluded when larvae were ~7 mm in length.

Larvae of West Australian Dhufish.

Investigations similar to those with snapper were conducted with the West Australian Dhufish (*Glaucosoma hebraicum*). Differences in procedure include; water temperature (to 22.5 ± 1°C), *N. oculata* density (to 0.5-1 million cells.m^{-1}), daily water exchange (to 150% from stocking to day 10 post-hatch, increasing to 220% from day 11 to 20 and 260% after day 21) and measurement of physical parameter each third day.

Larvae were obtained after fertilisation of eggs from a wild-caught female by sperm from captive broodstock held at ADU. Two day post-hatch larvae were stocked into each of 6 tanks at a density of 6.L^{-1}, and two diets were each randomly allocated to three replicate containers. The treatment group was fed a mixed diet consisting copepod nauplii (50%) and Super Selco® (SS) enriched rotifers (50%) The control diet comprised SS rotifers only. Feeding commenced on day 3 post-hatch with live prey items stocked at the rate of 10 ml^{-1}, replenished twice daily. Weaning onto SS enriched *Artemia* was conducted over a five day period when larvae had reached an average length of approximately 6 mm. The length of 3 larvae from each container was recorded every three days. At the conclusion of the trial, all surviving fish were counted. Larval length and survival were compared using oneway ANOVAs.

Western Australian Seahorse

The effect of including copepod nauplii in the diet of the Western Australian Seahorse (*Hippocampus angustus)* was investigated in two trials using a recirculating seawater aquarium system located at Curtin University. The system comprised six 15 L glass aquaria, each receiving gentle aeration and kept at 23 ± 0.5°C on a 12L:12D cycle. Water outlets were screened with 53 µm mesh. *Isochrysis galbana* was added once daily to each container to a density 30-50,000 cells.ml^{-1}. Daily water exchange in each tank occurred at the rate of approximately 480% throughout the trials. Substrate for seahorses was provided in each tank in the form of polypropylene rope weighted with bleached coral. Detritus was siphoned from the bottom of each tank daily. Dissolved oxygen, pH, ammonia and nitrite were measured in the system every 5 days. A UV water sterilisation unit (absent in the first trial) was fitted to the recirculation system in the second trial.

A single pair of adult *H. angustus* were captured from the wild and mated in captivity. Each trial was conducted using one batch of sibling juveniles from the same cohort. Each batch was split into six equal groups and stocked into the aquaria at a density of around 2 fish.L^{-1}. Two diets were each randomly allocated to three replicate aquaria; a treatment diet of copepod nauplii and a control diet of *Artemia* nauplii enriched with Super Selco® (SS). Feeding commenced on the day of stocking and live prey was added twice daily. Feeding rates were varied such that seahorses cleared almost all of the food before the next feed. For the copepod diet, nauplii were provided at 0.5 nauplii.ml^{-1} increasing to 1.2 nauplii.ml^{-1} For *Artemia*, the rate varied from 0.2 *Artemia*. ml^{-1} to 0.8 *Artemia*. ml^{-1}. In the first trial, the length and wet weight of 3 seahorses from each

aquaria was recorded every four days. All surviving fish were measured at the end of this trial (day 12). In the second trial, seahorse lengths and wet weights were recorded only at the conclusion (day 15). Mortalities were recorded throughout both trials. Length, wet weight and survival were compared using oneway ANOVAs.

Measurement of seahorse predation on copepod nauplii was conducted in 2 L plastic containers submerged in a water-bath maintained at 23±0.5°C. Each container was subject to gentle aeration and contained weighted rope as substrate. Five feeding seahorses (5 days old, length 9.4 ± 0.7mm) were placed in each of three containers. Copepod nauplii that had passed through a 149µm mesh were sorted into three size classes by selective sieving; 125-165µm, 166-215µm and 216-310µm in length. Equal number of each size class of nauplii were added to each container to a total density of approximately 5.25 nauplii.ml^{-1} Water samples were collected every 2 hours for a total of 8 hours and the number of nauplii present and the proportions of each size fraction was determined.

Clownfish

Newly hatched larvae of clownfish (*Amphiprion percula* and *A. clarkii*) were obtained and held in the recirculating aquarium system described above. Copepod nauplii from a continuous culture were added daily to a density of ~5 ml^{-1} from the day of hatching. Juveniles were weaned onto a commercial pellet at around 4 weeks old.

Hairy pipefish

Adult pipefish (*Urocampus carinirostris*), including males with embryo in the brood pouch were collected from the mouth of the Swan River estuary at Fremantle. Males were held in a 15 L tank in a recirculating aquarium system. Soon after juveniles were released by the males they were placed in a separate tank and provided with copepod nauplii. Casual observations continued over 18 months as juvenile pipefish grew to maturity with only copepods provided as food. Each new cohort of juveniles was removed and grown separately.

Biochemical profile of food and copepods

Fatty Acid analyses of algae used in copepod diets

Fatty acid profiles of algae used in copepod diet experiments were analysed using techniques modified from Dunstan *et al* (1992,1993). Details of procedures are presented in Appendix 3.

Fatty acid content of nauplii fed *Isochrysis galbana*

Nauplii were collected from the 500 L culture system and placed in clean water for 24 hours. Approximately 20% of the nauplii were separated for fatty acid analyses and the remainder fed *I. galbana* at the rate of 5x10^5 cells.ml^{-1} and subjected to gentle aeration at 23°C. After having had access to food for 0.5, 2, 4 and 6 hours, nauplii were collected for analysis. A separate cohort of nauplii were provided with a combination of *I. galbana* and *Nannochloropsis oculata* for 6 hours prior to analysis.

Nauplii were retained as water in which they had been feeding was passed through a 50 mm diameter disc of 44 μm nylon mesh. Animals were rinsed into a reaction tube with DCM:methanol:water (15 ml) and the sample homogenised and stored under nitrogen at 5 - 6°C overnight. Further extraction and analysis followed the same procedure as for algae (Appendix 3).

DETAILED RESULTS / DISCUSSION

Development of copepod cultures

Recirculating 500 L culture

Figure 1 shows the arrangement of component parts of the 500 L culture system, with notes on the function of each component. This culture system is also described in the culture manual (Appendix 6). Two 500L tanks (one shown in Figure 1) held different cohorts of copepods; one with adult animals from which nauplii were collected daily, the other with a cohort growing to maturity to eventually become the reproducing population. Each 500 L tank was thoroughly cleaned before being stocked with a cohort of nauplii taken from the other tank.

Application of PLC technology to the 500 L copepod culture system substantially reduced the human labour involvement in the daily routine of nauplius collection and water transfer. In addition, with PLC control of routines, each process or stage was repeated exactly on each occasion, giving more consistent results than would be likely with manual control. Details of the PLC programming and construction are given in Appendices 4 and 5.

Daily operation of the PLC cycle resulted in most of the nauplii which had been hatched in the culture tank during the previous 24 hours being collected in 10 L of water. Nauplii in a 0.2-0.5 ml sample of this water were killed by heat and counted under a microscope for estimation of the total numbers.

Some routine procedures were carried out manually, including daily provision of algal food for copepods and cleaning detritus from the bottom of the culture container. A coarse sponge filter suspended close to the bottom of the water column trapped faecal debris from suspension. This was cleaned daily. Culture water quality levels were as follows; dissolved oxygen 88-99% saturation, pH 7.8-8.3, ammonia <0.5 ppm and nitrite <0.6 ppm.

When a cohort of copepods reached maturity and commenced regular nauplius production the culture was kept for approximately 21 days with daily nauplius collection.

Copepods in circulating 500 L cultures showed varied reproductive performance between cohorts, especially during early trials. Not all cohorts of copepods grew to maturity with the same survival rate or maintained the same level of nauplius production once they reached maturity. Some cohorts were abandoned when poor performance was obvious. Table 1 shows the nauplius production from 16 different cohorts. Batches 5 and 11 failed to achieve nauplius production stage.

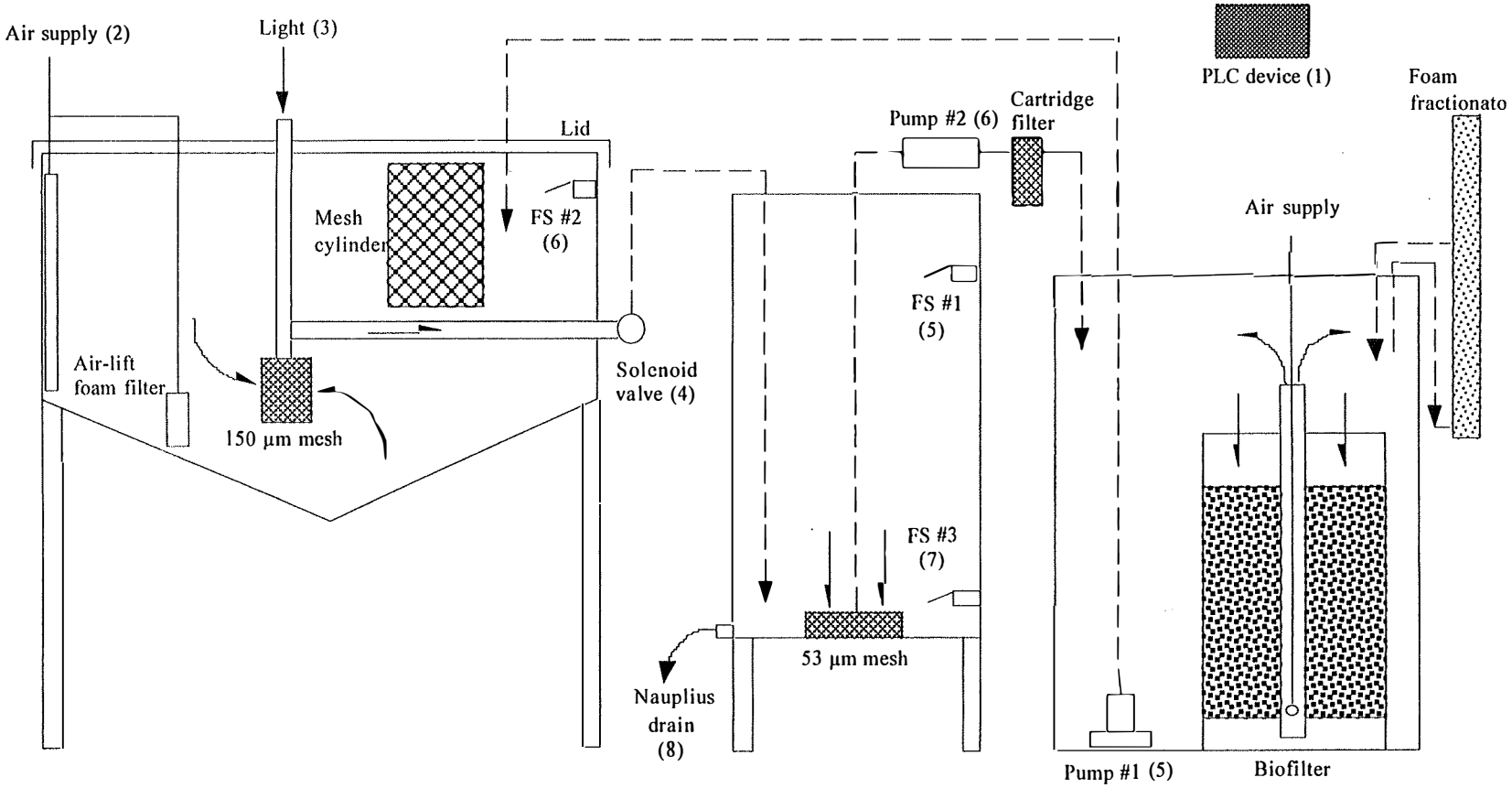

Air supply (2)
Light (3)
Lid
Mesh cylinder
FS #2 (6)
Air-lift foam filter
150 µm mesh
Solenoid valve (4)
Nauplius drain (8)
Pump #2 (6)
Cartridge filter
FS #1 (5)
FS #3 (7)
53 µm mesh
PLC device (1)
Foam fractionato
Air supply
Pump #1 (5)
Biofilter
500 l culture vessel
200 l collection vessel
500 l sump

Figure 1 (previous page). Schematic diagram of the automated 500 L copepod culture system. Nauplius collection and water recycling procedures are described as follows;

(1) Harvest sequence initiated by pressing 'start' button on the PLC device.
(2) Air supply to the culture vessels turned off for 5 min to stop water movement.
(3) Light turned on for 10 min to illuminate 150 μm mesh submerged in culture vessel.
(4) Solenoid valve opened so that water (containing nauplii) siphons through 150 μm mesh into harvest vessel.
(5) Float switch (FS) #1 activated which closes solenoid value, turns light off and turns pump #1 on.
(6) FS #2 activated which turns pump #1 off and pump #2 on (drawing through 53 μm mesh).
(7) FS #3 deactivated which turns pump #2 off and air supply on.
(8) Concentrated nauplii drained from harvest vessel.

Table 1. Summary of nauplius production from 16 separate cohorts of copepods maintained in a 500 L culture system.

Culture No.	Duration of culture (days)	Duration of nauplius collection (days)	Mean daily nauplius collection ($x10^3$)	Range ($x10^3$)	Total nauplii ($x10^6$)
1	62	28	441	112-1106	12.3
2	61	40	540	65-892	21.6
3	59	32	571	105-1140	18.3
4	57	33	424	71-767	14.0
6	25	12	304	74-636	3.6
7	41	24	153	70-233	3.7
8	46	29	466	79-1018	13.5
9	42	24	551	156-1113	13.2
10	41	21	605	280-966	12.7
12	46	27	423	144-713	11.4
13	42	21	498	290-648	10.4
14	38	22	477	90-925	10.5
15	39	22	312	20-546	6.9
16	45	25	431	174-640	10.8
17	52	29	584	173-912	16.9
18	43	20	413	164-1001	8.3
		Total = 409	Mean = 449		

Two related factors were probably responsible for poor performance by some cohorts of copepods; poor water quality and invasive organisms. Maintaining the copepods at high adult population density (~0.5 - 1 animal.ml^{-1}) and providing sufficient food to maintain a high rate of reproduction compromised water quality in the recirculating system. Poor performance followed failure of a protein skimmer, contamination of a gravel filter and protracted use of the same water. Adult copepods in cohorts with poor reproductive performance showed a high incidence of bacterial and protist infestation on the exoskeleton and showed abnormal behaviour by remaining in the water column rather than attaching to the internal surfaces in the culture tank.

Measuring nauplius production

Nauplius production was measured against a theoretical maximum production extrapolated from earlier feeding experiments in which fecundity of individual copepods was determined for animals maintained under optimal conditions. To enable comparison with published data on other copepod culture systems, a "standardised target production" was calculated as the number of nauplii produced per litre of copepod culture water per day.

Maximum production under optimum conditions	25 nauplii. female^{-1}.day.$^{-1}$
Production target under intensive conditions	10 nauplii. female^{-1}. day.$^{-1}$
Culture volume	500 L
Total culture population	500,000 copepods (1 ml^{-1})
Total females (1:1 sex ratio & 100% survival)	250,000 females
Target production	2,500,000 nauplii.day^{-1}
Standardised target production	5,000 nauplii.L^{-1}.day^{-1}

Relative success of the copepod culture system in terms of nauplius production was indicated by the realised standardised production (presented as nauplii.L^{-1}.day^{-1}) as a percentage of the standardised target production.

Table 1 shows that an average production of 449,000 nauplii.day^{-1} was maintained over 409 days. This represents a standardised production of 898 nauplii.L^{-1}.day^{-1}, which in turn represents 18% of the standardised target production. Maximum production was 1,140,000 nauplii.day^{-1} which equates to a standardised production of 2280 nauplii.L^{-1}.day^{-1} (46%). These percentages indicate nauplius production in the 500 L culture to be well below the reproductive potential of the animals.

These results do not take into account poor nauplius survival to maturity within the 500 L culture; approximately 29%. This reduces the standardised target production to 1,450 nauplii.L^{-1}.day^{-1}. Mean production of 898 nauplii.L^{-1}.day^{-1} now represents 62% of this figure. Factors resulting in poor nauplius survival are likely to contribute to poor health in the remaining broodstock copepods. This is the most probable cause of the shortfall in nauplius production and should be investigated. Simple solutions may be found for the current situation.

For cultures in which relatively high water quality was sustained with effective protein skimming, healthy gravel filtration and frequent recycling, nauplius production (nauplii.L^{-1}.day^{-1}) was high in comparison data published on the production of other calanoid copepod cultures.

Comparison with other work

Most published data on copepod production relates to harpacticoid copepods. Important distinctions must be made between calanoid and harpacticoid copepods. Calanoid copepods generally feed on small particles taken from the water column.

Calanoid nauplii swim continually in the water column where they are available as prey to fish larvae. Harpacticoids copepods are generally benthic detritivores. While they are relatively easy to culture in high numbers, their nauplii exhibit negative phototactic behaviour, do not swim in the water column and are therefore not generally available to pelagic larval fish. These differences make calanoid copepods generally better than harpacticoid as food for pelagic fish larvae. Production figures for copepod culture systems are shown in Table 2.

Table 2. Published data on nauplius production (nauplii.L^{-1}.day^{-1}) of calanoid and harpacticoid copepods compared to the current work.

Calanoids	Copepods.L^{-1}.day^{-1}
Ogle (1979)	91
Støttrup (1986)	213
Current work (mean)	898
Current work (max)	2280
Harpacticoids	
Kahan (1982)	706
Sun (1995)	1144
Nanton (1997)	1667
Støttrup (1997)	3333

Despite low nauplius survival to maturity, the nauplius production in the 500 L culture of the current work compares well with published data. Sustained production figures for calanoid copepods are almost double those of other work. The results also compare favourably with those for harpacticoid copepods. Comparison with production from intensive rotifer cultures is not warranted. The purpose of copepod cultivation is to provide alternative diets for those fish larvae that cannot be reared using rotifers.

For situations where high quality seawater is readily available, continuous flow-through, or regular replacement of water instead of recycling will provide for higher water quality than has been achieved in the recycling system. This is likely to improve both nauplius survival and adult fecundity, leading to much higher nauplius production per litre of culture. The principles of using PLC to automate routine procedures could readily be applied to a flow-through culture system, combining the benefits both. For situations where high quality seawater is not readily available, or is temporarily unavailable, a recirculating system under PLC is a realistic option for copepod production.

Flow-through 1000 L culture

Figure 2 shows the arrangement of component parts of a 1,000 L copepod culture with flow-through water and manual control of nauplius collection procedures. This culture system is also described in the culture manual (Appendix 6). During an initial trial of copepod production water temperature ranged 18-22°C, dissolved oxygen 76-93% saturation, pH 7.8-8.0 and ammonia <0.2 ppm. Nauplius production by the 1,000 L culture was not quantified. Large numbers of nauplii from this culture were used in feeding experiments with snapper and dhufish.

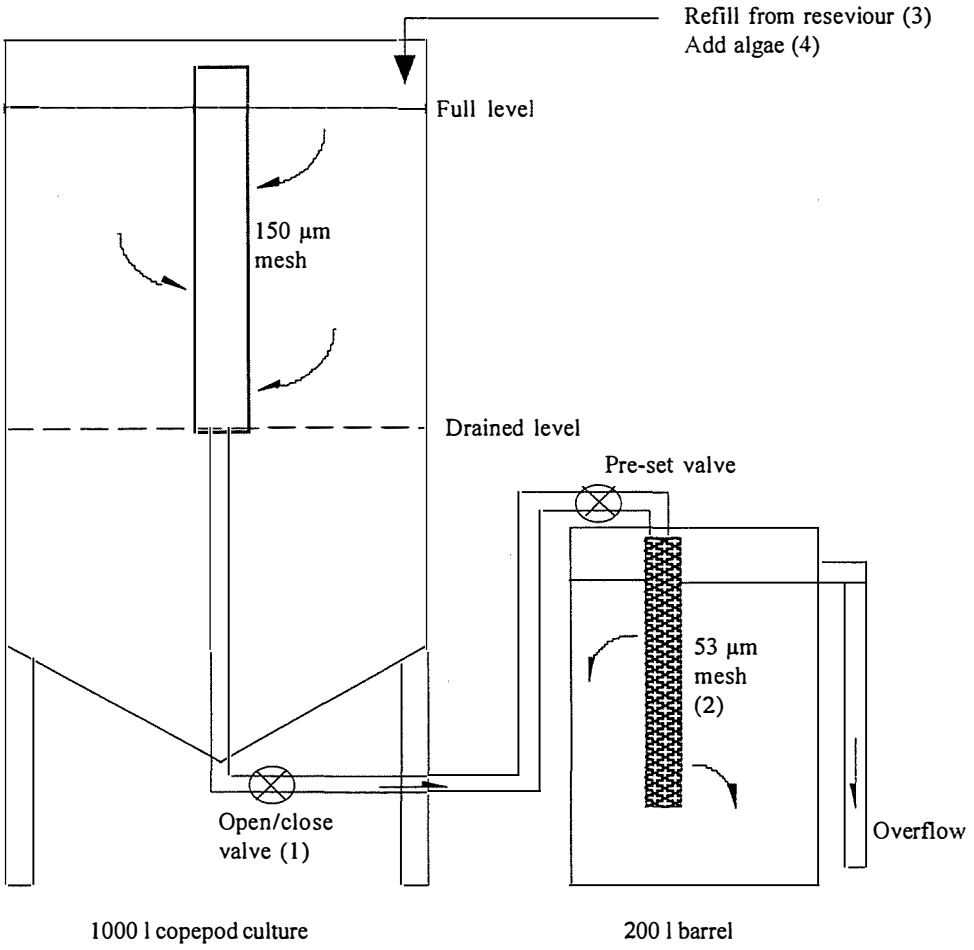


Figure 2. Schematic diagram of a 1000 L copepod culture system. Nauplius collection and water exchange procedures are described as follows;

(1) Valve opened allowing half the volume of 1000 L culture vessel to drain through 150 µm mesh into submerged 53 µm mesh. Pre-set valve ensures slow drainage (~10 l/min).
(2) When draining complete, valve closed and nauplii rinsed from 53 µm mesh.
(3) Culture vessel refilled with diluted seawater from reservoir.
(4) Copepods fed with algae.

Suitability of systems for culturing other live prey organisms

Both the 500 and 1000 L culture systems will likely be suitable for culturing other copepod species that carry their eggs in a sac prior to hatching of nauplii. This is particularly true for those copepods that occupy an ecological niche similar to *G. imparipes* in other part of Australia and the world (for example other *Gladioferens* spp, *Eurytemora* spp. and *Pseudodiaptomus* spp.). The system will unlikely be suitable for culturing those copepod species that broadcast eggs into the water column (eg. *Acartia* spp and *Calanus* spp.). For these species, culture methods must include techniques for the removal of these eggs from the bottom of the culture vessel.

The automated 500 L culture system may also have some use for culturing live food organisms other than copepods. Rotifer culture techniques may be simplified by the use of similar automatic harvest and water renewal techniques described in this report. The system may also prove useful for culturing mysid shrimp, an organism that has great value in the ornamental trade. Simple modification may be required in this case.

Effect of culture conditions on *G. imparipes*

Survival at low temperature

Figure 3 shows that at 8°C both adults and nauplii of *G. imparipes* had high percentage survival for 15 days. After 15 days adult survival percentage declined steadily to 75% after 39 days but nauplius survival declined more steeply. At 4°C both adult and nauplius survival declined steeply after 4 days.

Survival of *G. imparipes* at low temperature allows animals to be kept with minimum maintenance while metabolism and development is slow. If cultures are not required for intensive production, adult copepods can be stored at 8°C for 5 - 6 weeks. Survival and delayed development of nauplii during 2 weeks at 8°C allows large numbers of nauplii collected from broodstock cultures to be accumulated over a two week period prior to use. While the 500 L culture produced >500,000 nauplii.day^{-1}, nauplii were stored in large numbers for use in fish feeding experiments.

Data of Figure 3 suggest that little benefit would accrue from storing copepods at 4°C. Unless these data are unreliable, the high rate of mortality after 4 days would offset the benefit of slower development.

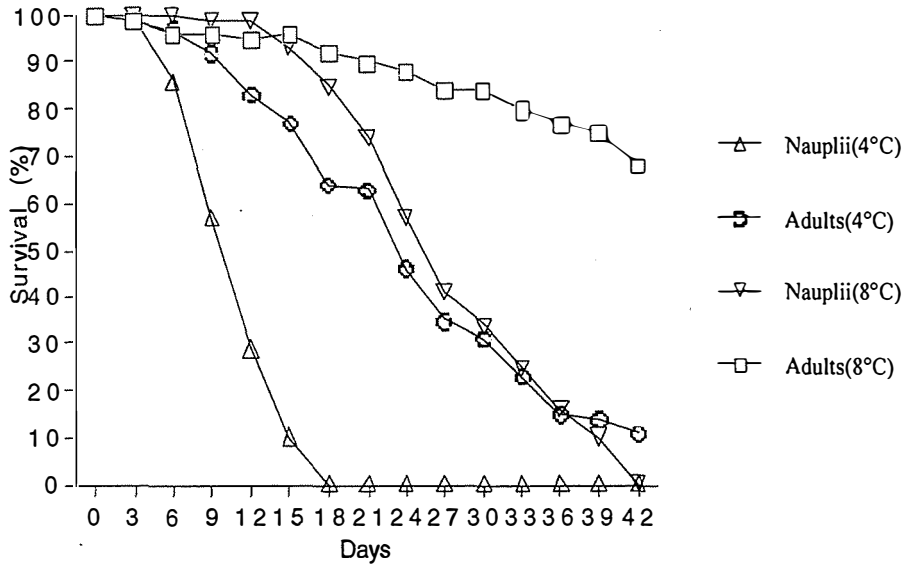


Figure 3. Percentage survival of *G. imparipes* nauplii and adults maintained at 4°C and 8°C

Effect of food type on survival and growth

Survival

Table 3 shows that copepod survival at 20°C and 25°C was greater than 90% with a diet of *Isochrysis* and between 50 and 90% with a diet of *Chaetoceros* and *Dunaliella*. At the lower temperature, between 10 and 50% of the nauplii reached maturity with a diet of *Nannochloropsis* . Copepod survival was negligible when fed with *Nannochloropsis* at 25°C and yeast at both temperatures. Table 4 shows that at 20°C survival was greatest with a diet of *Isochrysis* and lowest with a diet of *Nannochloropsis* . There was no difference in survival of copepods reared at 25°C or between the two temperatures.

Table 3. Subjective assessment of nauplius and copepodid survival with different diets at 20°C and 25°C. EP denotes the start of egg production.

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
20°C																			
Isochrysis	3	3	3	3	3	3	3	3	3	3	3	3	EP						
Chaetoceros	3	3	3	3	3	2	2	2	2	2	2	2	2	EP					
Dunaliella	3	3	3	3	3	3	3	3	3	3	3	3	2	2	2	EP			
Nannochloropsis	3	3	3	3	3	3	3	3	2	1	1	1	1	1	1	1	1	1	EP
Yeast	3	3	2	2	1	1	1	1	1	1	1	1	0						
Unfed	3	2	1	0															
25°C																			
Isochrysis	3	3	3	3	3	3	3	3	EP										
Chaetoceros	3	3	3	3	3	3	3	3	2	EP									
Dunaliella	3	3	3	3	3	3	2	2	2	2	EP								
Nannochloropsis	3	3	1	1	1	1	0												
Yeast	3	2	1	0															
Unfed	3	2	1	1	0														

3 = >90% survival
2 = 50-90% survival
1 = 10-49% survival
0 = <10% survival

Table 4. Number of copepods surviving to maturity (initial stocking≈70)with different diets at 20°C and 25°C. Column values with different superscripts are significantly different (P<0.05).

	20°C	**25°C**
Isochrysis	70.7 ± 8.4[a]	59.3 ± 8.1[a]
Chaetoceros	37.0 ± 7.9[b]	42.0 ± 11.3[a]
Dunaliella	48.7 ± 3.1[b]	51.0 ± 12.5[a]
Nannochloropsis	19.0 ± 3.6[c]	0

Maturation

Figure 4 shows that at 20°C the time taken for 50% of female copepods to mature was shortest when fed a diet of *Isochrysis* . Time to maturity increased with diets of *Chaetoceros*, *Dunaliella* and *Nannochoropsis* . The same trend occurred at 25°C (Fig. 5) however, results were not significantly different and no copepod maturation was recorded on a diet of *Nannochoropsis* . Copepods matured faster at 25°C than at 20°C.

The rate of maturation is indicated by the time period between the onset and completion of maturation in a cohort of copepods. In Figures 4 & 5, a faster rate of maturation is shown by a shortened period between the first and last regression points of each line, hence a steeper gradient. Copepods maturation rate was the same with different diets at 20°C (Fig. 4). At 25°C, this rate was significantly greater for *Isochrysis* fed copepods compared with those fed the other diets (Fig. 5).

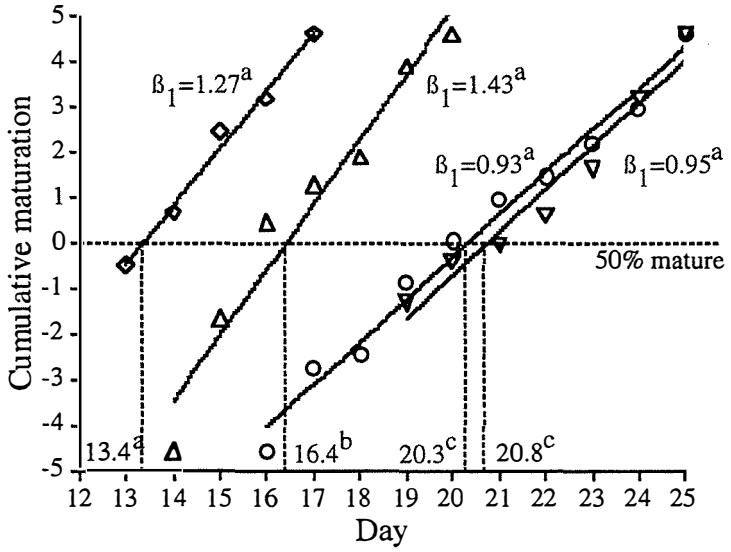


Figure 4. Cumulative maturation (logit transformed) over time of female copepods fed *Isochrysis* (◊), *Chaetoceros* (Δ), *Dunaliella* (O) and *Nannochloropsis* (∇) at 20°C. Dotted lines represent time (x axis) taken for 50% cumulative maturation (y axis). Days and gradients ($ß_1$) with different superscripts are significantly different ($P<0.05$).

