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Rearing of the Harpacticoid Copepod *Tisbe holothuriae* and its Application for the Hatchery Production of Atlantic Halibut *Hippoglossus hippoglossus*

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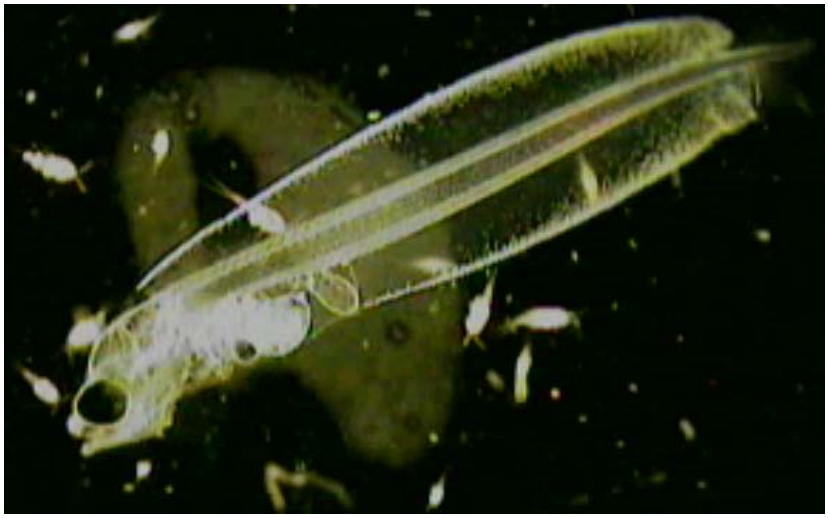


David Miles[1], Neil Auchterlonie[2], Christopher Cutts[1] & Carlos Mazorra de Quero[1]

Executive Summary

At the Marine Farming Unit, Ardtoe, a system was developed for the intensive culture of *Tisbe holothuriae*, based on the tray batch culture system described by Støttrup & Norsker (1997). Culture was carried out in 20l plastic trays stored in racks, lit by fluorescent lights and maintained at a temperature of approximately 20ºC.

Tisbe showed good survival in a range of salinity (25-38 ppt). However, wide fluctuations should be avoided. Ammonia appeared to be the most significant limiting factor for survival and production yield, and organic loading of the culture units should be carefully managed. Different substrates were assessed indicating possible increases in the naupliar production and the stocking density, however their use should be considered against the practicalities of husbandry.

Different harvesting regimes were evaluated, and their use depends on the needs of live prey size, and the management of ammonia levels in the cultures. Maximum productivity occured between days 4-10 in static cultures. Yields of over one million naupliae were obtained from static trays harvested at day 8, although productivity was generally highly variable with this harvest practise. Frequent harvest (daily to every three days) of established broodstock trays provided naupliae for on-grown in a separate tray to an appropriate size for the fish larvae to be fed. Best results were obtained stocking the trays with 40-50 thousand copepods as broodstock. The proportion of females would determine the culture naupliae production. Survivals of over 90% can be expected when on-growing naupliae for 6-8 days at 20^{o} C.

Copepods were fed with different algae species. A mixture of *Chaetoceros mulleri* and *Rhinomonas reticulata* provided the best naupliae production and would be used to feed the broodstock trays. On-growing trays would be fed with a mixture of *C. mulleri*, *R. reticulata* and *Isochrysis galbana*, which gave the best results for survival, and provided an adequate content of DHA (34.7% of fatty acids, compared to 8.3% provided by enriched *Artemia*) and a good DHA/EPA ratio (3.3). Supplementation of the algal diet with formulated diets can be carried out without detrimental effects on production provided that organic loading is managed appropriately.

The lipid composition of *T. holuthuriae* produced in the culture system proved to be of similar quality of other strains and species of copepods considered the natural nutrition for marine fish larvae. In particular, adult *Tisbe* showed very good contents of DHA (27-42% of fatty acids compared to 8.3% in enriched *Artemia*), EPA (6-12%) and ARA (1-3.8%).

Halibut were found to feed on *Tisbe* at least as readily as on *Artemia* or Rotifers. The differences in behaviour as far as mobility and association to the tank surfaces did not prevent the halibut larvae from preying on the copepods. The main obstacle appeared to be to make available enough numbers of the appropriate life stage to provide a useful nutritional supplement to the larvae. Other species of fish should benefit from the use of *Tisbe* in their diets.

[1] Seafish Industry Authority, Seafish Aquaculture, Ardtoe, Acharacle, Argyll, PH36 4LD
[2] Aquaculture Business Consultancy Ltd., 15 Cedarwood Drive, Afon Village, Gwent NP10 9JR

Contents

1 Introduction

The need to improve nutritional quality during the live-feeding stages in marine fin fish hatcheries is well documented. *Artemia salina* (brine shrimp) is a convenient and relatively economical product for hatcheries to utilise, but is known to be nutritionally sub-optimal given the prevalence of malpigmentation and morphology problems experienced particularly in flatfish hatcheries. Different stages of copepods, of various species, have been found to improve survival, pigmentation and morphology of marine fish larval feeding, but, as yet, industry take-up of this apparent nutritional advantage has been limited. Holoplanktonic calanoid copepods require high volumes for cultivation in captivity, and this is perceived to be too expensive and unreliable for most intensive hatcheries in the UK. Wild-caught calanoids can be obtained in some coastal regions, but variable seasonal availability and the risk of disease and parasite introduction precludes consideration of this route for commercial hatcheries. Intensive **harpacticoid** copepod production has been the subject of previous research, and appears to offer good prospects for large-scale controlled production. The principal considerations associated with use of harpacticoids can be simply listed:

- Harpacticoid copepods can be held and reproduced in captivity at high densities, and there are a number of published protocols for routine laboratory-scale culture.
- Harpacticoids are meroplanktonic with free-swimming early stages and benthic later stages.
- Despite their benthic mode of life as adults, there is some experimental evidence that these can be a suitable live feed for marine fish larvae, and that their use leads to the same types of improvements in larval performance as seen with calanoid copepods.

This demonstration project addressed the issue of copepods as a viable live food for UK marine fin fish hatcheries with two distinct objectives:

- Establish an intensive experimental scale harpacticoid production system and define the required culture conditions for the selected species of copepod.
- Assess the suitability of the selected harpacticoid copepod species as larval feed for halibut (*Hippoglossus hippoglossuss*), taking into account, larval prey requirements, feed availability, acceptability, survival, growth and all relevant condition factors.

At the Marine Farming Unit, Ardtoe, a system was set up for the intensive culture of *Tisbe holothuriae*, based on the tray batch culture system described by Støttrup & Norsker (1997). The present document describes the copepod culture system (Chapter 2), reports on the behaviour of *Tisbe holothuriae* populations under different husbandry parameters (Chapters 3, 4, 5 and 6) and recommends a production husbandry routine easily transferable to a commercial situation (Chapter 8). It also reports on the use of the copepod production for halibut larvae first feeding (Chapters 7) and discusses the possibilities for further development and use with other fish species.

The Culture System

In designing the culture system the principles outlined by Støttrup & Norsker (1997) were applied. Also, the arrangement of the production units and the husbandry procedures were intended to fit in a commercial hatchery situation. Algae were introduced in the trays as feed, without the intention of generating algal blooms as used in the extensive approaches in calanoid copepods production. The provision of light was intended to maintain the algae physiological activity to keep oxygen levels and low ammonia concentrations.

1.1 Tray Cultures

Culture was carried out in white PVC trays of approximate dimensions, 45cm x 10cm x 100cm, with a maximum volume of approximately 20l. The trays were stored on wheeled metal racks with approximately 2cm between them, which was found to reduce evaporation and consequent increase in salinity. A scale was written on to each tray, in order to record the volume of water to the nearest litre.

Each stack was illuminated and heated by six banks of fluorescent tube lights in close proximity to the trays, switched on permanently. The white trays allowed a considerable amount of light into the trays (approximately 1200 lux at the centre of any given tray). Air temperature was maintained at 20-22°C by a thermostat-controlled extractor fan, as several studies have found this temperature to be favourable for the culture of *T. holothuriae* from the western seaboard of Scotland (Heath 1994, Antonio 1999).

Seawater was filtered through a 5μm or a 1μm filter. Salinity ranged from 31-40‰, and was prevented from rising any higher by the addition of distilled water as required.

1.2 Harvest of Trays

The contents of the trays was passed through three filter bags, placed in a housing, with pore sizes of 150μm, 100μm and 45μm, which approximately sorted the tray contents into adults, copepodites and nauplii.

The contents of each fraction were placed in bowls at known volumes of seawater and stirred. Two 1ml sub-samples were taken and the number of copepods counted under a microscope. If necessary, the copepods were stained with a drop of Lugol's iodide solution, and the background destained with a drop of 150g l^{-1} sodium thiosulphate ($Na_2S_2O_3$).

All equipment that came into contact with the copepods was stored in disinfectant solution (Kickstart ®) when not in use, and rinsed with seawater before use.

1.3 Algal Culture

All algae were cultured in clear plastic bags or 10 l flasks at temperatures of approximately 23°C. Heat and light were provided by pairs of fluorescent tube lights. Airstones were used to ensure circulation. Flasks and media were disinfected by autoclaving. Bags were disinfected with chloros solution, which was neutralised by sodium thiosulphate before use. *Isochrysis galbana*, *Rhinomonas reticulata* and *Nannochloris atomus* were cultured in Walne's medium and *Chaetoceros mulleri* was culture in Guillard's $F/_2$ medium (Coutteau 1996). Walne's medium was made up of 90% v/v nutrient solution and 10% v/v vitamin solution, both of which were prepared in filtered tap water. The nutrient solution was 0.36 g l^{-1} $MnCl_2.4H_2O$, 1.38g l^{-1} $FeCl_3.6H_2O$, 33.6g l^{-1} H_3BO_3, 45.0g l^{-1} sodium EDTA, 20.0g l^{-1} $NaH_2PO_4.2H_2O$, 100.0g $l^{-1}$$NaNO_3$. The latter four components were added during heating with stirring. The nutrient solution was completed with the addition of 0.1% v/v of a trace metal solution prepared with 21g l^{-1} $ZnCl_2$, 20g l^{-1} $CoCl_2.6H_2O$, 9g l^{-1} $(NH_4)_6Mo_7O_{24}.4H_2O$ and 20g l^{-1} $CuSO_4.5H_2O$. The nutrient solution was allowed to stand for 4d before use, and subsequently stored at room temperature. The vitamin solution was 100mg l^{-1} vitamin B_{12}, 100mg l^{-1} vitamin B_1 and 2mg l^{-1} vitamin H. It was stored at 4°C before use.Guillard's $F/_2$ medium was prepared in filtered seawater with 0.075g l^{-1} $NaNO_3$, 0.00565g l^{-1} $NaH_2PO_4.2H_2O$, 0.1% v/v trace element solution and 0.1% v/v vitamin solution. The pH was adjusted to 8.0. The trace element solution was 4.160g l^{-1} sodium EDTA, 3.150g l^{-1} $FeCl_3.6H_2O$, 0.010g l^{-1} $CuSO_4.5H_2O$, 0.022g l^{-1} $ZnSO_4.7H_2O$, 0.010g l^{-1} $CoCl_2.6H_2O$ and 0.180g l^{-1} $MnCl_2.4H_2O$. The vitamin solution was 0.0005g l^{-1} Vitamin B_{12}, 0.1g l^{-1} Vitamin B_1 and 0.0005g l^{-1} Vitamin H.

Algal cell counts were carried out spectrophotometrically. Calibration curves were prepared by concentrating algae to approximately 20x their typical culture concentrations by centrifugation, and carrying out serial doubling dilutions in seawater. The highest dilution was counted with an improved Neubauer haemocytometer, and the concentration of cells in each aliquot was calculated.

A range of wavelengths between 400 and 750nm was used to assess the absorbance of each species, and regressed against cell concentration to find the calibration curve. The strongest regression for *I. galbana* and *C. mulleri* were obtained at a wavelength of 630nm, which is an absorbance peak for chlorophyll *a* (Hoff & Snell 1987). The strongest regression for *R. reticulata* and *N. atomus* were obtained at 440nm. In all cases, extremely precise calibration curves were obtained ($r^2 > 0.999$).

For subsequent measurements, the absorbance of the algae was measured and the equation of the calibration curve used to calculate the concentration of cells present.

1.4 Maintenance of Master Cultures

Copepod master cultures were maintained in 1l conical flasks. If the tray culture system collapsed or became contaminated, it could be restarted from these masters.

The cultures were prepared with 400ml seawater and 100ml of the algal mix described previously.

The master cultures were passage every two weeks by passing them through a 45μm filter, checking that no contaminants were present, and placing the copepods recovered in a clean flask. Care was taken not to passage the master cultures after trays had been harvested in order to avoid transferring contamination from the trays to the master cultures. Similarly, care was taken not to passage all the master cultures on the same day.

2.5 Contaminants

Other than the copepods and algae, a number of organisms were observed in the tray culture system in different ocasions. Most of these were innocuous, and small enough to be removed by sluicing water through the filter bags during harvest.

The most problematic contaminants were rotifers, as rotifer populations frequently grew to similar numbers to copepod populations when they appeared in a tray. The best defence against rotifers was found to be passing all incoming seawater through a clean 1μm filter. In order to prevent the spread of rotifers, it is advisable to immediately destroy the contents of any tray in which they are observed with kickstart.

If this is not possible because the copepods are required, they should be harvested with considerable care to prevent the contents of the tray being splashed into other trays. No copepods from that tray should be used for restocking even if there are apparently no rotifers in some of the fractions. All equipment used for the harvest should be disinfected overnight with kickstart before being used again, although vigorous scrubbing with kickstart solution was found to be sufficient to disinfect the tray itself.

Another contaminant that was found in two occasions was a filamentous green alga. Although it was not harmful to the copepods, and in fact was probably eaten by them, it interfered with harvests by blocking filter bags and making it difficult to homogenise copepods for counting if allowed to grow unchecked.

2 Assessment of Algal Mixes and biofilm supplementation

1.5 Introduction

Most of the research published on the nutritional requirements of harpacticoids has involved feeding on single species of algae, although Lee *et al.* (1985) reported that the best survival of *Tisbe carolinensis* was observed on a mix of algal feeds. Similarly, Kraul (1989) recommended mixing a diatom and a flagellate for the maximum production of the harpacticoid *Euterpina acutifrons*.

A feed mix of approximately 80% *R. reticulata* to 20% *I. galbana* by volume was adopted for starting growing the *Tisbe* populations at the initiation of the project. The mixture was added at approximately 1l per tray on alternate days. Observed over a 16d period, this regime resulted in the introduction of a total of 4.00×10^5 cells ml^{-1} (sd = 1.91×10^5), at a species ratio of approximately 1:1.

This ratio is substantially higher than the 1.5×10^5 that was recorded as the maximum ingestion rate for *T. furcata* fed on *R. reticulata* (Abu-Rezq *et al.* 1997), and exceeds Hoff & Snell's (1987) recommended maximum of 1.5×10^5 cells ml^{-1}. However, turbidity of the water in the trays was frequently observed to fall rapidly after feeding, suggesting that such high numbers are not maintained. Further, *R. reticulata* was observed to adhere to surfaces in the trays, so the actual concentration of algal cells in the suspension was probably considerably lower than the recorded numbers for most of the time.

Although previous studies with biomedia in harpacticoid culture have led to mixed results, harpacticoids feed by grazing as well as by filter feeding (Treece & Davis 2000). Consequently, they are likely to benefit from the rapid development of microbial biofilms to supplement an algal diet. While previous studies have considered the possibility of increasing the substrate for biofilm growth, none have attempted to introduce a developed biofilm, which may be beneficial in the early stages of the culture.

The purpose of this experiment was to assess several mixes of algae in order to establish whether it was possible to improve on the production levels achieved with the standard mix, and particularly to investigate Hoff & Snell's (1987) recommendation that harpacticoid feeds should be based on *Chaetoceros* sp. The effect of inoculating cultures with a developed biofilm was also observed.

1.6 Materials and Methods

1.6.1 Experimental Design

Experimental trays were arranged in the stack. Each experimental tray was prepared with 5l seawater and seeded with 3.5×10^5 copepods collected from the 150µl fraction of cultures kept under standard conditions (Section 1.1).

Two trays in each stack were randomly assigned to one of five feed mixes (Table 3.1). Of these two trays, two pebbles that had been kept in culture trays for four days were placed in one in order to inoculate it with a developed biofilm.

The populations were sampled every other day for 20 days.

1.6.2 Algal Preparation

Five algal mixes were prepared from the microalgae *R. reticulata*, *I. galbana* and *C. mulleri* in such a way as to add the same number of cells as in the standard feed to each tray. Cell numbers were recorded (Section 1.4) and the mixes were added to the trays every second day.

Table 0.1. Volumes and cell numbers of algal mixes added to trays on alternate days. Cell counts derived from mean across course of experiment.

		Mix 1		Mix 2		Mix 3		Mix 4		Mix 5	
Chaetoceros mulleri	Volume (ml) Cells					1100 1.97 10^9	x	1030 1.84 10^9	x	765 1.37 10^9	x
Isochrysis galbana	Volume (ml) Cells	200 1.42 10^9	x	400 2.84 10^9	x			170 1.21 10^9	x	85 6.03 10^8	x
Rhinomonas reticulata	Volume (ml) Cells	800 9.96 10^8	x	600 7.47 10^8	x	550 6.85 10^8	x			340 4.23 10^8	x
Total	Volume (ml) Cells	1000 2.42 10^9	x	1000 3.59 10^9	x	1650 2.65 10^9	x	1200 3.05 10^9	x	1200 2.40 10^9	x

1.6.3 Sampling Protocol

Sampling was carried out every second day. The volume of water in each tray was measured, and the contents mixed. 1ml samples were collected from two corners of the tray and the centre of the tray with a plastic Pasteur pipette. Four fractions of each sample were placed in the wells of a 12-well microplate, and the number of adults, copepodites and nauplii in each was counted. The counts were adjusted to the volume of the tray in order to find the total number of each stage present.

