UNIVERSITY OF SOUTHAMPTON

THE FEEDING AND REPRODUCTIVE STRATEGIES OF ACARTIDAE IN SOUTHAMPTON WATER

by

Fay Elizabeth Chinnery

A thesis submitted for the degree of Doctor of Philosophy

FACULTY OF SCIENCE

DEPARTMENT OF OCEANOGRAPHY

December 2002

Graduate School of the Southampton Oceanography Centre

This PhD dissertation by

Fay Chinnery

has been produced under the supervision of the following persons

Supervisor

Dr. John Williams

Chair of Advisory Panel

Prof. Paul Tyler

Member of Advisory Panel

Dr. Martin Sheader

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE
OCEANOGRAPHY

Doctor of Philosophy

THE FEEDING AND REPRODUCTIVE STRATEGIES OF ACARTIDAE IN SOUTHAMPTON WATER

By Fay Chinnery

This work looked at some of the environmental and biological aspects of the reproductive effort in Southampton Water Acartidae. Adult *Acartia* congeners *A. bifilosa, A. clausi, A. discaudata* and *A. tonsa* have distinct seasonal and spatial distribution patterns in Southampton Water, governed by temperature and salinity respectively. The effect of these factors on other life stages, hatch success and naupliar survival, was investigated by exposing the congeners to a range of salinity (15.5-33.3) and temperature (5-20°C). *Acartia clausi* is known to prefer more saline waters (Lance 1963, 1964) and showed highest hatch success at 33.3 salinity. *Acartia tonsa* is most tolerant to dilution and at 15.5 salinity it had the highest hatch success of all of the congeners. Hatch success in both *Acartia bifilosa* and *A. discaudata* was similar over the range of salinities investigated, confirming that they are intermediate species in terms of spatial distribution. However, nauplii of all species survived well at the higher salinities and best at 33.3, which allows for differential transport of the poor-swimming nauplii to the mouth of the estuary until size and swimming ability increase when they can then return to regions of preferred salinity (Villante *et al.*1993). The summer species, *A. clausi* and *A. tonsa* showed higher hatch success at 20°C, whereas *A. discaudata* which is present in the water column all year round showed no significant differences in hatch success at the three temperatures. *Acartia bifilosa,* which diapauses over summer showed significantly ($P<0.05$) higher hatch success at 10°C than 20°C. The physiological relationship between temperature and development time was clear with naupliar survival of all species highest at 20°C and all congeners reached CI significantly faster at 20°C, but no consistent pattern was seen for salinity.

Reflecting hatch and nauplii parameters, *Acartia bifilosa* Scope for Growth (SfG) at 10°C is twice that at 5°C and 20°C and since there is less energy available to it at 20°C the ultimate reason behind its oversummering in the egg phase is to avoid higher temperatures which decrease its competitive ability. Also, although its SfG at 5°C is equally low it is unfeasible to diapause twice in a year and competition is reduced during the winter months. Diapause egg production is triggered by a photoperiod of 13L:11D, which corresponds to an April light regime in the field. It took a minimum of two days for diapause eggs to be produced at this daylength. Temperature plays a small role in diapause induction as after 8 days at 14°C and 18°C diapause eggs were produced under 12L:12D photoperiod. However, significantly ($P<0.05$) higher numbers of diapause eggs were produced under 13L:11D and 14L:10D.There was no evidence of a spiny-type subitaneous egg.

Functional response patterns of the four species indicated that all of the *Acartia* congeners are opportunistic feeders and that feeding rate increased with initial food concentration. The ability to survive on a cosmopolitan diet is clearly an advantage in an estuarine environment. *Acartia bifilosa* is the most efficient feeder of the four congeners and its numerical dominance over winter can also be accounted for by it switching its diet focus successfully throughout the year. The fatty acid composition of adult female *A. bifilosa* was analysed over 12 months, as was the fatty acid content of the subitaneous and diapause eggs. April samples showed high levels of 16:(n-7), increased levels of C_{16} PUFAs and 20:5(n-3) suggesting that this species fed well on diatoms and did not augment its diet with bacteria. October females showed high levels 22:6(n-3) and 18:1(n-7) fatty acids indicating feeding on dinoflagellates and bacteria respectively. The winter samples (November to January) showed the highest levels of 18:1(n-7) where *A. bifilosa* was supplementing its diet with bacteria, probably from detritus. The diapause and subitaneous eggs were similar in fatty acid composition, but the eggs did not reflect the adult make-up. The different feeding rates on a variety of diets, the ability of *A. bifilosa* to feed well on detritus, and the different egg production rates, hatch success and naupliar survival at various temperatures and salinities help explain why the Acartidae congeners have different spatial and temporal distributions in Southampton Water.