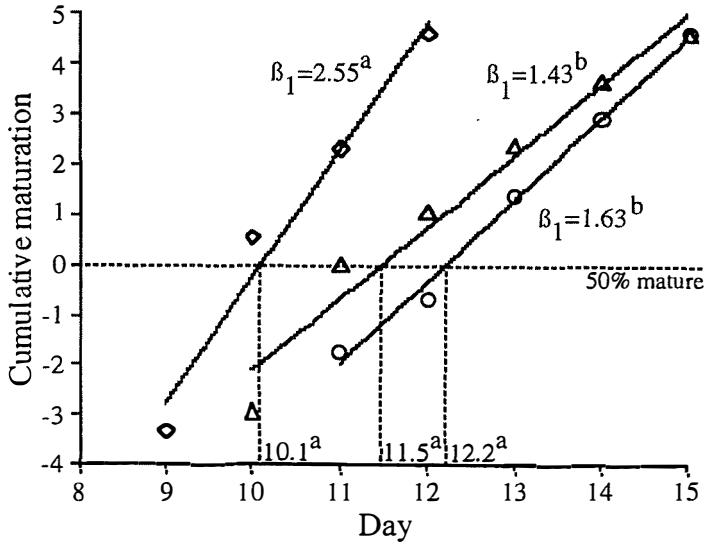


Figure 5. Cumulative maturation (logit transformed) over time of female copepods fed *Isochrysis* (◊), *Chaetoceros* (Δ) and *Dunaliella* (O) at 25°C. Dotted lines represent time (x axis) taken for 50% cumulative maturation (y axis). Days and gradients ($ß_1$) with different superscripts are significantly different ($P<0.05$).

Nauplius production and female length

Figure 6 shows that daily nauplius production per female at both temperatures was greatest ($p<0.05$) for animals with a diet of *Isochrysis*. At 20°C there was no difference in production with the other diets, whereas at 25°C production with a diet of *Chaetoceros* was greater than with *Dunaliella.*

Female prosome length was greatest for animals reared at 20°C with a diet of *Isochrysis* and shortest with a diet of *Dunaliella*. At 25°C, copepods fed *Isochrysis* and *Chaetoceros* had greater prosome length than those fed *Dunaliella.* There were no consistent trends in prosome length between copepods reared at different temperatures.

These data clearly indicate that *Isochrysis galbana* is the most appropriate diet for intensive culture of *G. imparipes*. Copepods fed this diet recorded the highest survival, maturation rate and nauplius production, and the fastest time to maturity. The next most successful diet was *Chaetoceros gracilis* followed by *Dunaliella tertiolecta*. Survival and nauplius production on both these diets were relatively high, hence they could be useful for feeding intensive copepod cultures. In contrast, *Nannochloropsis oculata* and yeast are of little value as food for *G. imparipes*.

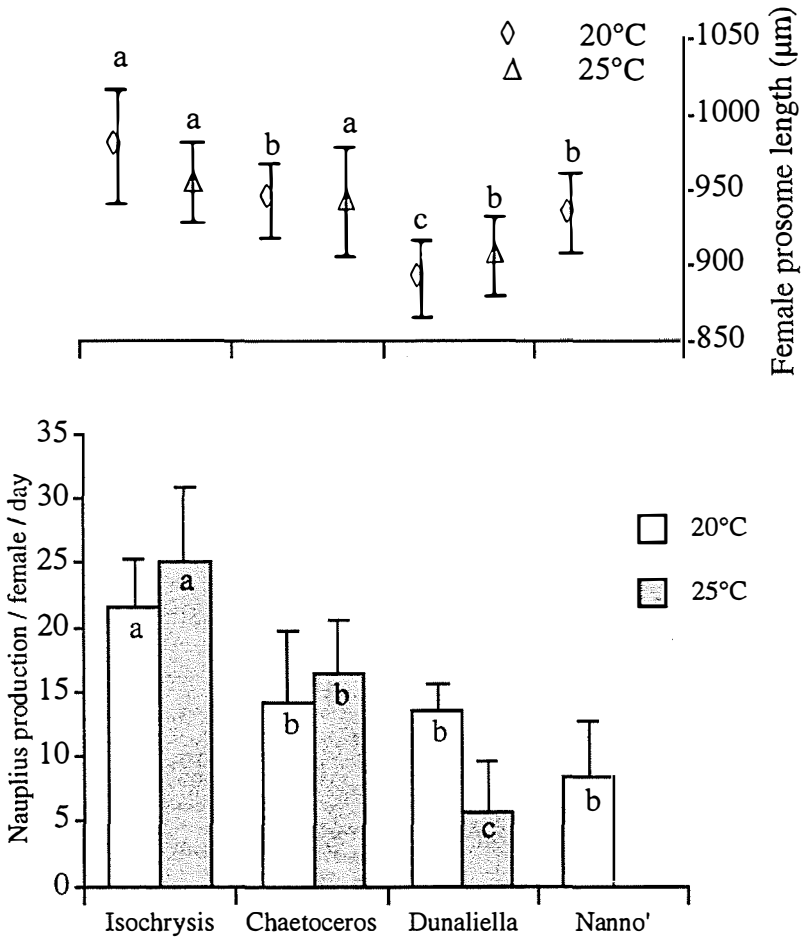


Figure 6. Nauplius production per female per day and female prosome length fed different diets at 20°C and 25°C. Bars indicate ±1 sd. Letters show significance of data at the same temperature, different letters indicate a significant difference ($P<0.05$).

Table 5 shows the fatty acid profile of algae used to feed copepods in this trial. Of the three efficacious diets used to rear copepods, *Isochrysis* contains the greatest proportion of 22:6n-3 (DHA). Adequate supply of this essential fatty acid is vital to high copepod fecundity (Norsker & Støttrup, 1994). *Chaetoceros* and *Dunaliella* contained little or no DHA, but did contain 20:5n-3 (EPA) and 18:3n-3 respectively. Both of these polyunsaturated fatty acids are important precursors to DHA in *de novo* synthesis reactions. These reactions are known to occur in some calanoid copepods (Moreno *et al*, 1979), but tend to be limited in marine invertebrates (Sargent & Whittle, 1981). Hence, these reactions may provide *G. imparipes* with some DHA (thereby increasing fecundity) when fed a diet deficient in this fatty acid.

Nannochloropsis contained a very high proportion of EPA. However, this was not a successful diet for copepods. This alga cannot be well digested by *Artemia* nauplii as it has a hard cell wall. Therefore, it is probably not digested by *G. imparipes*. Yeast, the least effective copepod diet, contained no polyunsaturated fatty acids or their precursors.

In this trial, monoalgal diets were examined. With knowledge of which algal species can be grazed by *G. imparipes* and the nutritional content of these species, diets

comprising two or more algal species can be examined with the aim of further increasing copepod production.

Table 5. Fatty acid content (% of total fatty acids) of diets used to rear copepods, mean of 3 replicates ± 1 sd. Tr indicates <0.1%.

Fatty Acid	*Isochrysis*	*Chaetoceros*	*Dunaliella*	*Nannochloropsis*	Yeast
C14:0	15.56 ± 0.42	22.98 ± 1.43	0.4 ± 0.19	4.57 ± 0.38	0.63 ± 0.06
C15:0	0.43 ± 0.03	0.32 ± 0.04		0.31 ± 0.02	0.23 ± 0.04
C16:0	13.08 ± 0.53	7.95 ± 0.25	13.48 ± 0.14	17.03 ± 0.4	8.83 ± 0.28
C16:1	6.05 ± 0.44	18.12 ± 0.65	1.72 ± 0.01	16.2 ± 0.28	34.25 ± 4.6
C16:2	0.39 ± 0.03		1.16 ± 0.07	0.25 ± 0.05	
C16:3		14.16 ± 0.3	3.96 ± 0.39		
C16:4			21.17 ± 0.52		
C17:0					0.16 ± 0.0
C18:0	0.38 ± 0.06	0.41 ± 0.03	0.72 ± 0.45	0.44 ± 0.08	2.86 ± 0.12
C18:1n7	2.48 ± 0.06	0.51 ± 0.15			1.85 ± 0.22
C18:1n9	16.69 ± 0.61	0.63 ± 0.01		5.48 ± 0.26	43.39 ± 2.75
C18:2n6	2.25 ± 0.23	0.69 ± 0.08	4.22 ± 0.05	1.73 ± 0.03	6.9 ± 1.59
C18:2n8			2.24 ± 0.06		
C18:3n3			47.25 ± 0.49		
C18:3n6		1.74 ± 0.25	3.2 ± 0.09	0.42 ± 0.08	
C18:4n3	26.03 ± 0.74	0.84 ± 0.12			
C20:0	tr				
C20:2n9				0.6 ± 01	
C20:3n3			tr		
C20:4n6		3.72 ± 0.14		7.58 ± 0.32	
C20:5n3	0.31 ± 0.21	25.15 ± 2.65		44.26 ± 0.53	
C22:0	0.1 ± 0.03				
C22:6n3	16.2 ± 0.93	2.44 ± 0.49			
C24		0.35 ± 0.08	0.43 ± 0.16	1.13 ± 0.69	0.9 ± 0.31
Total HUFA	16.51 ± 0.75	31.31 ± 2.1	tr	52.45 ± 0.49	0
DHA:EPA	52.3	0.1	0	0	0

Effects of water quality on survival and growth

Survival to maturity was around 90% for the copepods grown in clean seawater (Table 6). In contrast, copepod survival in reservoir or culture tank water was negligible. Frequency of water changes did not significantly affect survival (Table 6) or time to maturity (Figure 7) in those copepods grown in clean water. Approximately 75% of dead copepodids grown in reservoir and culture tank water were observed with either stunted or misshapen antennae. Approximately the same proportion of mature males grown in all clean water treatments displayed the same deformities.

Table 6. Survival to maturity (%) of late nauplii kept in water from different sources. Column value with different superscripts are significantly different ($P<0.05$).

	% survival to maturity
Clean water, two day intervals	91 ± 2.4^{1}
Clean water, six day intervals	90 ± 4.6^{1}
No water changes	88 ± 4.3^{1}
Reserviour water, two day intervals	5.7 ± 1.2^{2}
Culture tank water, two day intervals	3.2 ± 3.7^{2}

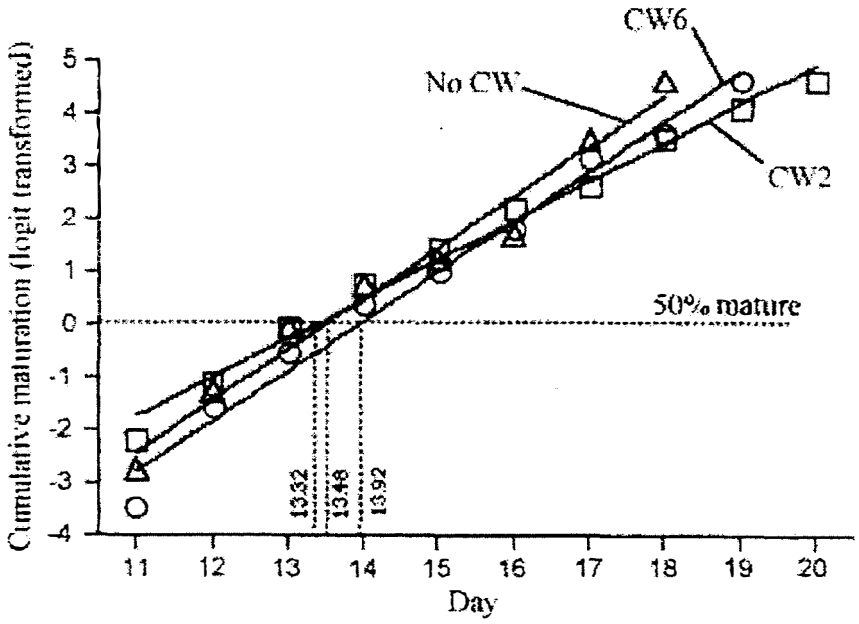


Figure 7. Maturation time of copepods grown in clean water with no water changes (NoCW) and water changes every 2 (CW2) and 6 (CW6) days. Dotted lines represent time at which 50% female copepods were mature.

During development of the 500 L culture copepod system, the procedure was to grow a cohort of nauplii to maturity in clean sea water prior to linking them to the system for nauplius collection. No water changes were made before these animals reached maturity. Typical survival of each cohort was approximately 29%. In this experiment, survival of copepods grown under the exactly the same conditions of water exchange was 88%. Negligible survival was observed for those copepods in reservoir and broodstock culture water. Therefore, poor survival in intensive cultures could be a result of toxins present in the system. Water changes did not improve either survival or time to maturity in this trial. Even if a toxin were not present in the intensive system, these results suggest that water changes would not improve survivorship. It is a matter of priority to determine if toxins detrimental to copepods are present in the culture system.

Development time to maturity in this trial was ~13.5 days at 23°C. Nauplii used in this trial were already 4 days old at stocking, hence time to maturity was actually 17.5 days, longer than the 13.3 days on the same diet at 20°C determined earlier in this project. Also, very high proportions of dead copepods and male survivors were observed with antennae deformities. One possibility is that water used for the current trial was contaminated with a pollutant. This water was collected from Fremantle harbour, where Tributyl Tin (TBT) is known to occur. Copepods are sensitive to this pollutant at very low concentrations (U'ren, 1983). Conditions of weather and tide at the time of water collection would affect the concentration of this, and other, pollutants it contains. The effect of pollution on copepod health could be the major limiting factor in this project and must be investigated further.

Gladioferens imparipes as food for fish

Size of copepods

Figure 8 shows that the various life history stages of *G. imparipes* are of body length between 120 - 800 μm when grown at 25°C. This provides a large range of sizes that can be offerred as food to growing fish larvae and indicates the age of a cohort at which animals of different size can be expected to occur. It is well known that prey size is a critical factor for succesful feeding by larval fish. The measurements given here are for copepod body length. For a predatory fish the body width of potential prey items may be the more important parameter imposing limits on the availability of the prey.

Animals which are commonly used as live prey for fish larvae include S-strain rotifers (~150 μm), L-strain rotifers (~250 μm) and newly hatched *Artemia* nauplii (~500 μm). *G. imparipes* has life cycle stages that correspond in size to each of these animals and may be useful to supplement or replace them as food in fish culture. Of more importance, early copepod nauplii are smaller than S-strain rotifers. Therefore they may be of use for feeding those fish larvae for which rotifers are too large.

G. imparipes nauplii commence exogenous feeding at N II. If they are able to feed on nutritious algae and then offerred as food for larval fish they may convey nutritionally valuable material to the fish.

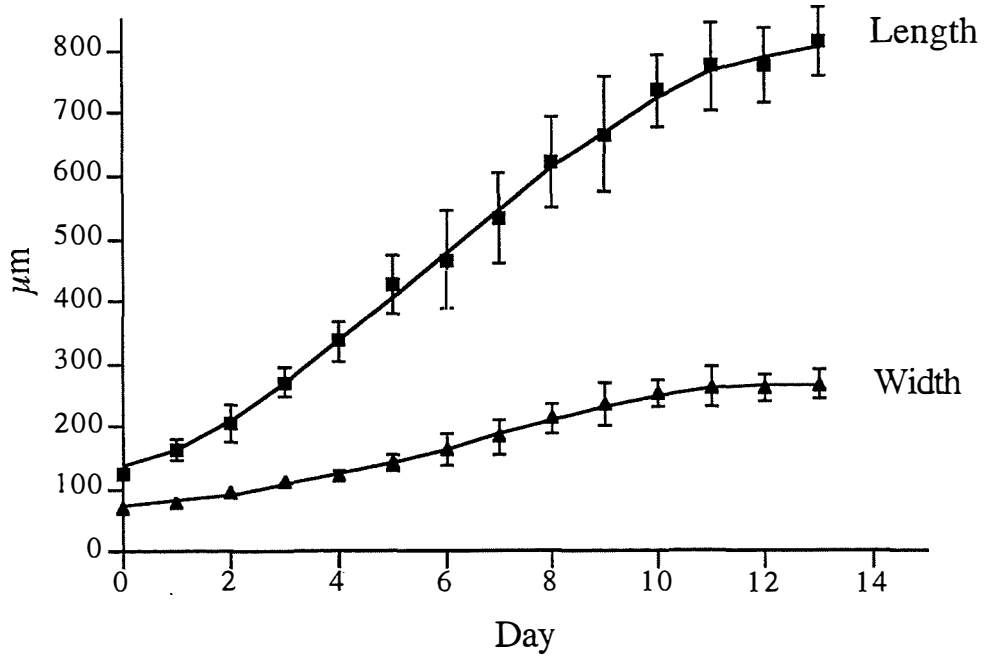


Figure 8. Body length (total length of nauplii, prosome length of copepodids) and width of *G. imparipes* grown at 25°C. (Mean ±1sd).

Larvae of snapper

Dissolved oxygen, pH and ammonia-nitrogen measured 88-95% saturation, 7.9-8.1 and <0.06 mg/l respectively in all containers throughout the trial.

Figure 9 shows that in the first trial, the length of snapper larvae with the treatment diet were significantly greater ($P<0.001$) from day 6 post-hatch. These larvae grew at a faster rate from day 4 to 10 and from day 22 to 25 (Figure 9). Growth rate was the same for the treatment and control groups from day 10 to 22. Substantial number of copepods were present in the treatment group throughout the trial despite no more copepod nauplii being added after day six. Growth in these uniform-sized copepods was rapid and they were observed being predated by snapper larvae from day 22 to 25. Figure 10 shows that survival and swim bladder inflation was greater in the larvae fed the treatment diet. However, these differences were not significant as there was large variation within replicates.

Figure 11 shows that larvae in the second trial were greater ($P<0.05$) in length from day 6 in the treatment group and that this group grew faster than the control group throughout the trial. Uneaten copepods grew rapidly in the treatment tanks. However, snapper larvae did not attain sufficient size to predate them and large numbers remained at the conclusion of the trial. Survival and swim bladder inflation was greater in larvae fed the treatment diet (Figure 12). However, again these differences were not significant as there was large variation within replicates.

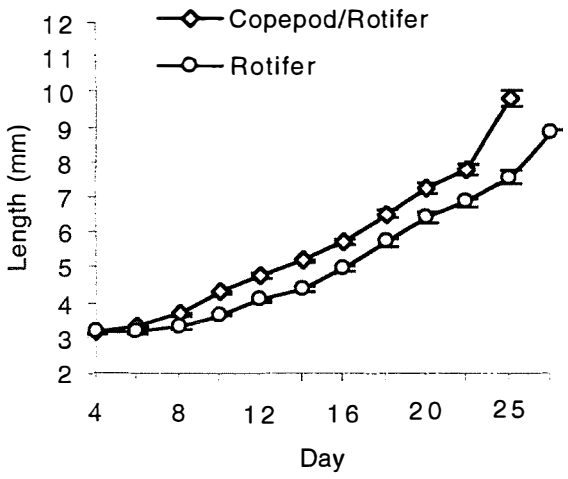


Figure 9. Growth of snapper larvae with a diet of copepod nauplii from days 4 to 10 post-hatch and then rotifers from day 11 onwards compared to those with a diet of rotifers only (mean±1se).

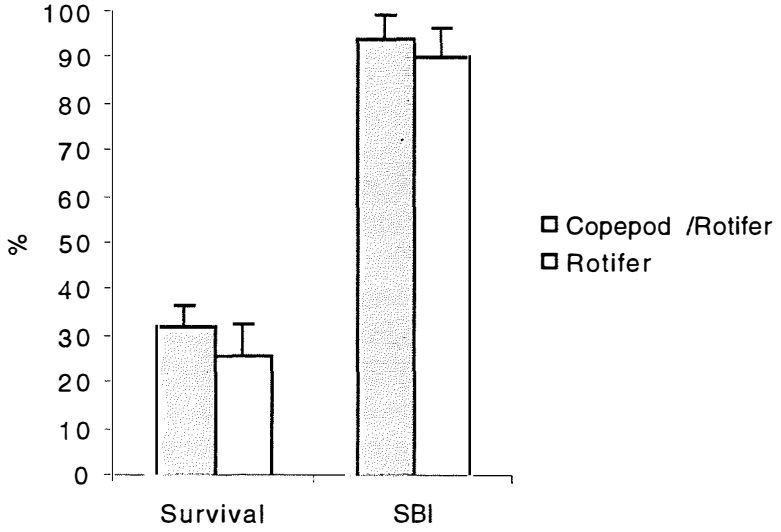


Figure 10. Survival and swim bladder inflation (SBI) of snapper larvae fed copepod nauplii from days 4 to 10 post-hatch and then rotifers from day 11 onwards compared to those fed rotifers only (mean±1sd).

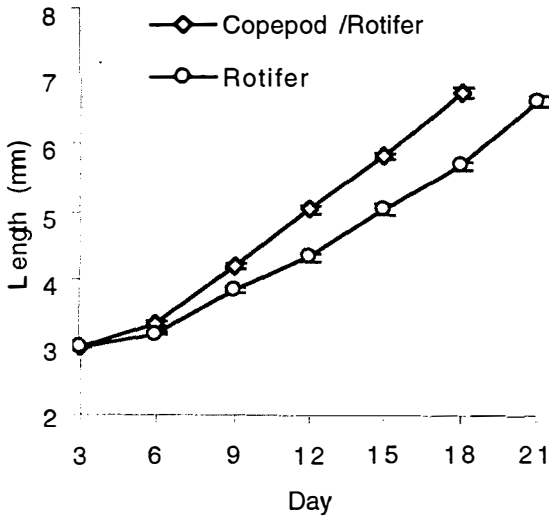


Figure 11. Growth of snapper larvae fed a mixture of copepod nauplii (20%) and rotifers (80%) and rotifers only (mean±1se).

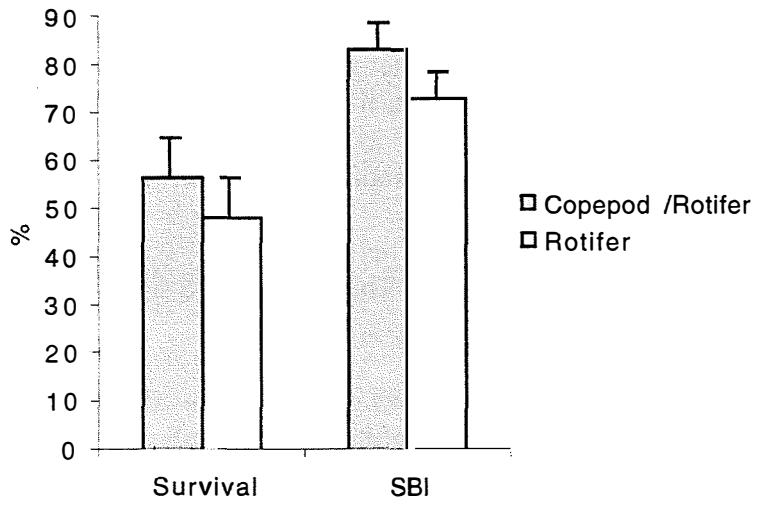


Figure 12. Survival and swim bladder inflation (SBI) of snapper larvae fed a mixture of copepod nauplii (20%) and rotifers (80%) and rotifers only (mean±1sd).

Growth rate of snapper larvae fed with the treatment diet was greater than those fed rotifers, but only while copepods were included in the larval diet. Once the copepods were replaced by rotifers in the diet after 6 days (trial 1), larval growth rate decreased, matching that of those fed only on rotifers. Growth rate in these larvae increased again from day 22 when they had grown sufficiently to predate the copepods which had survived earlier predation and grown beyond the size with which the younger fish could cope. When smaller numbers of nauplii were included in the larval diet for a longer period (trial 2), a faster larval growth rate was sustained. These results clearly indicate that growth in larval snapper can be increased by the inclusion of copepods in the diet. Subjective observations of gut contents revealed that larvae were predating large numbers of copepod nauplii in the treatment groups and that these nauplii were readily digested.

Survival and swim bladder inflation were both greater in the snapper larvae fed partly on copepods (trials 1 & 2), although not by a significant margin. Variation within replicates was large, probably reflecting the combined effects of small variations in light intensity, aeration and water exchange rate between the small containers. Overall survival was greater and swim bladder inflation lower in the second trial compared to the first as fish were younger when these data were collected.

These results show that snapper rearing practises will benefit from the inclusion of copepod nauplii into the larval diet. However, when compared with current practises that rely on enriched rotifers, these benefits are not substantial in the short term. Further work will determine if feeding snapper with copepods during the larval stage results in improved health and faster growth during grow-out.

Larvae of dhufish

Dissolved oxygen, pH and ammonia-nitrogen measured 89-95% saturation, 7.9-8.1 and <0.002 mg/l respectively in all containers throughout the trial.

Figure 13 shows that, from day 5 onwards, the length of dhufish larvae with copepods included in their diet was significantly greater ($P<0.05$) than the larvae with a diet of rotifers only. The larvae fed the mixed diet grew at a faster rate from day 5 to 23 and obtained a length of 11 mm 9 days earlier than the control group. Growth rates were the same between the two groups as they increased in length from 6 to 11 mm. There was a sharp decline in growth rate in the control group between day 20 and 23.

Survival in the larvae with copepods in their diet was 37% compared with 5% in the group fed with rotifers only (Figure 14). This represents a significant difference ($P<0.01$).

Growth and survival of dhufish larvae was greatly improved with the addition of copepods in the diet. Larvae which had been fed with rotifers and survived to 6 mm in length grew rapidly when they were weaned onto a diet of *Artemia*. These results clearly show that rotifers are a poor food source for larval dhufish. In contrast, copepod nauplii are an excellent food for these larvae. As for snapper larvae, gut contents showed an abundance of well-digested copepod nauplii in treatment larvae.

A sharp decrease in the growth rate in the rotifer-fed larvae before *Artemia* weaning commenced (day 20 to 23) suggests that these larvae had grown too large to benefit from a rotifer diet. Hence, an attempt to wean the larvae should be made before they obtain a length of 6 mm. It is also likely that the larvae with a copepod diet had grown too large to benefit from nauplii at around 6 mm. However, a decline in growth was not seen for these animals. Previously uneaten nauplii had survived and grown within the tanks and were available as larger prey items for the larger fish larvae.

It is clear that copepod nauplii offer great improvements to larval dhufish culture practises. Commercial viability of dhufish rearing will depend of cost effective mass culture of copepods or the establishment of semi-intensive green water techniques for larval culture.

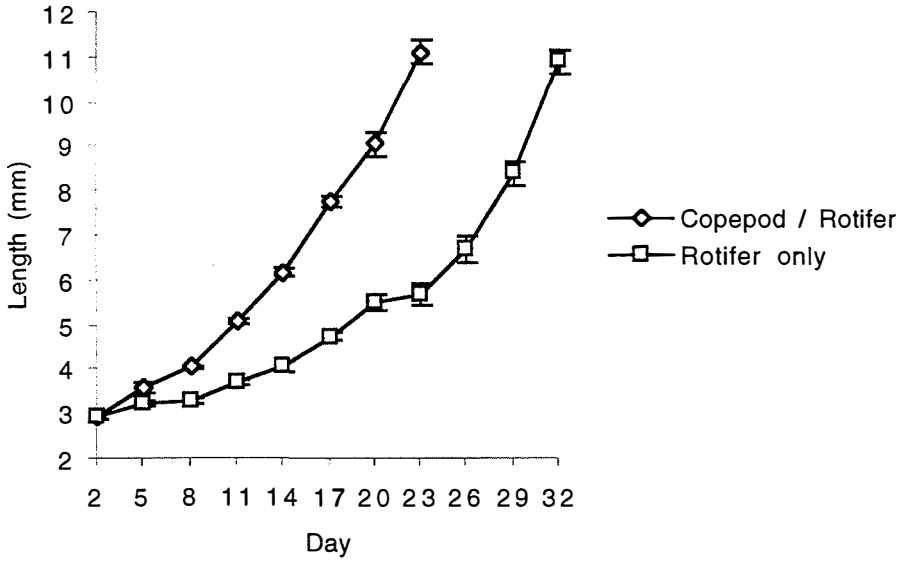


Figure 13. Growth of dhufish larvae fed a combination of copepod nauplii (50%) and rotifers (50%) and rotifers only (mean±1se).

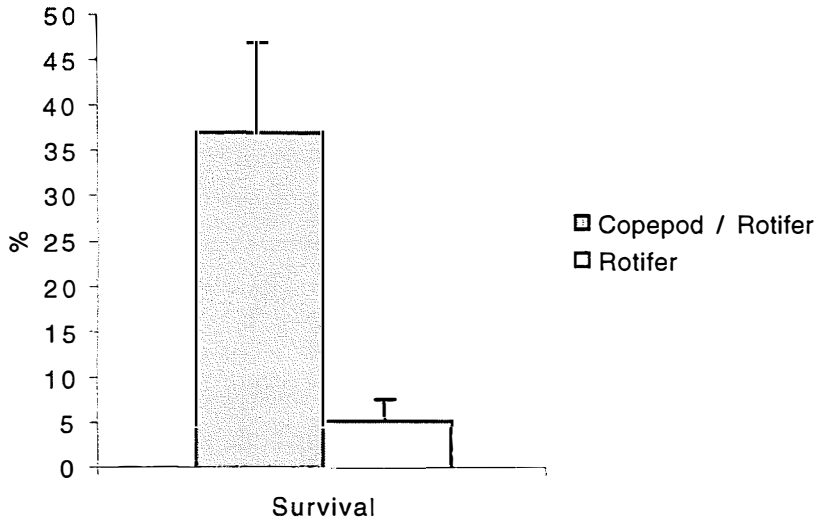


Figure 14. Survival of dhufish larvae fed fed a combination of copepod nauplii (50%) and rotifers (50%) and rotifers only (mean±1sd).