1.6.4 Environmental Monitoring

The temperature and salinity of each tray was regularly measured. Salinity was kept between 33.5‰ and 40‰ by adding distilled water to any tray recorded above 39‰.

1.6.5 Statistical Analysis

The counts of each fraction and of the total number of copepods present were analysed by ANCOVA. The position of the tray in the stack was the covariate, and feed mix, presence or absence of pebbles, sample day and stack were fixed, crossed factors. All possible interactions between sample day, feed mix and presence or absence of pebbles were investigated. Daily naupliar productivity was also analysed. Significant differences were further investigated using Tukey multiple comparisons. Significance levels of $p < 0.05$ were treated as unequivocally significant.

1.7 Results

1.7.1 General Observations

In the early stages of the trial, copepods tended to congregate on pebbles where they were available. Particularly high concentrations were observed on the underside of the pebbles, although such aggregations became less common later in the experiment.

As expected, the turbidity and volume of all trays increased steadily through the course of the experiment.

In addition to the copepods and algae, several other organisms were observed in the experimental treatments. Unidentified ciliates appeared within the first few days in all treatments. Nematodes were first observed on day 4, and were observed in 10 of the 20 trays by the end of the experiment. The nematodes were only present in small numbers, usually associated with clumps of algae.

Rotifers were far more prevalent, as they were first observed on day 6, and had infested 16 of the trays by the end of the trial. They were not counted, but usually attained high numbers, especially in the trays fed with mix 4.

A filamentous green alga was also prominent in all trays, and had grown to a considerable size by the end of the trial. It was observed that adult copepods appeared to congregate around the alga, and may have been feeding on it. While no obvious ill-effects on the copepods were observed, the alga hindered homogenisation of the trays for counting.

1.7.2 Copepod Production

Numbers of adult copepods, copepodites, nauplii and all stages pooled at day 20 were compared (Fig. 3.1). There were no significant differences between trays with and without pebbles, and no significant interactions of any kind ($p > 0.05$). Copepods fed on mix 4 consistently produced the highest number of nauplii and those fed on mix 5 consistently produced the lowest, although statistical significance between those two groups was equivocal ($q = 2.76$, $p = 0.052$). Conversely, it was mix 5 that gave the highest levels of copepodites and adults, while mix 4 gave the lowest ($p < 0.05$). The only other statistically significant difference was between the production of copepodites by mixes 5 and 2 on day 20 ($q = 2.85$, $p < 0.05$).

Daily naupliar productivity was also assessed. Unfortunately, there was no record of numbers of females in the populations. However, figure 3.2 plots the average number of nauplii on the sampling day (minus the number of nauplii on the previous sampling point) divided by the number of adults on the previous sampling point (2 days before) for each dietary treatment. This gives a measure of the productivity of the populations. There was a general peak of nauplii production between days 4 and 10. Productivity was much reduced from day 10 onwards. The ANCOVA of dietary treatment versus naupliar production per adult showed that day was the only predictor of naupliar production.

3.3.3. Fatty acid analysis:

Table 3.2 shows the results from the analysis of Tisbe (adults and copepodites) from the different dietary treatments. In general, adults showed a higher content of fatty acids than copepodites. *Rhinomonas* appeared to contribute with high levels of Polyunstarated fatty acids (PUFA), and in particular docosahexaneoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6). The addition of *Chaetoceros* to the diets increased the concentration of eicosapentaneoic acid (EPA, 20:5n-3) and ARA in the copepods. DHA/EPA ratios were also different in the different diest. In general, the introduction of *Chaetoceros* reduced the DHA/APA ratio from around 6 to values between 2.5 and 3.3. EPA/ARA ratios were similar (around 6) except for mix 3, *Chaetoceros & Rhinomonas*, which translated in much lower values (3.3). Table 3.3 shows the % fatty acid content of enriched Artemia for comparison. Of particular significance are the much higher concentrations of DHA and DHA/EPA ratios in copepods.

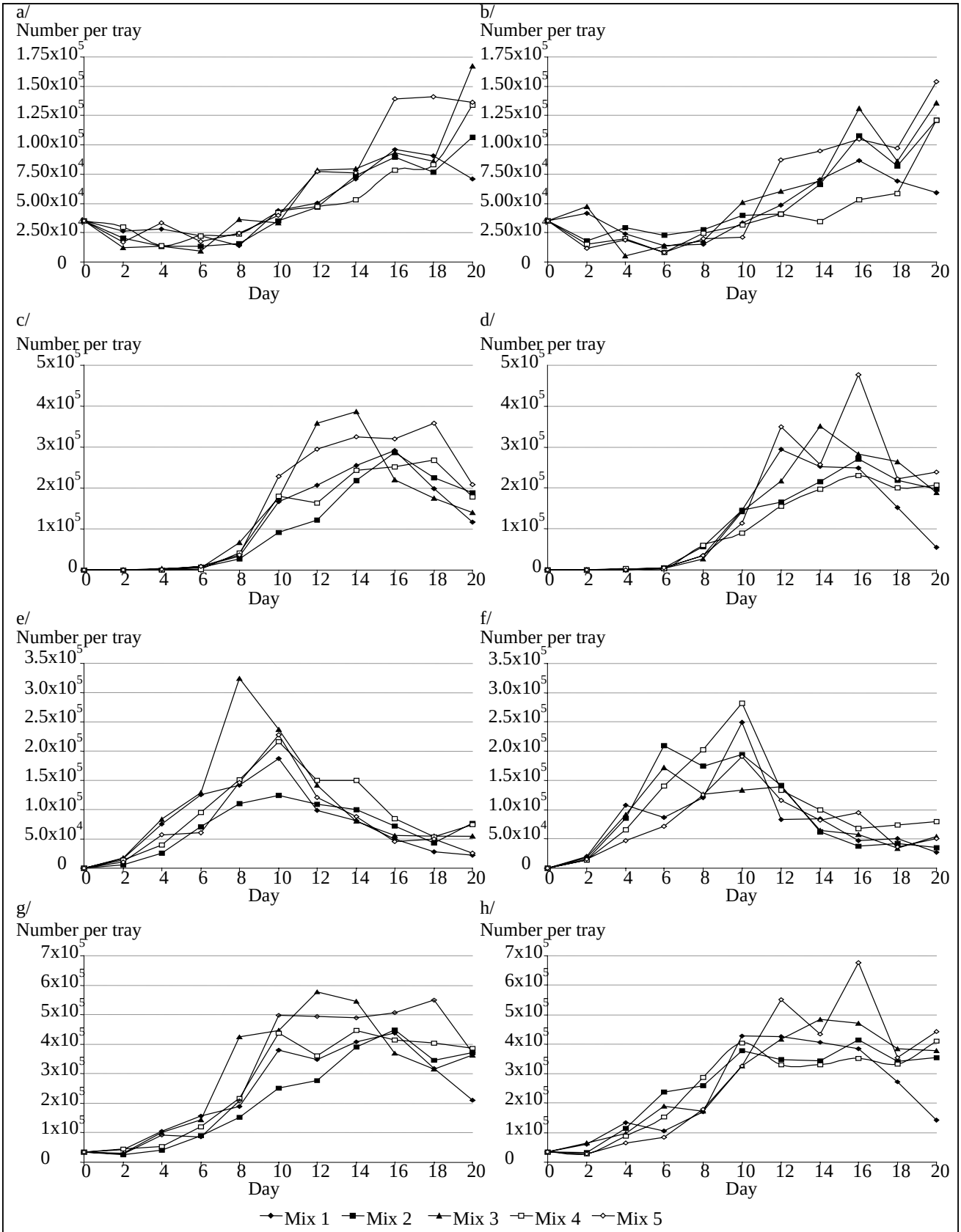


Figure 3.1. Numbers of *Tisbe holothuriae* per tray, fed on five different algal mixes. Counts are given for a/ adults under standard conditions and b/ with pebbles for biofilm inoculation, c/ copepodites under standard conditions and d/ with pebbles for biofilm inoculation, e/ nauplii under standard conditions and f/ with pebbles for biofilm inoculation, and g/ entire population and h/ with pebbles for biofilm inoculation.

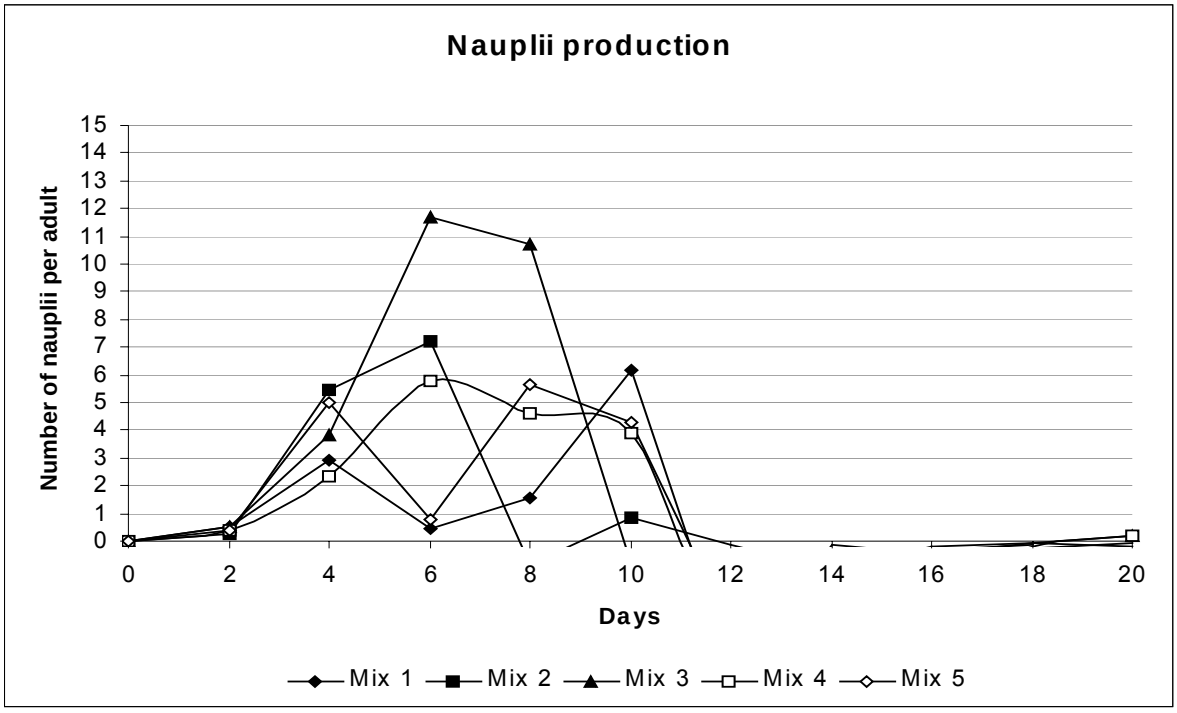


Figure 3.2: Nauplii production per treatment expressed as (number of nauplii present in the trays – number on the previous sampling day) / number of adults present in the previous sampling day.

Table 3.2: fatty acid analysis of adults and copepodite *T. holothuriae* fed three different algal mixtures (see Table 3.1).

Algal mix **Stage**	**1** **adults**		**2** **adults**		**3** **adults**		**4** **adults**		**5** **adults**	
	% fatty acid	**g/mg lipid**	% fatty acid	**g/mg lipid**	% fatty acid	**g/mg lipid**	% fatty acid	**g/mg lipid**	% fatty acid	**g/mg lipid**
14:0	1.4	**4.2**	1.4	**2.3**	2.1	**6.2**	4.2	**12.5**	1.6	**4.4**
15:0	0.4	**1.2**	0.0	**0.0**	0.5	**1.5**	0.5	**1.4**	0.3	**1.0**
16:0	14.6	**44.9**	14.2	**23.4**	14.0	**41.3**	14.2	**42.9**	14.8	**41.2**
i-17:0	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**
18:0	11.9	**36.8**	12.4	**20.3**	11.5	**34.0**	8.0	**24.1**	12.0	**33.2**
20:0	0.4	**1.1**	0.3	**0.5**	0.3	**0.9**	0.2	**0.5**	0.0	**0.0**
22:0	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**
Total saturated	28.6	**88.2**	28.3	**46.5**	28.5	**83.9**	27.0	**81.3**	28.8	**79.8**
16:1n-9	0.6	**1.9**	0.8	**1.3**	0.2	**0.6**	0.1	**0.4**	0.4	**1.0**
16:1n-7	4.6	**14.0**	3.7	**6.0**	9.3	**27.5**	10.2	**30.8**	8.2	**22.8**
18:1n-11	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**
18:1n-9	4.9	**15.2**	4.2	**6.9**	2.8	**8.1**	5.5	**16.6**	3.6	**10.0**
18:1n-7	2.6	**7.9**	1.8	**3.0**	3.0	**9.0**	2.8	**8.5**	2.4	**6.7**
20:1n-11	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**
20:1n-9	0.4	**1.2**	0.0	**0.0**	0.2	**0.6**	0.5	**1.4**	0.0	**0.0**
20:1n-7	0.0	**0.0**	0.0	**0.0**	0.2	**0.7**	0.3	**0.8**	0.0	**0.0**
22:1n-11	0.2	**0.8**	0.0	**0.0**	0.9	**2.6**	0.2	**0.5**	0.9	**2.4**
22:1n-9	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**
24:1n-9	0.3	**0.8**	0.0	**0.0**	0.0	**0.0**	0.1	**0.4**	0.0	**0.0**
Total monounsaturated	13.6	**41.8**	10.5	**17.3**	16.6	**49.0**	19.7	**59.4**	15.5	**42.9**
18:2n-6	2.6	**7.9**	2.5	**4.1**	1.9	**5.5**	2.8	**8.5**	2.1	**5.9**
18:3n-6	0.4	**1.3**	0.7	**1.1**	0.4	**1.2**	0.9	**2.6**	0.4	**1.1**
20:2n-6	0.3	**1.0**	0.3	**0.5**	0.2	**0.5**	0.2	**0.6**	0.0	**0.0**
20:3n-6	0.2	**0.7**	0.0	**0.0**	0.3	**0.8**	0.3	**1.0**	0.3	**0.7**
20:4n-6 **ARA**	1.0	**3.0**	1.0	**1.6**	3.8	**11.1**	1.6	**4.9**	1.9	**5.2**
22:5n-6	1.5	**4.6**	2.1	**3.5**	1.3	**3.7**	1.5	**4.5**	1.3	**3.6**
Total n-6 PUFA	6.0	**18.6**	6.6	**10.8**	7.8	**22.9**	7.3	**22.1**	6.0	**16.5**
18:2n-3	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.1	**0.4**	0.0	**0.0**
18:3n-3	3.5	**10.8**	3.5	**5.7**	1.6	**4.7**	2.1	**6.4**	1.9	**5.2**
18:4n-3	1.8	**5.6**	1.8	**3.0**	0.6	**1.8**	3.6	**10.8**	1.0	**2.8**
20:3n-3	0.2	**0.6**	0.0	**0.0**	0.0	**0.0**	0.1	**0.4**	0.0	**0.0**
20:4n-3	1.1	**3.3**	1.0	**1.6**	0.4	**1.2**	1.8	**5.6**	0.7	**2.0**
20:5n-3 **EPA**	6.3	**19.4**	6.3	**10.3**	12.2	**36.1**	9.0	**27.2**	10.4	**29.0**
22:5n-3	0.4	**1.1**	0.3	**0.5**	1.0	**3.1**	0.8	**2.5**	0.8	**2.3**
22:6n-3 **DHA**	38.7	**119.3**	41.7	**68.4**	30.7	**90.4**	27.1	**81.8**	34.7	**96.1**
Total n-3 PUFA	51.8	**159.9**	54.6	**89.6**	46.5	**137.2**	44.8	**135.1**	49.5	**137.4**
16:2	0.0	**0.0**	0.0	**0.0**	0.3	**0.7**	0.5	**1.4**	0.0	**0.0**
16:3	0.0	**0.0**	0.0	**0.0**	0.4	**1.1**	0.5	**1.7**	0.3	**0.8**
16:4	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.1	**0.3**	0.0	**0.0**
Total PUFA	57.9	**178.5**	61.2	**100.4**	54.9	**161.9**	53.3	**160.6**	55.8	**154.8**
Total	100.0	**308.5**	100	**164.1**	100	**294.9**	100	**301.4**	100	**277.4**
DHA/EPA		**6**		**6.6**		**2.5**		**3**		**3.3**
EPA/ARA		**6.5**		**6.4**		**3.3**		**5.5**		**5.6**