List of Contents

List of Tables

List of Figures

Acknowledgements

Many people have given me guidance and assistance over the course of this research, and I would like to take this opportunity to thank them for their help.

First of all I would like to thank my supervisor Dr John Williams for his advice, encouragement and patience during the past three years.

I would also like to mention Dr David Pond and thank him for his generosity in taking time to teach me the fatty acid analysis technique and for offering invaluable suggestions on the work. Thanks also to Kerry Howell for her assistance with the SIMPER statistical analysis of the fatty acid data.

I am very grateful to the various crew members of the *Bill Conway*, especially Graham Etheridge, Graeme Leggatt, Steve Hayward, Bob Wilkie, Matt O'Shaughnessy, Gary Fisher and Mike Crowfoot for their help in collecting samples from Southampton Water. Special thanks to Graham and Graeme for their priceless suggestions on how to make a decent cuppa!

I must also thank my good friend Erik Muxagata for his assistance with the boat work and for being a constant source of support.

I also wish to express my gratitude to the Natural Environmental Research Council (NERC) who provided funding for this research and paid my PhD fees.

Lastly I have to thank my Mum and Dad for not insisting that I go out and get a proper job!

Chapter 1:
General introduction and aims

1.1 General introduction

Estuaries are among the most productive of marine ecosystems owing to a nutrient supply from both fresh water and marine sources, and their circulation, which allows the retention of these nutrients. The continuous movement of the water complicates the study of planktonic copepods in estuaries. Populations at low tide are very different to those at high water (Ketchum 1954). If it was only circulation affecting the distribution of copepods, the pattern would be relatively simple. For a population originating at the seaward end of the estuary, the distribution would be proportional to the salt content of the water. In the case of a population that originates at the head of the estuary, the population numbers would be proportional to the fresh water content, or inversely proportional to the salt concentration of the water. This is never found in nature though, as organisms live and die in the estuary, and, because of a number of reasons, these rates are very different in different parts of the estuary. Estuaries are typically very productive with respect to high densities of phytoplankton and zooplankton, but the diversity is often relatively small compared with other marine habitats (Boaden and Seed 1985; Riley 1967). Few species are adapted to cope with the salinity, temperature and turbidity variations present in so stressful a habitat, and salinity tolerance plays a major role in the spatial distribution of a particular species within an estuary whereas temperature governs a species' seasonal occurrence (Kinne 1967).

Southampton Water comprises the north-western extension of the Solent estuarine system. It is 10km long, 2km wide, with a shipping channel that is dredged to a depth of 13m. It is a drowned river valley, with a triangular outline and a deeper and wider