West Australian seahorse

Dissolved oxygen, pH, ammonia and nitrite measured 95-99%, 8.1-8.3, <0.1mg/l and <0.1mg/l respectively throughout the trial.

Table 7 shows that length and wet weight were significantly greater in seahorses with a diet of copepod nauplii compared with those with an *Artemia* diet. The same result was obtained with and without UV water sterilisation. Figures 15 & 16 show survival was also significantly greater for seahorses reared with a copepod compared with an *Artemia* diet ($P<0.01$) without UV sterilisation and $P<0.05$ with UV sterilisation).

Final survival in seahorses fed both diets was greatest with UV water sterilisation despite these fish being older at the conclusion of the trial. Figure 17 shows that seahorse preferentially selected the largest size class (216-310μm) of nauplii for the first 4 hours. Between 4 and 6 hours feeding duration, seahorses selected the next largest size class of nauplii (166-215μm). Predation rate based on the depletion of nauplii between 2 and 6 hours of feeding was 214 nauplii.seahorse^{-1}.h^{-1}.

Table 7. Length and wet weight of seahorses with copepod and *Artemia* diets, without and with UV water sterilisation.

	Without UV (day 12)			With UV (day 15)		
	Copepods	*Artemia*	Sig	Copepods	*Artemia*	Sig
Length (mm)	21.3 ± 1.9	16.3 ± 1.5	P<0.001	27.5 ± 2.1	24.8 ± 1.9	P<0.01
Wet weight (mg)	26.4 ± 5.8	12.3 ± 4.3	P<0.001	38.6 ± 9.4	27.2 ± 6.2	P<0.01

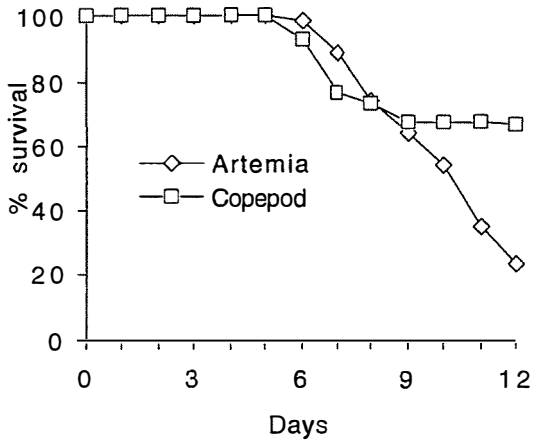


Figure 15. Survival of juvenile seahorses reared on copepod and *Artemia* nauplii without UV water treatment.

Seahorses fed with *Artemia* rapidly filled their gut. However, the intact condition of the bodies of *Artemia* in the faeces of these animals indicated poor digestion. In contrast, copepod nauplii were well digested. This apparent difference in prey digestibility probably explains the difference in growth and survival between the two diets. Care was taken to provide young seahorses with ample but not excessive quantities of food. Seahorses do not possess a true stomach and poor prey digestion (particularly of *Artemia*) is a likely problem. Excess feeding probably results in short retention time of food in the gut, poor food assimilation and malnutrition.

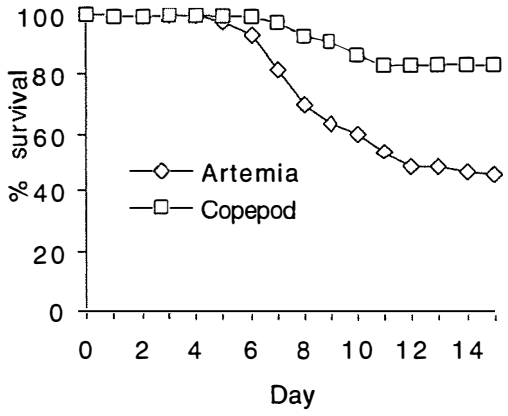


Figure 16. Survival of juvenile seahorses reared on copepod and *Artemia* nauplii with UV water treatment.

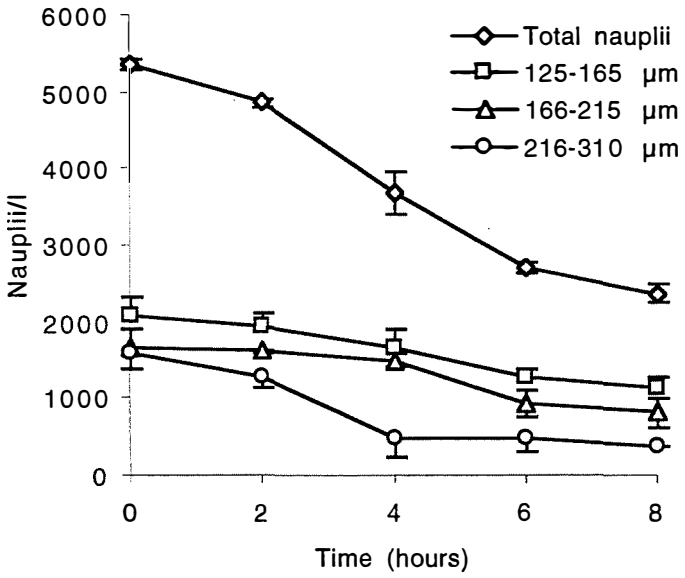


Figure 17. Numbers of copepod nauplii remaining (three size classes, mean±1sd) after predation by 5 day old seahorses.

Marked improvement in seahorse survival was recorded with the addition of UV water sterilisation. The recirculation system in which trials were conducted also contained several large seahorses and other fish. Pathogens that could affect young fish were probably present. UV sterilisation would reduce considerably the number of pathogens in the water.

Seahorses clearly selected the largest copepod nauplii as food. This was not surprising given that they are able to ingest *Artemia* nauplii, which are considerably larger than

copepod nauplii. Commercial scale seahorse production would be maximised if copepod nauplii were on-grown for a few days prior to being fed to juveniles. High predation rates by seahorses indicates that considerable quantities of prey are required to rear seahorses commercially.

Clownfish

Juvenile clownfish survived with a diet of early copepod nauplii. A small group of fish were kept after weaning onto commercial pellets. These grew to become healthy fish with strong colour development

Hairy pipefish

Pipefish reared with copepods as the only food provided remained actively reproducing through at least ten generations. Because no further collection from the wild was made, the fish were inbreeding within each age group and probably within each cohort of siblings. Although no measurements were made, observers reported a diminution of body length between the first and eighth laboratory generation. No experimental work was undertaken with these fish.

Other fish of the Family Syngnathidae; seahorses and sea dragons, are of substantial commercial value. That hairy pipefish could be successfully maintained through multiple generations suggests that inclusion of copepods in the diet of other species could increase the success of rearing them in captivity.

Biochemical profile of food and copepods

Fatty Acid Analyses of Copepod Diet

Table 5 shows that of the five diets used for growing copepods greatest proportion of DHA (22:6n-3) was found in *Isochrysis* followed by *Chaetoceros*. This fatty acid was not detected in the other diets. EPA (20:5n-3) was the most abundant fatty acid in *Nannochloropsis* and was also present in *Chaetoceros*. *Isochrysis* contained only a small proportion of EPA. The high proportion of EPA resulted in *Nannochloropsis* recording the largest proportion of HUFAs, followed by *Chaetoceros* and *Isochrysis*. In contrast, *Dunaliella* and yeast recorded negligible HUFA content. The most abundant fatty acids in these diets were 18:3n-3 and 18:1n-9 respectively.

Using growth and reproduction of *G. imparipes* as criteria, the value of five different diets can be ranked in descending order; *Isochrysis galbana*, *Chaetoceros gracilis*, *Dunaliella tertiolecta*, *Nannochloropsis oculata* and yeast The two most efficacious diets, *Isochrysis* and *Chaetoceros*, had high HUFA content. This is supported by Støttrup & Jensen (1990), who also found that copepod fecundity is greatest on diets with high HUFA content.. Whilst *N. oculata* contained considerable quantities of HUFAs, copepods appeared unable to digest algae of this species.

Fatty acid content of nauplii fed *Isochrysis galbana*

In *G. imparipes* nauplii enriched with *I. galbana* only, DHA (22:6n-3) content increased and EPA (20:5n-3) content slightly decreased after 4 h of enrichment (Table 8). After 6 h, DHA:EPA ratio increased to a maximum of 7.0 in these nauplii. In general, total fatty acid content also increased over this period. Arachidonic acid (AA; 20:4n-6) was not detected in nauplii enriched with *I. galbana* only. Nauplii enriched with a mixture of *I. galbana* and *N. oculata* for 6 h contained AA and a higher level of EPA relative to DHA. DHA:EPA and EPA:AA ratios of 3.6 and 3.1 were recorded in these nauplii, respectively.

Two HUFAs in particular, docosahexanoic acid (22:6n-3) and eicosapentanoic acid (20:5n-3), are essential in the early diet of marine fish larvae. Sargent *et al*, (1997) reports that a DHA:EPA ratio of around 2:1 is desirable for larval diets. Whilst the current work shows that *G. imparipes* nauplii fed only on *I. galbana* contains little EPA, DHA:EPA ratios in nauplii can be greatly improved with the use of *I. galbana* and *N. oculata* during enrichment.

Table 8. Fatty acid content of *G. imparipes* nauplii enriched with *I. galbana* for between 0 and 6 h and with a combination of *I. galbana* and *N. oculata* for 6 h. Mean of 3 reps ± s.d.

	Enriched with *I. galbana*					*I. galbana* and *N. oculata*
Fatty acid	0 h	0.5 h	2 h	4 h	6 h	6 h
14:0	3.0 ± 0.4	4.6 ± 0.6	5.1 ± 0.8	5.1 ± 1.0	5.6 ± 1.4	6.0 ± 0.9
16:0	11.8 ± 2.1	12.3 ± 1.9	11.2 ± 1.5	10.0 ± 1.2	11.3 ± 1.3	13.4 ± 1.6
16:1	1.8 ± 0.7	1.5 ± 0.3	0.8 ± 0.7	1.2 ± 0.3	1.2 ± 0.3	1.8 ± 0.4
18:0	2.9 ± 1.0	2.3 ± 0.6	1.9 ± 0.2	1.6 ± 0.1	1.5 ± 0.0	2.4 ± 1.2
18:1	9.6 ± 2.8	10.2 ± 2.5	9.1 ± 1.0	9.0 ± 2.4	8.2 ± 2.4	11.3 ± 2.0
18:2	1.5 ± 0.1	2.3 ± 0.4	2.3 ± 0.4	2.5 ± 0.5	2.4 ± 0.6	2.7 ± 0.4
18:3n-3	0.7 ± 0.1	1.2 ± 0.2	1.3 ± 0.3	1.6 ± 0.3	1.7 ± 0.3	1.7 ± 0.3
18:4n-3	1.8 ± 0.7	3.2 ± 0.7	3.4 ± 0.6	4.1 ± 0.8	4.4 ± 1.1	4.5 ± 0.7
20:2	1.4 ± 0.2	1.0 ± 0.2	1.2 ± 0.5	1.5 ± 0.3	1.2 ± 0.3	1.8 ± 0.7
20:4n-6	nd	nd	nd	nd	nd	0.9 ± 0.4
20:5n-3	1.4 ± 0.4	1.4 ± 0.3	1.4 ± 0.2	1.3 ± 0.1	1.3 ± 0.1	2.8 ± 0.2
22:6n-3	6.9 ± 0.2	7.8 ± 0.1	7.9 ± 1.2	8.6 ± 0.6	9.1 ± 0.2	10.1 ± 0.9
Σ fatty acid	42.9 ± 6.6	48.0 ± 7.2	45.7 ± 5.5	46.5 ± 6.6	47.9 ± 7.4	59.5 ± 6.6
DHA:EPA	4.9	5.6	5.6	6.6	7.0	3.6
EPA:AA	-	-	-	-	-	3.1

nd indicates not detected

- indicates cannot be calculated

Cost - benefit analysis of using cultivated copepods in fish larviculture

Calculations of cost-benefit of algae (Table 9) and copepods (Table 10) are based on working cultures located at the Aquaculture Development Unit (ADU) and Curtin University. Cost of materials, labour and power are similar to those that would be paid by a private aquaculture enterprise. Live food production costs for WA dhufish fingerlings are based on results of larval rearing trials conducted as part of this project.

Table 10 shows that labour costs are a major component of copepod production. By increasing automation and decreasing labour, nauplius production costs were reduced by 42%. However, it is unlikely that these costs can be reduced much further. Decreasing the cost of production must now be achieved by increasing copepod production from broodstock cultures. This in turn will be achieved by improving broodstock survival.

Table 9. Production cost of *Isochrysis galbana* in 800 L outdoor cultures at the ADU

Expense	Quantity	Cost/unit ($)	Cost ($)
f/2	800 l	7.88/1000 l	6.30
Hypochlorite	120 ml	8.40/20 l	0.05
Thiosulphate	14.4 g	57/20 kg	0.04
Labour			
Clean	0.25 hr	16/hr	4.00
Fill	0.25 hr	16/hr	4.00
Ster/Neut	0.25 hr	16/hr	4.00
Innoc	0.25 hr	16/hr	4.00
Power			
Aeration	3.2% of cost for 25,000 l*		0.48
Pump	3.2% of cost for 25,000 l*		0.02
Cost of innoc			8.50
Total cost			31.39
Cost / l			**0.04**

* based on production costs of *Nannochloropsis oculata* at ADU

Table 10. Copepod production costs of an automated and manual culture system, both producing 1 million copepods per 1000 L per day.

Automated system

Expense	Quantity	Cost/unit ($)	Cost ($)
Algae	50 l	0.04/l	2.00
Power			
Heating	12 kWh	0.16/kWh	1.92
Pump & Aeration	10% of cost for T-Iso		0.05
PLC & DC power supply	Est. from DC PS consumption		0.10
Labour			
Counting	0.17	16/hr	2.72
Feeding	0.17	16/hr	2.72
Cleaning	0.08	16/hr	1.28
Cost / million			**10.79**

Manual system

Expense	Quantity	Cost/unit ($)	Cost ($)
Algae	50 l	0.04/l	2.00
Power			
Heating	12 kWh	0.16/kWh	1.92
Pump & Aeration	10% of cost for T-Iso		0.05
Labour			
Harvest	0.5	16/hr	8.00
Counting	0.17	16/hr	2.72
Feeding	0.17	16/hr	2.72
Cleaning	0.08	16/hr	1.28
Cost / million			**18.69**

Results in Table 11 are presented as live feed cost per fingerling rather than total cost of production. Total cost was not calculated as dhufish rearing trials were conducted on a scale that was not representative of commercial production. Without the benefits of an 'economy of scale', expenses such as labour and power were disproportionately high. However, live feed cost comparison is valid as labour costs remain the same whether copepods are included in the diet or not.

Table 11 shows that the cost of live feed per dhufish fingerling was slightly lower when copepods were included in the diet. In this case, high survival of fish offset the increased cost of providing copepods. Also, recent observations of dhufish juveniles reared without copepods indicate poor neural development and some deformities, which have further decreased growth and survival. Hence, the difference in cost-benefit performance between fish that have and have not been fed copepods as larvae will continue to increase over time. Copepods can make a substantial contribution to the commercial viability of dhufish culture.

Table 11. Live food production costs of WA Dhufish fingerlings (11 mm TL) with and without copepods in the larval diet.

With copepods

	No. required (million)	Cost / million ($)	Total cost ($)
Copepods	17.65	10.79	190.44
Rotifers	30.87	0.74*	22.84
Artemia	4.49	4.30*	19.31
Cost of live food			232.59
No. dhufish produced			935
Cost / fingerling			**$0.25**

Without copepods

	No. required (million)	Cost / million ($)	Total cost ($)
Rotifers	49.81	0.74*	36.86
Artemia	0.63	4.30*	2.71
Cost of live food			39.57
No. dhufish produced			135
Cost / fingerling			**$0.29**

* obtained from Partridge *et al* (1998)

BENEFITS

Culture of other copepod species

This work has described culture techniques developed for the calanoid copepod, *G. imparipes*, which has a distribution limited to the south-west of Australia. It is unknown if translocation of *G. imparipes* (originating from Perth) will be permitted within Western Australia or Australia. If translocation is permitted, *G. imparipes* may be cultured in any location where water temperature can be maintained between 18-25° C and the tropical alga *I. galbana* can be grown. If translocation is not permitted, there are a number of embryo-carrying copepod species that occur throughout Australia which may be cultured using the techniques described for *G. imparipes*. This is particularly true of other copepods from the genus *Gladioferens*. Operating temperatures and food type may need to be altered to suit other species.

The culture systems described in this project may also prove useful for live prey organisms other than copepods. Rotifers may be cultured in a system similar to the automated 500 L recirculating system, thereby greatly reducing labour costs and the need for clean seawater. High value live feeds, such as mysid shrimp, may also be cultured in these systems.

Aquaculture of marine table fish .

The commercial feasibility of the dhufish (*G. hebraicum*) being succesfully cultured is substantially improved by this research. With a diet of copepods, larval dhufish have better survival and better health than with other diets.

For snapper (*P. auratus*), there appears to be no obvious immediate benefits to current larval rearing practises by the use of copepods. In the next year, there will be an opportunity to follow the progress of copepod reared snapper through the grow-out phase. This will determine if there are long-term benefits to using copepods in the larval diet of snapper.

At the World Aquaculture '99 conference in Sydney, many researchers reported substantial success using copepod nauplii for rearing larvae of fish species that previously could not be cultured. This was particularly true of the groupers, which include species with substantial commercial potential. There is a high likelihood that this project provides the means by which many such species can now be cultured.

Aquaculture of other fish .

High survival and healthy growth of WA seahorses (*H. angustus*) with a copepod diet indicates that some of the difficulties experienced elsewhere in rearing seahorses for commercial markets may be reduced by linking the technology of copepod production to the rearing of early juvenile sea horses. Benefits to that industry may be substantial.

Succesful rearing of clown fish (*A. percula* and *A. clarkii*) from egg to adult after an early diet of copepods and maintenance of a species of pipefish (*U. carinirostris*) through ten generations with a copepod diet indicates that economic benefits could

accrue to the trade in marine ornamental fish from application of copepod culture technology to fish rearing.

FURTHER DEVELOPMENT

Further research on the use of copepods for rearing WA dhufish will be undertaken with funds from Western Australia's Aquaculture Development Fund. The emphasis of this project will be the development of a standardised green water technique for rearing dhufish larvae that can be adopted by industry. It is likely that the technique will be applicable to a wide range of fish species.

Further research on the application of intensive copepod cultivation to the rearing of marine ornamental fish is now very feasible given the reliability of the copepod culture system.

One of the objectives of this project was to produce a manual 'Intensive Cultivation of a Calanoid Copepod, *Gladioferens imparipes*, A Guide to Procedures'. This is prepared as a separately bound Appendix to the present report and is available for distribution. This handbook provides sufficient basic information on the biology of copepods, together with details of procedures for cultivation of *G. imparipes*, to enable technical workers to maintain productive culture in aquaculture facilities which have access to seawater. The handbook could be used as the reference for an intensive course of technical training.

PLANNED OUTCOMES

The principal output from this project is the production of a manual of procedures (see Appendix 6) for culture of the copepod *G. imparipes*. The manual contains all the information on how to establish the copepod culture systems described in this report. Project outputs also include quantified production rates by copepod cultures and demonstrated improvements in larviculture practises for snapper, dhufish and seahorses. The planned outcome of the project is to increase the number of marine fish species that can be cultured in Australia, thereby enabling further expansion of the industry. Project outputs contribute directly to the planned outcome by providing commercial aquaculture facilities with detailed knowledge on how to culture copepods and how to use them to increase growth and survival in target fish species.

CONCLUSION

Objective 1
Determine the most effective procedures for increasing the scale of intensive production of *Gladioferens imparipes* to enable reliable supply of animals with minimum labour and maximum automation.

This objective was fulfilled. Two large-scale copepod culture systems have been developed and are fully described in this report. The 500 L system is fully automated and the reliability of nauplius production has been demonstrated. The 1000 L manual system require slightly more labour but is relatively simple and cheap to construct. Nauplius production by this system has not been presented in this report. However,

continued operation of this copepod culture system at ADU following the completion of this project has indicated that nauplius production is reliable. This work provides the means for commercial facilities to establish their own copepod culture systems using either *G. imparipes* or a similar species.

Objective 2
Assess the relative nutritional value of *G. imparipes* and other live food animals as food for various species of marine fish larvae through survival and growth trials of fish on different diets. This is to involve fish which are currently cultivated and other species for which eggs or larvae can be obtained.

This objective has been met through controlled growth trials with snapper, WA dhufish and WA seahorse larvae and juveniles. In all three species, inclusion of *G. imparipes* nauplii in early diets increased growth and survival compared to diets comprising rotifers. This was attributed to high nutritional content of nauplii, particularly in HUFAs, and greater selection of copepod nauplii by fish. Prospects for culture of selected marine species that are difficult to rear using standard larviculture technology are improved by development of *G. imparipes* culture systems.

Objective 3
Determine the diet of copepods which will provide a biochemical profile in the copepods which best serves the nutritional requirements of larval fish.

This objective was partly fulfilled .The HUFA content of *G. imparipes* nauplii was shown to be close to optimum when enriched with a combination of *Isochrysis galbana* and *Nannochloropsis oculata* compared to *I. galbana* alone. However, further work is require to examine the effect of a range of enrichment diets on both fatty acid and amino acid content of copepods.

Objective 4
Undertake a cost/benefit analysis of cultivated copepods in fish aquaculture.

A cost/benefit analysis of using copepods to rear WA dhufish larvae has been conducted. While copepods are certainly expensive to produce compared to rotifers, copepods greatly increased survival in dhufish larvae. Therefore, copepods are a cost-effective option for culturing dhufish. In addition, copepod-reared dhufish juveniles appeared somewhat healthier than their rotifer-reared counterparts, further indicating the benefits of using copepods in dhufish larviculture.

Objective 5
Produce a detailed manual of procedures for intensive cultivation of *G. imparipes* for distribution by sale to commercial hatcheries within Australia.

This objective has been fulfilled and this manual is now readily available throughout Australia.

REFERENCES

Dunstan, G.A., Volkman, J.K., Barrett, S.M., Garland, C.D., 1993. Changes in the lipid composition and maximisation of the polyunsaturated fatty acid content of three microalgae grown in mass culture. Journal of Applied Phycology 5. 71-83.

Dunstan, G.A., Volkman, J.K., Jeffrey, S.W., Barrett, S.M., 1992. Biochemical composition of microalgae from the green algal classes Chlorophyceae and Prasinophyceae. 2. Lipid classes and fatty acids. Journal of Experimental Marine biology and Ecology 161. 115-134.

Kahan, D., Uhlig, G., Schwenzer, D., Horowitz, L., 1981/82. A simple method for cultivating harpacticoid copepods and offering them to fish larvae. Aquaculture 26. 303-310.

Moreno, V.J., Moreno, J.E.A.d., Brenner, R.R., 1979. Fatty acid metabolism in the calanoid copepod *Paracalanus parvus* : 1. Polyunsaturated fatty acids. Lipids 14. 313-322.

Nanton, D.A., Castell, J.D., 1997. Mass culture of the harpacticoid copepod, *Tisbe* sp. International Council for the Exploration of the Sea. Meeting of the Marine Fish Culture Committee.

Norsker, N.H., Stottrup, J.G., 1994. The importance of dietary HUFAs for fecundity and HUFA content in the harpacticoid, *Tisbe holothuriae* Humes. Aquaculture 125. 155-166.

Ogle, J., 1979. Adaption of a brown water culture technique to the mass culture of the copepod *Acartia tonsa*. Gulf Research Reports 6. 291-292.

Partridge, G.J., Ginbey, B.M., Kennerly, D.V., Frankish, K.R. (1998). A comparison of semi-intensive larviculture techniques for the production of black bream (*Acanthopagrus butcheri*) (Information Series 1998/1). Perth: Aquaculture Development Unit, Fremantle Maritime Centre, South Metropolitan College of TAFE.

Payne, M.F., Rippingale, R.J., Longmore, R.B., 1998. Growth and survival of juvenile pipefish (*Stigmatopora argus*) fed live copepods with high and low HUFA content. Aquaculture 167. 237-245.

Sargent, J.R., McEvoy, L.A., Bell, J.G., 1997. Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. Aquaculture. 155. 117-128.

Sargent, J.R., Whittle, K.J. (1981). Lipids and hydrocarbons in the marine food web. In A. R. Longhurst (Ed.), Analysis of marine ecosystems (pp. 491-497). London: Academic Press Inc.

Støttrup, J.G., Jensen, J., 1990. Influence of algal diet on feeding and egg-production of the calanoid copepod *Acartia tonsa* Dana. Journal of the Marine Biological Association of the United Kingdom 141. 87-105.

Støttrup, J.G., Norsker, N.H., 1997. Production and use of copepods in marine fish larviculture. Aquaculture. 155. 231-248.

Støttrup, J.G., Richardson, K., Kirkegaard, E., Pihl, N.J., 1986. The cultivation of *Acartia tonsa* Dana for use as a live food source for marine fish larvae. Aquaculture 52. 87-96.

Sun, B., Fleeger, J.W., 1995. Sustained mass culture of *Amphiascoides atopus* a marine harpacticoid copepod in a recirculating system. Aquaculture 136. 313-321.

U'ren, S.C., 1983. Acute toxicity of bis, tributyltin oxide to a marine copepod. Marine Pollution Bulletin 14. 303-306.

APPENDICES

Appendix 1: Intellectual property

The only intellectual property issues associated with this project relate to procedures for intensive cultivation of copepods. These procedures are described in detail in the document 'Intensive Cultivation of a Calanoid Copepod, *Gladioferens imparipes* : A Guide to Procedures (Rippingale, R. J. & Payne M. F., 2001 ISBN 1 74067 070 1) which is published as a separate appendix to this report. The authors assign copyright for this document to the Fisheries Research and Development Corporation in the expectation that it be made available for sale at minimum cost. Electronic copies of the document may be distributed by the authors. No restrictions are to be imposed on any party intending to make use of the procedures described in the document.

Appendix 2: Staff

Curtin University

Rippingale, Robert J.

Payne, Michael F.

Corbett, John

Webb, Diane

Aquaculture Development Unit

Poller, Craig

Partridge, Gavin

Appendix 3. Procedure for fatty acid analysis of live food.

Adapted from Dunstan et al (1992, 1993) by Michael Payne and Dr Bob Longmore, Curtin University (28th May 1997).

Day 1

Algae

1. Concentrate 250-500 ml dense algal culture onto 90 cm GF/C Whatmann filter paper, using a gentle vacuum (for weight determination see below).
2. Wrap the filter paper in aluminium foil. If storing sample for a short time, place in sealed container under nitrogen gas and freeze.
3. Place sample into 50 ml beaker, add ~15 ml dichloromethane (DCM):methanol :water (1:2:0.8 v/v/v) and mash the sample with a glass rod.
4. Immerse an ultrasound probe in the sample (or place in u/s bath) for 10 min. On removal, ensure all of the sample is immersed in solvent, seal the beaker with foil and place in a cool, dark place overnight (eg fridge).

Rotifers, artemia & copepods

1. Determine sample volume that contained approximately 200,000 rotifers, 200,000 copepod nauplii, 50,000 artemia nauplii or 10,000 adult copepod. Concentrate sample on a disc of 44 um (rotifer, copepod nauplii) or 150 um (artemia, adult copepods) nylon mesh fitted to a millipore apparatus.
2. Using tweezers, remove mesh and place on the side wall of a breaker tilted at 45°. Rinse animals from the mesh using 15ml dichloromethane (DCM):methanol:water (1:2:0.8 v/v/v).
3. Homogenise sample, ensuring that it does not heat up above around 40°C.
4. Cover beaker with foil and place in a cool, dark place overnight (eg fridge).

Day 2

5. Transfer content of beaker into 25 mm millipore filter apparatus, rinsing beaker with DCM:methanol:water. Filter sample under vacuum, stopping occasionally to add more solvent and mash sample carefully with glass rod. Continue until sample has lost all pigment and the filtrate is clear (final volume >50 ml).
6. Set up two 100 ml separation flasks in fume hood, one above the other on the same stand. Rinse flasks with DCM or methanol. Add (separately) DCM and water (1:1 v/v) to both flasks, ~40 ml (total) in top flask and ~25 ml (total) in lower.
7. Transfer sample from millipore flask to top separation flask, rinsing millipore flask with DCM:meth:water. Stopper flask and shake gently releasing pressure frequently. Drain lower DCM layer into second separation flask, stopper and shake. Drain DCM layer into 100 ml round flask. Repeat process twice more.

Note : If separation between DCM and water does not occur:

1. Add more DCM
2. Add more water
3. Inject methanol into lower layer with pipette.

8. Fit round flask to Buchi rotary evaporator with clip. Set water temp to 50 °C, start vacuum with top tap open, close tap, start rotation and lower flask into

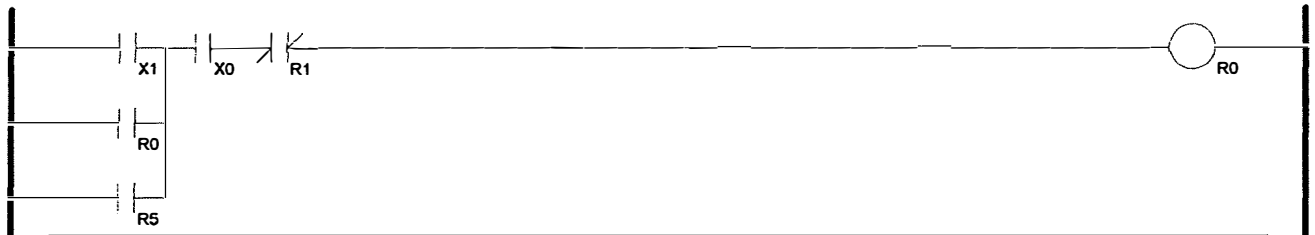
water bath. Continue until solvent no longer condenses in collector and sample is dry.