Algal mix Stage	**1 copepodites**		**2 copepodites**		**3 copepodites**		**4 copepodites**		**5 copepodites**	
	% fatty acid	**g/mg lipid**	% fatty acid	**g/mg lipid**	% fatty acid	**g/mg lipid**	% fatty acid	**g/mg lipid**	% fatty acid	**g/mg lipid**
14:0	1.9	**2.5**	1.9	**2.8**	4.8	**5.7**	5.0	**13.2**	3.9	**5.3**
15:0	0.5	**0.7**	0.5	**0.8**	1.4	**1.7**	0.5	**1.4**	0.5	**0.6**
16:0	16.3	**21.1**	17.0	**24.7**	17.4	**20.7**	15.2	**40.5**	16.4	**22.4**
i-17:0	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**
18:0	12.2	**15.8**	14.3	**20.7**	9.1	**10.9**	8.4	**22.4**	10.9	**14.8**
20:0	0.0	**0.0**	0.4	**0.5**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**
22:0	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**
Total saturated	31.0	**40.0**	34.2	**49.5**	32.7	**38.9**	29.1	**77.5**	31.7	**43.1**
16:1n-9	1.1	**1.4**	0.8	**1.2**	1.0	**1.1**	0.7	**1.8**	0.8	**1.1**
16:1n-7	5.1	**6.6**	2.9	**4.2**	9.4	**11.1**	9.7	**25.8**	6.6	**9.0**
18:1n-11	0.0	**0.0**	0.0	**0.0**	1.5	**1.8**	0.0	**0.0**	0.0	**0.0**
18:1n-9	4.0	**5.2**	4.1	**5.9**	4.7	**5.7**	5.4	**14.3**	3.8	**5.2**
18:1n-7	3.0	**3.9**	1.7	**2.4**	4.7	**5.6**	2.8	**7.5**	2.5	**3.5**
20:1n-11	0.0	**0.0**	0.0	**0.0**	1.1	**1.3**	0.0	**0.0**	0.0	**0.0**
20:1n-9	0.5	**0.6**	0.6	**0.9**	1.0	**1.2**	0.7	**1.8**	0.5	**0.7**
20:1n-7	1.9	**2.4**	0.0	**0.0**	0.7	**0.8**	0.3	**0.7**	0.0	**0.0**
22:1n-11	0.9	**1.2**	0.0	**0.0**	0.5	**0.7**	0.3	**0.8**	0.0	**0.0**
22:1n-9	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**
24:1n-9	0.0	**0.0**	0.5	**0.7**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**
Total monounsaturated	16.4	**21.3**	10.5	**15.2**	24.6	**29.3**	19.8	**52.7**	14.3	**19.5**
18:2n-6	2.5	**3.2**	2.5	**3.6**	4.2	**5.0**	2.8	**7.5**	2.5	**3.4**
18:3n-6	0.9	**1.2**	0.3	**0.4**	1.1	**1.3**	0.8	**2.2**	0.7	**0.9**
20:2n-6	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**
20:3n-6	0.4	**0.6**	0.3	**0.5**	0.7	**0.8**	0.4	**0.9**	0.0	**0.0**
20:4n-6 **ARA**	1.2	**1.5**	1.1	**1.7**	3.2	**3.8**	1.7	**4.6**	1.9	**2.6**
22:5n-6	1.4	**1.7**	2.2	**3.2**	0.6	**0.7**	1.4	**3.8**	1.4	**1.9**
Total n-6 PUFA	6.4	**8.3**	6.4	**9.3**	9.7	**11.6**	7.1	**19.0**	6.5	**8.8**
18:2n-3	0.0	**0.0**	0.0	**0.0**	0.5	**0.6**	0.0	**0.0**	0.0	**0.0**
18:3n-3	3.5	**4.5**	2.7	**4.0**	3.5	**4.2**	2.4	**6.3**	2.7	**3.7**
18:4n-3	2.2	**2.8**	2.1	**3.1**	1.0	**1.2**	4.2	**11.2**	2.9	**3.9**
20:3n-3	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**
20:4n-3	0.9	**1.1**	0.9	**1.3**	1.8	**2.1**	1.7	**4.6**	1.2	**1.7**
20:5n-3 **EPA**	6.7	**8.7**	5.7	**8.3**	8.9	**10.7**	9.5	**25.4**	10.7	**14.5**
22:5n-3	0.3	**0.5**	0.3	**0.4**	1.3	**1.6**	0.8	**2.1**	0.8	**1.0**
22:6n-3 **DHA**	32.6	**42.1**	37.1	**53.8**	15.0	**17.9**	24.1	**64.2**	28.8	**39.2**
Total n-3 PUFA	46.2	**59.8**	48.9	**70.9**	32.2	**38.3**	42.7	**113.8**	47.0	**64.0**
16:2	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.6	**1.5**	0.5	**0.6**
16:3	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0.7	**1.8**	0.0	**0.0**
16:4	0.0	**0.0**	0.0	**0.0**	0.9	**1.0**	0.0	**0.0**	0.0	**0.0**
Total PUFA	52.6	**68.0**	55.3	**80.2**	42.8	**51.0**	51.1	**136.0**	54.0	**73.5**
Total	100.0	**129.3**	100	**144.9**	100	**119.1**	100	**266.2**	100	**136.1**

Table 3.3: Fatty acid analysis of Artemia enriched with Algamac2000 (Aquafauna Biomarine®) or Super Selco (Inve®).

	Algamac2000	SuperSelco
	% FATTY ACID	% FATTY ACID
14:0	2.6	0.9
15:0	0.5	0.2
16:0	15.3	8.7
18:0	6.4	4.9
20:0	0.2	0.2
22:0	0.0	0.2
24:0	0.1	0.0
Total saturated	**25.2**	**15.2**
17:0		
16:1n-9	0.7	0.6
16:1n-7	3.2	2.7
18:1n-11	0.0	0.0
18:1n-9	17.2	17.5
18:1n-7	6.4	5.8
20:1n-9/n-11	0.6	1.3
20:1n-7	0.1	0.2
22:1n-11	0.4	0.3
22:1n-9	0.0	0.0
22:1n-7	0.0	0.0
24:1	0.0	0.0
Total monounsatd	**28.6**	**28.4**
18:2n-6	4.4	4.8
18:3n-6	0.4	0.2
20:2n-6	0.2	0.2
20:3n-6	0.2	0.2
20:4n-6 **ARA**	2.4	2.0
22:4n-6	0.0	0.0
22:5n-6	1.9	0.2
Total n-6 PUFA	**9.5**	**7.7**
18:3n-3	21.0	14.9
18:4n-3	2.1	1.8
20:3n-3	0.7	0.5
20:4n-3	0.6	0.9
20:5n-3 **EPA**	6.2	21.6
22:5n-3	0.1	0.5
22:6n-3 **DHA**	5.0	8.3
Total n-3 PUFA	**35.9**	**48.5**
16:2	0.1	0.1
16:3	0.7	0.1
16:4	0.0	0.0
Total PUFA	**46.2**	**56.5**
Total	**100.0**	**100.0**

1.8 Discussion

The best population growths, assessed as number of individuals from each size fraction of the population sampled from the trays on day 20, were associated with the use of *Chaetoceros.* Mix 5 appeared to be the best of the tested mixes as it continually produced more copepodites and adults than any of the others. Such a finding is in keeping with the suggestion that mixed diets are superior to monocultures for copepod production (Lee *et al.* 1985), as mix 5 included all the algal species used in the experiment. The fact that it includes a diatom and a flagellate bears out Kraul's (1989) recommendation that feeds should be made up of species from those two groups, although the addition of the chrysophyte *I. galbana*

gave consistently, although not significantly, higher survival than mix 3 which was based purely on *C. mulleri* and *R. reticulata*.

Although mix 4 gave rise to the highest final production of naupliae, it consistently gave the lowest number of adults and copepodites, suggesting that reproduction and survival are influenced by different factors. Also when analysing the numbers of the different size fractions of the populations through time, there was a clear single size class population developing, more apparent in mix 3. This treatment showed the highest numbers of naupliae early in the experiment up to a peak in day 8 (see figure 3.1 e). This population group developed into copepodites, and a peak of this size class for treatment 3 appeared between days 10-12 (figure 3.1 c). In figure 3.1 a, the final survival of this population into adults could be observed at around day 16. This pattern could be observed for every dietary treatment, and would be relevant when elucidating a best husbandry practise for the maximum production of copepods.

The analysis of the data of naupliae production (figure 3.2) shows that the highest yield was obtained between days 6 and 10. Productivity fell dramatically after day 10, probably due to the population stabilising and also to the build up of ammonia. When analysing the data in this way, mix 5 produced low yields of naupliae per adult. Best results were obtained with mix 3 (maximum average production 12 naupliae per adult on day 6).

The fatty acid content of *T. holothuriae* is influenced by the contents in its diet, although it does have the ability to synthesise such lipids if the diet is deficient in them (Nanton & Castell 1999). High levels of long chain fatty acids are essential for the development of larval fish (Sargent *et al.* 1997). For this reasons, the different content of PUFA between copepodites and adults, aside of the size difference, should be considered when choosing the stage of Tisbe to feed to the fish larvae. The fatty acid content of the naupliae is similar to that of the adults. It would be possible to define their fatty acid profile through the diet offered to the copepods, to address the needs of the fish species of choice. It is also possible to use a different diet for naupliae production and for on-growing of the naupliae to adult stage (see recommendations in final chapter).

In spite of the affinity that the copepods showed for the pebbles, their presence gave no improvement in the production of copepods. However, this experiment was intended to examine the effect of biofilm inoculation, trying to avoid any effect of increase of substrate surface. The copepods were observed gracing on the pebbles during the first days of the experiment. Communications by J. Støttrup suggest that the rate of grazing of the copepods on the biofilm would have soon out-compete the growing capacity of the later, with no nutritional advantage from the presence of the pebbles in the culture tray thereafter. She also described biofilm grazing occurring in set-ups that included biomedia with well developed biofilm. This can add a significant amount of nutrition to the cultures.

The number of rotifers present in the trays during the experiment was a cause for considerable concern. The 5μm filter that incoming seawater was passed through was replaced with a 1μm filter for subsequent experiments.

2 Salinity and Feed Supplementation

2.1 Introduction

Although *T. holothuriae* is a marine copepod, Heath (1994) reported that the best growth and survival rates were obtained at the relatively low salinities of 25-30‰. Other studies report optimal salinity as being much higher at around 38‰ (Miliou & Moraitou-Apostolopoulou 1991*a*, Miliou 1996), but those studies were carried out on populations collected from the Mediterranean, while Heath was using copepods from the same population as the present study.

Several studies have reported that the production of copepods is enhanced when algal diets are supplemented with yeast (Carli *et al.* 1995) or the extracts of animals such as bivalves or fish (Miliou & Moraitou-Apostolopoulou 1991*b*, Heath 1994). The quantities of supplements added vary widely, from 1-3mg l^{-1} (Hoff & Snell 1987) to 100mg l^{-1} (Carli *et al.* 1995). It is uncertain whether the copepods ingest the feeds directly, or whether their benefit is conferred by enhancement of the microbiota available to the copepods.

2.2 Materials & Methods

2.2.1 Experimental Design

Six trays from each stack were used for the experiment, paired by row. Half from each stack were assigned to a low salinity treatment and half to a high salinity treatment. Similarly, the algal mix added to half was supplemented, and half was unsupplemented. Treatments were assigned in such a way as to ensure due separation between replicates of the same treatment. Each tray was stocked with 60,000 copepods from the 150μm filter bag.

The low salinity treatment was maintained at 25-30‰ by regular addition of distilled water. This treatment was compared to a normal salinity of 31-38‰.

Trays were fed with 1l of the mix of *C. mulleri*, *R. reticulata* and *I. galbana* designated mix 5 in Chapter 0. The feed supplement (8.5% lipid, 54 % protein) used was a starter feed used for the weaning of fish larvae (Nippai, Yokohama, Japan), added at 50μg l^{-1} at the same time as the algal mix. The trays were fed on days 0, 2, 5, 7, 9, 12, 14, 16 and 19, which was less often than in the previous trial (Chapter 0), due to the high turbidity observed in the latter stages of that experiment.

Otherwise, culture was carried out under standard conditions with seawater passed through a 1μm filter. Sequential sampling of copepod numbers was as previously described.

2.2.2 Environmental Monitoring

Salinity and temperature were measured as previously described, and salinity adjusted as necessary. In addition, the turbidity and ammonia concentration were measured on days 1, 6, 12, 16 and 20 of the experiment.

Ammonia was measured using the phenate method (Eaton *et al.* 1995)**.** Samples of 15ml from each tray were collected and 1ml 100g l^{-1} phenol in ethanol and 5g l^{-}

[1]sodium nitroferricyanide in deionised water were added to each. An oxidising solution of 80% v/v alkaline solution (200g l^{-1} sodium citrate and 10g l^{-1} sodium hydroxide in deionised water) and 20% v/v of 5% w/v sodium hypochlorite solution was prepared immediately before use, and 2.5ml were added to each sample. A control sample was prepared using deionised water to validate the reagents, and all samples were incubated at a room temperature of 22-26°C for 50min. After incubation, the samples were poured into 15ml glass vials and their absorbance at 640nm measured, using a blank of deionised water. The optical densities were compared to a calibration curve, and the concentrations of ammonia were mesured.

Turbidity was measured by filling a 4ml cuvette with water from each tray and measuring its absorbance at 750nm, blanking on seawater (Hoff & Snell 1987).

2.2.3 Statistical analysis

All variables were compared by ANCOVA, using the row as the co-variate. The stack, salinity treatment, presence or absence of supplements and sample day were fixed crossed factors, and all possible interactions between salinity treatment, presence or absence of supplements and sample day were included. Daily naupliar productivity was also analysed.

Tukey multiple comparisons were used to elucidate differences where they were found.

2.3 Results

2.3.1 Copepod numbers

The number of adult copepods, copepodites, nauplii and the total number of copepods of all stages were compared between treatments. All variables covaried with the position of the tray in the stack ($p < 0.05$), except for the number of copepodites where the significance of the covariate was equivocal ($p = 0.078$). The most likely reason for that is the difference in temperature across the stacks.

There were significantly more adults on day 20 in the high salinity treatments ($F = 12.75$, $df = 1$, $p < 0.005$), but there was also an interaction between salinity treatment and sample day (3.48, $df = 10$, $p < 0.005$) and multiple comparisons revealed that the differences were only significant on days 18 ($q = 5.50$, $p < 0.0005$) and 20 ($q = 3.72$, $p < 0.05$). The use of feed supplements made no significant difference ($p > 0.05$).

The salinity treatments had no effect on the number of copepodites on day 20 ($p > 0.05$), but there were significantly less in the treatments that were supplemented with the weaning diet ($F = 5.88$, $df = 1$, $p < 0.05$). There was also a significant interaction between the salinity and the presence or absence of the supplement ($F = 28.96$, $df = 1$, $p < 0.0005$), and multiple comparisons indicated that significantly less copepodites were present at day 20 at low salinity with supplementation than with any other treatment ($p < 0.005$).

Although neither treatment had a significant effect on the total numbers of copepods across the whole experiment ($p > 0.05$), there were significant interactions between salinity treatment and sample day ($F = 3.75$, $df = 10$, $p < 0.0005$) and between the salinity treatment and the presence of the weaning diet ($F = 21.74$, $df = 1$, $p <$

0.0005). Multiple comparisons revealed that there were more copepods present in the unsupplemented cultures on day 20 (q = 5.29, p < 0.0005) but no other

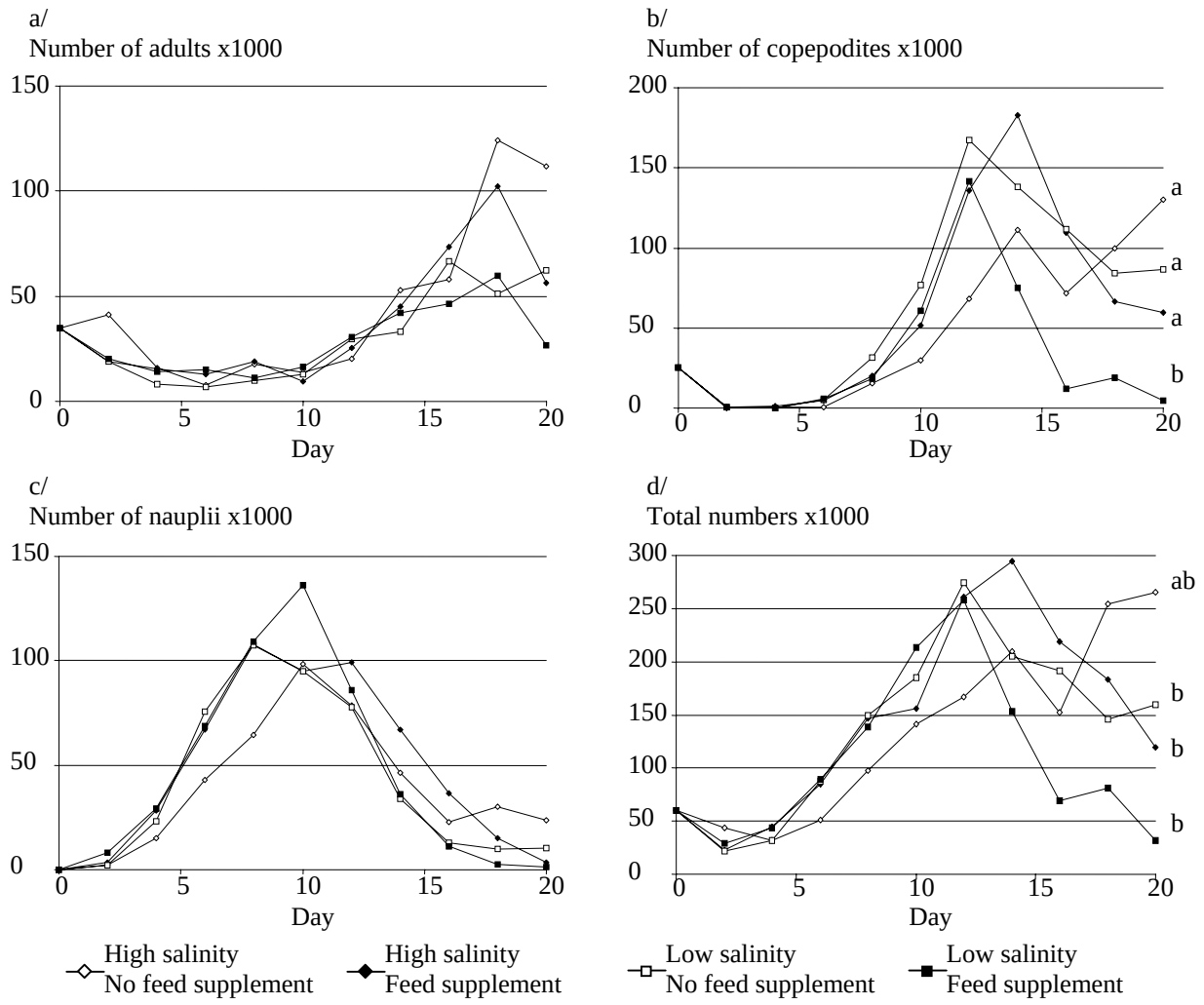


Fig. 4.1. Mean number of a/ adult copepods, b/ copepodites, c/ nauplii and d/ total population of three trays cultured at two salinity levels and with or without feed supplements.

differences related to the supplementation.