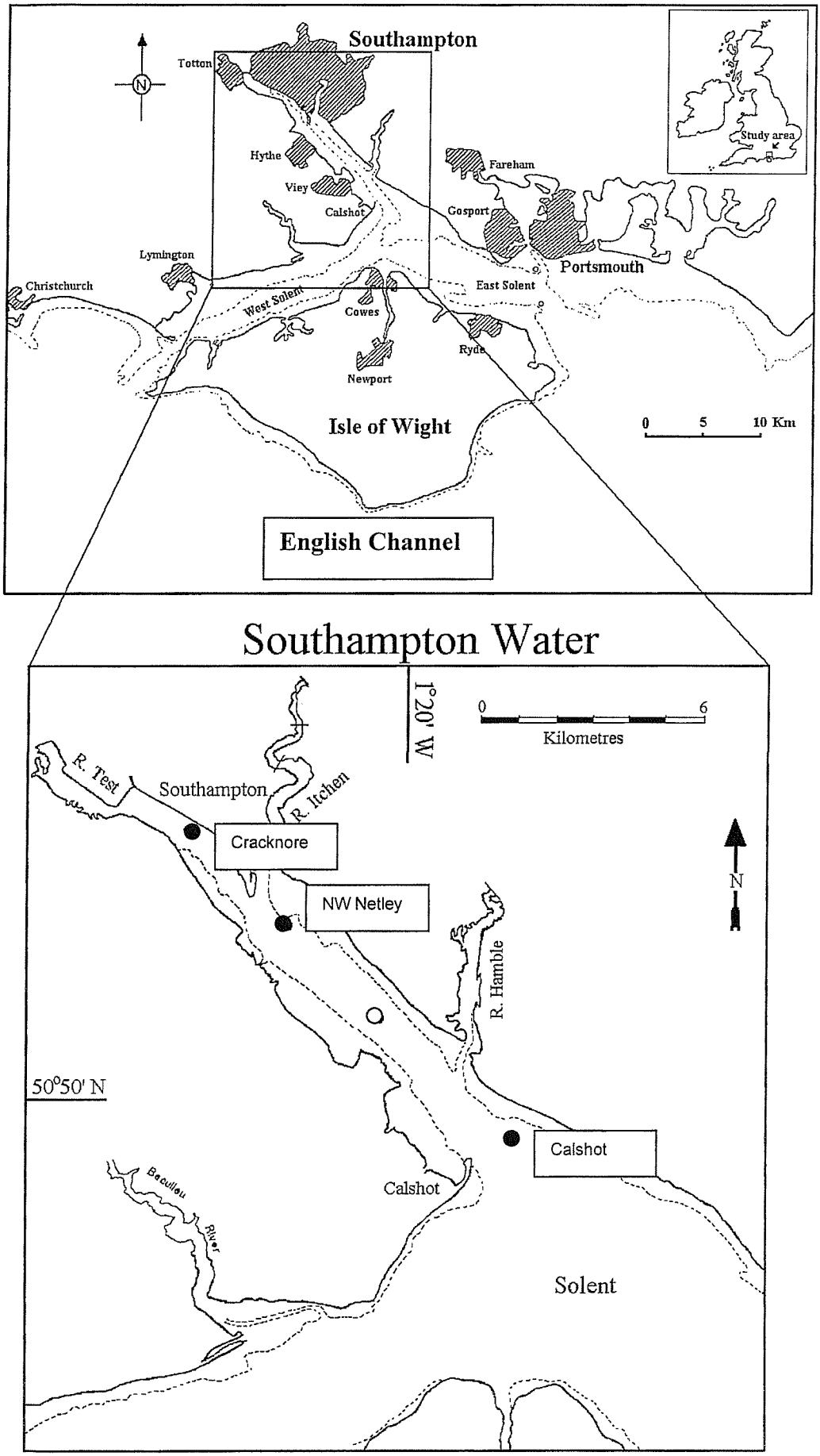


Figure 1.1: Map of Southampton Water. ● = Sampling sites in the present survey.

cross-section at the mouth, (Dyer 1973). Southampton Water is described as a partially mixed estuary (Dyer 1973), being vertically homogenous towards the mouth, with stratification occurring only in the upper reaches of the estuary where a sizeable river enters at the head, i.e. the Test, or in times of peak fresh water discharge (Webber 1980). There is a tendency for upward flow on the western side, and downward flow, of fresher water, on the shallower eastern side (Dyer 1973). This is the opposite to what one would expect from the action of Coriolis force. Topography plays an important part in the water flow, and the effect is caused by the abrupt widening of Southampton Water towards the mouth of the estuary and the significant input of fresh water from the Test, Itchen and Hamble rivers (Dyer 1973). The tidal prism for the Solent is estimated at $540x10^6$ m^3 for spring tides and $270x10^6$ m^3 for neaps, with Southampton Water contributing to 20% of these volumes (Webber 1980).