9. Rinse pigment from round flask with a small volume of methanol, pipette into 5 or 10 ml volumetric flask. If necessary, warm flask briefly in water bath to dissolve lipid. Continue rinsing, using small volumes of methanol, to make up the 5 or 10 ml volume of extract. Store sample under nitrogen gas in fridge.
10. Place half the volume of extract into a 30 ml capped tube. Add 1 ml standard (1 mg/ml C19:0 in hexane), 2 ml Toluene and 4 ml acidified methanol (1% conc sulphuric acid by vol).
11. Place sealed tubes in covered 50 °C water bath overnight.

Day 3

12. Fill a 100 ml separation flask with ~30 ml deionised water. Add sample to the flask and rinse sample tube with ~10 ml hexane into the flask. Shake, releasing pressure frequently.
13. Allow phases to separate and then drain lower water layer to waste. Wash the sample twice more by adding ~30 ml DI to the flask, shaking, separating and draining.
14. Add two scoops of anhydrous sodium sulphate to the flask and shake. Filter (Whatman 1) the contents of the flask into a round flask. Rinse separator immediately. Use the Buchi to reduce the volume down to ~10 ml. Do not use temperatures over 50°C or allow all the hexane to evaporate.
15. Transfer sample to sealed tube ready for GC analysis. Store under nitrogen gas in the fridge.

Appendix 4. PLC ladder logic for 500 L automated copepod culture system.



X0 - N/C Push Button (Stop)
X1 - N/O Push Button (Start)
R0 - Harvest System Activated Indicator
R1 - Harvest System Reset
R5 - Harvest Reactivate

When the Start Button (X1) is pressed R0 is turned on and remains on when X1 is released. R0 is turned off when either the Stop Button (X0) or R1 is activated.

Y0 - N/O Air Valve
R0 - Harvest System Activated Indicator
R3 - Refill Complete Indicator
When the Harvest System (R0) is turned on the Air Valve (Y0) is turned on thus stopping the air flow to the tanks until R3 is activated.

T0 - 5min Timer (Tank Settle Time)
R0 - Harvest System Activated Indicator

When the Harvest System (R0) is turned on Timer 0 (T0) is activated allowing a 5min settle time.

T0 - 5min Timer (Tank Settle Time)
T1 - 10min Timer (Nauplii Attraction Time)
R0 - Harvest System Activated Indicator

When the Harvest System (R0) is turned on and Timer 0 (T0) is complete activate Timer 1(T1) allowing a 10min attraction time.

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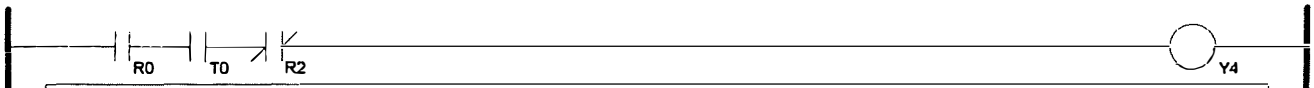
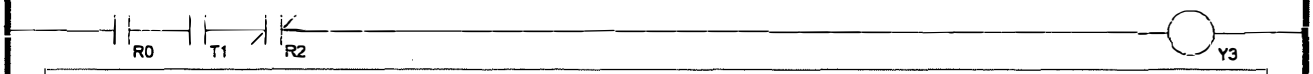


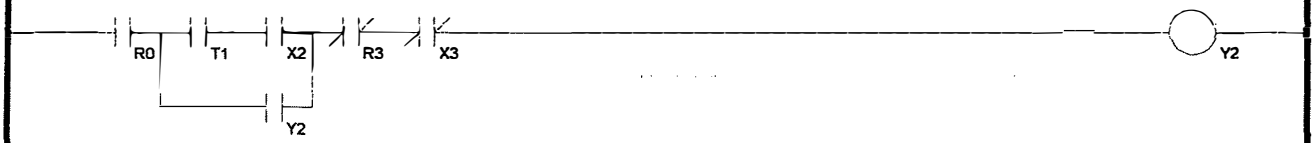
Y4 - Attraction Light
T0 - 5min Timer (Tank Settle Time)
R0 - Harvest System Activated Indicator
R2 - Harvest Complete Indicator (Locks out Y3 & Y4)

When the Harvest System (R0) is turned on and Timer 0 (T0) is complete activate the Attraction Light (Y4) until R2 is activated.



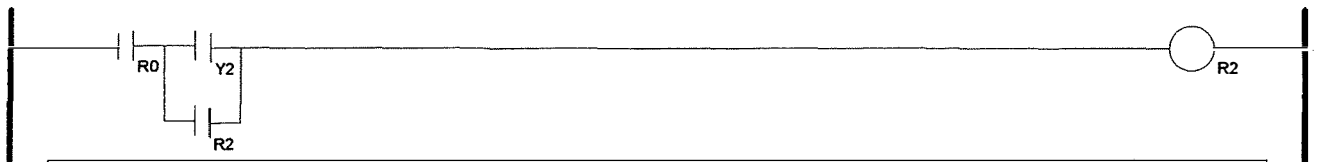
Y3 - Extraction Pump Relay Coil (N/O Relay)
T1 - 10min Timer (Nauplii Attraction Time)
R0 - Harvest System Activated Indicator
R2 - Harvest Complete Indicator (Locks out Y3 & Y4)

When the Harvest System (R0) is turned on and Timer 1 (T1) is complete activate the Extraction Pump Relay to turn on it's Pump until R2 locks out the Y3.



X2 - Harvest Tank Level Switch (N/O)
X3 - Culture Tank Level Switch (N/O)
Y2 - Fresh Water Pump Relay Coil (N/O Relay)
T1 - 10min Timer (Nauplii Attraction Time)
R0 - Harvest System Activated Indicator
R3 - Refill Complete Indicator (Locks out Y0 & Y2)

When the Harvest System (R0) is turned on, Timer 1 (T1) is complete and the Harvest tank is full (X2) activate the Fresh Water Tank Relay to turn on it's Pump until the Culture Tank is full (X3). Lock out Y2 with R3.

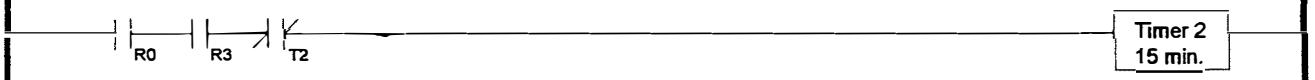


Y2 - Fresh Water Pump Relay Coil (N/O Relay)
R0 - Harvest System Activated Indicator
R2 - Harvest Complete Indicator (Locks out Y3 & Y4)

When the Harvest System (R0) is turned on and the Fresh Water Pump Relay (Y2) is activated, turn on R2. R2 holds itself on when Y2 goes off and thus locks out Y3.

X2 - Harvest Tank Level Switch (N/O)
X3 - Culture Tank Level Switch (N/O)
T1 - 10min Timer (Naulpii Attraction Time)
R0 - Harvest System Activated Indicator
R3 - Refill Complete Indicator (Locks out Y0 & Y2)

When the Harvest System (R0) is turned on, Timer 1 has expired and both tanks are full, turn on R3. R3 holds itself on when X2 goes off and thus locks out Y2.

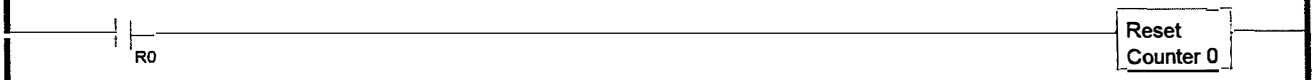


T2 - 15min Timer (Harvest Tank Drainage Time Fraction)
R0 - Harvest System Activated Indicator
R3 - Refill Complete Indicator (Locks out Y0 & Y2)

When the Harvest System (R0) and R3 are both on, Timer 2 is activated, resetting itself upon completion.

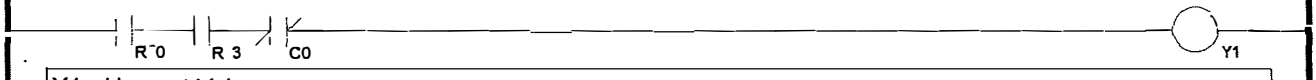
T2 - 15min Timer (Harvest Tank Drainage Time Fraction)
C0 - 8 Pulse Counter
R0 - Harvest System Activated Indicator

When the Harvest System (R0) and Timer 2 (T2) completes timing Counter 0 (C0) increments by one. 8x15min=2hrs drainage time.



RC0 - 8 Pulse Counter Reset
R0 - Harvest System Activated Indicator

When the Harvest System (R0) is deactivated Counter 0 (C0) is reset for the next cycle.

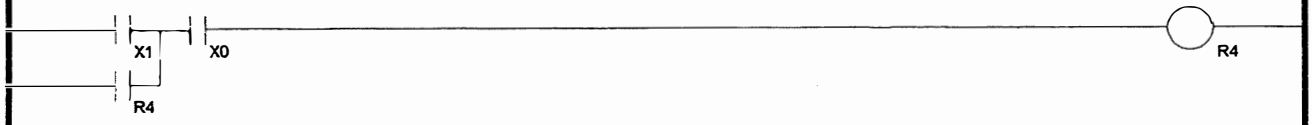


Y1 - Harvest Valve
C0 - 8 Pulse Counter
R0 - Harvest System Activated Indicator
R3 - Refill Complete Indicator (Locks out Y0 & Y2)

When the Harvest System (R0) and R3 are on, Harvest Valve (Y1) remains on until C0 is activated.

C0 - 8 Pulse Counter
R0 - Harvest System Activated Indicator
R1 - Harvest System Reset

When the Harvest System (R0) is on and Counter 0 (C0) has counted to 8 the Harvest System is reset by R1.

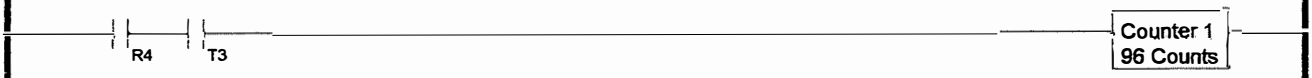


X0 - N/C Push Button (Stop)
X1 - N/O Push Button (Start)
R4 - 24hr Cycle Activated Indicator

When the Start Button (X1) is pressed the 24hr Cycle Activated Indicator (R4) is turned on and remains on when X1 is released. R4 is turned off when the Stop Button (X0) is pressed

T3 - 15min Timer (24hr Cycle Time Fraction)
R4 - 24hr Cycle Activated Indicator

When the 24hr Cycle Activated Indicator is on, Timer 2 is activated and resets itself upon completion with T2.

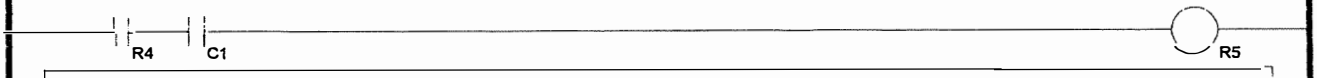


T3 - 15min Timer (24hr Cycle Time Fraction)
C1 - 96 Pulse Counter
R4 - 24hr Cycle Activated Indicator

When the Cycle (R4) is on and Timer 3 (T3) completes a timing Counter 1 (C1) increments by one. 96x15min=24hrs cycle time.

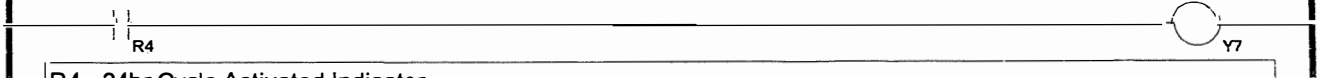
C1 - 96 Pulse Counter
RC1 - 96 Pulse Counter Reset
R4 - 24hr Cycle Activated Indicator

When the 24hr Cycle (R4) is deactivated or Counter 1 (C1) completes it's count reset the counter for the next cycle.



C1 - 96 Pulse Counter
R4 - 24hr Cycle Activated Indicator
R5 - Harvest Re-activate

When the Cycle (R4) is on and Counter 1 (C1) completes counting reactivate the Harvest System with R5.



R4 - 24hr Cycle Activated Indicator
Y7 - 24hr Cycle Indiactor

When the Cycle (R4) is on indicate the backgorund timing with Y7.

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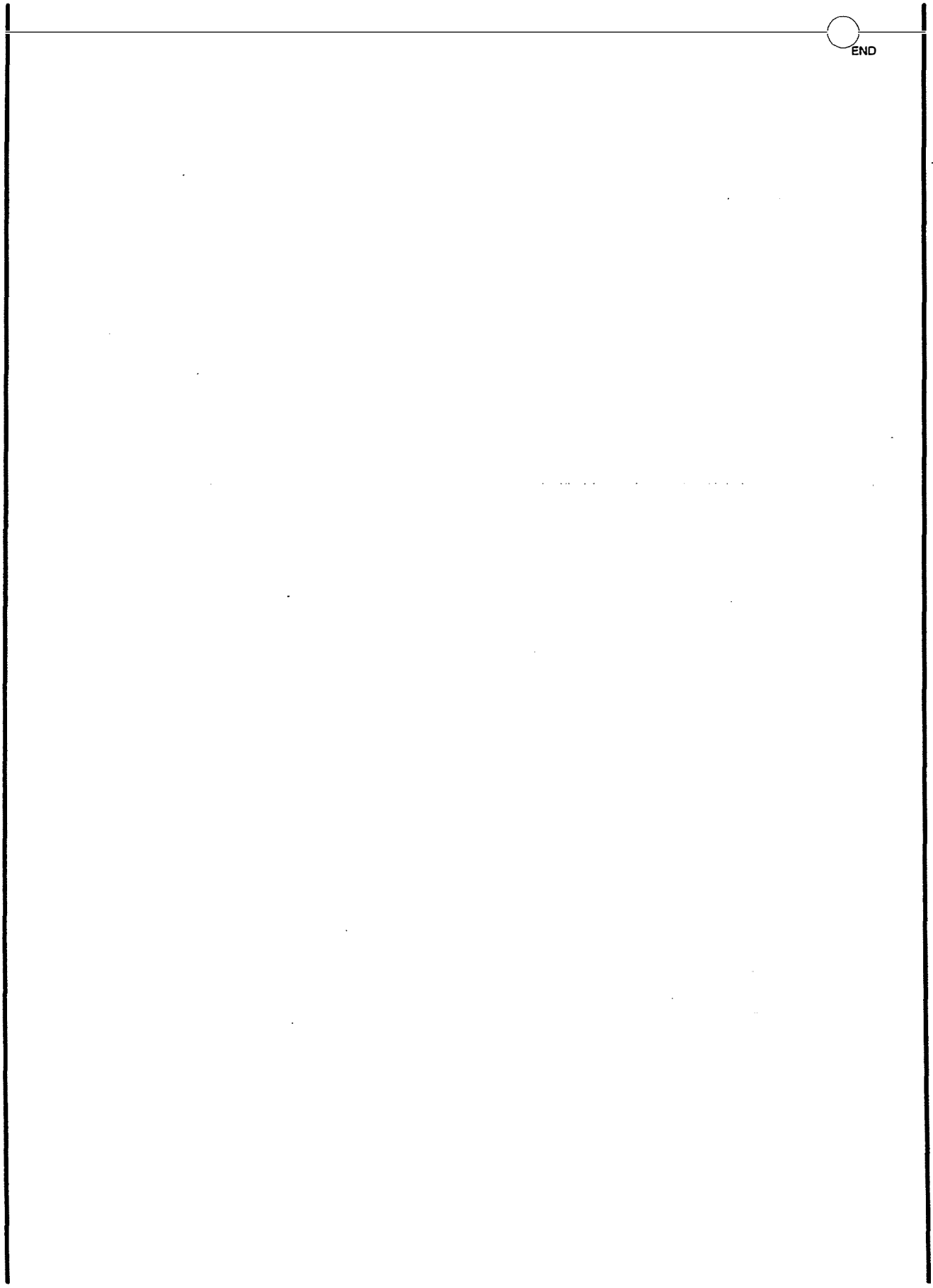

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List of Memory Bits Used

Inputs :

X0	-	Stop Button (Normally Closed Push Button)
X1	-	Start Button (Normally Open Push Button)
X2	-	Harvest Tank Float Switch (Normally Open)
X3	-	Culture Tank Float Switch (Normally Open)

Outputs :

Y0	-	Air Valve (Normally Open)
Y1	-	Harvest Valve (Normally Closed)
Y2	-	Fresh Water Pump Relay (Normally Open)
Y3	-	Extraction Pump Relay (Normally Open)
Y4	-	Attraction Light (Normally Off)
Y7	-	24hr Cycle Indicator

Timers :

T0	-	Settle Time (5 minutes)
T1	-	Attraction Time (10 minutes)
T2	-	15 minute timer
T3	-	15 minute timer

Counters :

C0	-	8 Pulse Counter
C1	-	96 Pulse Counter

Internal Bits :

R0	-	Harvest System Activated Indicator
R1	-	Harvest System Reset
R2	-	Harvest Complete Indicator
R3	-	Refill Complete Indicator
R4	-	24hr Cycle Activated Indicator
R5	-	Harvest System Reactivate

Appendix 5. Cost and suppliers of electrical components for PLC copepod culture system.

All suppliers are listed in the Perth telephone directory.

No.	Item	Cost/item ($)	Total cost ($)	Supplier
1	Toshiba EX20 PLC	1069.00*	1069.00	Control & monitoring Services
1	12V Power Supply (11 Amp)	170.00	170.00	Electronic Parts (WA)
1	12V Diaphram pump	149.00	149.00	The 12 Volt Shop
1	12V Bilge pump	59.79	59.79	Wilson Marine
2	12V Pneumatic solenoid valve (water)	58.00	116.00	Technical Irrigation Imports
1	12V Solenoid valve (air)	83.50	83.50	SMC Pneumatics
2	12V 20W halogen lamp + holder	11.75	23.50	Pro-Lamps
4	Nylon float switch	23.39	93.56	RS Components
2	12V Relay + base	36.02	72.04	RS Components
10	10A fuse	0.30	3.00	RS Components
10	2A fuse	0.32	3.20	RS Components
7	Fuse holder	1.93	13.51	RS Components
20M	Red/Blk cable (50x0.12mm)	0.75/M	15.00	Dick Smith Electronics
5M	Red/Blk cable (26x0.3mm)	1.35/M	6.75	Dick Smith Electronics
12	6.3mm QC connector housing	1.11	13.32	Coventrys
12	Connector receptacle and tab	0.49	5.88	Coventrys
	Total		**1897.05**	

* This price does not include programming software

Appendix 6. Intensive cultivation of a calanoid copepod *Gladioferens imparipes* : A guide to procedures

This is a separate document with ISBN 1 74067 070 1.

INTENSIVE CULTIVATION OF A CALANOID COPEPOD

Gladioferens imparipes

A GUIDE TO PROCEDURES

Prepared by

R. J. Rippingale & M. F. Payne
Department of Environmental Biology
Curtin University of Technology
GPO Box U1987,
Perth WA 6845

ISBN 1 74067 070 1

June 2001

Foreword

Keeping animals in intensive cultivation has many benefits for humans. In some cases the benefits are obvious; if animals provide food or labour or companionship but in other cases, especially for small obscure creatures with no direct charismatic appeal, the benefits of cultivation are less obvious. In the case of small aquatic crustaceans which can barely be seen and have no common name, the time, effort and expense involved in developing techniques for cultivation may require some explanation.

Breeding of marine fish for food or recreation is a large industry and since growth in the world catch of wild ocean fish is unlikely, effective techniques in aquaculture will become more and more important. Although some marine fish will breed and grow quite readily in captivity, others are more problematic. One of the major difficulties in breeding these fish is the supply of suitable food for the larvae. In nature, many marine fish depend on copepods when they commence feeding, but few species of marine copepod have been successfully cultivated on a scale that is suitable for use in aquaculture.

This manual describes procedures that have been developed by the authors for intensive production of the calanoid copepod *Gladioferens imparipes*. The procedures were developed during extensive studies of the biology and ecology of the animal, both in the laboratory and in the natural environment. The procedures are based on a thorough understanding of the life requirements of the animal and a practical understanding of the realities of the cost of equipment, space and labour. Procedures are described for different scales of production, ranging from low-level maintenance of a few animals in small volumes of water, through production units of different volume up to 5,000 litres and with different levels of automation in the procedures.

Copepod cultivation may make an important contribution in aquaculture but this is not the only area in which the animals may be useful. A reliable supply of healthy copepods at known ages and life stages can be valuable for research in a variety of fields. Studies of ecological processes in marine and estuarine environments can be made using copepods as model animals involved in the movement of energy, of specific chemicals and of nutrients. Copepods may be used as model animals on which to measure the effects of environmental stressors or be used as food in research which focuses on marine or estuarine animals which require live prey.

The potential benefits to aquaculture were a major motivating force in recent development of the procedures for cultivating *G. imparipes*. It must also be said that much of the early work arose from attempts to understand the place of the animal in the functioning ecosystems of estuaries in south west Western Australia. We hope that this manual of procedures will be of use to people involved in a wide range of work. This includes aquaculturists, applied researchers, curiosity motivated researchers, educators wanting to give their students experience with live animals and home aquarists wanting to provide an interesting diet for their ornamental fish.

Acknowledgements

This project was funded equally by Curtin University and the Fisheries Research and Development Corporation (Project No. 96/398).

The bulk of this work was conducted at Curtin University of Technology on the premises of the Department of Environmental Biology. The assistance and interest of staff and students alike at this school is gratefully acknowledged. In particular, we would like to thank both technicians who where directly involved with the project, John Corbett and Diane Webb.

Studies on large-scale copepod cultures were conducted at the Aquaculture Development Unit in Fremantle, Western Australia. For this, we would particularly like to thank Greg Jenkins and Ken Frankish for their generous co-operation. The assistance of all the ADU staff, particularly Gavin Partridge, Craig Poller and Bruce Ginbey, is also acknowledged.

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Introduction

Human fishing has made serious impact on the wild populations of many species of fish and in many parts of the world aquaculture is seen as an alternative way of producing fish in commercial quantities. Although some species of fish can be cultivated with relatively straightforward procedures, others require particular conditions or specific foods which present a challenge to the aquaculture industry.

Many marine fish release very large numbers of small eggs which hatch into small larvae with very low survival prospects in their natural environment. To replace the parent fish, only a small fraction of one percent of the reproductive output of a female fish must survive. Early death is the normal occurrence, coming to the fish as they are taken by a range of predators or as they fail to find appropriate food. Most of the attrition occurs at the critical stage after yolk reserves are depleted and the fish need to take food from their environment.

It is well known that for many species of fish **live** food is essential at the critical stage of first feeding. In the sea, the potential food items most likely to be encountered by fish larvae are the nauplius stages of calanoid copepods. For many older fish, adult copepods are very important diet items. Copepods have probably been important in the diet of many marine fish during their evolution and effective predation strategies have evolved for their capture. Some fish may have developed a partial dependence on copepods.

With aquaculture it is possible to reduce the high mortality of early larval fish both by timely provision of adequate and appropriate food and by removing the risk from predators.

Marine calanoid copepods that feed primarily on phytoplankton have the biochemical composition of their diet reflected in their body tissues and storage compounds. Hence, fatty acids such as EPA and DHA, which are essential in the diet of marine fish larvae (and other vertebrates), can be provided in nature through a food chain leading from phytoplankton through herbivorous copepods to fish.

Herbivorous calanoid copepods are particularly suitable as food for fish. The size range (~100 µm nauplii to ~1,000 µm adult) fits the gape of many larval fish, the nauplii elicit a strong feeding response from many fish larvae and they have naturally high levels of essential fatty acids.

Different approaches are made for the supply of copepods for fish larviculture. In areas with productive waters, plankton, including copepods, can be collected from the wild. This material may be used directly as food or may be transferred to ponds with enriched water to allow populations to develop to high densities for later collection. Difficulties may be encountered in the reliability of wild collection. Natural communities of zooplankton fluctuate in abundance, species composition, nutritional state and health, and animals may carry parasites or other diseases.

Intensive cultivation of copepods for use as food for larval fish may be an attractive alternative to wild capture if it can be cost effective. For cultivation to be successful, the quality, quantity and timing of the product must be reliable and must be tuned to the requirements of the larval fish which are to be fed.

This manual has been produced to provide practical information regarding the production and use of a copepod for aquaculture in marine and estuarine waters of temperate and sub-tropical Australia.

The Copepoda

The Copepoda are a Class of animals within the larger group, the Phylum Crustacea. The group is very diverse, with more than 10,000 different species in many different ecological niches. Copepods occur in most bodies of marine and fresh water, including inland saline lakes and estuaries. Some even occur in water trapped in the soil. Many are parasitic, others swim freely as part of the plankton while others are benthic (bottom dwelling) or live on or around other organisms. Other copepods live in the spaces between sediment particles. Few free-living copepods exceed two millimetres in length as adults although some ecologically important copepods in cold marine water reach 10mm. Three major groups of free living copepods are identified; the Calanoida, mainly free swimming planktonic animals, the Cyclopoida, which may be planktonic or demersal and the Harpacticoida, which are almost entirely benthic.

Copepods pass through very distinct life history stages. They emerge from the egg as a nauplius, usually 100-150 µm in length. After six nauplius stages (referred to as stages N1 to N6), with growth between each stage, the body shape changes and a series of usually six copepodid stages follow (referred to as stages C1 to C6). The last of these is the adult in which the separate sexes can be identified. Reproduction is sexual. In parts of the sea the nauplius larvae of calanoid copepods are the most abundant metazoan animal. Figures 1, 2 &3 illustrate calanoid and harpacticoid copepods.

Copepods are ecologically important. Planktonic calanoids in the sea are significant grazers of phytoplankton, converting primary production (algae) into secondary production (animal tissue) and faecal debris. The rate at which grazing copepods feed, grow, reproduce and produce faeces depends on the abundance and quality of the algae on which they feed. When the species of algae available are highly nutritious and abundant the rate of growth and the production of faeces is maximised (Figure 4). At lower levels of food availability growth may be equally high and feeding efficiency much higher with less of the surplus food going to faeces. Copepods contribute to the biological activity of microbes in the water. They damage algal cells as they feed, causing cell debris and cell contents to leak into the water. This, along with the faeces, is a resource for bacteria, protists and other small detritus feeders. Through their excretion, copepods return soluble nutrients to the water and make those nutrients available for uptake by algae. Copepods are of great importance in many marine ecosystems and are a major food source for many fish and other animals in the sea.

Benthic (bottom dwelling) harpacticoid copepods are probably important components of the diet of many small bottom feeding fish. Planktonic (free-swimming) calanoid copepods are certainly important for many pelagic fish, especially during early stages of the fishes growth.

Nutritional content

All calanoid copepods are not of equal value in the diet of fishes. Larval fish require particular long chain highly unsaturated fatty acids (HUFAs) in their diet to ensure normal development of their nervous system. These HUFAs are not synthesised by animals but are produced by some species of phytoplankton. Well fed copepods which feed primarily by grazing on phytoplankton are likely to have stores of HUFAs and therefore to be beneficial in the diet of fish. Those copepods which feed by scavenging on detritus or by predating on ciliates and rotifers have a larger proportion of fatty acids in their stores which have been synthesised by bacteria rather than phytoplankton. These are less valuable in the diet of fishes.

Calanoid copepods vary in the total amount of energy stored as lipid in their body. Cold water copepods from high latitudes (e.g. *Calanus* sp.) tend to breed annually, timing their breeding with the seasons. During the spring, phytoplankton is abundant and these animals store large reserves of lipid which they later use in reproduction. Calanoid copepods from coastal waters (e.g. *Acartia* sp) which are more continually productive, tend to breed more opportunistically, converting food energy to reproduction without a lot of intermediate energy storage. For most of these animals, females release a few embryos at a time. For some calanoids, especially those which occur in the turbid waters of estuaries (e.g. species of the genera *Diaptomus, Pseudodiaptomus, Eurytemora,* and *Gladioferens*), a large store of lipid can be produced which is then converted to a large number of eggs which develop simultaneously. Fertilised eggs are then held as a clutch, external to the body of the female, as the embryos develop (Figure 5).

If copepods which store high levels of lipid and which carry embryos in a clutch have suitable phytoplankton in their diet, their value as a food item for fish can be great. A healthy population of these copepods will include adult females with fresh algal food in their gut, lipids in storage, eggs developing in the reproductive tract and a clutch of embryos attached (Figure 6). Studies have shown these animals to be preferentially selected by feeding fish. In nature, especially in turbid estuarine water, the diet of these grazing copepods may not always provide for optimal copepod growth rate, lipid store, reproduction or gut content. This is not so in a laboratory, where the quantity and quality of algal food can be controlled, along with such other conditions as temperature, salinity, water quality and photoperiod.

Copepod culture

Continuous cultivation of marine copepods has been achieved relatively recently, but only for a small number of species (see Further Reading). Easiest to cultivate are the harpacticoid copepods, especially *Tisbe* spp. and *Tigriopus* spp. These can be grown at high densities but being benthic, with benthic nauplii which tend to avoid the light, the use of these animals in fish larviculture is most effective for those fish which feed primarily from bottom sediments. Some marine calanoid copepods have been maintained in cultivation and have been used as food in larviculture. Various species of *Acartia* have been maintained in intensive cultivation and in extensive pond cultures. However, most of the free swimming calanoid marine copepods do not respond well to being kept in high population densities. Figure 7 provides a summary of the differences between some copepods which have been cultivated for use in fish larviculture.