When analysing daily nauplii production per adult, no significant difference was found between treatments, and only day was a significant predictor (F = 11.65, df = 9, p < 0.0005). However, there was a significant negative relation between ammonia concentration and daily naupliar production per adult (F = 4.91, p < 0,05).

2.3.2 Fatty acid analysis

Table 4.1 shows the fatty acid analysis and total lipid content of adult copepods collected at the end of the experiment on day 21. The total content of lipids of the copepods fed the supplemented diet was higher (362 ± 33.3 µg/mg lipid) than that of the algae only fed copepods (298 ± 23.2 µg/mg lipid). However, this was achieved through an increase in the % of saturated and monounsaturated fatty acids, and a decrease in the content of polyunsaturated fatty acids, in particular of the n-3 series. Algae only fed copepods had higher contents of both PUFA and n-3 PUFA.

Table 4.1: Fatty acid composition of adult copepods fed on standard algal diet with and without the supplementation of a fish larvae weaning diet.

	Supplement				No supplement			
	% FA	sd	g/mg lipid	sd	% FA	sd	g/mg lipid	sd
14:0	4.8	2.5	18.0	10.6	3.1	0.3	9.1	0.8
15:0	0.3	0.1	1.2	0.4	0.4	0.1	1.1	0.2
16:0	21.0	6.1	77.2	28.5	15.6	1.0	46.3	1.8
i-17:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:0	10.6	1.2	38.6	7.6	11.0	1.4	32.5	1.6
20:0	0.3	0.1	1.0	0.3	0.2	0.0	0.7	0.1
22:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total saturated	37.0	9.8	136.1	46.8	30.2	2.6	89.6	2.6
16:1n-9	0.6	0.2	2.2	0.4	1.6	0.8	4.7	2.3
16:1n-7	5.8	2.0	20.5	4.9	7.2	0.9	21.4	3.3
18:1n-11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:1n-9	7.9	3.0	29.2	13.2	4.2	0.2	12.6	1.3
18:1n-7	4.3	1.9	16.1	7.8	2.6	0.3	7.6	0.5
20:1n-11	0.2	0.2	0.6	0.6	0.0	0.0	0.0	0.0
20:1n-9	1.0	0.7	3.7	2.9	0.3	0.1	0.9	0.4
20:1n-7	0.2	0.1	0.9	0.4	0.2	0.1	0.6	0.3
22:1n-11	0.4	0.4	1.6	1.5	0.4	0.5	1.0	1.4
22:1n-9	0.4	0.4	1.6	1.5	0.0	0.1	0.1	0.2
24:1n-9	0.4	0.3	1.5	1.1	0.1	0.1	0.2	0.3
Total monounsaturated	21.2	4.8	77.9	23.6	16.5	0.9	49.0	1.8
18:2n-6	2.1	0.2	7.5	1.0	2.4	0.3	7.2	0.8
18:3n-6	0.4	0.2	1.4	0.5	0.7	0.1	2.1	0.2
20:2n-6	0.2	0.0	0.6	0.1	0.1	0.1	0.3	0.2
20:3n-6	0.2	0.1	0.8	0.1	0.3	0.0	0.8	0.1
20:4n-6	1.6	1.0	5.6	2.9	2.3	0.2	7.0	1.2
22:5n-6	0.7	0.7	2.5	2.3	1.4	0.1	4.1	0.4
Total n-6 PUFA	5.2	1.9	18.4	5.2	7.2	0.1	21.4	1.4
18:2n-3	0.2	0.0	0.6	0.2	0.1	0.1	0.3	0.2
18:3n-3	1.7	0.8	5.9	2.4	2.8	0.4	8.3	1.5
18:4n-3	2.1	0.7	7.6	1.8	3.6	1.0	10.7	3.2
20:3n-3	0.0	0.1	0.1	0.2	0.0	0.0	0.0	0.0
20:4n-3	0.6	0.2	2.2	0.7	0.8	0.1	2.5	0.3
20:5n-3	9.3	1.8	33.1	3.1	10.4	1.1	31.3	5.5
22:5n-3	1.0	0.0	3.7	0.3	1.1	0.1	3.2	0.4
22:6n-3	21.0	9.5	73.9	26.8	26.9	2.6	80.5	13.5
Total n-3 PUFA	35.9	12.8	127.1	33.4	45.7	3.4	136.8	20.7
16:2	0.2	0.1	0.8	0.3	0.2	0.0	0.6	0.1
16:3	0.2	0.1	0.6	0.2	0.2	0.0	0.6	0.1
16:4	0.3	0.3	1.1	1.0	0.0	0.0	0.0	0.0
Total PUFA	41.8	14.4	148.0	37.4	53.3	3.3	159.4	22.2
Total	100.0	0.0	362.0	33.3	100.0		298.1	23.2
mg Lipids per sample			4.1	1.6			1.7	0.8

2.3.3 Environmental monitoring

Turbidity and ammonia were monitored throughout the experiment. Across the whole experiment, the high salinity treatments were more turbid than the low ($F = 10.41$, $df = 1$, $p < 0.005$), and trays that had received the weaning diet were more turbid than those that had not ($F = 4.74$, $df = 1$, $p < 0.05$). The two treatments had a

significant interaction ($F = 4.48$, $df = 1$, $p < 0.05$), and multiple comparisons revealed that the high salinity treatment that had received the weaning diet generated more turbidity than any other treatment ($p < 0.05$) and there were no other differences. TAN (figure 4.1) was increased by both low salinity ($F = 9.54$, $df = 1$, $p < 0.005$) and by the presence of the weaning diet ($F = 22.02$, $df = 1$, $p < 0.0005$).

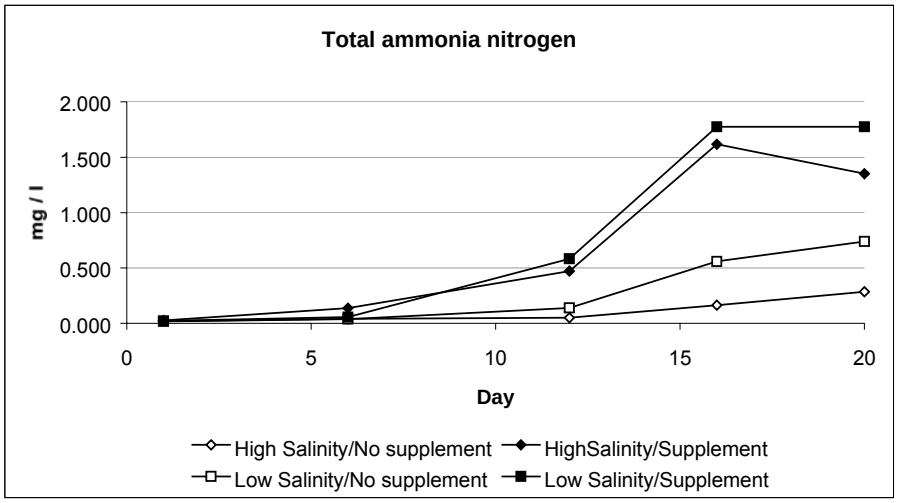


Figure 4.2: TAN in the trays during the experiment. Treatments combined Low and High salinity, with and without nutritional Supplementation.

2.4 Discussion

Salinity had no significant effect in itself on production. This suggests that this strain of *T. holothuriae* is tolerant to a relatively wide range of salinity.

The benefits of animal-based feed supplements in experimental copepod rearing systems had been previously reported (Miliou & Moraïtou-Apostolopoulou 1991*b*, Norsker & Støttrup 1994). The weaning diet used in the present study did not affect production until the end of the experiment. The build up of TAN in the trays fed the nutritional supplement is the more likely explanation for the late decline in numbers of copepods. It is probably significant that most reports of copepod production systems that use animal-based feed supplements refer to systems with continuous exchange of water (Kahan *et al.* 1982, Zhang & Uhlig 1993). The results confirm ammonia levels as one of the major constraints for naupliar productivity. Observations by Støttrup indicate that diets with high lipid content (60%) used in combination with water exchange gave good production results. These could be used to partially replace the algae, saving some of the operational costs associated with their production. However, attention should be paid to the nature of the supplement diet, as the analysis showed that the balance of PUFA in the copepods can be altered by it, and hence their nutritional value for the fish larvae.

3 Substrate Enhancement

3.1 Introduction

As adult *T. holothuriae* are demersal, it is possible that the area of substrate available for them to attach to may limit the capacity of a culture system. It is also possible that increasing the surface area available in a system may allow the development of a larger amount of biofilm, which the adults may graze.

Heath (1994) found that placing plastic biofilter material in culture tanks increased the productivity of *T. holothuriae*. Støttrup & Norsker (1997) found that bioreactors containing polypropylene balls gave a poorer productivity than a tray culture system, but believed that the limitation was nutritional rather than a constraint of the system design.

Some studies have found that substrates comprised of small particles of less than 1mm in diameter are ideal for the long-term culture of demersal copepods (Hockin 1981, Chandler 1986). However, substrates of this size are rarely used in systems used for aquaculture, due to the difficulty in separating the copepods from the substrate during harvest. Chandler (1986) suggested that mud could be used as a substrate and separated from adult copepods using a 125μm filter, but that would entail the loss of nauplii and early copepodites, which may be required for restocking if the larger fractions are used as fish food (Chapter 4). Consequently, small particle sizes are not favoured in production systems, in spite of their larger surface area.

The present study considered the possibility of enhancing the standard culture system with pebbles of a size that would increase surface area for adult settlement without making harvesting unduly difficult.

3.2 Materials and Methods

3.2.1 Experimental Design

Eight trays were used, equally split between the two stacks and paired by row. One tray on each row was assigned as a treatment and the other as a control.

Culture was under standard conditions, with seawater passed through a 1μm filter Temperature and salinity monitored regularly and the latter maintained at between 33-40‰. All trays were fed with the algal mix of *C. mulleri*, *R. reticulata* and *I. galbana* (Chapter 0).

Each tray was filled with 5l of seawater and stocked with 2.8×10^5 aults.

3.2.2 Preparation of Substrate

The substrate used was made up of small pebbles of 5-10cm in length. They were all autoclaved before use to ensure sterility. The pebbles were then kept in buckets of seawater inoculated with the algal mix for 4d to allow the development of a biofilm.

Before the trays were stocked with copepods, a measuring jug was used to place 3l of pebbles in each treatment tray, which displaced approximately 1l of water.

3.2.3 Sampling

Sequential sampling was not possible because it was not possible to homogenise the trays with the substrate in place, so sampling was carried out by harvesting (Section 1.2), with the addition of a grill to hold the pebbles while they were sluiced with seawater. Half of the experiment was harvested on day 10, and half on day 14.

3.2.4 Environmental monitoring

Turbidity and TAN were measured (Section 2.2.2) on day 9. Temperature and salinity were measured regularly throughout the study.

3.2.5 Statistical analysis

The effect of the presence of pebbles on the numbers of copepods recovered from the 45μm, 100μm and 150μm filter bags was compared by ANOVA. Sample day and the presence of pebbles were crossed factors, and with the position in the stack nested within sample day. The same analysis was applied to the sum of the copepod population.

Turbidity and TAN were compared using the two stacks, the position in the stack and the presence or absence of pebbles as crossed factors in an ANOVA. Numbers of nauplii per adult were also analysed.

3.3 Results

3.3.1 Copepod numbers

Significantly less adult copepods were collected from the trays with a pebble substrate ($F = 11.92$, $df = 1$, $p < 0.05$) on days 10 and 13, indicating poorer survival. However there were no differences in the numbers of nauplii or copepodites, or in the total population numbers ($p > 0.05$).

Numbers of nauplii per adult were analysed for each sampling day (Figure 5.1). There was a trend towards better production of the trays with pebble substrate on both sampling days and also when sampling days were pooled together. However this trend was not quite significant ($p = 0.09$).

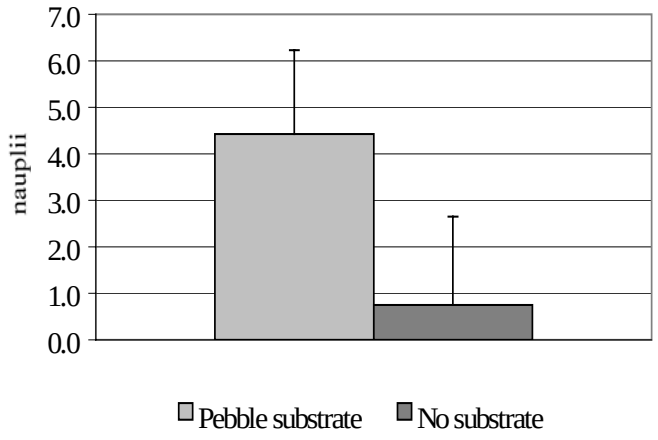


Figure 5.1: Mean number of nauplii per adult from trays with and without pebbles. Data are pooled from both sampling days; bars are s.e.m.

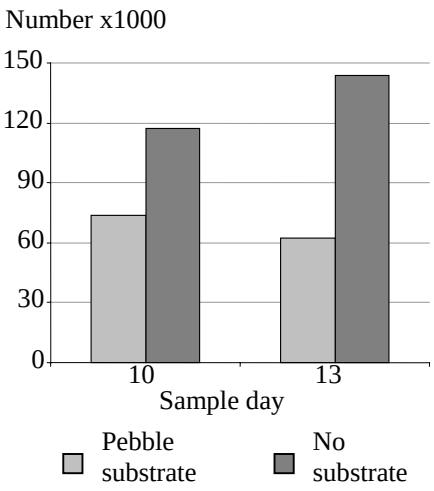


Fig. 5.2. Mean number of copepods harvested from 150µm fraction in the presence or absence of a pebble substrate.

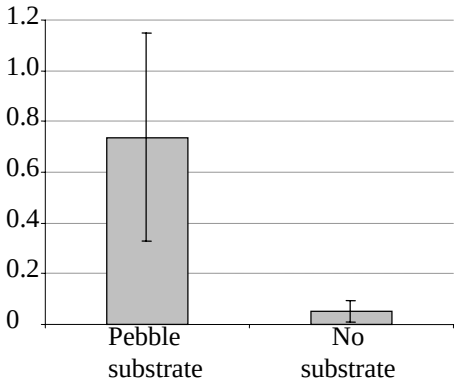


Fig. 5.3. Total ammonia nitrogen from trays with or without a pebble substrate.

3.3.2 Environmental monitoring

The presence of pebbles made no difference to turbidity ($p > 0.05$). However, TAN was considerably higher in the case of trays with the substrate ($F = 137.66$, $df = 1$, $p < 0.01$).

3.4 Discussion

The reduced number of copepods collected from the trays with a pebble substrate was a similar result to that obtained by Støttrup & Norsker (1994), although it is probably not a result of insufficient food in this case as large amounts of algae were added regularly. It is more likely that their reduced survival was a result of the higher levels of waste products present in those trays, as evidenced by the higher levels of ammonia on day 9. Even with the much higher levels of ammonia, trays with the enhanced substrate presented a trend towards higher numbers of nauplii per adult. These results strongly indicate that the accumulation of waste products is more important than physical crowding in limiting the productivity of the tray culture system. This again suggests that the combination of substrate enhancement plus control of ammonia levels (water exchange) would provide increased naupliar production.

4 Comparison of Stocking Regimes

4.1 Introduction

Results from preliminary work on the tray culture system suggested that the highest yields were obtained from trays stocked with relatively low numbers of copepods. Similar results were recorded by Zhang & Uhlig (1993), who found that high stocking densities of *T. holothuriae* increased larval mortality and development time.

During the present experiment, a range of stocking regimes, including different numbers and life stages, were tested.

4.2 Materials and Methods

4.2.1 Experimental design

Copepods were cultured under standard conditions, using seawater filtered at 1μm and fed with the *R. reticulata*, *I. galbana* and *C. mulleri* mix on days 0, 3, 6, 8 and 10.

Five replicates of four stocking regimes (Table 0.1) were randomly arranged in the two stacks. Copepods and rotifers from various aliquots were counted (Section 1.6.3) on every second day from day 4 to day 16.

Table 6.1. Number and size of copepods used in the four stocking regimes.

Regime	1	2	3	4
Number of copepods	2500	15000	50,000	70,000
Size fraction	>150μm	>150μm	100-150μm	45-100μm

4.2.2 Environmental monitoring

Turbidity and total ammonia nitrogen were measured (Section 2.2.2) on days 7, 10, 13 and 16. Temperature and salinity were monitored throughout the trial, and salinity was maintained between 33-40‰.