Temperature ranges from ~2°C in winter and exceeds 20°C in summer in the estuary, with a seaward decrease in temperature (Raymont and Carrie 1964). The homogenous vertical distribution of temperature at high water in the middle of Southampton Water indicates the complete mixing of the water column (Antai 1989). Although Southampton Water is classed as an estuarine environment, it is essentially marine in character (Raymont and Carrie 1964). As mentioned, the upper part the estuary is stratified with salinities ranging from 28 - 34.3 (Lucas 1993) and the salinity pattern depends on tidal state and seasonal fresh water flow. There is then a typical longitudinal salinity gradient to the mouth (Westwood 1980) where salinity values peak at 35 (Lucas 1993).

The tides of Southampton Water are complex and are primarily controlled by the tidal effects of the English Channel (Webber 1980). The estuary is known for its pronounced double high water, and there is a "stand" of about 3 hours around high water. This causes faster ebb currents, as the water must leave the system in only 4 hours as opposed to 6 (Webber 1980).

The Southampton Water phytoplankton community is characterised by a protracted spring-summer bloom which usually occurs between May and July (Kifle 1992). Williams (1980) found that chlorophyll *a* concentration varied from 1-2 $\mu g\ l^{-1}$ during winter to 10-20 $\mu g\ l^{-1}$ during summer, and can exceed 40 $\mu g\ l^{-1}$ at the peak of the bloom (Williams 1980). Kifle (1992) reports the year-round importance of *Skeletonema costatum* in the estuary. It is the dominant phytoplankton species in the winter and spring because of its adaptation to low light and temperature conditions; *S. costatum* has a higher growth rate and shorter lag-phase than other phytoplankton species, which enables it to out compete other diatoms, and dinoflagellates and hence develop into a bloom. *Skeletonema costatum* numbers peak in the early spring (March), followed by a later, smaller bloom in mid-April. The summer phytoplankton crop is usually dominated by the dinoflagellate *Scrippsiella trochoidea.* The dinoflagellates are successful in the warmer months as they can maintain themselves in the water column, take up nutrients at depth during vertical migration and, most importantly, utilise the low concentrations of nutrients usually found in summer conditions.

In short, the winter-spring, (cold, turbulent water) phytoplankton community in Southampton Water is predominantly made up of diatoms, whereas the summer waters, (calm and warm) are dominated by dinoflagellates and *Mesodinium.* Motile microflagellates, like *Isochrysis galbana*, are abundant throughout the year, although

their cell maxima is usually associated with the cell maxima of the dominant diatom species. This pattern is typical of a broad spectrum of estuarine environments, giving the overall pattern seen in figure 1.2.

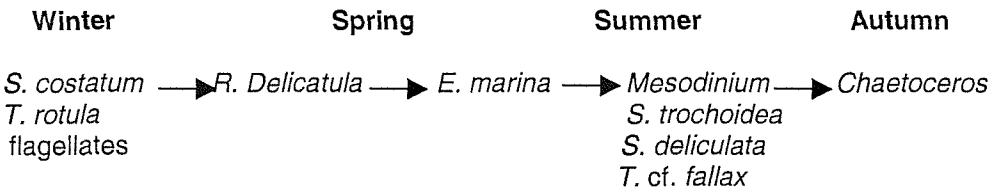


Figure 1.2: Seasonal succession of the suite of phytoplankton species at NW Netley (After Kifle 1992).

Some aspects of the pelagic mesozooplankton biology of Southampton Water have been covered extensively (e.g. Antai 1989; Conover 1957; Crawford 1989; Hirst 1996; Lance 1964; Leakey *et al.* 1992; Lucas 1993; Raymont and Carrie 1964; Williams 1980; Williams 1996; Zinger 1989), including specific work on the *Acartia* congeners of the estuary, (Castro-Longoria 1998; Castro-Longoria and Williams 1996, 1999; Lance 1963).