The following sections describe aspects of the biology of a calanoid copepod which occurs naturally in the estuaries of South West Western Australia. *Gladioferens imparipes,* can be maintained in cultivation, will survive at artificially high population densities and produces free swimming nauplii which are taken as food by pelagic fish larvae.

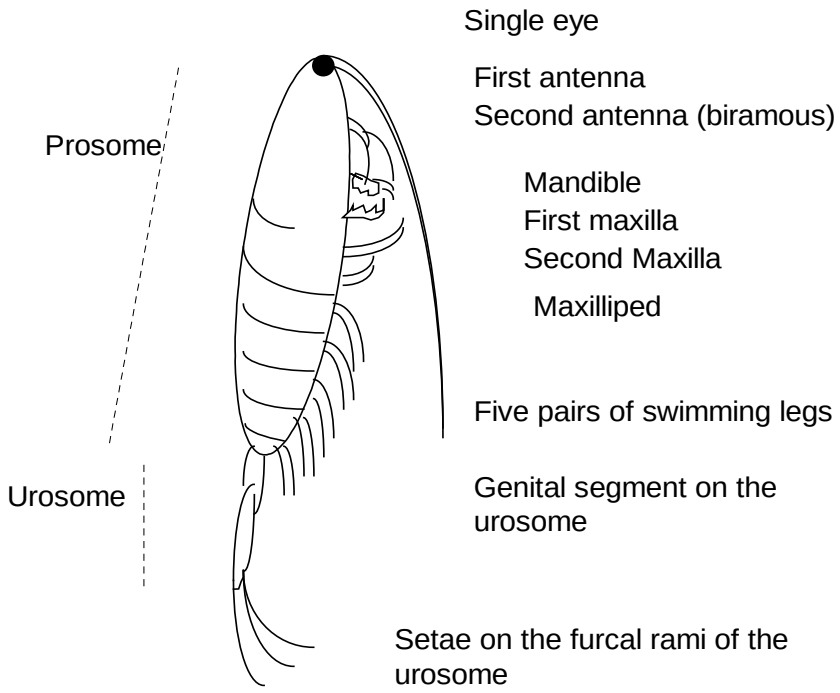


Figure 1. Diagram of the main anatomical features of an adult calanoid copepod.

Figure 2. Photomicrographs showing adult *Gladioferens imparipes*; a) male with asymmetrical first antennae, b) female with symmetrical first antennae, c) female with a clutch of embryos.

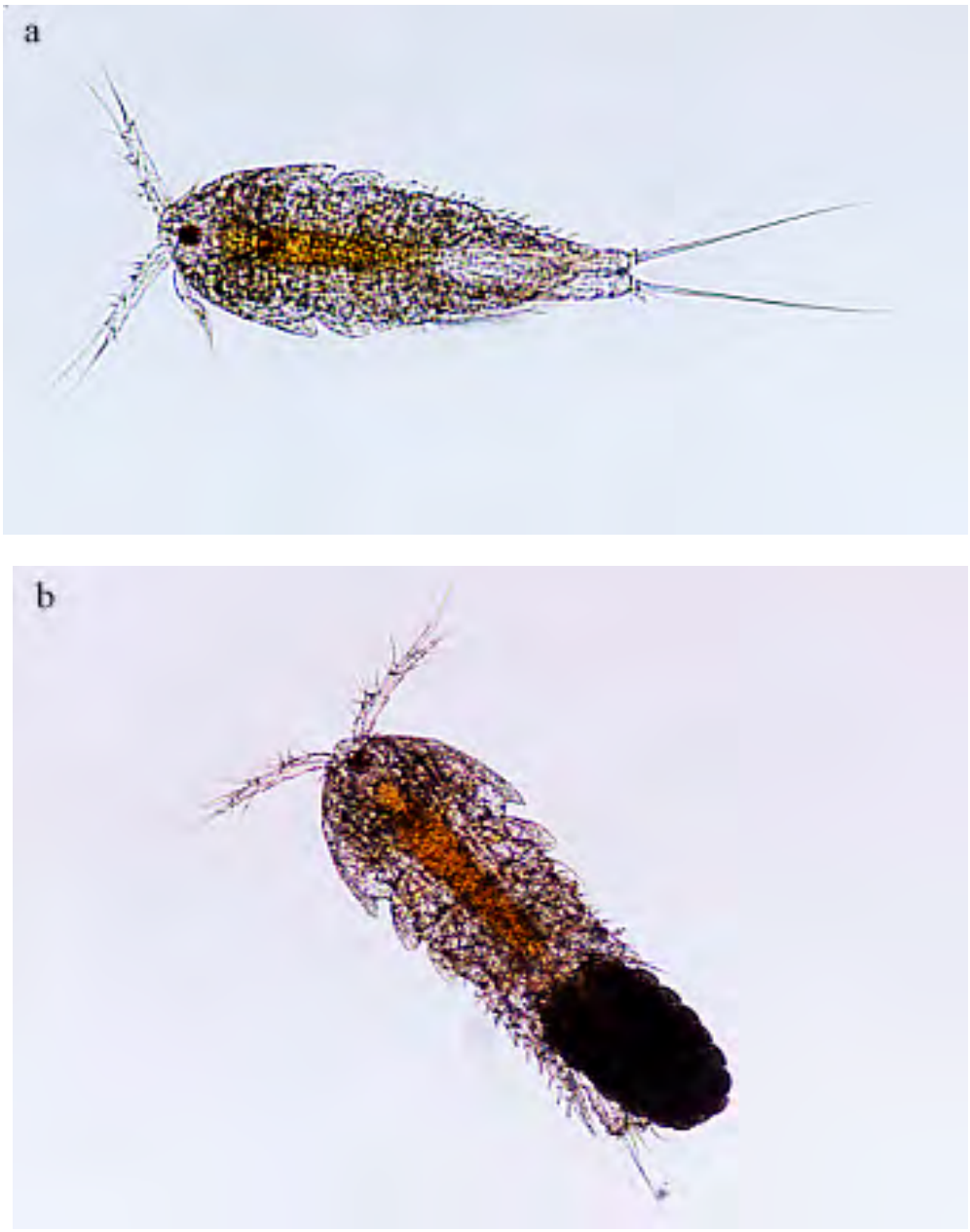


Figure 3. Photomicrographs showing, a) adult female of the harpacticoid copepod, *Tisbe* sp, b) adult female with a clutch of embryos.

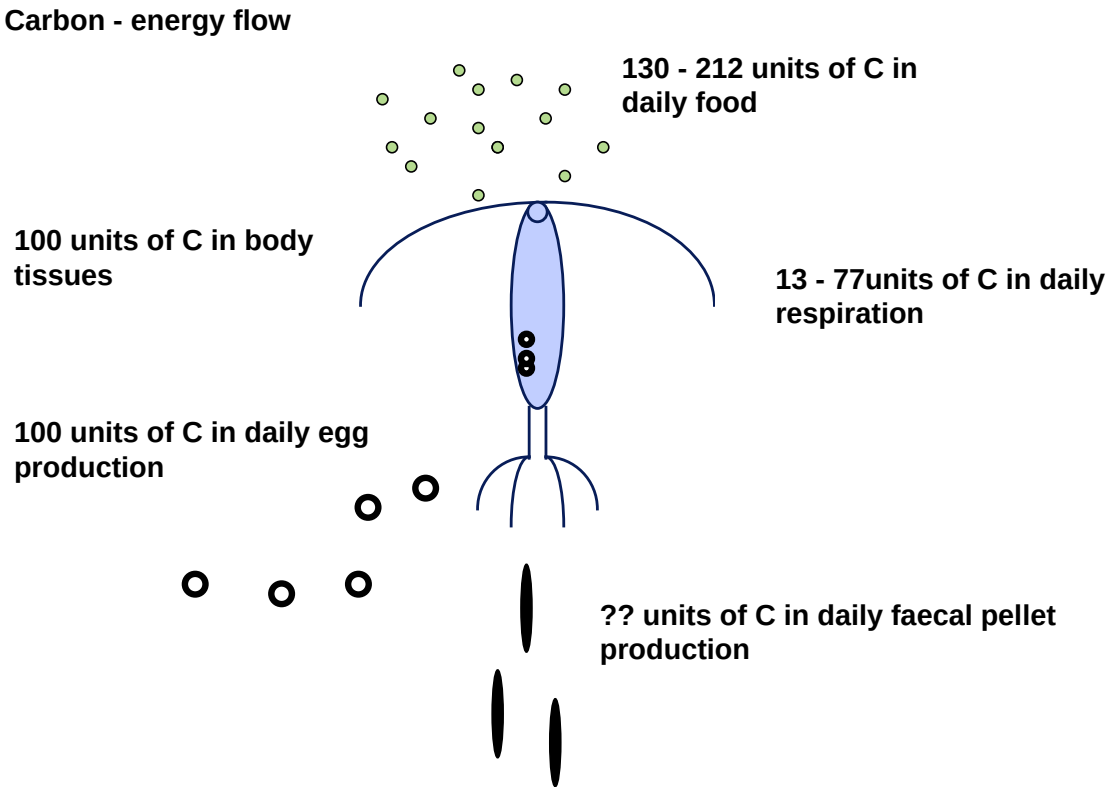


Figure 4. Diagrammatic representation of the energy (as carbon) movements associated with the life activities of a copepod (data from various sources).

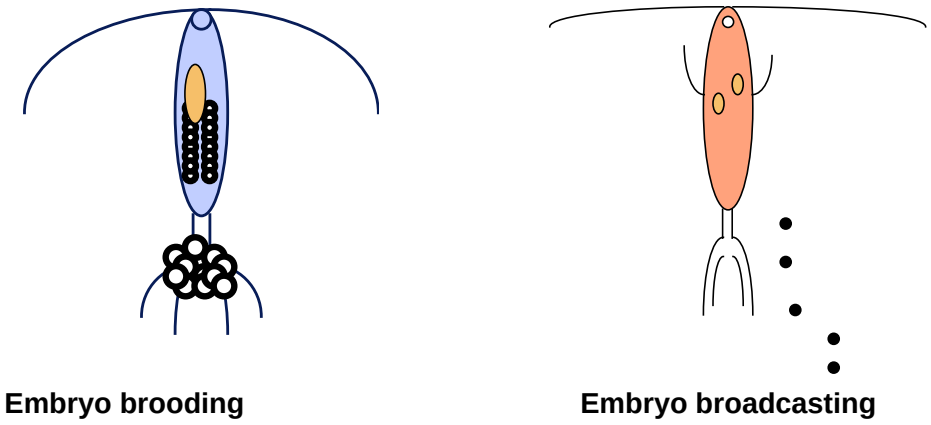


Figure 5. Diagrammatic representations of different reproductive strategies in copepods with regard to maternal protection of eggs.

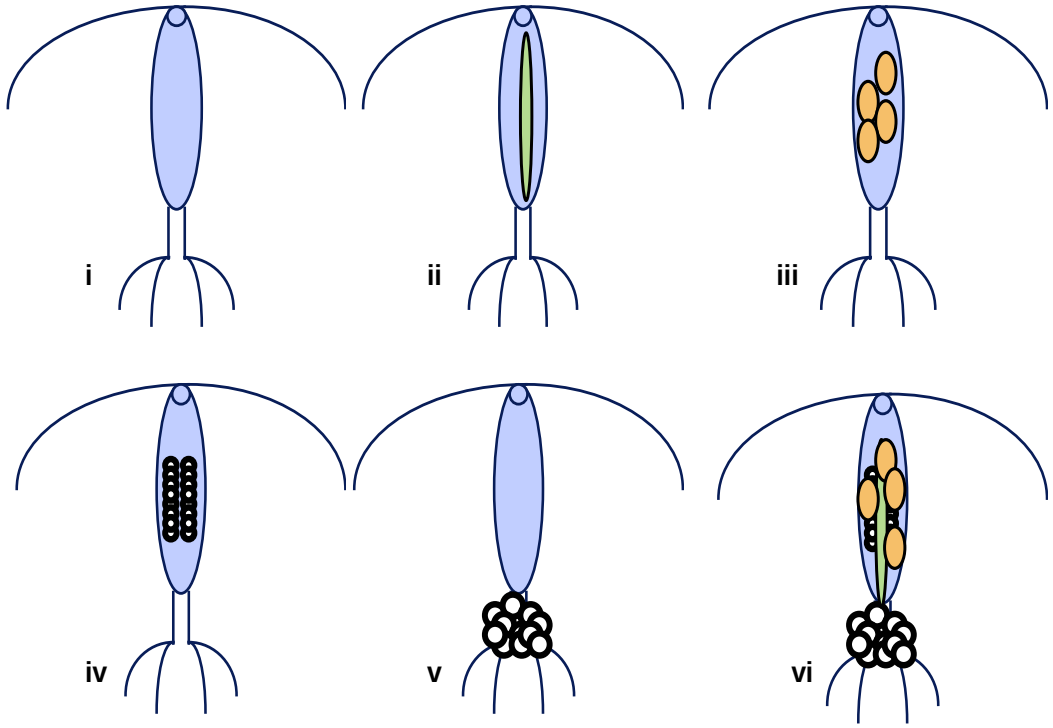
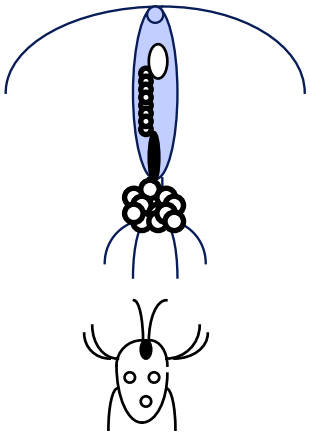


Figure 6. Diagrammatic representations of a calanoid copepod e.g. *G.imparipes* in different conditions of feeding and reproductive state and representing different food value to a predator.

i. non reproductive, unfed.
ii. non reproductive, with food in the gut.
iii. non reproductive, unfed, with stored lipid.
iv. with eggs in the reproductive tract.
v. with a clutch of embryos
vi. as a maximum value food item.

A calanoid copepod, *Gladioferens imparipes*. Australia



Adult female with internal eggs, external embryos, large lipid store and phytoplankton food in the gut.

Length ~ 1mm

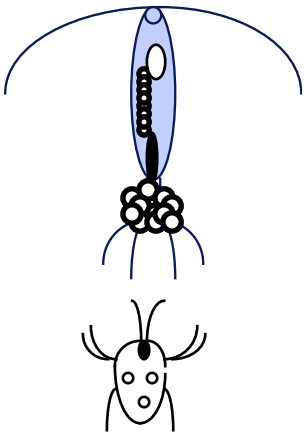
Embryos held in a clutch until they swim free

Free swimming nauplius stage, with lipid droplets and food in the gut.

Length 125 - 300 µm.

G. imparipes **grows well in sea water and at lower salinity.**

A calanoid copepod, *Eurytemora affinis,* Northern hemisphere.



Adult female with internal eggs, external embryos, large lipid store and phytoplankton food in the gut.

Length ~ 1mm

Embryos held in a clutch until they swim free

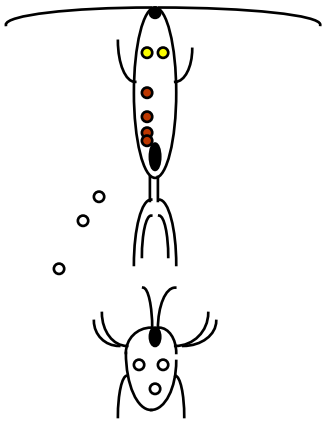
Free swimming nauplius stage, with lipid droplets and food in the gut.

Length 125 - 300 µm.

E. affinis **grows well in low salinity water but is stressed in sea water.**

Figure 7. Comparison of some different copepods grown in culture.

Calanoid copepods, genus *Acartia*



Adult female with a few internal eggs, small lipid store. Phytoplankton food in the gut.

Length ~ 1mm

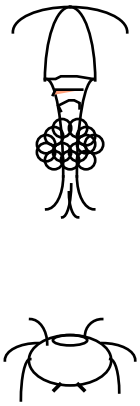
Embryos released singly or in small groups. These sink and hatch after some hours.

Free swimming nauplius stage, with lipid droplets and food in the gut.

Length 100 - 300 µm.

Acartia **grow well in sea water. Adults are omnivorous and may eat some of their own nauplii in crowded conditions.**

An harpacticoid copepod, *Tisbe*, from coastal marine water



Adult female with external embryos, small lipid store. Food in the gut.

Length ~ 6-700 µm

Embryos hatch and are released from the female. Adults are mainly benthic, feeding on sediments.

Free moving benthic nauplius stage, strongly photo negative.

Length 90 - 200 µm.

Tisbe **grow well in sea water. Adults are omnivorous detritivores.**

Figure 7 continued.

Biology of *Gladioferens imparipes*

This section describes aspects of the biology of *G. imparipes*. It is provided as background to help the non-specialist reader to understand the animal.

Although the copepods can be seen with the naked eye, any reference to their appearance assumes that a microscope is used. A dissecting stereoscopic microscope (7x - 40x) allows observations of whole animals and is used to locate and manipulate animals. A compound microscope (40x - 400x) is necessary for close observation of anatomy.

Classification

Gladioferens imparipes (Thomson 1946)

Family Centropagidae
Genus *Gladioferens*
Gladioferens imparipes

Type locality; Swan River estuary, Western Australia, collected from among vegetation in shallows.

Adult sex differences and reproduction

Figures 1 & 2 show male and female adult *G. imparipes* with labels indicating some anatomical features. Males are identified by the geniculate (jointed) left first antenna, which gives them an asymmetry. Females have symmetrical first antennae and may carry clutches of embryos. Other differences between the sexes are less obvious but differences in the structure of the fifth pair of legs are important.

Mating occurs as a male locates a female and grasps her with his geniculate first antenna. He then uses his fifth pair of legs to hold the female in a very precise way while he extrudes a spermatophore from his body cavity (Figure 8) and attaches it close to the genital opening on her urosome. Sperm from the spermatophore enter the reproductive tract of the female and fertilisation of her eggs is achieved. In *G. imparipes* fertilised eggs are released from the female into a sac which is held by the female until it is ruptured by the emergence of free swimming nauplius larvae.

Life history and development

Fertilised eggs are held in a sac against the urosome of the female. When first released the eggs appear dark brown. As embryos develop the colour and shade lightens until the mature embryos appear light brown with a dark eye spot just visible in each.

Nauplius larvae emerge from the egg sac and swim freely. Newly released nauplii have up to four or five small lipid droplets regularly arranged in their body cavity (Figure 9). The first nauplius stage (N1) is very brief (hours) before the animals metamorphose to N2, then with progressive growth to N6.

Following N6, the first copepodid stage (C1) occurs (Figure 9). By this stage the overall body form has changed from the 'pear shape' of the nauplius to the general form of the adult with conspicuous first antennae and a distinct division between the prosome and the urosome. As the animal develops through stages C1 to C6, the number of pairs of swimming legs increases from one to five and the total size increases. Between each developmental stage the animals shed the previous exoskeleton. By the stage C5 the geniculate antennae of males can just be detected but by C6 (adult) this feature is conspicuous. The prosome length of females is slightly larger than that of males. When the final (C6 or adult) stage is reached, no further moulting occurs.

Development time is temperature dependent. At 25°C, embryo and nauplius stages are completed in 4 - 5 days and full maturity (embryo - adult) in a total of 10 - 12 days.

Behaviour of *G. imparipes* alters as the animals develop. Nauplii swim continually and are attracted to directional light. Copepodid stages are progressively less attracted to light and by stage C4 start to hold to substrates (described below). Mature animals attach to substrates for lengths of time varying between seconds and minutes.

Body size

Animals at the first nauplius stage are ~125µm long and ~65µm wide. These grow to ~310µm long by N6.

For copepodids and adults the length of the prosome is the most convenient descriptor of size. Adult *G. imparipes* have prosome length of 750µm - 950µm depending on the temperature at which they developed. As for other invertebrate animals, growth rate is depressed at the low end of the tolerable temperature range but the final body size of adults is larger for those grown in cooler than those grown in warmer water.

Feeding

Appendages on the anterior part of the prosome, on the ventral side, are used in food collection. When the animals feed the second antennae sweep backwards and forwards very rapidly to generate a current of water which flows through combs of fine setae on the first and second maxilla. These remove potential food particles from the water. Food is then transferred to the mouth.

G. imparipes feeds readily on many species of small unicellular algae. In animals that have been actively feeding, the gut appears coloured by the ingested food.

When animals have access to abundant food they produce faecal pellets at intervals of ~20 minutes. Faecal pellets can usually be seen in the lower gut of well-fed animals. Each pellet is contained within a membrane of chitin, which is secreted by the animal. This causes the pellets to retain their shape after they are released.

Lipid stores

If *G. imparipes* are provided with abundant food they develop stores of liquid lipid in the body cavity. In newly released nauplii, four or five lipid droplets usually occur. These are spherical, similar in size and symmetrically arranged (Figure 9). In copepodid and adult animals, lipid stores form as one or more unstructured globules, or pools, of various sizes that appear to be loose within the body cavity, usually within the prosome but sometimes in the legs. Lipid stores can be made conspicuous by staining with Sudan IV (Figure 10, see Appendix 1 for methods). Extensive stores of lipid allow *G. imparipes* to survive periods without food. This is probably an advantage to an animal living normally in estuaries where microalgal productivity is irregular. It makes for convenience in laboratory maintenance because animals can be left without food for periods up to 2 weeks (longer at low temperature) if high production is not required. As food for predators, an adult *G. imparipes* with lipid reserves presents a valuable food item. The food value is even greater if the copepod is an adult female carrying a large clutch of embryos and having well developed eggs in the reproductive tract (Figure 6).

Locomotion

G. imparipes, like many other calanoid copepods, has two different modes of locomotion. A smooth, gliding, swimming motion is achieved by the force produced as the second antennae beat at high frequency. This movement achieves both feeding and swimming. The typical orientation when swimming is with the anterior ventral surface uppermost and the axis of the body ~ 45° to the horizontal.

More rapid movement through the water occurs as the animals ‘row’ with the five pairs of legs, resulting in a brief moments of jerky movement through many body lengths.

G. imparipes displays a behaviour which is unusual among calanoid copepods. Late copepodid and adult animals attach to firm substrates. This occurs as fine `hair sensillae’ on the dorsal surface of the prosome make contact with the substrate (Figure 11). While attached, the animals may be inactive or they may generate feeding currents with their

second antennae. Attached animals are not dislodged by gentle water currents and may remain in one position for many minutes before they detach themselves and either swim or relocate elsewhere. When food is abundant, healthy animals remain attached for extended periods of time. When food is sparse, more time is spent moving in the water column, apparently “searching” for food.

The exact mechanism by which *G. imparipes* attach to surfaces is not understood. It probably involves contact between the tips of many sensillae and irregularities in the substrate surface. Slight flexure of the prosome may result in a sideways force at the tip of the sensillae providing some grip on the surface.

Ecology of *Gladioferens* in estuaries

The genus *Gladioferens* is represented by five species which occur in inland coastal waters of Australia and New Zealand; *G. symmetricus, G. pectinatus, G. inermis, G. spinosus* and *G. imparipes*. Of these, *G. imparipes* is known to occur in the estuaries of southwest Western Australia around the coast from the Moore River (31.25S, 115.30 E) to the King and Kalgan Rivers (35S, 118E). Populations are influenced by the patterns of productivity, by the hydrological regime and by the ecological relationships that occur in each estuary. Most of these estuaries are dominated by a seasonal hydrological regime in which fluvial flow follows winter rain. During the flow regime phytoplankton production and zooplankton activity are both low. With reduction or cessation of fluvial flow at the end of winter those estuaries which are not cut off from the sea by a sand bar are influenced by small scale tidal movement and saline water gradually intrudes into the estuary as a horizontal and vertical salt wedge. Under these conditions phytoplankton productivity is stimulated and *G. imparipes* has been recorded as a pioneer herbivore, successful over a wide salinity range (<2 - 35ppt) and the first of the crustacean zooplankton species to exploit the new primary production. In some estuaries an ecological succession occurs following the recovery of saline conditions each year. *G. imparipes*, the pioneer herbivore is replaced by other species of omnivorous calanoid copepods which predate the nauplius larvae and restrict the distribution of *G. imparipes* to zones of the estuary which are inaccessible to the predators. The predator species, *Sulcanus conflictus* and *Acartiura* sp., where they occur, occupy zones of different salinity. *S. conflictus* from 2 - ~20ppt and *Acartiura* sp. from 20 - 35ppt. In the Swan estuary, where the distribution patterns have been most closely studied, at the end of summer *G. imparipes* is restricted to an upstream section of the estuary beyond the upstream distribution of *S. conflictus*. Further downstream *G. imparipes* is excluded as the other copepods occupy estuary sections of different salinity. Although *G. imparipes* can tolerate the physical conditions which occur throughout the estuary, it has a distribution pattern dictated by ecological interactions with other species.

The distribution pattern described above is a simple summary. In any year the pattern is affected by variations in hydrological regime, productivity and algal blooms.

G. imparipes is successful in a natural environment that is characterised by variability. Populations persist in habitats where salinity ranges from almost fresh to >35ppt, temperature ranges from 10 - 28°C and water quality and productivity vary.

The estuarine habitat has selected for tolerance of changing and possibly stressful conditions. *G. imparipes*, as individual animals, can withstand salinity changes over a wide range. They can survive in temperatures from ~6°C to 28°C, can tolerate periods without food and can survive in water of low quality. Adult animals can store energy in large lipid reserves and persist without additional food. Embryos are protected by being carried until free swimming nauplii are hatched and survival rates of juveniles are maximised by parental investment of food reserves in the embryo. Rapid development to maturity and repeated reproduction provide for a high intrinsic rate of population growth that enables rapid exploitation of available resources.

These various attributes of *G. imparipes*, which equip the animal for survival in the rigorous conditions of the estuaries, also allow it to tolerate the conditions of intensive cultivation.

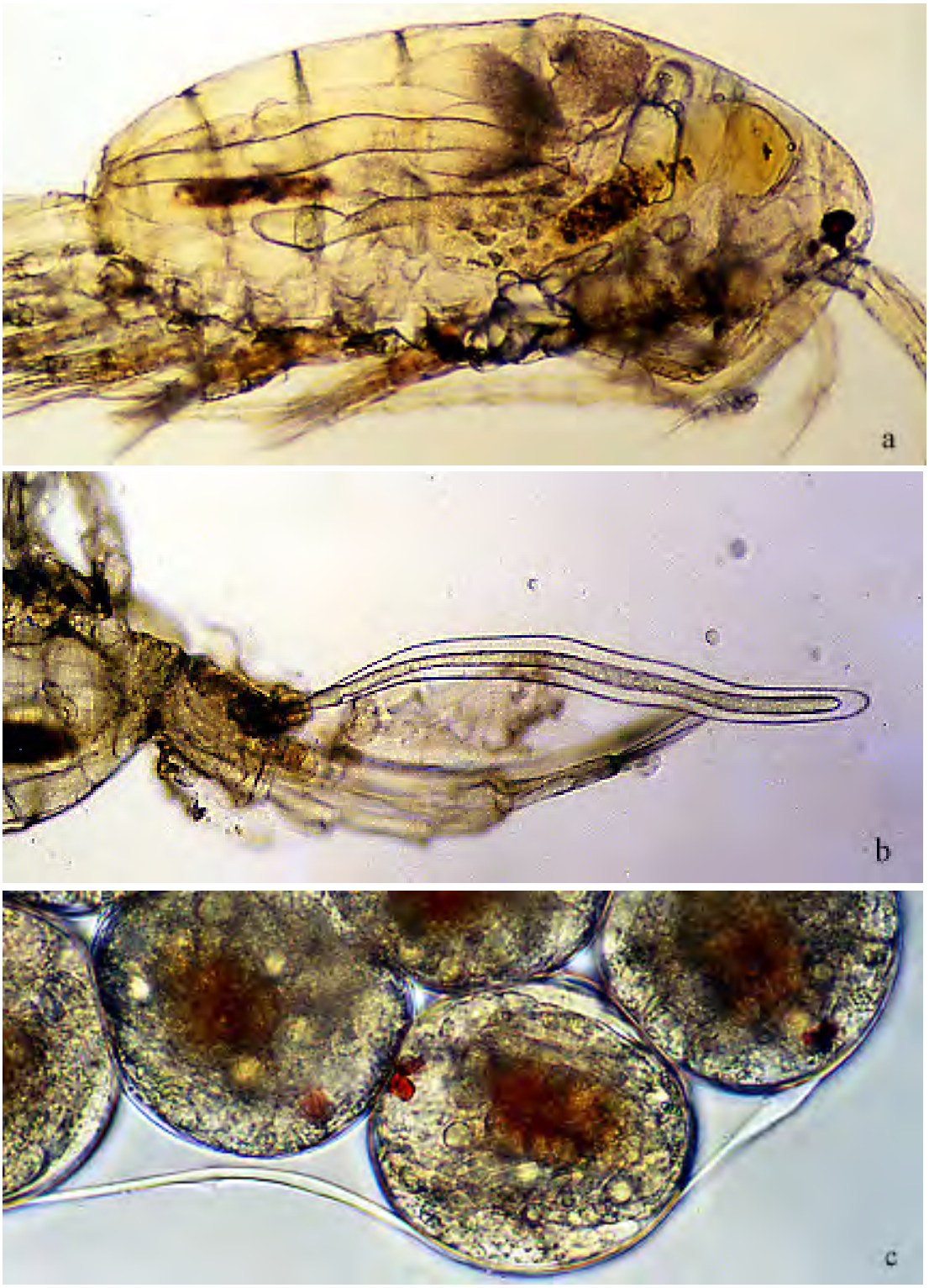


Figure 8. Photomicrographs showing aspects of reproduction and development in *G. imparipes*, a) adult male showing a spermatophore within the prosome, b) spermatophore attached to the genital segment of an adult female, c) developing embryos held together in a clutch by a membrane.