4.2.3 Statistical analysis

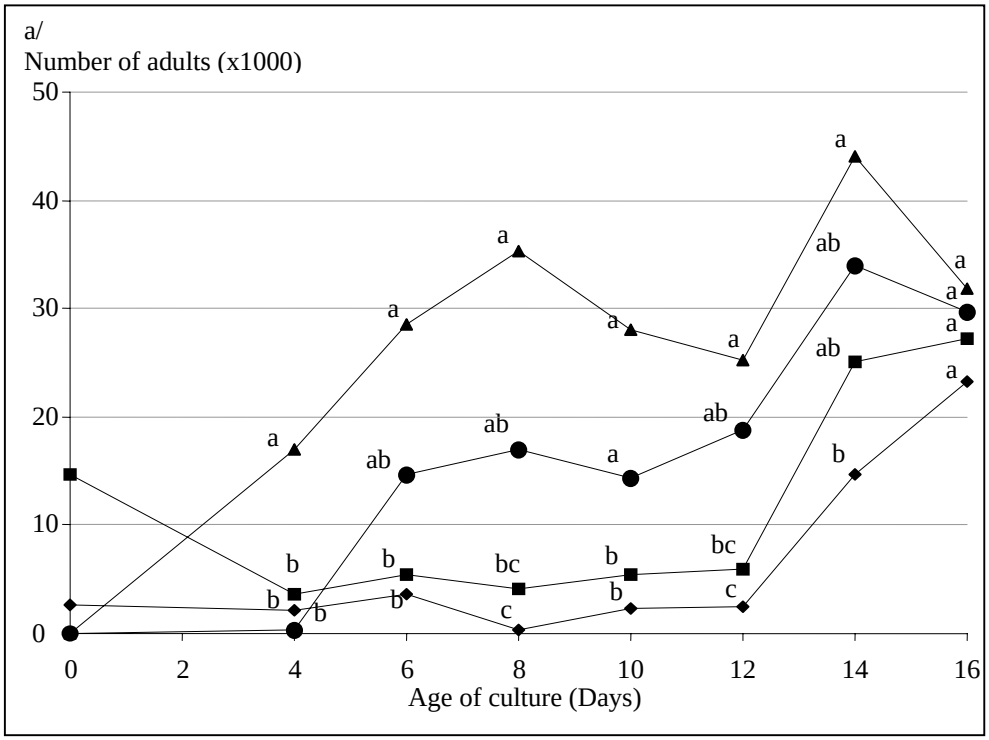
All data were analysed by ANCOVA, using position in the stack as a covariate. The two stacks, day and stocking regime were crossed fixed factors, and the interaction between regime and day was considered. Significant results were followed by Tukey multiple comparisons.

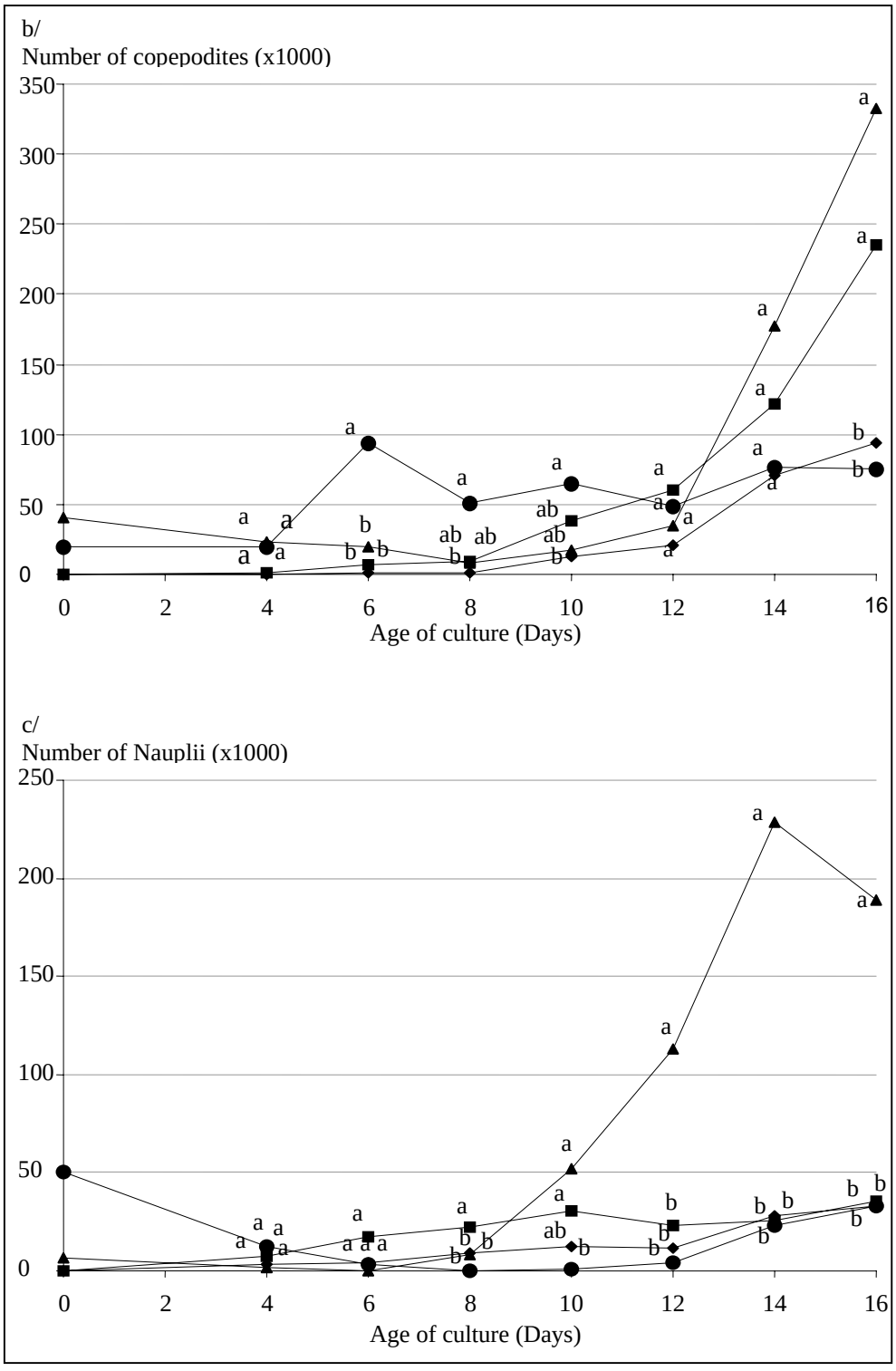
4.3 Results

4.3.1 Copepod numbers

Comparison of the four stocking regimes revealed significant differences between them and interactions between stocking regime and sample day in the case of all life cycle stages ($p < 0.0005$). The number of copepods covaried with the position in the stack in all cases ($p < 0.005$) (Fig. 6.2).

Trays stocked with the 100-150μm fraction gave the highest number of adults from an early stage of the trial, and also the highest total number of nauplii after day 10. Trays stocked with the 45-100μm fraction gave high numbers of adults and copepodites, although very few nauplii were produced until day 12. The trays stocked with the >150μm gave generally low total numbers of all categories, with those stocked with only 2,500 such copepods being particularly low, although those stocked with 15,000 gave numbers of copepodites and adults that were comparable with those produced by the best stocking regime towards the end of the trial.




b/
Number of copepodites (x1000)
350
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
Age of culture (Days)
c/
Number of Nauplii (x1000)
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
Age of culture (Days)

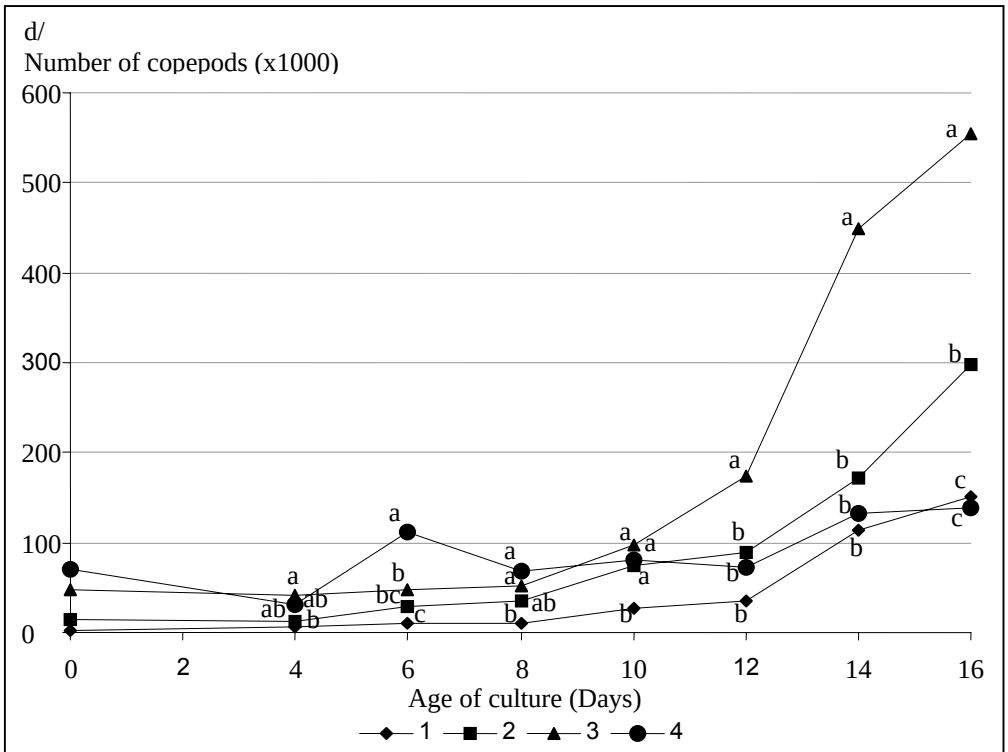


Fig. 6.2. Numbers of a/ adult copepods, b/ copepodites, c/ nauplii and d/ copepods of all stages in trays stocked with four different regimes. Letters indicate significance groups of comparisons on each day.

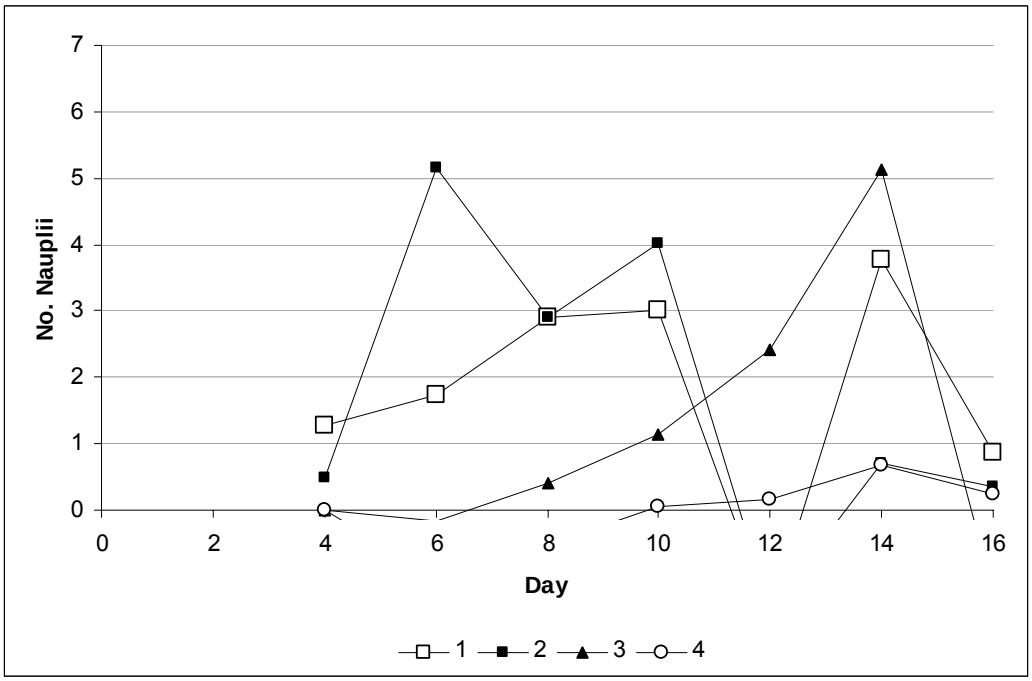


Figure 6.2: Average Nauplii production from each stocking regime.

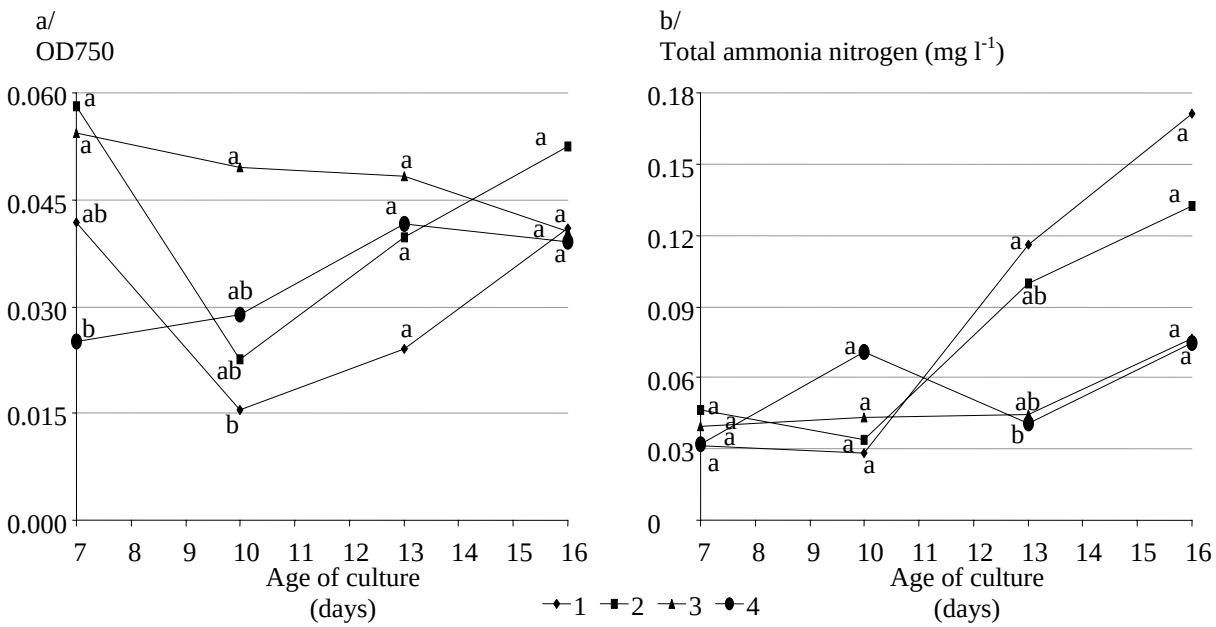


Fig. 6.4. Plots of a/ turbidity and b/ total ammonia nitrogen in tray cultures stocked with four different cultures. Letters indicate significance groups on each day.

4.3.2 Environmental monitoring

As with copepod numbers, both turbidity and total ammonia nitrogen showed significant differences between stocking regimes and significant interactions between stocking regime and day ($p < 0.05$). However, neither showed significant covariance with the position of the tray in the stack ($p > 0.05$).

Turbidity remained low in all treatments, although it was consistently higher in the trays stocked with the 110-150µm fraction (Fig. 6.4a). Ammonia levels showed a similar trend, staying generally low until late in the trial, but with the trays stocked with the 100-150µm fraction having consistently, if rarely significantly, higher levels (Fig. 6.4b).

4.4 Discussion

The most successful stocking regime of those tested was 50,000 copepods derived from the 100-150µm fraction. A high number of adults was obtained after a relatively short space of time, and sufficient copepodites were produced within 12 days to ensure that a tray could be restocked in a similar way if harvest was carried out at that point. Trays stocked with 70,000 nauplii produced large enough numbers of adults to suggest much higher survival rates than those observed in previous experiments.

Many previous studies relied on restocking with adult copepods (Støttrup & Norsker 1997). Those trays stocked from the >150µm fraction produced the best nauplii production (Number of nauplii per adult) early in the trial. The good survival and production in the trays stocked with copepodites indicate that higher concentrations of adults can be achieved. This, combined with frequent water exchange (daily to a few days) to attain low ammonia and intense harvest, will provide bigger harvests of

nauplii. The harvest could then be on-grown to the optimal live stage to suit the needs of the hatchery.

5 First Feeding of Halibut

5.1 Introduction

Copepods have been used successfully for the rearing of haddock *Melanogrammus aeglefinus* (Nanton & Castell 1998), Dover sole *Solea solea* (Heath & Moore 1997) and Atlantic halibut (McEvoy *et al.* 1998). The latter study found that copepods had considerably higher levels of polar lipids than enriched *Artemia*, which are usually used, and attributed the better pigmentation of copepod-fed halibut to that fact.

The intention of the present study was to evaluate the potential of cultured copepods in the hatchery production of Atlantic halibut and compare it to the standard regime based on enriched *Artemia salina*. Two trials were carried out in 100 l tanks, allowing for high replication. Two further runs were carried out in two commercial size 1300 l tanks each.

5.2 100 l tanks trials.

5.2.1 Materials and Methods

Halibut larvae were placed in 100l black plastic bin-shaped tanks, 200 larvae per tank. Each tank had an airstone, and incoming seawater was passed through a u.v. and a 5μm filter. The water was 'greened' by adding 1l of *Nannochloris atomus* from a standard culture system (Section 1.3) every day. Water was exchanged by passing a flow of approximately 0.3l min^{-1} through the tanks for 2h per day. The tanks were lit from above at 500-1000 lux. Temperature ranged between 6 and 9°C from start to end of each experiment. All treatments were fed with 20,000 prey items per day. *Artemia salina* were hatched from cysts and enriched with Algamac 2000®. *Tisbe holothuriae* were derived mainly from the 100-150μm fraction, supplemented from the >150μm fraction where necessary to make up numbers.

Sampled fish were culled with MS222 and digitally photographed at 2.5x magnification. Total length and myotome height were measured from the images recorded. Larvae were placed in Eppendorf tubes of known weights. The tubes were stored at -20°C until they were freeze dried for 72h and re-weighted. The weight of the tube was subtracted from the weight of the tube and the sample to calculate the sample dry weight.

The numbers of survivors from each of the four treatments of trial 1 was compared by 1-way ANOVA, while the numbers on the final day of trial 2 were pooled for each treatment and compared by Yates-corrected λ^2 analysis. Lengths, myotomes and dry weights were compared by 1-way ANOVA in all cases. Significant ANOVAs were followed by Tukey multiple comparisons.

5.2.2 Trial 1

Trial 1 compared the survival and dry weight of halibut fed on 100% *T. holothuriae*, 100% enriched *Artemia salina*, 75% enriched *Artemia* and 25% *T. holothuriae*, or a 50:50 mixture of the two prey items. The number of survivors in each tank were counted on day 22, and five fish were sampled from each tank and placed in a vial for dry weight analysis.