Raymont and Carrie (1964) noted that the zooplankton community of Southampton Water was unusual because it was dominated for much of the year by meroplankton species, specifically cirripede larvae. *Balanus balanoides* and *Balanus crenatus* are abundant during early spring and can represent over 60% of the zooplankton population at Calshot and 40-50% at Marchwood. By summer *Elminius modestus* is extremely important, accounting for 40-50% of the zooplankton total at Calshot and is almost solely responsible for the massive rise in zooplankton numbers at Marchwood where it can make up to 85% of the zooplankton community. More recent work has determined that calanoid copepods have a more dominant role in the mesozooplankton community of Southampton Water (Zinger 1989; Lucas 1993; Hirst 1996; Castro-Longoria 1998). Low densities of calanoids are usually found at Calshot in winter, but they can account

for over 90% of the zooplankton total at Marchwood. Numbers of calanoids increase over spring and by April they represent over 70% of the zooplankton in the estuary (Raymont and Carrie 1964). The population falls sharply in May to its lowest density in June (<30% of the population at Calshot) and at Marchwood depradations made by *Aurelia aurita* and *Pleurobranchia pileus* (Lucas 1993) reduced the calanoid population to less than 1% of the total. Reproduction commences in July-August and a fairly high density is maintained throughout autumn along the length of Southampton Water and calanoids account for 54-73% of the zooplankton community from September-December (Raymont and Carrie 1964). The calanoid community is more diverse at the mouth of the estuary because of its more marine character.

The Acartidae are by far the most dominant copepod genus in Southampton Water and are the only calanoids to appear throughout the year. Other species found at Calshot include *Centropages hamatus,* which is moderately abundant, *Temora longicornis,* which occurs in lower numbers, as well as the sporadic visitors *Pseudocalanus elongatus* and *Paracalanus parvus* (Raymont and Carrie 1964; Hirst 1996). These last four species are considered to be neritic not estuarine (Perkins 1974; Raymont 1983), even though they are found in Southampton Water and so must enter the estuary from coastal waters (Castro-Longoria 1998). Consequently, the calanoid presence at the head of Southampton Water consists almost entirely (>90%) of *Acartia* congeners and none of the other species appear with any significance (Raymont and Carrie 1964). Understanding this congeneric association is important because it clearly plays a major role in zooplankton population dynamics and the transfer of energy through the Southampton Water estuarine system

The environmental conditions of the marine area of coexistence determine the mechanisms that decrease any interspecific competition. That includes seasonal succession of the dominant species, and the spatial segregation depending on the degree of heterogeneity in the time and space of the ecological factors. Zooplankton can reduce the degree of direct competition in a number of ways, including spatial differentiation, functional differences e.g. selective feeding preferences and temporal differentiation i.e. species reach their maxima at different times of the year. Greenwood (1981) investigated the distribution of two sympatric *Acartia* congeners, (*A. tranteri* and *A. pacifica*) in Moreton Bay, Queensland and looked for these techniques. In this case, the two sympatric species coexist within the total range of salinity and temperature conditions in the Bay, but have different optimal ranges. *A. tranteri* is more eurytopic and hence considered a "generalist". In contrast, *A. pacifica* prefers conditions towards one end of the range and so is a "specialist". Close competition occurs during the spring and autumn when temperature is suboptimal for both. The result of this competition depends on whether the temperature is increasing or decreasing. There are also salinity effects. *A. pacifica* is less euryhaline but is thermophilic and, consequently, only competes successfully within its optimal temperature range at reduced salinities. So, the *A. pacifica* population is more variable from year to year than that of *A. tranteri*, depending on the degree of summer, (wet season) dilution. Competition is undoubtedly present, but the environment is partitioned both temporally and spatially by factors correlated with temperature and salinity differences. Thus, selective pressure alternates to favour first one, then the other species. This permits sympatry without prolonged competitive coexistence. The *A. tranteri* – *A. pacifica* pairing appears to have one species that has a plasticity in dealing with its environment and is probably more phylogenetically ancient (*A. tranteri*), and one which has evolved towards greater

efficiency under more specified conditions (*A. pacifica*), at which times it supplants the generalised form. However, at suboptimal conditions it is out-competed.