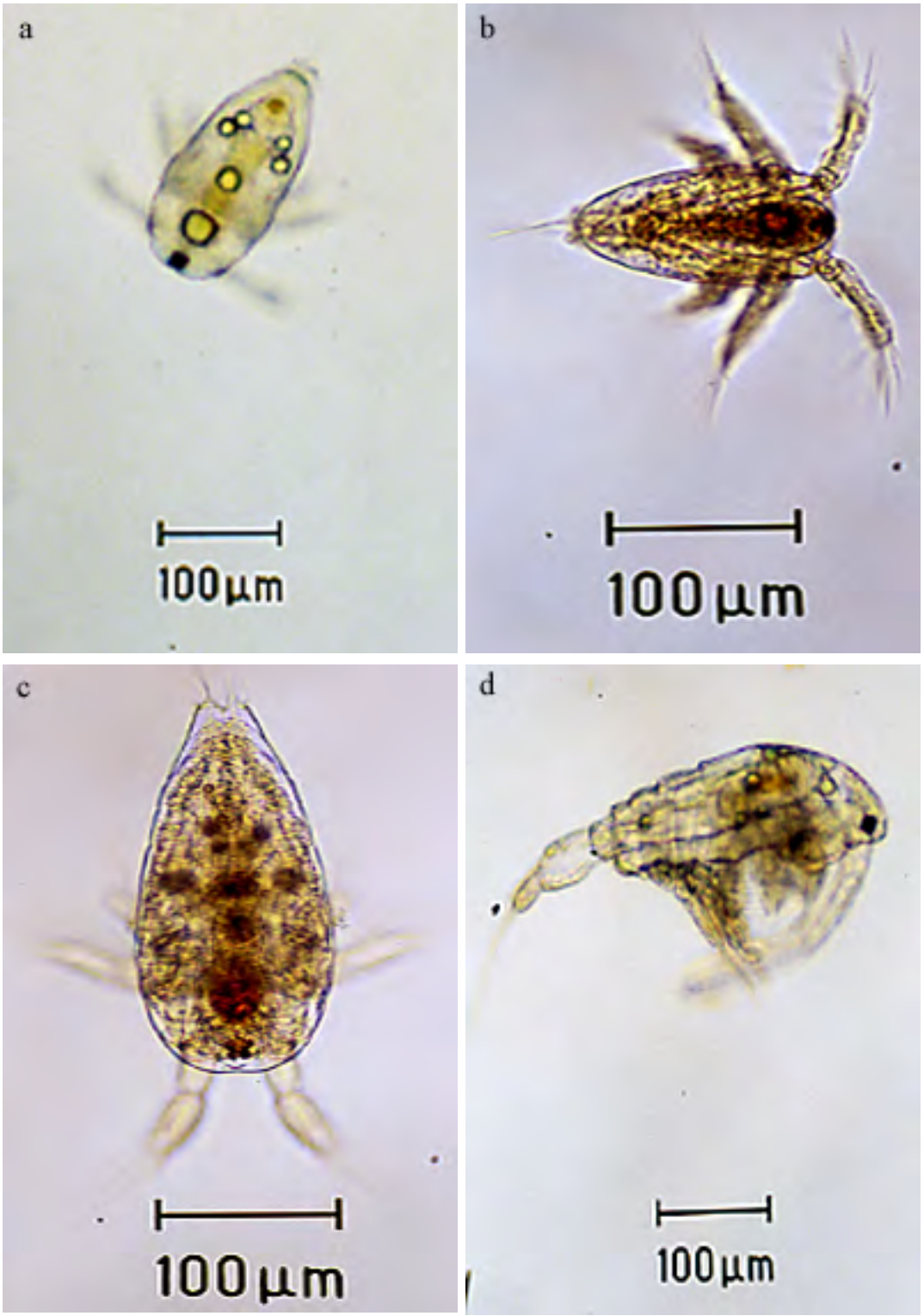


Figure 9. Photomicrographs showing developmental stages of *G. imparipes*, a, b &c) early stage nauplii, d) early copepodid.

Figure 10. Photomicrograph of adult *G. imparipes* showing lipid stores stained with Sudan IV.

Gladioferens imparipes in laboratory cultivation

Natural selection in the estuarine environment has resulted in *G. imparipes* having many attributes which make it suitable for intensive cultivation and particularly useful as a species to use as food for larval fish. The section below describes some aspects of *G. imparipes* biology, which should be understood if cultures are to be established.

Physical tolerances

Salinity

Tolerance of a wide range of salinity and tolerance of sudden salinity change are not essential for effective cultivation because salinity can easily be controlled. However, this tolerance is convenient. By routinely keeping copepod cultures at 27 ppt. some protection is afforded from adventitious marine invader species. If cultures become contaminated by unwanted invaders, e.g. some species of harpacticoid copepods, ciliates, rotifers, worms (Figure 12), the invaders may be removed by subjecting the culture to a sudden salinity change which causes death of the invaders but is within the range of tolerance of *G. imparipes*.

Temperature

Although *G. imparipes* can survive through a wide temperature range (6 - 28°C) the best balance of animal health and culture production occurs between 20 - 25°C. At lower temperature growth and egg production rate decreases and at higher temperature water quality in cultures is difficult to maintain. Animals can be maintained within the recommended temperature range and then used at higher temperature.

Tolerance of low temperature (~6°C) by *G. imparipes* through all life history stages allows convenient refrigerator storage of cultures which are not currently required for full production and allows for the development of nauplii to be deliberately delayed while animals are stored for later use.

Dissolved oxygen

Adult *G. imparipes* are able to tolerate at least brief exposure to low levels of dissolved oxygen (DO). At salinities of 17.5 and 9 ppt., 50% of adult animals will survive for 48 hours in water with DO at 20% and 15% of saturation, respectively. Even low aeration in intensive copepod cultures prevents DO from falling below these stressful levels. Loss of intensive *G. imparipes* from falling DO levels during power failures is unlikely.

Water quality

Water quality is a major issue whenever aquatic animals are kept in a confined volume. Animal excretory products and decomposition products from unused food and faeces result in rising levels of dissolved organic compounds and nitrogenous wastes. When fish or other macro fauna are kept, water quality can be maintained by frequent exchange or by a high rate of flow-through. When micro fauna are kept e.g. copepods of 1mm adult body length and 125 µm nauplius length, any water removed from the container must be passed through retaining screens down to 50 µm. Maintaining high flow through fine screens is not compatible with dense cultures of copepods. For most copepod cultures, water remains as a batch volume for extended periods. In these circumstances some water quality deterioration is inevitable.

Precise tolerance of *G. imparipes* to various nitrogenous waste products is not known. Rates of water exchange described in later sections allow for high production of nauplii for feeding to larval fish. However, as for the majority of aquatic animal maintained in culture, health of copepods will decrease gradually as the concentration of nitrogenous (and other) waste products increase. Thus, higher rates of water exchange will improve water quality and result in higher rates of nauplius production. Concentration of nitrogenous wastes should be kept as low as clean water supply and/or water conditioning equipment allows.

Food requirements

G. imparipes, at all life history stages, feeds by removing small particles from suspension. In the natural habitat food particles probably include micro-algae and organic debris. In cultivation many species of salt water micro-algae are an adequate diet for animal survival, although not all allow for maximum growth and reproduction rates of the copepods.

If copepods are to be used as food in fish production their biochemical composition must include those compounds which are essential in fish nutrition, especially the essential unsaturated fatty acids (HUFA). With small particle feeding (herbivorous) copepods, the composition of the body lipids reflects that of the algae on which they have been feeding. *G. imparipes* cultures can be maintained at high production by using species of algae which can be reliably grown and contain the appropriate biochemical profile, e.g. *Isochrysis galbana* (T-Iso), *Chaetoceros muelleri*, *Pavlova* sp. *Rhodomonas* sp. *Heterocapsa* sp. Of these, T-Iso and *Pavlova* sp. are recommended because they are widely used in aquaculture, are relatively simple to culture and provide a suitable diet for the copepods.

Faecal waste

When food is available *G. imparipes* produce faecal waste as membrane bound pellets that sink to the bottom. With abundant food, pellet production may be up to 3 pellets.copepod^{-1}.hr^{-1}, resulting in accumulation of debris on the bottom of the container. Decomposition of faecal pellets by bacteria and other micro-organisms releases dissolved organic matter and soluble material (especially nitrogenous products) into the water and compromises water quality. Most of each individual faecal pellet decomposes within 3 days. To maintained water quality for dense cultures of copepods, accumulated faecal debris should be regularly

removed from the bottom with a siphon and/or a continuous foam filter (described in a later section) should be used to trap debris before it sinks to the bottom.

It is important to achieve a balance between providing adequate food for animal production and excess food that is converted to faecal debris and soluble wastes.

Embryo clutch retention

In different species of calanoid copepods the females either broadcast fertilised eggs into the water column (e.g. *Acartia*, *Labidocera*, *Centropages* spp.) or they hold them together in a sac located on the ventral surface of the urosome while the embryos develop (e.g. *Gladioferens*, *Eurytemora*, *Diaptomus* spp. Figure 5). In the natural habitat, different risks and benefits are probably associated with each of these strategies.

Eggs from broadcast spawners will normally settle with faecal and exoskeleton debris on the bottom of culture vessels. These eggs risk predation during the incubation period by any non target benthic fauna which may have invaded the culture. Unless debris can be left to accumulate for the duration of the incubation period, eggs will be removed with the debris during cleaning operations. Either way, some eggs will be lost or must be kept for hatching in a separate culture container.

For *G. imparipes*, and other calanoid copepods that retain embryo clutches, nauplii are free swimming almost immediately following hatching. Eggs and nauplii are at no risk of being smothered by sedimented debris, and debris can be removed from the bottom of containers with no loss of unhatched embryos and only minimum loss of nauplii.

Cannibalism by adult copepods.

Some species of calanoid copepods have feeding mechanisms allowing collection of a wide size range of food particles. Food for these omnivorous copepods (e.g. *Acartia* spp), may include micro-algae, ciliates or small organisms such as copepod nauplii. With these copepods, cannibalism of free eggs or nauplii may occur in crowded conditions. Where these copepods are kept in cultivation, losses to cannibalism must be accepted or provision must be made to maximise the proportion of eggs that hatch separately from the adults.

G. imparipes, as a strictly small particle feeder, can coexist at high densities with nauplii and not cause them damage.

Nauplius response to light.

The nauplii of *G. imparipes,* as with most other calanoid copepods, are strongly photopositive (ie swim towards directional light). In the natural environment, nauplii stay in the photic zone of the water column by day. In artificial conditions they have a strong behavioural response towards directional light.

In intensive cultivation *G. imparipes* should be kept in complete darkness. This results in nauplii being evenly distributed through the water and avoids them continually using metabolic energy by swimming towards a light source and congregating in one section of the container.

The positive response to light can be exploited in the collection of nauplii. A light positioned in the water of a culture, behind a 150 µm screen will attract nauplii but exclude later life history stages. Nauplii can then be ducted from the zone in which they congregated to a separate container.

Free swimming nauplii which move towards the surface of containers which are illuminated from above are visible to pelagic fish larvae and therefore vulnerable to being taken as food.

Nauplii of harpacticoid copepods e.g. *Tisbe* & *Tigriopus* spp. generally display negative responses to directional light. These nauplii tend to swim to the bottom of culture vessels where they feed amongst settled detritus. In this location, they are not readily available as food for pelagic fish larvae that most often feed in the open water.

Holding behaviour

The behaviour of late copepodid and adult stage *G. imparipes,* which has them holding to surfaces for extended periods of time, may contribute to their success in cultivation. With a large proportion of the population holding to the sides of the container and only swimming occasionally, minimum damage to fragile appendages is caused by contact between individuals.

Holding behaviour may also contribute to efficient conversion of food energy to growth. While animals hold to the sides of the container energy is not being expended in continual swimming. As attached animals resist the gentle water currents rather than swimming within the currents their body surfaces are effectively ventilated and suspended food particles are brought within reach for collection with less energy expenditure. Gas exchange may also be more efficient with the animals remaining stationary against a moving current of water. In large volume copepod culture, internal surface areas should be increased by the addition of synthetic substrate, such as plastic mesh.

Exoskeletons and ecdysis

During development *G. imparipes* undergoes ecdysis (moulting) between each stage. The moults contribute, with faecal pellets, to the load of debris in a culture. Because the nauplius and copepodid life history stages each last only a few days at most there is little opportunity for invasive organisms to foul the exoskeletons and cause harmful infestations. Once the adult stage is reached there is no further moulting. If an adult animal lives for weeks in a culture with accumulated debris and low water quality it is at risk of becoming infected by micro-organisms growing on the exoskeleton (Figure 13).

Maintaining cultures in darkness minimises the risk of infection from surface growing algae but other infections can occur. It is best to terminate old cultures containing invasive micro-organisms and establish new cultures using nauplii or early copepodids.

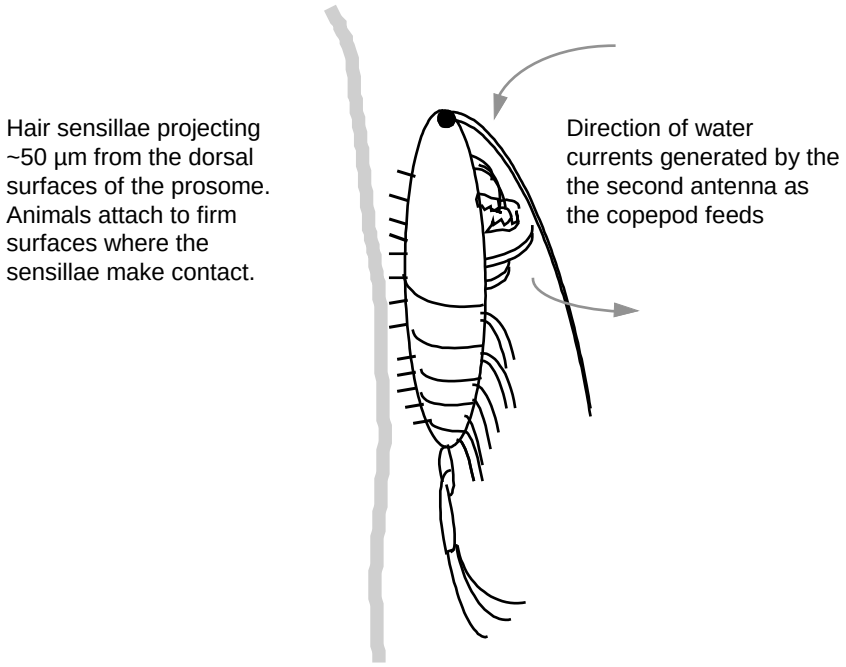
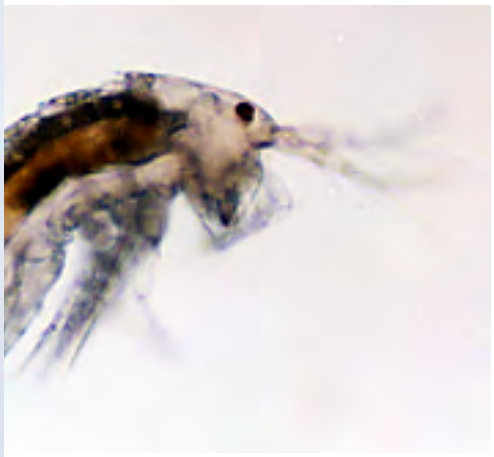
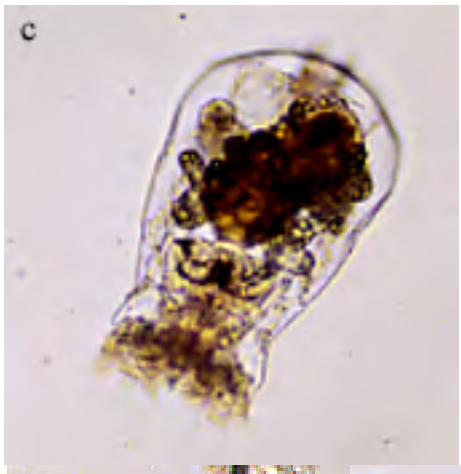


Figure 11. Diagram showing how hair sensillae on the dorsal surface of the prosome of *G. imparipes* are involved in attaching the animal to firm surfaces. Feeding currents continue while animals are attached.


b




c


a
10µm


b
10µm


c

Figure 13. Photomicrographs showing the exoskeleton of adult copepods with infestations of ciliates; a) apostome ciliates, b) stalked peritrichous cilliates, c) severe infestation of stalked ciliates that have accumulated debris.

Culturing unicellular algae

Reliable supply of algae must be arranged before copepod cultures can be established. As mentioned previously, algal species recommended for culturing *G. imparipes* are *Isochrysis galbana* (T-Iso strain) and *Pavlova lutheri* or *P. salina*. T-Iso is the preferred choice. Starter cultures of algae can be purchased from the CSIRO Marine Research laboratory (ph: (03) 6232 5316, email: microalgae@marine.csiro.au) in Hobart. Alternatively, algae may also be obtained from local aquaculture facilities. Recent advances in the preparation of algal concentrates may reduce the need for continuous supply of fresh algal if the concentrate proves to be of acceptable quality.

This section is intended only as a guide to culturing algae for the purpose of maintaining copepod cultures. There are some excellent publications that discuss the topic in much more detail and these are included in the Further Reading section.

Culture volumes and vessels

The quantity of algae needed to maintain copepod cultures depends on the level of copepod production required. As a general rule, the total volume of algal cultures must be at least 25% of the volume of copepod culture if high copepod production is required. This rule applies only when algal cultures are grown to a high density (>5 million cells per ml for T-Iso). If algal cultures are lower than this, greater volumes are required. The table below indicates the volume of algal culture required to supply copepod cultures of different volumes.

Volume of copepod culture	Minimum volume of algal culture required
0.1-0.5L	2L (2x1L)
30L	8L (2x4L)
60L	16L (4x4L)
500L	80L (5x20L)
1000L	250L (5x50L)

Glass conical flasks are ideal (but expensive) for volumes of algal culture up to 5 L. A much cheaper option are clear softdrink (PET) bottles, which are very effective. Standard glass vessels are generally not suitable. Larger culture volumes can be grown in any food-grade quality translucent plastic containers, such as those used for bulk filtered water (eg Aquavital). White cylindrical drums are also suitable, although they require strong lighting to achieve good algal growth. Clear plastic bags, supported in cylinders of steel mesh, can also be used. Bags are generally used once only.

Good hygiene is essential for successful culturing of algae. Vessels can be cleaned by swirling a clean wet kitchen scourer inside and by vigorous hosing. Avoid harsh scratching of the inside of plastic vessels with the scourer. After mechanical cleaning flasks should be soaked with a solution of cleansing agent or bleach. Household soap or detergent must not be used. Persistent scale deposits can be shifted with a brief soak in acid (5% nitric, hydrochloric or oxalic) followed by very thorough rinsing. Store vessels dry with a lid to exclude dust. Aeration tubing must also be kept very clean, using the above techniques.

Culture media

Algae differ in their requirements. Some species are very robust and can be grown with fertilisers thar are formulated for horticulture (eg Aquasol). Others have very specific requirements and are difficult to maintain. For many species, a chemically defined medium developed by Guillard provides for strong growth. This medium, very widely used, is referred to as Guillard's f/2, often abbreviated to f/2. Guillard originally formulated Medium f, but found it more effective with half of the nutrient concentration, hence f/2.

Various procedures can be used for preparing f/2. The most important requirements are that the chemicals end up in the correct proportions and that cleanliness is maintained at all stages of the process. The procedure that is described in Appendix 2 has been used very effectively to maintain algae in culture volumes ranging from 200 ml - 20 l. The procedures have been simplified, where possible, to keep labour to a minimum.

In principle, the procedure involves preparing the nutrients for f/2 as a concentrated solution and freezing 20 ml aliquots in snap top plastic vials. These aliquots can be placed directly into exact volumes of prepared seawater to give f/2 media. The size of the algal culture vessel will determine the concentration of the nutrients in the frozen aliquot. The recipe given in Appendix 2 is for making 50 vials, each containing enough nutrients to make 2 L of f/2 media. The quantities should be adjusted if larger algal cultures or more than 50 aliquots are needed. For example, to make 50 aliquots with each aliquot containing nutrients for 20 L of f/2 media, use 10 time the amounts listed in Appendix 2.

The 'ice block' procedure for Guillard's f/2 has a number of advantages. Weighing of chemicals and preparing the concentrated nutrient solution can be completed in one session, providing nutrients for an extended period of time. Storing prepared medium in a frozen state minimises the chance of fungi or bacteria causing contamination because each frozen aliquot remains sealed until it is used. It takes very little time to add an ice block to water and have f/2 ready for use.

Preparation of culture water

Seawater can be collected from any convenient clean coastal place. Calm clear water is best. Water with a high load of particulate matter should be avoided, as should polluted locations. All containers for carrying or storing seawater must be clean. It is best to use dedicated containers as you can be sure that they have not been used to store harmful chemicals. Collected seawater should be stored in darkness for at least 10 days before being used for algal culture. Synthetic seawater, available as commercially packaged salts, has been used successfully for algal culture. Many algae that normally grow in seawater will also grow successfully in seawater that has been diluted by 50% with carbon filtered tap water. This may be useful if seawater is not readily available.

Seawater must be filtered (~ 5µm) and sterilised before being used in cultures. Volumes up to 5 L can be heated over a gas flame or hot plate to ~ 80°C in a glass flask with a

dust proof cover. Aluminium foil is effective as a cover. Seawater should not be boiled as this will alter the chemistry. Small volumes can be heated in a microwave oven. Some literature suggests autoclaving but this is only needed for specialised work with very sensitive species of algae. If water is heated twice, at 24 hour intervals, it will definitely be clean enough for general algal culture. In areas with strong sunlight, glass or PET plastic containers of seawater in direct sunlight will receive UV light and radiant heat. If this water reaches ~50°C it can be used for algal culture when it has cooled.

After sterilisation it is best to allow water to cool slowly by standing for at least 24 hours. This will allow some CO_2 to diffuse from the atmosphere into the water.

For larger volumes of water, heating is inconvenient and uneconomical Chlorination/dechlorination is an effective and easier way to sterilise. For this technique, bleach (sodium hypochlorite) is use to kill all life in the culture water. Sodium thiosulphate is then used to deactivate the remaining bleach.

Liquid pool chlorine is the most appropriate source of bleach as it contains a high concentration of active chlorine (125g/L) and has no added surfactants or perfumes. Sodium thiosulphate should be prepared as a 100 g/L stock solution. To sterilise algal culture water, add liquid pool chlorine at the rate of 1.5 ml per 10L of water (20 drops ~ 1 ml). It is best to place the culture water in the culture vessel and then add the aeration tubing so that both are sterilised along with the water. Cover the vessel and leave for 24 hours without aeration. After 24 hours, add the thiosulphate solution at the same rate (ie 1.5 ml per 10L) and aerate vigorously for an hour before adding nutrients and innoculating with algae.

Culture procedure

Each species of algae must be kept as a small volume (e.g. 150 ml) #1 culture showing the date when it was set up. These #1 cultures should be grown with f/2 at low light intensity in a clean location. Under these conditions the algae will grow slowly and will last at least one month before it is necessary to sub culture them. These #1 cultures are only to be opened under very clean conditions to inoculate a new #1 culture which, in turn, will not be opened until it is time for the next sub culture. Old #1 cultures can be used as the inoculum for starting larger cultures.

Prepared culture vessels containing water should be inoculated from a clean #1 algal culture and an ice block added with the correct f/2 nutrients for the final volume. Cultures grow rapidly if the initial inoculum is 10-20% of the final volume. A 4 L culture should be inoculated with 0.4 - 0.8L of culture if maximum growth is required. A smaller inoculum will take longer to produce a dense culture.

If large volumes are needed, increase the volume from small cultures in a series of steps, using the smaller cultures to inoculate (at the rate of 10-20%) progressively larger volumes of medium. Thus to establish a 50 L algal culture, it is necessary to grow (in sequence) cultures of approximately 150 ml, 1 L, 10 L and 50 L in volume, using each culture to inoculate the next.

Culture conditions

Aeration

Algae require CO_2 to photosynthesise and grow. For cultures in small flasks, enough CO_2 will diffuse from the atmosphere across the surface if the flask is only 1/3 full. Cultures larger than 500 ml must be aerated to provide movement and uptake of CO_2 if maximum growth is required.

Air for algal cultures can be supplied by any standard diaphragm-type air pump. Air should be passed through a cotton wool filter to minimise the entry of dust particles into cultures. Black capillary poly-pipe (0.025" ID) is effective for aerating small cultures. Glass tubes with flow control valves or taps are best for cultures >2 L. If glass tubes are used they should be suspended just above the bottom of flasks, or a short length of silicone tubing should be put on the tip to prevent the glass tube from scratching the flask. Use of airstones, particularly those that produce fine bubbles, are not recommended as they will produce foam on the surface of the culture. This foam will trap algal cells.

Lighting

Most marine algae grow well with light from cool white or ‘plant light’ fluorescent tubes. Metal halide lights are also suitable. Natural sunlight can be used very effectively to grow algae, although care must be taken not to over-heat the cultures. Quartz halogen and standard tungsten lights are NOT suitable. Lights should be placed to the side or above cultures and ventilated sufficiently to avoid cultures being overheated. The intensity of light received by a culture depends on the number of fluorescent tubes, the distance of the tubes from the culture, the transparency of the culture vessel and the cell concentration in the culture. To achieve dense cultures (which block the passage of light) in 4L flasks, two fluorescent tubes are needed, ~25 cm from the flask and the culture must be vigorously aerated. High density cultures can be achieved in 20L vessels with 6 fluorescent tubes illuminating one side of the vessel. Larger cultures are best grown using either multiple fluorescent tubes, metal halide lamps or sunlight. Lighting can be continuous or intermittent, with 16 hours light: 8 hours darkness.

Temperature

Growth rate of algae is temperature dependent between ~15 °- 25°C. It is best to keep #1 cultures at lower temperatures to reduce growth and minimise maintenance.

Isochrysis is of tropical origin and grows well at higher temperatures but culture conditions are more difficult to maintain at >25°C.

Use of cultures

When an algal culture is at high cell density it can be used to feed animals. If the entire volume is not used on one day, the remainder can be used later. When removing algae from cultures, take care not to contaminate the remainder. Pour algae from small containers into a clean jug rather than directly into copepod cultures to avoid the risk of copepod water splashing up into the algal culture. For large algal cultures, use a clean siphon or pump.

Generally, it is not recommended that cultures be 'topped up' with new medium. However, on a day by day practical level, topping up a culture may be successful for a short time. If cultures are dense, all of the nutrients have been taken up from the medium and the top-up water must contain enough nutrients for the entire volume. For example, if a 2 L dense culture is to be increased to 4 L, add sufficient nutrients for a 4 L culture. If sediment has accumulated, the culture should not be topped up. It is time for a new culture in clean glassware.

Trouble shooting

Most problems with algal culture can be traced to errors in preparing the culture medium or to lapses of hygiene.

If the balance of nutrients is incorrect, one essential chemical may be depleted. The algal cells may continue to photosynthsise but may be unable to undergo cell division. Under these conditions energy rich compounds leak from the cells and the culture medium becomes an ideal environment for bacteria. A smelly bacteria culture replaces the algae. Always double check the quantities and calculations when preparing chemicals for culture media

Unless conditions are very clean, which is rarely possible in a busy working environment, it is difficult to completely prevent cultures from occasionally becoming contaminated. This is why working cultures should not be repeatedly topped up. The chances of contamination increase each time the culture is opened and, especially with air being blown in, the longer a culture lasts the longer a population of unwanted organisms has to grow. Cultures should be used, the residues discarded and the culture vessels thoroughly cleaned and stored clean. Maximum care must be taken with the #1 cultures so that they do not become contaminated.

Culture procedures for *Gladioferens imparipes*

Procedures used to maintain *G. imparipes* in artificial conditions must meet the life requirements of the animals but can, within limits, be modified according to the objectives of the program. Descriptions are given below of small scale culture procedures using static batches of water, larger scale procedures in which water is used, reconditioned and then re-used, and for situations in which clean sea water is readily available, a large scale flow-through system.

Source of copepods

G. imparipes may be collected from the natural environment or obtained from established cultures (see Appendix 3).

Static cultures of different volumes

G. imparipes can be conveniently maintained in static (batch) cultures of different volumes with either replacement of water (for small volume cultures) or recirculation of water (larger cultures). Maintenance procedures for cultures of different volume are described below. For all culture volumes, animals may be kept in any salinity within the range 5 - 35ppt. A salinity of 27ppt (75% of normal seawater) is convenient. Temperatures within the range 15 - 25°C are acceptable and should be chosen according to the rate of growth that is required. Many species of marine unicellular algae (e.g. *Isochrysis* (T-Iso), *Chaetoceros*, *Rhodomonas*, *Pavlova*, *Heterocapsa*) are effective as food. T-Iso and *Pavlova* are recommended.

0.1 - 0.5L Static Cultures

Small cultures of 100ml - 500ml can be used to maintain a population with minimum maintenance or to observe the growth, reproduction or health of a small group of animals.

Culture vessels

Plastic containers manufactured for single use food packaging are effective. From new, these can be washed and reused but are cheap enough to discard.

Contents of clear or translucent round 150ml containers can be examined with a conventional stereo microscope by using transmitted light (from beneath the subject).

Stock density

Nauplii stocked at ~1.ml^{-1} will growth to maturity in 150ml containers. Adults at this density will remain alive and, if fed, continue breeding and eventually become stressed through crowding.

Sub Culture

Non quantitative sub culture can be achieved by pouring ~20 ml from an advanced culture into a clean or new container (ensuring that some copepods are transferred) and filling the new container with clean water.

Slower culture growth, better hygiene and therefore less maintenance, can be achieved by reverse filtration of culture water through a 125 - 150 µm screen to remove small nauplii into a new or clean container but leave adults and copepodids behind (Figure 14). Water from the second container is then reverse filtered to waste through a 50µm screen, leaving the nauplii in a small volume of water. Clean water and food is then added to the nauplii.