The survival was very low in all treatments (8-9%), and 1-way ANOVA found no difference between them on day 22 ($p > 0.05$). There were no differences between the dry weights of the fish sampled on day 22 ($p > 0.05$), although the low number of survivors rendered the statistical power very low.

5.2.3 Trial 2

The two treatment groups used were fed with either 100% enriched *Artemia* or 100% *T. holothuriae*. Ten fish were sampled for length and myotome height at the start of the experiment, and a further ten from each treatment on day 9. Samples were collected for dry weight analysis at the same time, with three tubes containing five fish each sampled from each treatment. The experiment was terminated on day 15, and the number of survivors per tank was counted.

Only three survivors were recovered from the copepod-fed halibut on day 15, while there were 635 survivors from the *Artemia*-fed fish ($\lambda^2 = 744.86$, df = 1, $p < 0.00001$). *Artemia*-fed halibut were significantly longer (19.4 mm ± 1.2) than copepod-fed halibut (17.8 ± 1.5) on day 9 ($q = 2.94$, $p < 0.05$). The lengths of neither were significantly different to the halibut at the time of stocking ($p > 0.05$). *Artemia*-fed halibut on day 9 (2.1 mg ± 0.7) were heavier than at the time of stocking ($q = 4.14$, $p < 0.05$). No significant increase of weight was observed in the copepod-fed halibut (1.4 mg ± 0.1, $p > 0.05$). There were no differences in myotome heights (*Artemia* fed 1.4 mm ± 0.3, Copepod fed 1.3 mm ± 02, $p > 0.05$).

5.3 Commercial scale runs

5.3.1 Materials and Methods

Two separate runs were carried out. For each run, two black plastic 1300l tanks were prepared following standard husbandry procedures (algae *N. atommus* addition, 0.5 l/min u.v. filtered water through-flow, 500-1000 lux). The halibut larvae were aged approximately 270°d at the time of stocking. In each run, the control tank was fed with the standard regime of enriched marine rotifers *Brachionus plicatilis* for the first seven days, and enriched *Artemia* for the rest of the trial. The treatment tanks were fed on *T. holothuriae* from the 100-150µm fraction for the first seven days, and a 50:50 mixture of *T. holothuriae* and enriched *Artemia* until day 20. *Artemia* was fed on demand to all tanks thereafter until metamorphosis, when the final sampling was carried out.

Fish were regularly sampled for length, myotome height and dry weight composition during both trials (see table 7.1). Length and myotome height were initially measured from photographs or with Vernier callipers when they grew too large to be photographed. Dry weight was measured as previously described.

Table 7.1. Number of samples collected for each parameter measured. Figures for dry weight indicate number of samples collected, with the number of fish per sample in parentheses.

Run	Day	Length	Myotome height	Dry weight
1	17	5	5	3 (5)

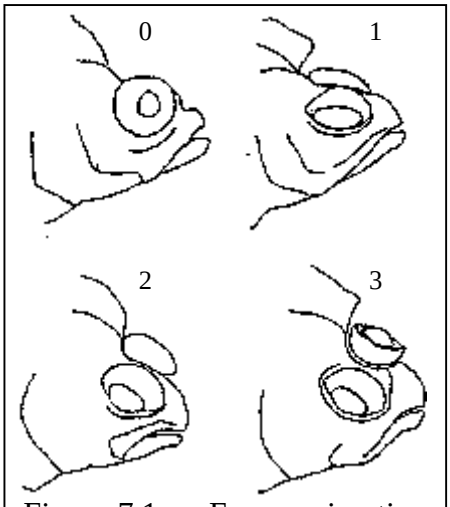


Fig. 7.1. Eye migration categories.

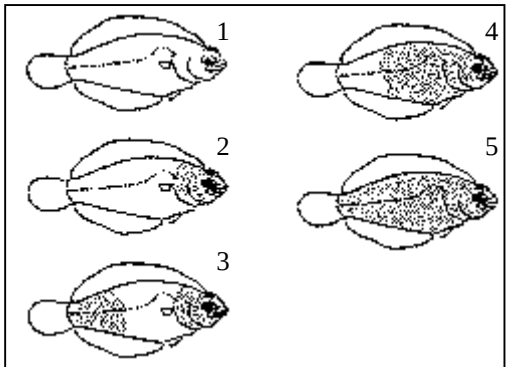


Fig. 7.2. Halibut pigmentation categories.

1	31	10	10	3 (5)
1	44	10	10	3 (5)
1	72	20	20	10 (1)
2	11	10	10	3 (5)
2	31	10	10	3 (3)
2	74	20	20	10 (1)

Twenty fish were sampled for pigmentation and eye migration on the final day of each trial. Eye migration was measured by assigning each fish to one of four categories (Fig. 7.1), and pigmentation was sampled by assigning each fish to one of five categories (Fig. 7.2).

Survival data were compared by Yates-corrected λ^2 analysis. Lengths and myotome heights were compared by ANCOVA using feed treatment as a fixed factor and sample day as a covariate. All dry weight data from run 2, and dry weight data collected on days 17, 31 and 44 of run 2 were compared by 2-way ANOVA with interaction, using day and feed as crossed factors. Dry weight data from run 1 collected on day 72 contained a different number of fish to those collected on other days, and were analysed separately by t-test. No fish was recorded as pigmentation category 3, so both pigmentation and eye migration data were regarded as ordinal and analysed by Mann-Whitney U test. All significant ANOVAs and ANCOVAs were followed by Tukey multiple comparisons.

5.3.2 Run 1

Each tank was stocked with 4460 halibut larvae. The tanks received 5.5 x 10^6 prey items during the first 7 days, and 0.4 x 10^6 per day until day 20 (100% *Artemia* or 50:50 Copepods: *Artemia*). Survival was assessed on days 22 and 72.

5.3.3 Run 2

Each tank was stocked with 2350 halibut larvae. The tanks received 2.5 x 10^6 prey items during the first 7 days and 0.2 x 10^6 per day until day 20 (100% *Artemia* or 50:50 Copepods: *Artemia*) Survival was assessed on days 31 and 74.

5.3.4 Results

Survival rates in both treatments of both runs ranged from 5.5% to 14.4% of the initial stock at the end of the experiments (see table 7.2). In run 1, there were nearly 50% more halibut left of the copepod fed treatment after 72 days ($\lambda^2 = 32.53$, df = 1, $p < 0.00001$). Survival until day 31 in Run 2 was similar for both treatments. However, survival of the control larvae was better by day 74 ($p < 0.05$).

Table 7.2. Numbers of fish remaining on sample days, with percentage of original number stocked in parentheses.

Run	Day	Control	Copepod-fed halibut
1	22	508 (11.4%)	864 (19.4%)
1	72	246 (5.5%)	384 (8.6%)
2	31	490 (20.9%)	434 (18.5%)
2	74	338 (14.4%)	197 (8.4%)

Both runs showed considerable variation in the stage of eye migration at the end of the experiment, but there were no significant differences between the treatments ($p > 0.05$).

There were no differences in the level of pigmentation between the treatments in run 1. However, halibut on the control treatment had more complete pigmentation in run 2 (W = 265, p < 0.0005). Halibut from the Control treatment were longer and had broader myotomes and higher dry weights in both runs (p < 0.05) (Fig. 7.3).

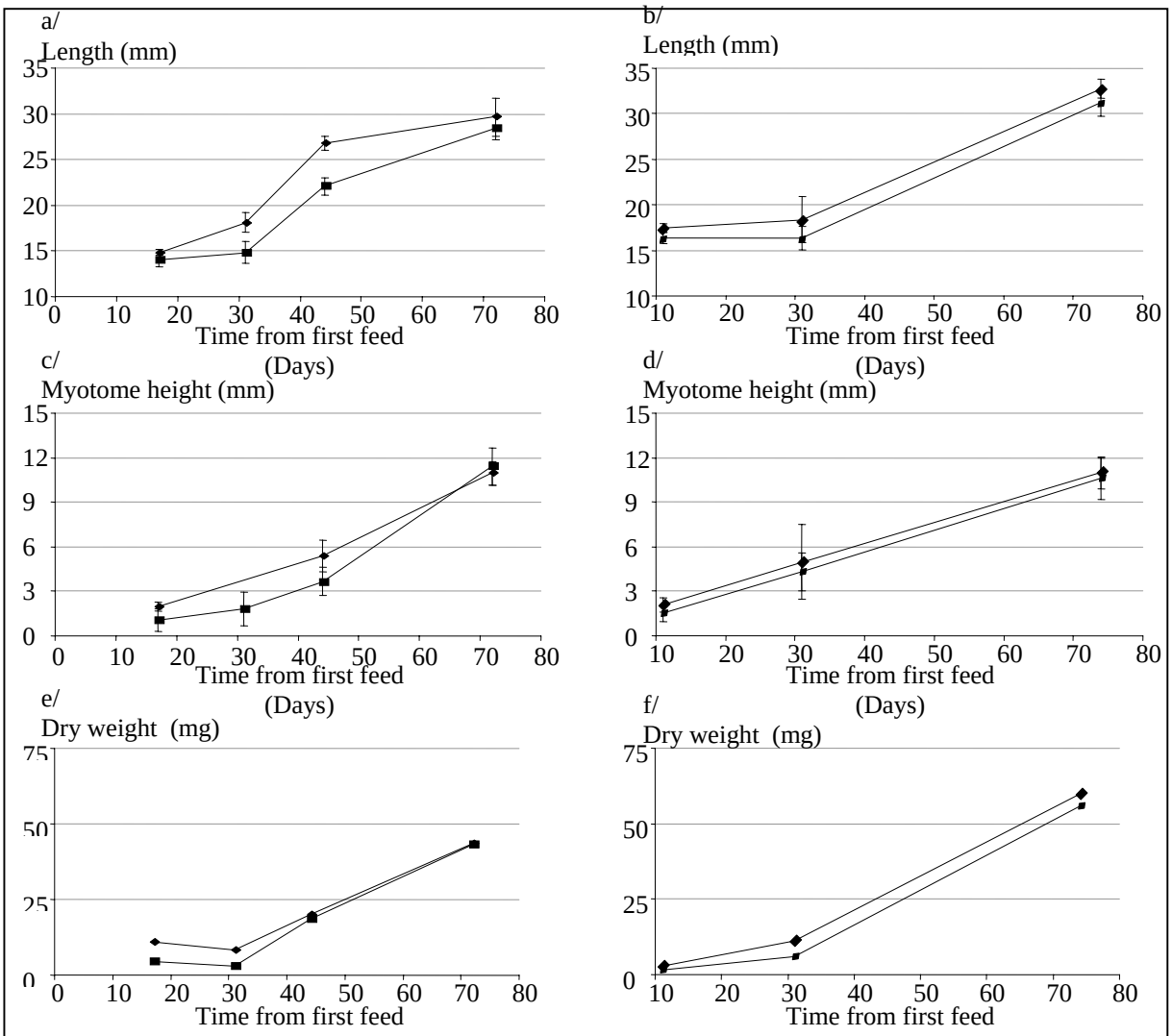


Fig. 7.3. Mean lengths of halibut cultured in 1300l tanks in a/ run 1 and b/ run 2, myotome heights in c/ run 1 and d/ run 2 and dry weights of halibut in e/ run 1 and f/ run 2. Bars indicate 95% confidence intervals where present. *holothuriae* (■).

7.4 Discussion

The poor survival in most treatments in the 100 l tanks replicated trials makes interpretation of results difficult. However, the only treatment to give a reasonable survival was fed on *Artemia* rather than copepods, which differs from the results of McEvoy *et al.* (1998). That study used wild copepods caught with meshes with pores of over 250μm, so the biomass of the copepods was likely to be considerably more than those used for this study, which were collected from the 100-150μm fraction. A decision was made early in the experiments to maintain equal numbers of prey rather than biomass between treatments. The biomass per unit prey of *Artemia* (average size fed to the larvae 500 □m) appeared to make *Artemia* feeding much more energy efficient than *Tisbe* feeding (100-150 □m). This was more apparent

when comparing the commercial size runs. When offering preys of comparable sizes (rotifers and copepods) during the first 7 days at sub-optimal prey concentration (Run 2), the survival of the copepod fed larvae was comparable to that of the rotifer-*Artemia* treatment. When offering preys of comparable sizes (rotifers and copepods) during the first 7 days and in sufficient numbers (5.5 million during that period in Run 1), the survival of the copepod fed larvae was better, and the effect lasted well beyond totally replacing the copepods with *Artemia*.

Further, many of the wild caught copepods used in previous studies were pelagic calanoids, and did not show the demersal behaviour of *T. holothuriae*. Consequently, they may have been more available to the halibut larvae in the water column. In the present work, halibut larvae were observed to feed on the copepods added to both the 100l experimental tanks and the commercial scale system, and feed was present in the guts of the larvae in the copepod only treatments from early in the experiments. Furthermore, there was a very distinct behaviour, more apparent in the commercial size tanks, of the larvae fed on *Tisbe*. Larvae remained associated to the surfaces of the tanks, mainly to the bottom, to the point of making them very difficult to be seen in the well-greened tanks. This behaviour continued until the introduction of *Tisbe* copepods was interrupted, when the larvae could be seen through the water column and closer to the surface, preying on *Artemia*. Samples taken from the commercial size tanks showed that the majority of copepods congregated in the lower levels of the tank, as would be expected from knowledge of life history patterns. The halibut larvae appeared to follow the prey items, with the larvae in the copepod fed tank generally deeper in the water column than those in the control tank. Another observation probably worth mentioning is the fact that the walls of the tanks fed on *Tisbe* remained distinctively cleaner, most likely due to grazing by *Tisbe*.

More surprising is the fact that there were no differences in pigmentation between copepod fed fish and control fish in the commercial size Run 1. However the better pigmentation of the Control fish in Run 2 can be explained by their improved nutritional status and better feeding rate due to the higher biomass per prey unit, the composition of copepods and its sufficient numbers should have provided improved pigmentation to the larvae fed *Tisbe* in Run 1, if indeed the numbers were adequate. More work is required to establish the prey needs of halibut larvae when using different developmental stages of *Tisbe*.

8 Conclusions and system husbandry

A number of husbandry protocols were used to run the copepod culture system. The results of these, combined with the results of the population experiments described above give the basis for a recommended husbandry practise.

8.1 Environmental parameters

8.1.1 Temperature

Throughout the experiments, a persistent feature was the significance of the position of the tray in the stack in determining its productivity. The most likely reason for this effect is the temperature gradient, which ranged from 16°C, recorded at the bottom of the stacks, to 24°C, recorded at the top, in separate runs. Temperatures of 20-21°C gave best results.

8.1.2 Ammonia

Ammonia appeared to be the most significant limiting factor for survival and production yield. Build up of waste products has been reported as the limiting factor in harpacticoid systems, and some descriptions of commercially operated systems recommend using aeration or recirculation to prevent build up of toxins (Kahan *et al.* 1982, Hoff & Snell 1987). Harvest cycles (see below) or partial water changes should be planned to maintain ammonia concentrations as low as possible, and certainly below 0.5 mg/l.

8.1.3 Light

The effect of light was not systematically investigated. The light arrangement in the system was intended to serve as a heat source and to maintain the functionality of the algae while in the water column. However the initial set up was over-dimensioned, and the light requirements of the system could be reduced to keeping normal light levels in the room. Copepods did seek shelter under substrates when provided, and copepodite and adult forms are known to present negative phototaxys. Although there was no apparent direct negative influence of light on production yields, once the benefit of the light to maintain algae activity is disregarded, avoiding excessive light levels is preferred. Thus, the use of opaque trays, rather than translucent white trays would be effective.

8.1.4 Salinity

Tisbe showed good survival in a wide range of salinity (25-38 ppt). However, evaporation from the trays was very significant, specially when these were maintained static for a number of days. The fluctuations in salinity due to evaporation or bulk distilled water addition are likely to impose unnecessary stress on the animals. Transparent Perspex or other lids were eventually used to avoid this problem.

8.1.5 Substrate

Different substrates were evaluated, including three different kinds of biomedia (Trickle Filter Media – RMP, Fluidised Filter Media – FM, and Degassing Media – RS, all obtained from Aquasystems U.K. Ltd.) and pebbles. The use of substrate

enhancement indicated the possibility of increasing the naupliar production and the stocking density. However, the substrates used also posed the disadvantage of making the harvesting operation more difficult. Although it was considered that the inconveniences out weighed the advantages in the case of the trays, the use of adequate substrate enhancement in bigger volume rearing units (e.g. tanks) would be of interest. Firstly, the increase in surface to volume would facilitate higher concentrations of copepods, provided that the ammonia levels were kept low. Secondly, the development of a biofilm on the media would supplement the nutrition of the copepods.

8.2 Harvest

Different harvesting regimes were assessed, and their use depends on the needs of live prey size, and the management of ammonia levels in the cultures.

Maximum productivity occured between days 4-10 in static cultures. Yields of over one million naupliae were obtained from trays harvested at day 8, although productivity was generally highly variable with this harvest practise. A number of trays were set up on this regime at different days, so as to provide adequate daily numbers to the hatchery. Best naupliae yield on this regime, expressed as number of nauplii divided by number of adults, was 28 nauplii per adult at day 6.

However, when harvest is more frequent, values of daily naupliar production per female of 5-10 are generally to be expected. Trays can be harvested, daily to every three days, and naupliae on-grown in a separate tray to an appropriate size for the fish larvae to be fed (considering also the nutritional differences between the life stages of *Tisbe*; see table 3.2). Survivals of over 90% can be expected when on-growing naupliae for 6-8 days at 20^{o} C. A tray can be run as a naupliae production unit, or broodstock tray, in this way for approximately 20 days. After this time, production starts to decay as the population ages. At this stage, the production role can be taken over by an on-growing tray previously set up to that effect.

8.3 Stocking

Best results were obtained stocking the trays with 40-50 thousand copepods as broodstock. The proportion of females will determine the naupliae production. Sieving methods like utilising 250 □m sieves to separate gravid females can prove useful to select the initial population.