Aeration

Small cultures remain healthy without aeration of the water. Very gentle aeration from a capillary tube just breaking the water surface will prevent a surface skin of organic material. Aeration will increase evaporation, thus vigorous water movement from aerators should be avoided.

Water renewal

At a stocking density up to 1 adult.ml^{-1}, a culture will remain viable with weekly exchanges of water and container. If biological activity has resulted in an obvious surface skin of organic material, this can be removed by stretching a folded paper tissue across the surface of the water from one side of the container to the other and drawing it across the surface. This skims the accumulated material from the surface.

Temperature control

Fluctuation in temperature can be minimised by suspending containers in a water bath. For small round containers, holes cut in a floating sheet of styrofoam will hold the containers securely in position (Figure 15).

An immersion heater in the water bath can be used to control temperature above ambient. In this case, the water should be stirred using a pump or airstone so that a layer of heated water does not form at the surface and overheat the animals. The water level within the animal containers should be slightly higher than that of the heated waterbath. This causes gentle stirring of the water in the animal containers as the surface loses heat to the atmosphere and convection currents are established.

Food

The frequency and quality of food will control the population growth rate. For minimum maintenance at low temperature, weekly feeding is adequate. Maximum growth can be achieved with daily feeding. Cultured algae should be added as food until water is just discernibly coloured. For T-Iso and *Pavlova*, this gives a cell concentration of 1-$2x10^5$cells.ml^{-1}. To determine whether adequate food is being provided, see 'Assessing copepod cultures'.

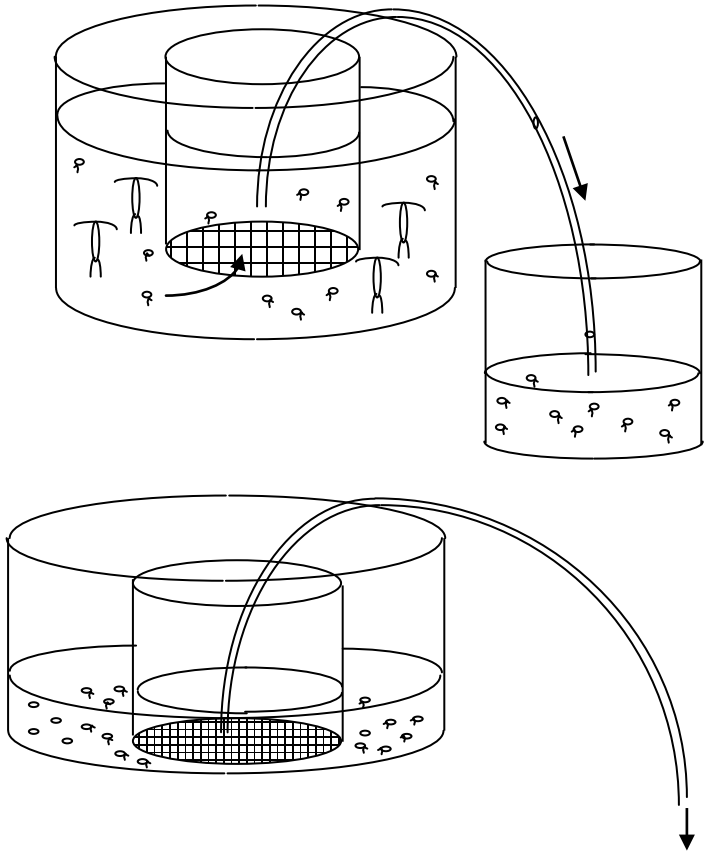


Figure 14. Top, reverse filtration through a screen (125 - 150 µm) to remove nauplii and leave adult copepods. Bottom, reverse filtration through a 50 µm screen to reduce the water volume and concentrate nauplii.

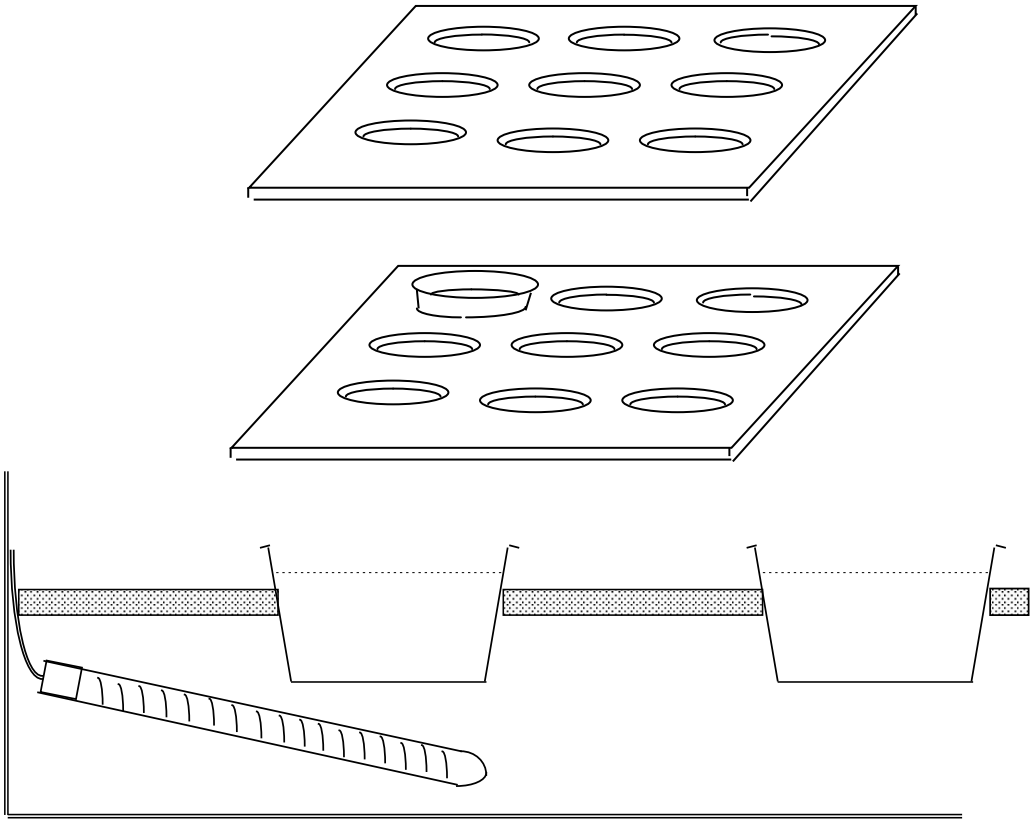


Figure 15. Top, 10 mm styrofoam sheet with holes to support 150 ml bowls in a water bath. Bottom, cross section diagram showing an arrangement of 150 ml bowls supported on a styrofoam sheet in a water bath with an immersion heater.

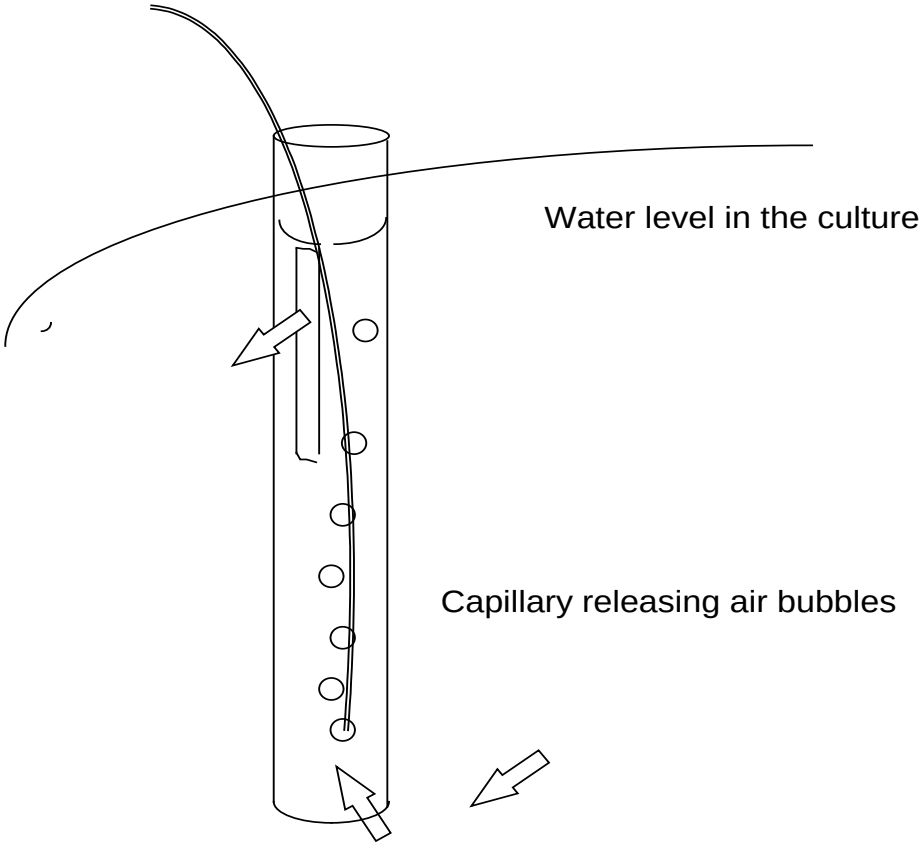


Figure 16. Vertical stand pipe providing for water circulation by air lift. Arrows show the direction of water movement.

1 - 60 L Static Cultures

Copepod cultures in the volume range 1 - 60 L can be kept to provide up to a few thousand adult animals or a few tens of thousands of nauplii for research or to provide initial stocks for larger culture vessels.

Very healthy cultures are obtained by growing a single cohort of nauplii together to maturity. After these animals reproduce, cohorts of nauplii can be removed regularly. Unless nauplius removal is efficient a mixed age culture will result.

Culture vessels

Successful cultures can be kept in high quality plastic containers with the proportions of buckets or drums. Opaque black containers with loose fitting lids are effective. Commercially produced containers of capacity 10L, 30L and 60L have been used successfully. Some plastic bags of high quality can be used to line culture vessels. Bags holding ~ 4L, produced for storing frozen food, are effective for small cultures. Some high grade white rubbish bin liners are acceptable for larger volumes. Lining vessels with disposable plastic reduces the labour of cleaning vessels between cultures.

Stock density

Adult copepods at ~1.ml^{-1} will remain healthy and productive. Nauplius production is likely to be less at lower and at higher adult stock density.

Aeration

A vertical stand pipe with air injected via a capillary will move water and cause oxygenation. Figure 16 shows a standpipe with a vertical slit directing air lifted water to cause a rotational current in the container. A single air lift is adequate for cultures up to 60L.

Water renewal and nauplius collection

Daily replacement of ~ 30% of the water volume with clean water will provide adequate water quality for healthy animals. Less frequent water renewal may reduce production but animals will survive, especially if stock density is low. Water quality will be improved if faecal debris is removed with a siphon every 2-3 days. Some copepods will be removed in this process. If the water and debris that was removed is allowed to stand for a few hours, debris will settle and most of the copepods can be poured back into the culture.

Nauplii can be collected from the culture vessel at the same time as removing dirty water. By reverse siphoning through a submerged 150 µm screen (Figure 14), water containing nauplii only can be collected. Nauplii can then be concentrated using the method illustrated in Figure 14. *G. imparipes* nauplii are attracted towards directional light. By turning the aeration off and shining a light onto the surface of the culture water for 10 mins, most of the nauplii will congregate near the surface. These can then be removed in the top ~30% of the water volume in the manner described above.

Sub culture

For optimum culture health it is best to work with animals of known age which have grown together from a single cohort of nauplii.

Cultures should be recommenced with clean containers at least monthly and preferably after two weeks. Any culture containing animals which have been adult for four weeks or more will have debris from dead copepods.

New cultures should be inoculated with a cohort of nauplii collected from the previous culture.

Temperature control

Temperature above ambient can be maintained with thermostatically controlled immersion heaters as used in aquaria. Prolonged temperature above 25°C should be avoided by the use of air conditioning.

Food

Food rations of unicellular algae can be quantified by cell counts using a compound microscope. The amount of food required depends on the copepod biomass present in each culture. When cultures are stocked with nauplii at a density of 1/ml, feed rates generally follow a standard protocol; young nauplii are fed at the rate of $2x10^4$ cells/ml/day increasing gradually to $1x10^5$ cells/ml/day when copepods have reached maturity. This higher feed rate is maintained during the adult life of the copepods while nauplii are being produced. However, this higher feed rate can be varied daily in response to subjective assessment of water clarity in copepod culture vessels prior to the addition of food (see 'Assessing copepod cultures'). If the water appears turbid, the feed rate is decreased to 6 - $8x10^4$ cells/ml/day and if it appears very clear, the rate is increased to 1.2 - $1.4x10^5$ cells/ml/day. Feed rates can be increased further if a very high density of adult copepods is present. However, in this case decreasing water quality will necessitate more frequent water exchanges.

Exact feed rates are not essential. Operators will quickly learn to estimate the cell density of algal cultures from their apparent colour and the amount of algae required by copepod cultures from subjective assessment of copepod biomass and pre-feeding water clarity. This will enable an appropriate feed ration to be calculated.

500L culture with recycled water

In locations where clean sea water cannot be supplied reliably and at low cost, dense populations of *G. imparipes* can be maintained in reconditioned water. Regular collection of nauplii involves removal of, for example, half of the water from the culture vessel. This may be reconditioned before being re-used.

A culture of 500 L can produce 0.5- $1x10^6$ nauplii daily but requires regular monitoring to maintain animal health and production. Procedures for collection of nauplii and recycling water can be carried out manually or can be controlled electronically. Figure 17 is a summary diagram of a culture system based on a 500 L vessel, recycled water and Programmable Logic Control (PLC) of procedures to collect nauplii and recondition the water. A list of procedures for establishing a 500 L culture is provided in Appendix 4.

Culture vessels

Five hundred litre cylindrical containers with a conical base (Figure 17) have been used effectively. When combined with a circular flow of water, a conical base serves to concentrate settled debris in the centre of the vessel. It can then be easily removed.

Stock density

Nauplii stocked at 1 nauplius.ml^{-1} as a single cohort will produce an adult population at close to 0.5 copepods.ml^{-1}. Higher stock density does not appear to increase nauplius production.

Internal surface

The ratio of internal surface area / total water volume is much smaller for a large volume container than for a small container. Because adult *G. imparipes* hold to internal surfaces high stock density in large volume containers results in very high number of animals per unit of internal surface area. This may be deleterious. Provision of addition internal surface area has been beneficial in 500 L vessels. This has been provided by suspending cylinders (~25cm diameter, 40cm height) of black plastic mesh (4mm aperture and 2-3mm strand thickness) in the culture container.

Aeration

Air injected into two vertical standpipes with vertical slits close to the water surface provides for oxygenation, vertical circulation and for a gentle rotational current. Air is turned off before collection of nauplii to allow internal currents to stop and debris to settle. Air supply can be turned off by electronic control of a solenoid side valve. The valve is normally closed, directing air to the capillaries. When open, air is released.

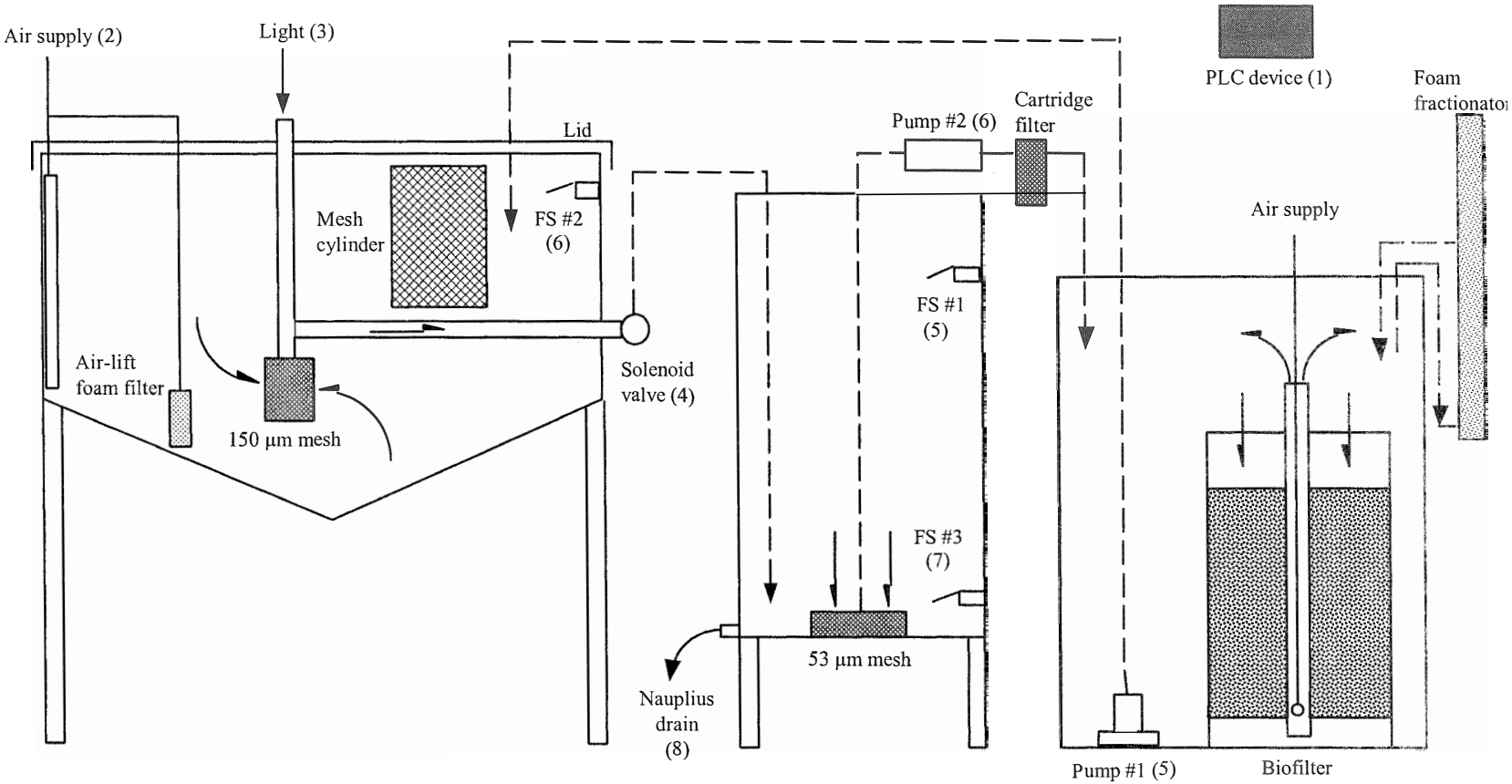

Air supply (2)
Light (3)
Lid
Mesh cylinder
FS #2 (6)
Air-lift foam filter
150 µm mesh
Solenoid valve (4)
Nauplius drain (8)
Pump #2 (6)
Cartridge filter
FS #1 (5)
FS #3 (7)
53 µm mesh
PLC device (1)
Foam fractionator
Air supply
Pump #1 (5)
Biofilter
500 l culture vessel
200 l collection vessel
500 l sump

Figure 17 (previous page). Schematic diagram of the automated 500 L copepod culture system. Nauplius collection and water recycling procedures are described as follows;

(1) Harvest sequence initiated by pressing 'start' button on the PLC device.
(2) Air supply to the culture vessels turned off for 5 min to stop water movement.
(3) Light turned on for 10 min to illuminate 150 µm mesh submerged in culture vessel.
(4) Solenoid valve opened so that water (containing nauplii) siphons through 150 µm mesh into harvest vessel.
(5) Float switch (FS) #1 activated which closes solenoid value, turns light off and turns pump #1 on.
(6) FS #2 activated which turns pump #1 off and pump #2 on (drawing through 53 µm mesh).
(7) FS #3 deactivated which turns pump #2 off and air supply on.
(8) Concentrated nauplii drained from harvest vessel.

Sub culture

Maximum production has been achieved from cultures which were started with a cohort of nauplii and kept for ~21 days after commencing daily collection of the next generation of nauplii. Cultures left for longer will contain mixed age adult animals with increasing chances of debility from infections. If two 500 L culture vessels are kept in parallel, a cohort of nauplii collected from newly mature adults in vessel can be used to commence a culture in the second. Alternatively, nauplii from a smaller culture can be used to initiate a new 500 L culture.

Water renewal and nauplius collection

New copepod cultures that have been stocked with nauplii can be maintained as static cultures until the copepods have reached maturity and commence reproduction. In mature cultures, nauplius collection should be carried out either daily or every second or third day. If left static for longer than this, deteriorating water quality will begin to have an adverse effect on copepod health.

Each nauplius collection removes water from the main culture vessel. Although the volume removed is not critical, 200 L is very effective at removing most nauplii and maintaining water quality. After nauplius collection, the volume can be made up with clean water, either freshly diluted seawater (27 ppt) or water previously treated for 24 hours with a protein skimmer and biological filtration.

In a recirculation system water can be cycled (as in Figure 17) from the main culture vessel, through nauplius collection to a renewal system and then back to the culture tank. An in-line filter (1 µm) between the nauplius collection tank and the water renewal system reduces the organic load in the culture. If clean seawater is readily available, a preferable protocol is to refill the main culture vessel with clean seawater after nauplius collection.

The total load of organic debris and soluble wastes in a culture can be minimised by daily removal of accumulated debris from the bottom with a siphon and by collecting fresh faecal

pellets from suspension by air lifting water continually through a coarse sponge filter (Figure 18) which is cleaned daily. The sponge filter should be located near the bottom and close to the centre of the culture vessel, where the circular movement of water will cause debris to accumulate.

Food

Food rations of unicellular algae can be quantified by cell counts using a compound microscope. The amount of food required depends on the copepod biomass present in each culture. When cultures are stocked with nauplii at a density of 1/ml, feed rates generally follow a standard protocol; young nauplii are fed at the rate of $2x10^4$ cells/ml/day increasing gradually to $1x10^5$ cells/ml/day when copepods have reached maturity. This higher feed rate is maintained during the adult life of the copepods while nauplii are being produced. However, this higher feed rate can be varied daily in response to subjective assessment of water clarity in copepod culture vessels prior to the addition of food (see 'Assessing copepod cultures'). If the water appears turbid, the feed rate is decreased to 6 - $8x10^4$ cells/ml/day and if it appears very clear, the rate is increased to 1.2 - $1.4x10^5$ cells/ml/day. Feed rates can be increased further if a very high density of adult copepods is present. However, in this case decreasing water quality will necessitate more frequent water exchanges.

Exact feed rates are not essential. Operators will quickly learn to estimate the cell density of algal cultures from their apparent colour and the amount of algae required by copepod cultures from subjective assessment of copepod biomass and pre-feeding water clarity. This will enable an appropriate feed ration to be calculated.

PLC Control

Procedures for regular (daily) collection of nauplii and renewal of water can be under PLC control. This involves PLC control of 12V circuits which operate a solenoid air valve, solenoid water valves, two water pumps and a light. These are individually timed, in sequence, to bring about the procedures shown in Figure 17. A list of components comprising the PLC system are provided in Appendix 5.

Nauplii which collect in 10 1 of water at the bottom of the nauplius container are removed by manually operating a tap. A 1 ml sample of this is collected for counting and estimation of total numbers.

On completion of the nauplius collection cycle, algae is added manually to the main culture vessel. With *Isochrysis* as food, cells from a dense culture are added to give final cell numbers of approximately 1-$2x10^5$ cells.ml^{-1}. Visual estimates of algal cell density are simpler and effective for routine nauplius production.

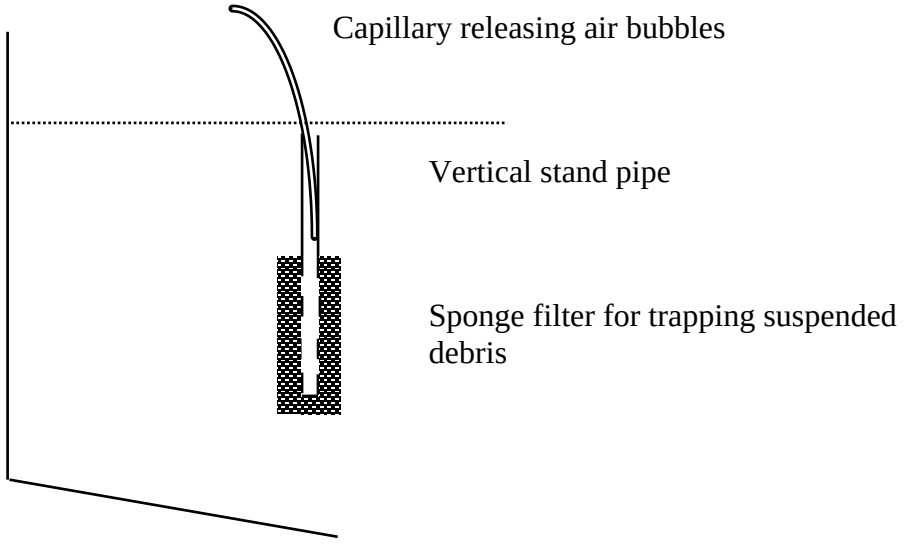


Figure 18. Diagram of a coarse sponge filter fitted to an air lift for removal of suspended debris from a culture vessel.

500 - 1000L culture with flow-through water

Where a large number of copepods are required from a culture and clean sea water is readily available, nauplius collection and water quality maintenance can be achieved by controlled flow-through of water with no recycling. Most of the culture procedures are the same as for a recycling system. Differences relate to the procedures for nauplius collection and water exchange. Figure 19 shows the nauplius collector linked to an overflow pipe. Natural light serves to concentrate nauplii in the top half of the culture vessel. These nauplii are drained into a 50 µm "sock" submerged in a separate tank with overflow.

Water renewal and cleaning

Intermittent flow-through exchanges water from the main culture vessel with new seawater. As with 500 L cultures, new culture can be left static until copepods mature. In mature cultures, nauplius collection/water renewal is best performed daily, although every second or third day is sufficient to maintain water quality. Do not leave mature cultures static for longer than this.

As for a recirculating system, the total load of organic debris and soluble wastes in a culture can be minimised by daily removal of accumulated debris from the bottom with a siphon and by collecting fresh faecal pellets from suspension by air lifting water continually through a coarse sponge filter (Figure 18) which is cleaned daily. The sponge filter should be located near the bottom and close to the centre of the culture vessel, where the circular movement of water will cause debris to accumulate.

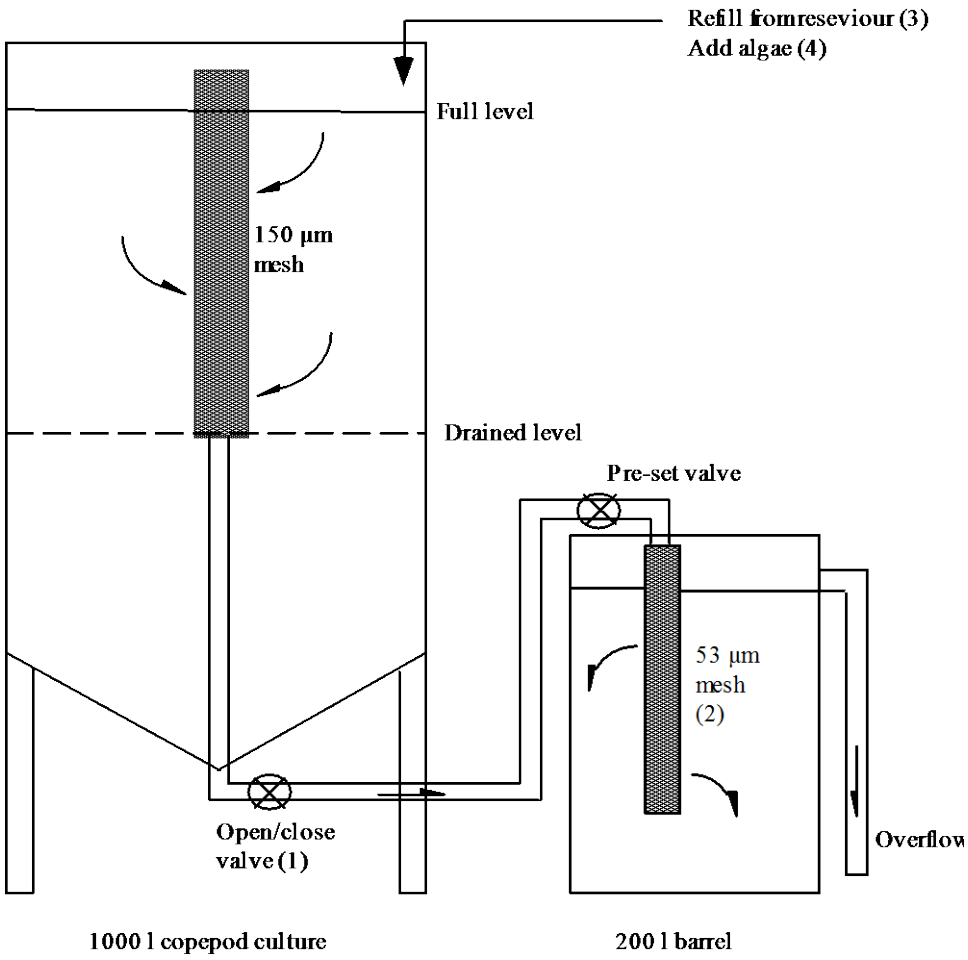


Figure 19. Schematic diagram of a 1000 L copepod culture system. Nauplius collection and water exchange procedures are described as follows;

(1) Valve opened allowing half the volume of 1000 L culture vessel to drain through 150 µm mesh into submerged 53 µm mesh. Pre-set valve ensures slow drainage (~10 l/min).
(2) When draining complete, valve closed and nauplii rinsed from 53 µm mesh.
(3) Culture vessel refilled with clean seawater from reservoir.
(4) Copepods fed with algae.

Assessing production by a copepod culture

The rate of nauplius production in a culture is an effective measure of the health of adult copepods in a dense culture. Expressed as nauplii.l^{-1}.day^{-1}, production can be compared at different times during the life of a culture and indicates when a culture has passed maximum productivity.

If newly collected nauplii are held in a known volume of water (e.g., 10 l) a random sub sample of known volume (e.g. one or two mls) can be heated to 45 - 50°C in a clear tray or trough and the dead nauplii counted with a stereo microscope. Total numbers collected can be estimated from the number in the volumetric sub sample (Figure 20).

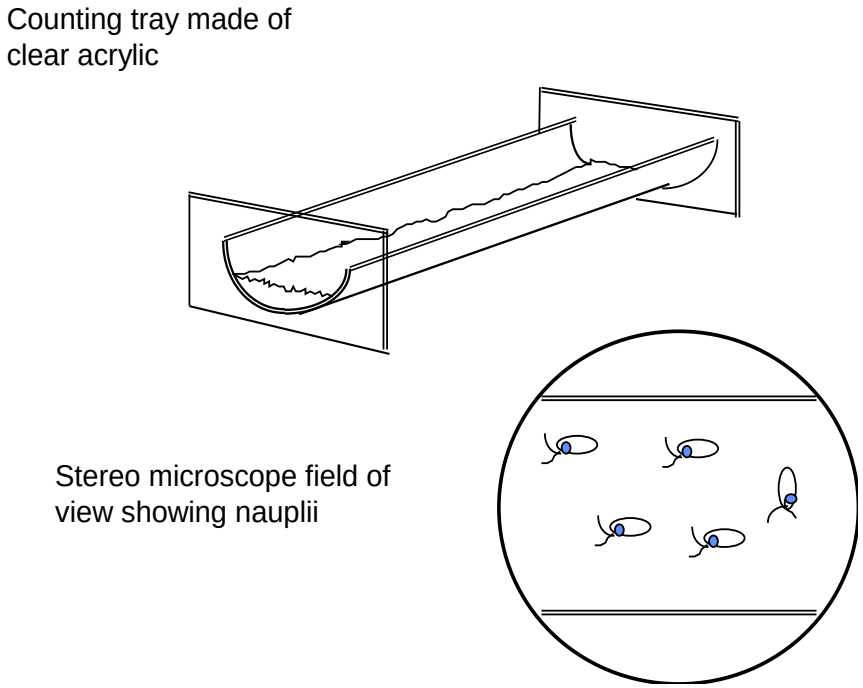


Figure 20. A tray cut from clear acrylic tubing used for counting copepod nauplii under a stereo microscope.

Assessing health of copepod cultures

Assessing the copepods

Simple procedures allow for rapid assessment of copepod health and reproduction and rapid assessment of whether adequate food is being provided.

Rapid attachment behaviour by adults

In culture vessels, healthy adult *G. imparipes* usually occur in large numbers attached to the walls close to the water surface. A clean glass beaker (e.g. 400 ml) pushed quickly beneath the water surface close to the wall of the vessel, can be swept parallel to the surface such that water flows from close to the wall into the beaker. This will collect copepods. This procedure allows for inspection of animals through the side of the beaker, with a hand lens.

If a large proportion of the animals attach to the sides of the beaker within ~ 1 minute, they are likely to be healthy. If the culture is unhealthy most of the animals in the culture vessel were swimming in open water and not vulnerable to collection by this technique. Those that are caught are likely to continue swimming rather than attach to the side of the beaker.

A common symptom of unhealthy *G. imparipes* is an infestation of micro-organisms on the exoskeleton (Figure 13), especially in the grooves between segments. This causes damage to the prosomal sensillae and subsequent failure of the mechanisms by which the sensillae attach the animal to surfaces.

Feeding by adult animals

Animals collected from a culture as described above can be individually inspected with a 10x hand lens as they remain stationary on the walls of the beaker. If food has been available, healthy animals will have the gut conspicuously darkened with compacted food and faecal pellets will be seen in the hind part of the gut.

Rapid movement of the anterior feeding appendages may also be seen but this is not continuous.Unhealthy animals which are not feeding will not show a dark gut, faecal pellets in the hind gut or rapid movement of the anterior feeding appendages.

Proportion of adult females which are reproductively active

With the same method of observation, adult female animals can be distinguished. A high level of reproductive activity is indicated by the proportion of females with conspicuous development of eggs in their reproductive tract (Figure 6), the proportion with clutches of embryos attached and the size of the embryo clutches.

A population with high productive activity will show ~50% of females with eggs in the reproductive tract and >50% with embryo clutches that are wider than the animal's prosome. Low reproductive activity is indicated by <50% of females either with eggs in the reproductive tract or with embryo clutches. If present, the embryo clutches are small.
Nauplius numbers

A healthy population of *G. imparipes* from which nauplii are not regularly removed will show active copepodids and nauplii over a full size range. If nauplii are regularly collected, small nauplii will be present in a healthy culture. If nauplius production is quantified, substantial decline in production indicates poor culture health or inadequate food.

Nauplius activity

Direct observation of nauplii can be used to judge the level of nauplius activity. Healthy nauplii make almost continuous vigorous jerky movements and are strongly photo positive. Unhealthy nauplii are inactive or sluggish in movement and display a weak or no response to directional light.

Symptoms of deterioration of copepod health

Depressed nauplius numbers

Low number of nauplii may occur in a culture of healthy animals receiving low levels of food or it may indicate poor health of well-fed animals.

Low fecundity

Linked with low nauplius numbers, poor health may be detected from indicators of low reproductive activity in adult females; few small embryo clutches and low numbers of females with eggs in the reproductive tract.

Damage to the furcal rami of adults

At high stock densities adult *G. imparipes* may sustain damage to the furcal ramii, possibly from frequent mating encounters. Ramii may appear shortened or asymmetrical from breakage. Although implications for animal health are not known, nauplius production (i.e. adult fecundity) does not continue to rise with adult stock density.

Low proportion of adults showing attachment behaviour

In healthy cultures with abundant food most adult animals remain attached to underwater surfaces for most of the time. If in a well-fed culture, many animals seem to be swimming, or if in a beaker animals are slow to attach to the side, poor health may be indicated. When animals are mainly swimming rather than attached, stalked ciliates have been seen living in the intersegmental grooves of the prosome.

Damage to sensillae

Healthy adult *G. imparipes* have many fine sensillae on the dorsal and lateral segments of the prosome (Figure 11). These can be observed with a compound microscope at 100x if the animal is positioned on its side and the microscope has adequate resolution. The sensillae are involved in the normal behaviour of attachment. Unhealthy animals from crowded cultures have been seen to have damaged sensillae; either broken off and absent or bent.

Unhatched embryos in sediments

The embryo sacs of healthy animals rupture when the nauplii are fully developed. Nauplii swim freely from the ruptured embryo sac while it is still attached to the female or very soon after. Poor health is indicated if embryo sacs are shed before the nauplii begin to emerge. Unhatched embryo sacs on the bottom of the container are not usual in a healthy culture.

Antennae

Copepodid and adult *G. imparipes* have been observed with abnormally short or distorted first antennae. For adult males that use the geniculate first antenna in sexual encounters, this may prevent effective mating. The condition has been observed in some very old cultures and in some cultures exposed to possible chemical pollutants.

Multi-segmental arthropod appendages form from flat concentric structures in the cuticle of the embryo. During development the elongate appendage forms as the central disc produces the terminal segment and the rest of the appendage pushes away from the body surface, analogous to an expanding telescope. Successive concentric rings of cuticle elongate and form the exoskeleton of each segment of the appendage. The first antennae of calanoid copepods comprise many segments. For *G. imparipes* , 24 segments. The developmental process involving 24 concentric structures in the cuticle of the juvenile copepod must be under very delicate physiological control. Poor health or physiological disturbance may be reflected in disruption of the finely co-ordinated processes involved in forming the multisegmental antennae.

Assessing the culture

Rapid assessment of whole culture health may be made using various criteria. Symptoms of poor health can be detected in the animals, as described above, or may be seen in the conditions of the culture as a whole.

Smell

No particular smell is associated with a healthy culture of *G. imparipes*. An obvious and unpleasant smell is usually indicative of bacterial activity. This is associated with a high load of organic debris in the water, high levels of dissolved organic compounds and probably low dissolved oxygen.

Algal cultures can become unhealthy, usually through nutrient imbalance, and contamination with bacteria or cyanobacteria. Organic matter released from cells in unhealthy algal cultures will favour bacterial growth. If this algae is added to copepod cultures a heavy bacterial load, smell and unhealthy copepods will result. Only healthy algal cultures should be used as food for the copepods.

Water clarity before feeding

Algae added as food to copepod cultures will cause water turbidity. This will clear as the algal cells are removed by the animals. If excess algae is added the copepods will not clear it within a few hours and it will provide a resource or substrate for invasive organisms.

Uneaten algae cells may senesce, die and add to the organic load of the culture. This will favour bacteria. If the water is not relatively clear prior to feeding, the culture is likely to have an unhealthy load of bacteria or debris. The water should be changed and less food given until water clarity improves.

Water colour

A high level of dissolved organic material in water is indicated by green/yellow discoloration. Water change will correct this. If water is being recirculated, a protein skimmer should be used as part of the water reconditioning procedure. This will remove organic material from solution.

Dissolved oxygen

Although individual *G. imparipes* can survive dissolved oxygen levels down to 20% of saturation, levels above 80% saturation are probably better for culture health. If DO drops below 80% of saturation culture conditions are probably less than optimum.

Nitrogenous wastes

The normal excretory product of most aquatic animals is ammonia (NH_3 / NH_4^+). Bacterial activity converts this to nitrite (NO_2) and then to nitrate (NO_3). Of these nitrogen compounds, NH_3 is the most toxic to animals and should be monitored. If NH_3 exceeds 0.5 $mg.l^{-1}$ water exchange is required. The effects of less toxic nitrite and nitrate on copepod health are unknown.

Invasive fauna

Poor copepod culture health is indicated by conspicuous populations of ciliates, rotifers, nematode worms, gastrotrichs or harpacticoid copepods. These various organisms may be inadvertently introduced to a culture during water exchange. They exploit the resources of algae added as food or the faecal debris in the culture vessel. Invasive fauna may exert deleterious effects on the copepods in a culture through competition or injury or they may reduce the nutritional value of the copepods as food for fish.

Rotifers, e.g. *Brachionus sp*. may be inadvertently introduced to a copepod culture if cultures of both animals are kept in close proximity and hygiene lapses. Rotifers will compete with *G. imparipes* for food.

Ciliates which grow as epizooites have injurious effects on *G. imparipes*. Ciliates are generally ubiquitous and will only become a problem when water quality deteriorates.

Nematode worms and gastrotrichs may develop populations in accumulated sediments of faecal debris in a culture container. These are probably introduced during water changes. Although these worms are not harmful to the copepods they may reduce the value of a copepod culture if the worms are perceived to be potentially harmful to other animals.

Harpacticoid copepods (e.g. *Tisbe* spp.) may thrive in accumulated sediments of a *G. imparipes* culture. These copepods are primarily detritus feeders and contribute to the physical and metabolic breakdown of the sediments. This may accelerate the release of

dissolved organic and inorganic matter and reduce water quality. In low numbers, harpacticoid copepods are not harmful but unless debris accumulation is kept to a minimum their population can grow rapidly such that they compete with *G. imparipes* for internal surfaces.

Simple remedial treatment procedures

If copepods are surviving but infected

Most infections of *G. imparipes* in culture involve epizooites on the exoskeleton. Since adults do not ecdyse these cannot removed.

It is most simple to isolate a cohort of nauplii and grow them to maturity with regular water changes and clean containers. Any epizooites will be shed with each ecdysis.

Infected cultures should be discarded

If poor water quality persists

Implement all of the procedures for ensuring water quality:

Daily renewal of at least 1/3 of the water volume with clean water,
Protein stripping and biological filtration if water is to be reused,
Daily removal of conspicuous accumulated faecal debris,
Continuous trapping of suspended faecal pellets in a coarse filter and daily cleaning of the filter,
Continuous water movement and oxygenation through air lift,
Addition of sufficient food to maintain copepods but not pollute the water,
Keeping stock density equal or below 1 adult $.ml^{-1}$,
Regular nauplius collection to maintain the adult population as the main biomass,
Replacing a culture with a younger cohort before adults die from old age.

Invasive harpacticoid copepods

Marine harpacticoid copepods such as *Tisbe* spp. can be removed from a *G. imparipes* culture, or at least stressed and reduced in number by rapidly reducing the salinity to a level below their tolerance. *G. imparipes* will tolerate sudden salinity changes from 30 to 15ppt. and then, after 10 minutes, from 15 to 5ppt. This treatment will kill almost all *Tisbe* and most other invasive species of marine origin. *G. imparipes* occurs naturally over a wide salinity range and will survive the sudden changes. After a few hours at the low salinity, culture conditions can be restored. In returning to high salinity sudden changes of more than 15ppt. should be avoided.

Invasive rotifers

Contamination of copepod cultures with rotifers may occur if the two organisms are used in close proximity. *Brachionus* rotifers will compete with *G. imparipes* for algae and copepod production will be reduced. Rotifers tolerate rapid salinity changes and so cannot be removed by salinity stress. Theys can be separated from adult copepods by passing them

through a 150 µm mesh. This allows the passage of rotifers and copepod nauplii but not adult copepods. Daily removed of ~30% of the culture volume, coupled with collection of nauplii through a 150µm mesh has successfully controlled invasive rotifers.

Relevant literature

Gladioferens imparipes

Bayly, I. A. E. (1963). A revision of the coastal water genus *Gladioferens* (Copepoda: Calanoida). Australian Journal of Marine and Freshwater Research 14: 194-217.

Payne, M. F. and R. J. Rippingale (2000). Evaluation of diets for culture of the calanoid copepod *Gladioferens imparipes*. Aquaculture 187: 85-96.

Payne, M. F. and R. J. Rippingale (2000). Rearing West Australian seahorse, *Hippocampus subelongatus*, juveniles on copepod nauplii and enriched *Artemia*. Aquaculture. 188: 353-361.

Payne, M. F., R. J. Rippingale, et al. (2001). Cultured copepods as food for West Australian dhufish (*Glaucosoma hebraicum*) and pink snapper (*Pagrus auratus*) larvae. Aquaculture 194: 137-150.

Payne, M. F. and R. J. Rippingale (in press). Effects of salinity, cold storage and enrichment on the calanoid copepod *Gladioferens imparipes*. Aquaculture.

Payne, M. F. and R. J. Rippingale (in press). Intensive cultivation of the calanoid copepod *Gladioferens imparipes*. Aquaculture.

Rippingale, R. J. (1994). A calanoid copepod *Gladioferens imparipes*, holding to surfaces. Hydrobiologia 292/293: 351-360.

Rippingale, R. J. and E. P. Hodgkin (1974). Population growth of a copepod *Gladioferens imparipes* Thomson. Australian Journal of Marine and Freshwater Research 25: 351-360.

Rippingale, R. J. and E. P. Hodgkin (1974). Predation effects on the distribution of a copepod. Australian Journal of Marine and Freshwater Research 25: 81-91.

Rippingale, R. J. and M. G. MacShane (1991). A calanoid copepod for intensive cultivation. Memoirs of the Queensland Museum 31: 457.

Culture of other copepod species

Delbare, D., P. Dhert, et al. (1996). Zooplankton. In: Manual on the production and use of live food for aquaculture. P. Lavens and P. Sorgeloos (eds). FAO Fisheries Technical Paper. No. 361. FAO, Rome: 252-282.

Fukusho, K. (1980). Mass production of a copepod *Tigriopus japonicus* in combination with a rotifer *Brachionus plicatilits* fed w-yeast as a food source. Bulletin of the Japanese Society of Fisheries Science 46: 625-629.

Hoff, F. H. and T. W. Snell (1987). Plankton Culture Manual. Florida Aqua Farms Inc, Dade City.

Kahan, D., G. Uhlig, et al. (1982). A simple method for cultivating harpacticoid copepods and offering them to fish larvae. Aquaculture 26: 303-310.

Klein Breteler, W. C. M. (1980). Continuous breeding of marine pelagic copepods in the presence of heterotrophic dinoflagellates. Marine Ecology Progress Series 2: 229-233.

Schipp, G. R., J. M. P. Bosmans, et al. (1999). A method for hatchery culture of tropical calanoid copepods, Acartia spp. Aquaculture. 174: 81-88.

Støttrup, J. G. and N. H. Norsker (1997). Production and use of copepods in marine fish larviculture. Aquaculture. 155: 231-248.

Støttrup, J. G., K. Richardson, et al. (1986). The cultivation of *Acartia tonsa* Dana for use as a live food source for marine fish larvae. Aquaculture 52: 87-96.

Sun, B. and J. W. Fleeger (1995). Sustained mass culture of *Amphiascoides atopus* a marine harpacticoid copepod in a recirculating system. Aquaculture 136: 313-321.

Zillioux, E. J. (1969). A continuous recirculating culture system for planktonic copepods. Marine Biology 4: 215-218.

Algal culture

Guillard, R. L. (1972). Culture of phytoplankton for feeding marine invertebrates. In: Culture of Marine Invertebrate Animals. W. L. Smith and M. H. Chanley (eds). Plenum Press, New York: 29-60.

Hoff, F. H. and T. W. Snell (1987). Plankton Culture Manual. Florida Aqua Farms Inc, Dade City.

O'Meeley, C. and M. Daintith (1993). Algal cultures for marine hatcheries. National Key Centre for Teaching and Research in Aquaculture, University of Tasmania, Launceston.

Wilkerson, J. D. (1998). Clownfishes: A guide to their captive care, breeding and natural history. Microcosm Ltd, Shelburne.

Appendices

Appendix 1. Staining lipids

Procedure to stain lipids of zooplankton, e.g. copepods, with Sudan IV.

1. Concentrate living zooplankton in the corner of a mesh so that ~10 animals can easily be removed with a pipette.

2. Place these animals in a 5 ml vial or depression. Add 50% alcohol. When animals are dead, use a pipette to replace the 50% alcohol with new. Leave 5 minutes.

3. Pipette away nearly all of the alcohol and replace it with a solution of Sudan IV (5g in 95ml of 70% alcohol). Avoid skin contact or inhalation. Irrigate the animals with Sudan IV, cover and leave 1 hour.

4. After 1 hour, pipette the Sudan IV to waste and irrigate the animals with 50% alcohol for 30 seconds. The timing is quite critical.

5. Transfer animals to a slide, use a paper tissue to remove XS alcohol. Cover animals with 50% glycerol and a cover glass.

6. Observe at 100x for the presence of lipids, shown Red by Sudan IV.

7. Use cheap cosmetic nail varnish to seal the edge of the cover glass, this will allow the specimen to be stored for a few weeks or more.

Appendix 2. Guillard's f/2 medium for culturing unicellular algae

Shopping list for f/2 media

These chemicals are available from most suppliers, eg Sigma Chemicals. Be prepared to shop around to get the best price. Chemicals should be stored dry in a sealed container with a desiccant. Some of the chemicals tend to absorb water from the atmosphere (especially Ferric Chloride) and if this has happened, they cannot be weighed accurately.

Chemical	Amount required
$NaNO_3$	250-500 g
$NaH_2PO_4.H_2O$	100-250 g
$Na_2SiO_3.9H_2O$	250-500 g
Na_2EDTA	100-250 g
$FeCl_3.6H_2O$	100-250 g
$CuSO_4.5H_2O$	10-50 g
$ZnSO_4.7H_2O$	10-50 g
$CoCl_2.6H_2O$	10-50 g
$MnCl_2.4H_2O$	10-50 g
$Na_2MoO_4.H_2O$	10-50 g
Thiamine HCl	5 g
Biotin	500 mg
B_{12}	500 mg

Preparing media

These instructions are for making 50 aliquots of concentrated medium, each of 20 ml, which are to be frozen. Each 20 ml will contain f/2 nutrients for 2 L of culture. The quantities should be adjusted if larger cultures or more than 50 aliquots are needed. For example, to make 50 aliquots with each aliquot containing nutrients for 20 L of f/2 media, use 10 time the amounts given below.

1. Trace elements

The first step is to prepare a standard stock solution of concentrated trace elements. Only a portion of this is used for each batch of f/2, the remainder can be frozen for later use.

Trace element	Weigh out*	Volume of water*
$CuSO_4.5H_2O$	100 mg	100 ml
$ZnSO_4.7H_2O$	220 mg	100 ml
$CoCl_2.6H_2O$	100 mg	100 ml
$MnCl_2.4H_2O$	1800 mg	100 ml
$Na_2MoO_4.H_2O$	60 mg	100 ml

*If this amount cannot be weighed exactly, weigh an amount close to this and calculate the volume of water required to end up with the same concentration.

e.g., if 112 mg of $CuSO_4.5H_2O$ is weighed, add it to 112 ml of water

There are now five solutions, each of a trace element.

Take exactly 50 ml of each solution and pool them in a 500 ml cylinder or volumetric flask and make the volume up to 500ml with water.

The pooled solution of trace elements is more than needed to prepare 50 x 20 ml ice blocks, each for 2 L of culture. Only 10 ml is required.

Keep 10 ml of this trace element solution. Freeze 10 ml aliquots for later use.

2. Vitamins

Prepare standard stock solutions of concentrated vitamins. As for the trace elements, most of this can be frozen for later use.

Vitamin	Weigh out*	Volume of water*
Thiamine HCl	500 mg	50 ml
Biotin	50 mg	1000 ml
B_{12}	50 mg	1000 ml

* as above, but 50 ml of Thiamine HCl is needed do not weigh out less than 500 mg.

Take 50 ml of each of the three vitamin solutions and pool them in 500 ml of water.

Keep 10 mls of this vitamin solution. Freeze 10 ml aliquots for later use.

3. Silicate (use for culturing diatoms only)

Silicate is only required for growth of diatoms. It can be omitted from f/2 unless diatoms are grown. Therefore it is convenient to keep silicate separate from the other components of f/2.

Disolve 3.0 g in 100 ml of water.
Distribute 2 ml aliquots into vials or keep as a liquid, using 1 ml per litre of culture medium as required.

4. Major chemicals

Weigh exactly each of the following. Keep them in separate labelled vials

$NaNO_3$		8.3g
$NaH_2PO_4.H_2O$	0.5g	
$FeCl_3.6H_2O$		0.315g * caution, hygroscopic.
EDTA		0.436g

5. Preparation of 20ml aliquots

To about 500 ml of distilled or deionised water,

Add 0.436 g of the EDTA . Stir to dissolve. N.B. EDTA must be added first.
Add 0.315 g of the iron chloride. Dissolve.
Add 0.5 g of the phosphate salt
Add 8.3 g of the nitrate salt
Add 10 mls of the pooled trace metal solution
Add 10 mls of the pooled vitamin solution

Pour 20 ml aliquots of this solution into snap top vials, seal and freeze. These will keep almost indefinitely. If culturing diatoms, do not add silicate to the frozen aliquots. Add it directly to culture media along with frozen aliquot.

Appendix 3. Sources of copepods.

Field Collection.

Gladioferens imparipes may be collected from estuaries in the south west of Western Australia. Given the ecological dynamics that influence the distribution and abundance of estuarine copepods, times and locations for successful field collection cannot be guaranteed.

As described earlier, the distribution of *G. imparipes* within an estuary is influenced by the seasonal hydrology, the pattern of productivity and the distribution and abundance of the main predators. They may occur where neither of the main copepod predators have established populations (*Acartiura* sp. in salinity >25 or *Sulcanus conflictus* in salinity <5), where flushing from fluvial flow is minimal and where phytoplankton production occurs.

Net collection.

Night plankton samples can be taken with a 150 µm mesh net fitted with a cod end funnel or collecting jar which retains water. Collected material should be transferred to a few litres of the water from which they were collected and kept cool with no more than ~ 1 animal per ml. If collected material is placed in a transparent container or a clear plastic bag *G. imparipes* may be seen and recognised by the characteristic shape of females carrying clutches of embryos. If these are not present it is unlikely that *G. imparipes* have been collected.

Daylight samples from open water plankton with a finer net (100 - 125µm) may collect copepodid and nauplius *G. imparipes* . These are difficult to recognise in the field without a microscope.

Collection from vegetation.

Where loose submerged peripheral vegetation (e.g.*Potomageton* sp, macroscopic algae) occurs in the zone of an estuary where a *G. imparipes* population can be expected, the copepods may be collected by pushing a hand held net vigorously through the vegetation or by pushing handfuls of the vegetation into a container underwater and bringing it to the surface. Vigorous stirring or shaking of the vegetation within the container will dislodge the copepods and the vegetation can then be discarded. Copepod fauna should be kept in water from the place of collection and sorted before deoxygenation occurs.

Separation of *G. imparipes* from other fauna.

Different techniques and combinations of techniques may be used to separate *G. imparipes* from other fauna in newly collected material.

Reverse filtration of collected material using a 150 µm mesh allows animals to be concentrated into a volume of water which convenient to examine with a stereo microscope.

Using a stereo microscope, individual copepods can be removed with a wide mouthed pasteur pipette and transferred to a few mls of clean water of appropriate salinity. A pastuer pipette fitted with a rubber teat and with the narrow tube broken off is suitable for collecting

individual animals. If other fauna are inadvertently collected, the procedure is repeated with a second transfer from the first volume of clean water to a second. If animals are difficult to catch they may be cooled to ~5 - 8°C in a refrigerator for a few minutes. They are easier to pick up with a pipette when inactive.

G. imparipes may be separated from many other planktonic organisms by exploiting their tolerance of salinity change. If unwanted animals are from a low salinity habitat, a sudden salinity increase of ~15, followed by another increase after 10 minutes will kill them but leave *G. imparipes* intact. Dead animals can be siphoned from the bottom and / or live animals decanted to a clean container. The same process in reverse will kill most other fauna from a high salinity habitat.

After being completely or partially separated from other fauna, newly collected adult *G. imparipes* may be kept in clean water (salinity 15 - 35ppt, temperature 20 - 25°C) and provided unicellular algae as food. Within three days nauplius production can be expected. Newly released nauplii can be removed by reverse filtration through a 125µm mesh and grown to maturity as a single species cohort. Depending on the apparent health and quality of the collected adults they may be kept for continued nauplius production or discarded after the first nauplii are obtained.

Working cultures can be gradually built up from newly collected material.

Established cultures

As at January 2001, *G. imparipes* were available from the following institutions.

Curtin University of Technology
School of Resource Science and Technology,
GRO Box U1987,
Perth WA 6845
Contact: Dr Rob Rippingale
Ph: (08) 9266 7922
Email: r.rippingale@info.curtin.edu.au

Challenger TAFE
Fremantle Maritime Centre
1 Fleet St,
Fremantle WA 6160
Contact: Mr Greg Jenkins
Ph: (08) 9239 8030
Email: jenkig@fleetst.training.wa.gov.au

Appendix 4. Establishing a 500 L copepod culture

Preparation and stocking

1. Fill culture vessel to the 300 L mark with 5 µm filtered seawater dilluted to 27 ppt with carbon filtered tap water water. Add 25 ml hypochlorite (12.5%) and leave overnight without aeration.

2. Deactivate hypochlorite with 25 ml Sodium Thiosulphate solution (100 g/l). Turn air-lift circulation on and leave for 1 hr. Test for residual chlorine.

3. Stock culture vessel with 500,000 nauplii (1 copepod/ml when full). Ensure that aeration is gentle and culture vessel is covered to exclude light. Add T-Iso as food at the rate of 30,000 cells/ml.

4. Increase feed rate by ~10,000 cells/ml every 2 or 3 days until the feed rate is 100,000 cells/ml. Feed rate may be further increased if cultures remain healthy (see below). Do not exceed 200,000 cells/ml.

5. Monitor copepod health daily by observing copepod activity and gut content under a stereo microscope. Use a torch to determine culture water clarity. Turbid water is usually a result of a bacterial bloom and is associated with an unpleasant smell and decreased copepod activity. If this occurs, do not increase feed rate and effect an 80% water change.

6. Almost all nauplii should have metamorphosed into copepodids by day 5-6. When this has occurred, increase air-lift circulation, add plastic mesh substrate and add foam filter. Ensure that the filter is placed centrally near the bottom of the vessel and operates with very low aeration.

7. Commence daily nauplius collection when adults carrying egg sacs are abundant in a sample taken from the culture vessel. Continue collection until nauplius numbers decrease.

Appendix 5. PLC components

Cost and suppliers of electrical components for PLC copepod culture system. All suppliers are listed in the Perth telephone directory.

No.	Item	Cost/item ($)	Total cost ($)	Supplier
1	Toshiba EX20 PLC*	1069.00#	1069.00	Control & monitoring Services
1	12V Power Supply (11 Amp)	170.00	170.00	Electronic Parts (WA)
1	12V Diaphram pump	149.00	149.00	The 12 Volt Shop
1	12V Bilge pump	59.79	59.79	Wilson Marine
2	12V Pneumatic solenoid valve (water)	58.00	116.00	Technical Irrigation Imports
1	12V Solenoid valve (air)	83.50	83.50	SMC Pneumatics
2	12V 20W halogen lamp + holder	11.75	23.50	Pro-Lamps
4	Nylon float switch	23.39	93.56	RS Components
2	12V Relay + base	36.02	72.04	RS Components
10	10A fuse	0.30	3.00	RS Components
10	2A fuse	0.32	3.20	RS Components
7	Fuse holder	1.93	13.51	RS Components
20M	Red/Blk cable (50x0.12mm)	0.75/M	15.00	Dick Smith Electronics
5M	Red/Blk cable (26x0.3mm)	1.35/M	6.75	Dick Smith Electronics
12	6.3mm QC connector housing	1.11	13.32	Coventrys
12	Connector receptacle and tab	0.49	5.88	Coventrys
	Total		**1897.05**	

* This is an outdated and expensive PLC unit. Modern PLC's suitable for this task cost less than half this amount.
# This price does not include programming software.