Maximum stocking density for on-growing was not identified. Populations of over one million copepods (i.e. over 220 individuals cm^{-2}) were maintained in the trays for a number of days.

8.4 Feeding

The best copepod nutritional profile and performance, as well as water quality in the cultures, was obtained with the use of algae.

A mixture of *Chaetoceros mulleri* and *Rhinomonas reticulata* provided the best naupliae production and would be used to feed the broodstock trays. On-growing trays would be fed with a mixture of *C. mulleri*, *R. reticulata* and *Isochrysis galbana*, which gave the best results for survival, and provided an adequate content of DHA

(34.7% of fatty acids, compared to 8.3% provided by enriched *Artemia*) and a good DHA/EPA ratio (3.3).

Supplementation of the algal diet with formulated diets can be carried out without detrimental effects on production. Observations by Støttrup indicate that diets with high lipid content (60%) used in combination with water exchange gave good production results. These could be used to partially replace the algae, saving some of the operational costs associated with their production. However, attention should be paid to the nature of the supplement diet, as the analysis showed that the balance of PUFA in the copepods can be altered by it, and hence their nutritional value for the fish larvae.

In any case, over feeding should be avoided. The appearance of ciliates and nematodes in the culture trays was associated with poor water quality and high organic loading. It was preferred to feed the trays more frequently than to add a higher volume of algae mixture on a single feeding episode. Typically, 500 ml of the algae mix of choice would be added to the trays every other day, reducing or increasing this volume according to the appearance of the tray (colour, turbidity, presence of ciliates…). If necessary, a tray would be emptied and its contents passed through a 45 □m sieved, rinsed and restocked.

8.5 Feeding of halibut larvae

The lipid composition of *T. holuthuriae* produced in the culture system proved to be of similar quality of other strains and species of copepods considered the natural nutrition for marine fish larvae. In particular, adult *Tisbe* showed very good contents of DHA (27-42% of fatty acids compared to 8.3% in enriched *Artemia*), EPA (6-12%) and ARA (1-3.8%). Fatty acid profiles were also altered with the diet, providing the possibility of tailoring the nutrition to be delivered to fish larvae.

Halibut were found to feed on *Tisbe* at least as readily as on *Artemia* or Rotifers. The differences in behaviour as far as mobility and association to the tank surfaces did not prevent the halibut larvae from preying on the copepods. The main obstacle appeared to be to make available enough numbers of the appropriate life stage to provide a useful nutritional supplement to the larvae. Halibut larvae are big enough to feed on *Artemia* from the start of first feeding. Although *T. holuthuriae* can be identified and pursued as a prey item, the energy balance could be at the edge of profitability for halibut because of the size. More work should be carried out on this matter.

8.6 Commercial Application and future work

Tray incubation of copepods appears to have two important associated factors that are likely to be attractive to the industry: relatively low running costs and ease of operation. A cost/benefit analysis for this system showing a comparison with rotifer and *Artemia* production may be easily constructed, and this information would be of importance to the operation of a commercial hatchery. The operation of the system described does appear to be somewhat more labour intensive than the other live feed production, however scaling up the process will help to redress this imbalance.

Commercial hatcheries are likely to require a greater level of production than the 1 million copepods d^{-1} per tray obtained during the present work. Current research

efforts already underway at Seafish Aquaculture are focusing on improving productivity per rearing unit through improvements in husbandry and the use of tanks and substrate enhancement.

The use of *T. holothuriae* as first feeding prey supplement, in combination with Rotifers or *Artemia*, would be beneficial for a number of other finfish species. Haddock larvae, in particular, have often been observed grazing on benthic copepods off the walls of rearing tanks. This suggests that the natural behaviour of haddock larvae would include grazing on plankton associated with surfaces, making *Tisbe* a very promising candidate for haddock first feeding

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Appendix I: Copepod Culture: A Review

Christopher J. Cutts

Abstract

The suitability of copepods as prey for marine fish larviculture, in terms of both nutrition and ease of culture, is reviewed. Harpacticoid copepods are favoured over calanoids, since harpacticoids (due to their benthic ecology) can be reared at much higher densities. However, their benthic nature also makes mass culture more difficult, due to their requirements for large surface areas. Within harpacticoida, *Tisbe* spp. seem most favourable due to their overall high fecundity, and positive phototaxis of the nauplii. Harpacticoids can biosynthesise *de novo* several nutritionally important essential fatty acids (EFA), making them desirable as marine fish prey items. However, a diet rich in EFAs (e.g: animal derived feed) will improve the productivity of copepod cultures, suggesting that biosynthesis is rate-limiting for reproduction. Substrate is also important in maintaining a good population, since copepod biomass is more dependent on surface area than volume of a culture. Heterogeneous substrates can support large cultures due to their high surface area, but efficient cleaning methods must be devised. Frequent harvesting of populations will maintain good water quality and an overall low density of sexually mature copepods, raising naupliar productivity overall, but over-harvesting will naturally deplete the population. Harpacticoids are generally tolerant of environmental fluctuations but temperature and salinity optima do exist, and these will be species- and strain-dependent. These copepods are nutritionally superior to *Artemia*, due to their ability to biosynthesise EFAs such as DHA, EPA and AA. However, the nauplii are energetically poor but appear to have an appetite-stimulatory effect. Furthermore, uneaten nauplii will grow within the rearing tank and graze on the walls, thus maintaining their own nutritional value and tank hygiene.

Introduction

This paper is a review of the intensive culture of copepods. It will synthesise the findings of previous reports on their suitability as food for marine fish larvae, in terms of nutritional quality and ease of culture. It also summarises the different techniques used in copepod culture (e.g.: diet, rate of harvest), and investigates the suitability of different species for the intensive culture of live feed on the basis of their life-history.

The larviculture of most marine fish species depends on the provision of live prey during the larval stage. The success of mass culture techniques for both rotifers and *Artemia* have resulted in their widespread use as food for larvae. Both species provide a wide size range suitable for most marine fish larvae, but are inherently nutritionally inadequate. Specifically, essential fatty acids such as docosahexaneoic acid (22:6n-3, DHA), eicosapentaneoic acid (20:5n-3, EPA) and arachidonic acid (20:4n-6, ARA) are important for normal development in several marine species. Both the absolute levels of these fatty acids and the dietary ratios between them are known to be important in maintaining larval viability. Although enrichment regimes to improve the nutritional value of rotifers and *Artemia* are well established, the rearing success of species such as Atlantic halibut (*Hippoglossus hippoglossus*) is still limited.

Evidence suggests that copepods may serve as superior prey for fish and crustaceans in intensive systems (Watanabe *et al.*, 1983; Sun & Fleeger, 1995; Stottrup & Norsker, 1997). Of the order copepoda, the sub-classes calanoida and harpacticoida have been most studied in this respect. For example, when offered a mixture of prey items simultaneously, turbot larvae (*Scophthalmus maximus*) selectively ingested the nauplii of harpacticoids (Van der Meeren, 1991). Furthermore, copepods are known to have greater digestability (Schipp *et al.*, 1999) and a relatively high caloric content per unit weight (Kahan *et al.*, 1982; Sun & Fleeger, 1995). They are also more nutritional than some strains of *Artemia*. Specifically, harpacticoids are rich in essential fatty acids, most notably 22:6ω-3 and 20:5ωn-3 (Leger *et al.*, 1986; Norsker & Stottrup, 1994; Nanton & Castell, 1998*a*), which are vital for marine larval development (McEvoy *et al*., 1998; Shields *et al*., 1999). In addition, both calanoids and harpacticoids, from first nauplius to adult, exhibit a broad spectrum of prey sizes (80 to >900 µm in length and 3-5 µg in dry weight). This makes them suitable for ingestion by a similarly broad range of developing fish sizes (Gee, 1989; Sun & Fleeger, 1995; Schipp *et al*., 1999). For example, *Acartia* has been sucessfully fed to red snapper (*Lutjanus argentimaculatus*) since rotifers are too large (Schipp *et al*., 1999), and Kahan *et al*. (1982) has suggested that *Artemia* may be too large for larvae with especially small gapes. In addition to their nutritional and physical superiority as live feed, they are also highly suitable for culture due to their eurythermal and euryhaline characteristics. This gives them tolerance to large environmental fluctuations (Miliou & Moraitou-Apostolopoulou, 1991*a*; Carli *et al*., 1995).

Although copepods in general make good prey items, it is important to consider certain *inter*-species characteristics if they are to be intensively cultured in sufficiently large numbers. The life-histories of calanoids and harpacticoids are fundamentally different. Calanoid copepods are entirely planktonic, so their cultivation environments are homogenous and relatively easy to operate on different scales (Stottrup & Norsker, 1997). However, calanoid production is limited by difficulties in maintaining broodstock at high densities. Stress caused by overcrowding decreases fecundity in the calanoid *Centropages typicus* (Miralto *et al*., 1996), and cannibalism of nauplii by adults can also occur (Ohno *et al*., 1990). Conversely, harpacticoids are benthic or epibenthic copepods that sometimes enter near-bottom waters. They often serve as obligate prey for wild marine fish larvae (Sun & Fleeger, 1995). Since they are benthic, their population growth rate depends on the area of solid substrate but they can be produced in volumetrically much denser cultures than calanoids. However, such rearing environments are not homogenous and are correspondingly harder to scale-up and manage (Stottrup & Norsker, 1997). The two contrasting life-histories in culture is emphasised in two separate studies, where a large volume culture of the calanoid *Acartia tonsa* produced 530 eggs.L^{-1} (Stottrup *et al.*, 1986), and a small volume culture of the harpacticoid *Tisbe holothuriae* produced 100,000 nauplii.L^{-1} (Stottrup & Norsker, 1997).

Irrespective of life-history, desirable characteristics for the mass culture of copepods are high reproductive potential, short turnover time (from egg to egg), and fast individual and population growth rates. Other requirements are a diet flexible enough to allow good growth on a variety of food sources and a tolerance of a wide range of environmental factors such as temperature and salinity (Sun & Fleeger, 1995).

Although *Acartia* species (calanoida) have been cultured successfully for many generations in the laboratory (Stottrup *et al.*, 1986) and in large outdoor tanks (Ohno *et al.*, 1990), the low culture densities make calanoids inappropriate for intensive mass cultivation. Harpacticoid species are therefore the preferred organisms for the development of an intensive copepod culture system (Stottrup & Norsker, 1997). Several harpacticoid species have been studied in this respect, under a variety of regimes. This paper presents a review of the different species and rearing techniques used, with regard to species' life-history (reproductive potential, turnover time, *etc*). The influence of different diets and substrate are summarised, as are the relative merits of batch *versus* continuous culture and the effects of the rearing environment (e.g.: salinity and temperature). Data on optimal harvesting techniques is also investigated. Furthermore, the suitability of harpacticoid copepods as a diet for marine fish larvae is also reviewed.

Harpacticoid life-histories

After stocking trays with 40,000 adult *Tisbe holothuriae* harpacticoids (predominantly oviger females), Stottrup & Norsker (1997) harvested a daily average yield of 300 000 nauplii per tray (see below for details of the cultivation system), or 125 nauplii.cm^{-2}.d^{-1}. This relatively high production is due to the high reproductive capacity and short life-cycle of *Tisbe*. For example, Miliou & Moraitou-Apostolopoupou (1991*a*,*b*) reported reproductive characteristics for *Tisbe* of 6.14 d for larval development, 1.40 d for egg sac maturation, up to 76 offspring per female, and a longevity of as little as 14.67 d. Due to longevity and the time for larval development, inter-generation time was on average, 8.11 d. Gaudy & Guerin (1982) reported similar findings.

However, species of *Tigriopus* are considered by some to be more suitable for mass culture. Lee and Hu (1981) reported that female *Tigriopus japonicus* can produce up to 204 nauplii in two weeks, and Harris (1973) reported a total production of 301 eggs per female. Therefore *Tigriopus* is more fecund than *Tisbe*. However, generation time for *Tigriopus* is longer than for *Tisbe* (ca. 14 d) (Takano, 1971; Carli *et al*., 1995). Although more fecund, the longer generation time of *Tigriopus* means that *Tisbe* can achieve greater maximum yields in mass culture (Miliou & Moraitou-Apostolopoupou, 1991*b*). Furthermore, within the *Tisbe* genus, *T. Holothuriae* has higher values of intrinsic rate of natural increase (r_m) than other *Tisbe* species (r_m is the replacement rate of ovigerous females by their female progeny over time). There is also experimental evidence that *Tigriopus* spp. show maternal hatching inhibition at high population densities (Kahan *et al.*, 1988). As well as evidence of hatching inhibition from *Tigriopus*, there is evidence of sex ratio regulation in *Tisbe*. Zhang & Uhlig (1993) discuss possible causes of sex ratio (the female percentage) regulation: crowding may cause changes in the sex ratio, with low female percentages occurring at high stocking densities.

In addition to favourable reproductive characteristics, *Tisbe* also demonstrate changes in behaviour which make them suitable as prey for marine fish larvae. Although harpacticoids are benthic, *Tisbe* nauplii will collect in the upper water layers of first-feeding tanks after *ca.* 17 h *post*-introduction. Prior to this they exhibit benthic behaviour and are found predominantly at the tank walls. This change in behaviour makes them available to foraging fish larvae (Stottrup & Norsker, 1997). Furthermore, *Tisbe* nauplii exhibit positive phototaxis, making them relatively easy to

harvest from the upper water layers of a culture. Adult and copepodite *Tisbe* exhibit more benthic behaviour, and are negatively phototactic (Stottrup & Norsker, 1997).

However, the negative phototaxism of adults and copepodites has also been used as a harvesting tool in a third genus, *Amphiascoides*. Sun & Fleeger (1995) collected older stages of copepod by harvesting from the dark end of a rearing tank. They also reported harvests of 70 copepods.$cm^{-2}.d^{-1}$ from their culture system. This respectable harvest included all stages of the copepod life-cycle, but no effort was made to maximise yield, so many more could probably have been collected. However, the initial population was much larger than the starting population reported by Stottrup & Norsker (1997): four million compared to 40,000. Despite longer generation times than *Tisbe*, this population of *Amphiascoides* produced a consistently good harvest. This may be due in part to the larger initial population, which was also allowed to grow for one month unharvested.

Sexual dimorphism is apparent in many harpacticoid species. For example, male *T. carolinensis* are 50 to 60% of the female length (Lee *et al.*, 1985). This will influence the choice of food particle size offered to a culture of harpacticoids. Since males are smaller, with smaller feeding appendages, they will benefit from efficient use of food under a regime of small food particles. Dimorphism will reduce intraspecific competition for food sources, but may also account for differential sex mortality under particular dietary regimes (Lee *et al*., 1985).

The effects of diet on harpacticoid nutritional quality and population growth

Harpacticoids are widely distributed in coastal waters and particularly abundant in the near shore benthic marine environment (Hicks, 1980). In their natural habitat, they feed on algae and settled organic particles (Coull & Wells, 1983). In culture, a combination of different algae has been shown to be a better diet than monoxenic algal cultures, presumably due to the mix of vitamins, minerals and trace elements that are vital for the survival, growth and reproduction of animals (Lee *et al*., 1985). Furthermore, as mentioned above, a range of food sizes will reduce differential sex mortality. However, copepod diet (in culture) is not necessarily limited to algal species. Carli *et al.* (1995) compared *Tigriopus fulvus* culture performance between an algal (*Monochrysis lutheri*, 70,000 cells.ml^{-1}) and yeast diet (*Saccharomyces cerevisiae,* 0.1 mg.ml^{-1}). The study found that yeast-fed copepods had a lower daily production of nauplii than copepods fed algae, but naupliar production was spread over a longer time period. Algae-fed copepods also exhibited lower survival and a higher incidence of infertile females. The authors concluded that both diets were beneficial: algal food provided a high daily naupliar production, but yeast-fed copepods had higher overall production over a longer period of time. Therefore diet can have a big influence on the reproduction and life-history of harpacticoids.

However, it must be stressed that copepod productivity is directly correlated with several life-history parameters: survival, sex ratio, the number of egg-sacs per female, the number of offspring per female and the development rate. Furthermore, different diets may favourably affect different parameters. For example, Miliou & Moraitou-Apostolopoulou (1991*b*) tested the influence of different diets on several life-history parameters. They reported that the seaweed *Ulva* increased the developmental rate of nauplii and copepodids, also increasing the survival of nauplii,

whereas the artificial compound feed Fryfood® (Waterlife) increased offspring production and increased the survival of copepodids.

Generally, the suitability of different diets depends on their digestibility and how they fulfil the nutritional requirements of the species. The free amino acid content does not seem to be very important in the effectiveness of food, since the diet richest in amino acids tested by Miliou & Moraitou-Apostolopoulou (1991*b*) was also the least efficient. Despite this, free amino acids are generally very abundant in copepods (Fhyn, 1989). However, total protein content does seem to be important. Lee *et al.* (1985) found that the algal diet with the highest protein content provided the best copepod reproductive performance. Similarly, Guidi (1984) found survival and naupliar development rate related to the protein content of the diet. The paucity of information on proteins and amino acids in copepod diet reflects the importance of essential fatty acids (EFA) in marine fish nutrition, with a corresponding interest in their role in copepod nutrition.

It is well known that coldwater marine fish larvae have a nutritional requirement for live food with high concentrations of the long chain n – 3 essential fatty acids such as eicosapentaenoic acid (EPA; 20:5n – 3) and docosahexaenoic acid (DHA; 22:6n – 3) (Watanabe, 1982). Arachidonic acid (10:4n – 6; AA) is also considered to be essential for marine fish, but at a lower demand (Castell *et al*., 1994). Marine fish require these fatty acids since they lack the desaturase enzymes necessary to convert short chain fatty acids into their long chain EFA end-products (Tocher, 1989). Harpacticoid copepods can produce significant amounts of EPA and DHA when fed diets deficient in these EFA. Furthermore, copepods have a high proportion of DHA in their polar membrane lipids, as opposed to their neutral lipids (cf. *Artemia*). Polar lipids are involved in the formation of lipid emulsions which facilitate lipid digestion. The increased amounts of DHA in the polar lipid fraction therefore increases the digestibility of DHA in marine fish larvae fed copepods (Nanton & Castell, 1999). Although copepods are capable of *de novo* fatty acid biosynthesis, it ceases in the presence of a substantial input of dietary fatty acids. However, the fatty acid content of food will influence copepod productivity, namely improving copepodid survival and egg number (Miliou & Moraitou-Apostolopoulou, 1991*b*). Therefore animal-derived feeds have proved to be more efficient than compound vegetarian feeds (e.g: soya and yeast), and Guérin & Gaudy (1977) have suggested that *T. holothuriae* demonstrates a higher productivity and lipid content when fed on animal-derived compound artificial diets. *Tisbe* species are attractive live food organisms for marine fish culture since their high desaturase activity ensures they produce large quantities of favourable fatty acids (such as DHA) even if the EFA composition of their diet is poor (Nanton & Castell, 1999). Since fatty acid biosynthesis ceases under a large input of dietary fatty acids, the fatty acid distribution in copepods largely reflects that of their diet (Norsker & Støttrup, 1994). Biosynthesis of EFAs may be rate-limiting for reproduction, so the dietary supply of fatty acids would enhance naupliar production (see above). Furthermore, diets rich in animal-derived fatty acids increase the carbon content, C:N ratio, and the energy content of *T. holothuriae* (Miliou, 1996). Despite improved productivity under such regimes, final body size remains almost constant. It is hypothesised that harpacticoids, since they are not normally subjected to periodic variation in food supply, can utilise excess energy for reproduction at the expense of storage. This will keep final body size constant (Miliou, 1996).

It must be stressed that the suitability of diet does not rely on chemical composition alone. The seaweed *Ulva*, as well as improving developmental rate, increases the substratum available to *Tisbe*, and an increase in the surface to volume ratio in rearing tanks can result in an increase in production (Miliou & Moraitou-Apostolopoulou, 1991*b*). Furthermore, live seaweed can supply oxygen and absorb toxic compounds (Harlin, 1978). As mentioned earlier, a large range of particle sizes is necessary to match the changing size of copepod mouthparts during development. The success of a diet will also rely on its capability of allowing bacteria to proliferate. Harpacticoids will assimilate bacteria, and bacteria will also serve as food to microzooplankton on which the copepods feed (Rieper, 1978; Miliou & Moraitou-Apostolopoulou, 1991*b*). Finally, the optimum diet should be cheap and simple, such as an easily available animal-derived compound diet, used in conjunction with an algae (rich in fatty acids) or a seaweed to increase surface area (Guérin & Gaudy, 1977; Miliou & Moraitou-Apostolopoulou, 1991*b*).

The effects of different substrates on harpacticoid populations

The importance of substrate has already been stressed above. Choosing a suitable substrate is vital for good productivity in a copepod culture. Since harpacticoids are benthic, a large surface area to volume ratio is vital. Støttrup & Norsker (1997) investigated two different methods in achieving this. One method employed large, shallow trays, to which fresh seawater and algae (*Rhodomonas baltica*) were added daily, after removing *Tisbe* nauplii through a mesh. This is a batch culture method, and is rather labour intensive. Therefore Støttrup & Norsker (1997) designed a continuous culture system, to compare efficiency and productivity of that system with batch culture. The continuous culture system consisted of a closed bioreactor continuously supplied with algae. Positively phototactic nauplii were harvested from the upper water layers. In order to provide a large surface area, the bioreactor was filled with polypropylene balls. All the balls developed a thin biofilm of sediment. By calculating the surface areas of both systems, the authors deduced that productivity in the bioreactor was only 32 $mg.m^{-2}.d^{-1}$, compared with 100 $mg.m^{-2}.d^{-1}$ in the tray system. The authors surmised that the relatively poor productivity was due to insufficient capacity of the algal dosing system. The addition of more food did improve productivity in the continuous system (Støttrup, unpublished data).

It is precisely their preference for sand or mud substrates of a particular size, texture and faunal composition which precludes the easy cultivation of harpacticoids in a laboratory (Hockin, 1981; Chandler, 1986). Although *Tisbe* spp. are semi-planktonic, a substrate providing a large surface area will be important for the adults. Chandler (1986) experimented with mud and sand substrates and found that cleaned and sorted mud was superior because all the sediment particles were less than 0.125 mm. Adult and late-stage copepodites are usually larger than 0.125 mm, allowing them to be easily separated in a sieve. Care must be taken to prevent excessive bacterial films overgrowing the substrate, entrapping and killing the copepods. Chandler (1986) prevented fouling by not feeding cultures until accumulated food on the substrate disappeared.

Although most studies attempting large scale harpacticoid production have eliminated the use of natural substrates, and use glass or plastic surfaces, Sun & Fleeger (1995) used limestone cobbles in a system that produced in excess of one

million indivivduals (5 g dry weight biomass) of *Amphiascoides atopus* per day. They covered a surface area of 2 m^2 with cobbles (1 cm in diameter) to 2 cm in depth. Air lifts were used to increase gas exchange in the limestone. Sun & Fleeger (1995) attributed the large daily harvests to the large surface area available for growth, and to the biofilms and bacteria coating the limestone cobbles. The system only ran for 17 weeks, and the authors stressed the need to devise a low-maintenance method to clean the culture and prevent the build-up of faeces and decomposing algae. Kahan *et al.* (1982) used a novel technique, bypassing the use of trays or natural substrate. They employed floating net-bottomed trays with an appropriate pore size, allowing *Tisbe* nauplii to pass directly into the fish tank. This system allowed close observation and control of conditions, particularly of excessive bacterial growth. The total number of nauplii produced per tray (200 cm^2) per day was 132,000, giving a density of 10 nauplii per ml for the whole tank volume. The floating trays also removed the need to harvest copepods and then offer them to fish larvae.

Effects of harvesting

Harvesting, or the regular exploitation of copepod cultures will act on the biomass and the production of the population. Therefore a good knowledge of the conditions of exploitation is required to guarantee the maintenance of a good yield. Using *T. holothurieae*, Gaudy & Guerin (1982) maintained populations for 70 days, and found that mean production was highest in the most frequently harvested culture (weekly exploitation, with 50% of the tank volume being removed). The frequent harvesting increased the ovigerous rate in the population (the percentage of females with egg sacs), demonstrated by the high production. By citing a paper by Alessio (1974), where a population of *T. furcata* were exploited daily at 40% with no decrease in yield, Gaudy & Guerin (1982) surmised that the maximum yield of their own cultures was probably not reached. However, they caution that too rapid a frequency will decrease the yield, citing a study on *A. tonsa* (Heinle, 1970). This study reported that a harvesting frequency of four days was too rapid to maintain the population level. By also varying the amount of water removed at harvest, Gaudy & Guerin (1982) hypothesised that the increase in production was due to the improvement in water quality and the decrease in density brought about by the harvest. Crowding can lower naupliar production and survival (Zhang & Uhlig, 1993). Sun & Fleeger (1995) also reported no population decline, even with a high level of exploitation (more than one million individuals per day).

These studies complement earlier work by Hoppenheit (1975, 1976). He reported low naupliar mortality at high exploitation rates, attributable to the subsequent low densities. He also proposed that sex ratio was influenced by high exploitation rates, or low densities: a surplus of males was found at an exploitation rate of 90%, while more females were found at an exploitation rate of 10%. However, the paucity of explicit studies on the effect of exploitation rates on standing biomass demonstrates that this important factor has been relatively neglected.

Environmental effects on harpacticoid quality and growth

It has already been stated that the density of a population can affect population growth by modulating survival, development and fecundity. This is an important observation, especially since the mass culture of *Tisbe* spp. is related to the available substrate area, rather than the available water volume (Zhang & Uhlig, 1993). Female fecundity is influenced by both the density of breeding females and

nauplii. It has been suggested that complex chemical compounds may be produced by the animals as a result of crowding, allowing them to perceive and respond to different crowding levels (Zhang & Uhlig, 1993). Alternatively, it has also been hypothesised that direct close encounter may change the behaviour of copepods, and even their development (Brand, 1985). Since fecundity decreases with increasing density, Zhang & Uhlig (1993) suggested a density of 40 female *T. holothuriae* per cm^2 to achieve maximum daily yield of nauplii.

In addition to biotic environmental factors, abiotic factors will also affect the population dynamics of harpacticoids. Harpacticoids are very tolerant to environmental fluctuations, but discerning the most favourable conditions for mass culture is paramount. Using a Greek strain of *T. holothuriae*, Miliou & Moraitou-Apostolopoulou (1991) found an optimum salinity of 38 ‰ for offspring productivity. The decreased fecundity beyond 38 ‰ suggested inhibition of an enzymatic mechanism in salinities different from those to which the strain is genetically acclimated. Furthermore, in extreme salinities (20 and 48 ‰), the strain tested was not able to survive. Interpopulation tolerance differences are therefore of considerable importance, both for their ecology and for mass culture. Generally *Tisbe* has limited euryhalinity, and can only reach its maximum development in culture within a narrow range of salinity (Miliou & Moraitou-Apostolopoulou, 1991). Moreover, sub- and supranormal salinities will cause a decrease in total body length. Extreme salinities require extensive adjustments in osmoregulation and will affect structural properties of aquatic invertebrates (Miliou, 1996). Estuarine harpacticoids, such as *Amphiascoides subdebilis*, are able to survive in a wider range of salinities, but will still show a preferred optimal salinity for fecundity and longevity (Ingole, 1994). Salinity appears to affect mainly longevity and the number of offspring per egg sac. Temperature appears to have a more pronounced effect (Miliou & Moraitou-Apostolopoulou, 1991).

Miliou & Moraitou-Apostolopoulou (1991) state an optimal culture temperature of 19°C for their strain of *T. holothuriae*. Lower temperatures caused longer development and maturation, but an increase in the number of offspring per egg sac. Higher temperatures caused an accelaeration of development and maturation but a reduction in the number of offspring per egg sac. In both cases the number of egg sacs and offspring decreased. Therefore temperature had a more pronounced effect than salinity. There is a well documented inverse relationship between temperature and body size of zooplankton (McLaren, 1965; Corkett & McLaren, 1978). Fecundity increases with increasing body length (McLaren, 1965). The increase in total body length of *T. holothuriae* at low temperatures is associated with an increase in egg production per egg sac (Miliou & Moraitou-Apostolopoulou, 1991). The two factors of temperature and salinity act on populations independently, as there was no significant interaction between the two variables (Miliou, 1996).

In addition to affecting population variables, temperature can also have a marked effect on the nutritional quality of copepods. Nutritionally important long chain EFAs, such as DHA, EPA and AA are incorporated into the cell membranes of animals, where they help maintain membrane fluidity. Nanton & Castell (1999) hypothesised that in response to lower temperatures, copepods would increase the amounts of long chain EFA to maintain a standard membrane fluidity (homeoviscous adaptation; Sinensky, 1974), since they can synthesise fatty acids *de novo*. Using two genera of

harpacticoid, *Amonardia* and *Tisbe*, the authors indeed found higher levels of DHA, EPA and AA at 6ºC compared with 15ºC. However, DHA and EPA were also high at 20ºC. This may have been due to the selective oxidative catabolization of neutral lipids rather than phospholipids, raising the relative amounts of DHA and EPA accordingly. Although satisfying the hypothesis of homeoviscous adaptation, the high levels of DHA and EPA (and high DHA:EPA ratio) at the higher temperature precludes the need to coldwater-enrich these harpacticoids.

Harpacticoids such as *Tisbe* are semi-planktonic, so it is worth noting the effects of turbulence on copepod productivity. The first limiting step for feeding and meeting is the initial encounter of prey or mate. Under turbulence, the contact rates are higher than predicted when only densities and relative velocities of predator and prey are considered (Alcaraz, 1997). Although food intake rates can be increased by turbulence, it can also decrease the number of eggs laid per female. This is due to an increase in metabolic rate caused by the turbulent conditions, and a concomitant decrease in the energy allocated to egg production (Saiz & Alcaraz, 1992). Although this work was carried out on calanoid copepods, it will apply to zooplankton in general. Working on *Acartia grani*, Alcaraz (1997) observed a reduction in the development time for the different instars under turbulent conditions. Life-span was also shortened, and both effects may be due to higher metabolic expenses. Although feeding is increased, turbulence tends to reduce overall biomass due to the decrease in size and fecundity. Therefore the turbulence characteristics of a culture can be a significant factor in the modulation of copepod populations.

Harpacticoids as prey for marine fish larvae

In a study comparing wild copepods with enriched *Artemia*, McEvoy *et al*., (1998) found much greater proportions of polar lipid in the copepods (66% compared with 36% in the enriched *Artemia*). This was reflected by significantly higher proportions of polar lipid in the copepod-fed fish. It has been suggested that polar lipids are more readily digested by larvae, and may also facilitate the digestion of other lipids in the digestive tract of larval fish (Koven *et al*., 1993). Furthermore, halibut larvae fed copepods were heavier from 30 days *post* first-feeding than their *Artemia*-fed conspecifics (McEvoy *et al*., 1998).

Harpacticoids such as *Tisbe* have great potential as live food, since they contain large amounts of EFAs and have consistently high DHA:EPA ratios even when fed on EFA-poor food such as yeast. Furthermore, newly hatched nauplii are 90 µm long and reach 2 mm as adult females, so they could potentially replace both rotifers and *Artemia* throughout the first-feeding stage of marine fish (Nanton & Castell, 1998*a*). However, a potential drawback would be their benthic nature. *Tisbe* tends to stay near the sides and the bottom of the tank, rather than swimming freely where the larval fish can feed upon them (Nanton & Castell, 1998*a*). Nevertheless, a later study by the same authors demonstrated improved growth in haddock larvae (*Melanogrammus aeglefinus*) fed on *Tisbe* at 19 days *post*-hatch, compared with those fed rotifers (Nanton & Castell, 1998*b*). Furthermore, Støttrup & Norsker (1997) demonstrated that, despite their benthic nature, *Tisbe* nauplii were available to fish larvae. Nauplii were present in the water column from ten hours *post*-introduction, and successful first-feeding of turbot larvae could be established with *Tisbe* nauplii alone. *Tisbe* nauplii are half the size of rotifers, and have a caloric content of 0.00147 J per individual, compared with 0.0036 J for rotifers. Despite this, the

introduction of *Tisbe* nauplii seemed to have an appetite stimulatory effect, and fish co-fed with *Tisbe* and rotifers grew better than those fed rotifers alone (Støttrup & Norsker, 1997). A study using larval Dover sole (*Solea solea*) showed similar results. Including *Tisbe* in the dietary regime resulted in improved appetite and growth rate compared to sole reared on *Artemia* alone (Heath & Moore, 1997). Furthermore, pigmentation is improved in both halibut and sole larvae, possible due to the high levels of DHA present in copepod diets (Heath & Moore, 1997; McEvoy *et al*., 1998).

As well as providing a superior diet, harpacticoid nauplii which are not eaten would be able to find nourishment in fish-rearing tanks by feeding on detritus, the biofilm, and bacteria, maintaining their nutritional value as well as keeping the tank clean. These are both important factors in the successful rearing of marine larvae (Norsker & Støttrup, 1994).

Conclusions

By comparing harpacticoid life-histories, *Tisbe* spp. seem more suitable for mass culture due to their short generation time and intrinsic rate of natural increase. Although *Tigriopus* spp. are generally more fecund, their longer generation time precludes their suitability as species for mass culture. Although benthic, *Tisbe* nauplii will exhibit positive phototaxis, enabling their easy harvest and availability to foraging fish larvae. In terms of suitable diet for mass harpacticoid culture, the importance of a large size range of food particles must be stressed, since sexual dimorphism occurs, as does changing mouthpart size during copepod development. Of several diets tested, animal derived compound feed seems to perform best. Although harpacticoids can biosynthesise nutritionally important essential fatty acids, their productivity is rate-limited by this biosynthesis, so cultures fed with EFA-rich diets usually show enhanced productivity. However, seaweed diets, as well as providing food, also provide a large area of substratum. This is an important point, as harpacticoid productivity is related more to surface area than volume, and seaweed will supply oxygen and absorb toxins. Although most cultures have been raised using glass or plastic surfaces, good results can be obtained using more natural substrates, such as limestone cobbles. This technique provides a large surface area for copepod growth, and also for biofilm and bacteria deposition. However, effective methods of cleaning are still to be developed.

Harvesting will also affect productivity, by modulating the standing density of the culture. Frequent harvesting (with the addition of fresh seawater to replace that abstracted by the harvest) maintains a low standing density and high water quality, with subsequent high naupliar productivity. However, too frequent a harvest will eventually reduce the yield, by lowering the population level. A density of 40 female *T. holothuriae* per cm^2 has been suggested to achieve a maximum yield of nauplii. Although *Tisbe* are tolerant of environmental fluctuations, optimal salinities and temepratures of 38 ‰ and 19^{o}C have been suggested for *Tisbe* cultures, although there will be *inter*-strain differences in performance. Temperature will also increase the amounts of EFA in copepods, with high amounts at low temperatures. However, there seems to be no need to coldwater-enrich harpacticoids, as high levels of EFA are also present at higher temperatures due to selective oxidative catabolization of neutral lipids. Turbulence will also affect productivity in contrasting ways. Although it can increase feeding rates, turbulence can also increase metabolic expenses, lowering productivity and life-span in copepod cultures. Finally, harpacticoids seem

suitable as a diet for marine fish larvae due to their relatively high levels of essential fatty acids, even when fed on an EFA-poor diet. The nauplii are available to fish larvae in the water column, although the adults are benthic. Furthermore, although small in comparison to rotifers, *Tisbe* nauplii appear to have an appetite-stimulatory effect on fish larvae when co-fed with rotifers. Uneaten harpacticoids will also graze on rearing tank walls, maintaining their own nutritional value and tank hygiene.

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