Similar to the situation at Moreton Bay, *Acartia clausi* and *Acartia tonsa* compete for resources in New England and Middle Atlantic estuaries (Jeffries 1962). *Acartia clausi* is a winter-spring copepod, and *A.tonsa* is regarded as a summer-autumn form. Consequently, there are alternate cycles of dominance. Temperature keeps the overlapping niches of *A. clausi* and *A. tonsa* separate during the major portion of the year, but intermediate temperatures lead to competition within the niche and succession of species. Salinity also plays a part in the copepod distributions with *A. tonsa* evidently more tolerant of low salinities than its winter-spring counterpart. The distribution of *Acartia* congeners in the *ria* of Vigo in Spain indicated that r-strategist species occupied the less stable areas, thus *Acartia margalefi* and *Acartia grani* were found at the head of the *ria*. As environmental stability increases, so too does the efficiency of the species that occupy the area and *A. discaudata* is found in the intermediate zone and *A. clausi* in the most stable area (Alcaraz 1983). Lakkis (1994) investigated the coexistence and competition within *Acartia* congeners from Lebanese coastal waters. Although both spatial and temporal segregation occurred between congeners, there was found to be a degree of niche overlap. This overlap can be used to determine the relative amounts of inter- and intraspecific competition. Two groups of Acartiidae were present; the spring collection of *A. clausi, A. discaudata* and *A.grani*, as well as the summer assemblage of *Acartia italica, Acartia josephinae* and *Acartia latisetosa*. The high amount of overlap, and resulting dominance in these waters, is due to incomplete partitioning of a poor habitat. The dynamic equilibrium between reproduction rates, trophic competition and environmental variables explains the short life cycle and low numbers of the species in the water column. Despite this however, all of the *Acartia* species were present in

highest numbers in the more disturbed and variable habitats indicating that they are r-selected species.

As mentioned, the Acartiidae distribution in Solent-Southampton Water system is very important because this calanoid genus dominates the zooplankton community (Conover 1957; Raymont and Carrie 1964; Castro-Longoria 1998; Hirst *et al.* 1999). Castro-Longoria (1998) reports that three *Acartia* species, (*A. bifilosa, A. discaudata* and *A. clausi*) occur in the Solent and Southampton Water, with *A. tonsa* and *A. margalefi* mainly restricted to the upper reaches of Southampton Water. The seasonal occurrence of these species is predominantly controlled by temperature, while the distribution patterns are related to horizontal salinity gradients. Predation and competition for food play a lesser role in determining species distribution, as do water movement/flow patterns and gyre formations. Castro-Longoria (1998) recorded that, in Southampton Water, *A. bifilosa* was found throughout the entire system. It was numerically dominant during winter and reached its seasonal maximum in April-May with a density of 4791 individuals m^{-3}. There was a sharp drop in numbers in June to less than 5 individuals m^{-3} and it disappeared from the water column come July, and reappeared in November. In contrast, *A. margalefi* was present in the water column all year round, though in very low abundances. It reached its maximum in June and underwent a sharp decline in numbers in July. *Acartia discaudata* was also seen virtually all through the year, but was more common in Southampton Water than the Solent. Its highest numbers were recorded in May and the lowest in June. Again, a sharp fall in numbers was seen in July. *Acartia clausi* was actually more abundant in the Solent than in Southampton Water. It has been classified as both an estuarine and a marine animal, but from the patterns observed in the Southampton water-Solent system, Castro-Longoria (1998) described it as being more neritic than estuarine. This copepod appeared from June to November in

Southampton Water, after which it left this part of the system. *Acartia clausi* disappeared from the Solent in Feb/March and reappeared in May. Like *A. margalefi, A. tonsa* was essentially confined to the lower salinities of Southampton Water (Jeffries 1962). It was present from April to February and disappeared in March. It exhibited a similar temporal pattern of abundance to the other species; low numbers in the early part of the year, followed by a peak in density from August to October. The *Acartia* copepodites, (groups III to V) were similar in density throughout Southampton Water. The numbers were low in autumn and winter and increased in spring and summer, mirroring the peaks in adult densities. The high density in April were *A. bifilosa* copepodites, and the smaller peak in July/August was composed of *A. clausi* and *A. discaudata* copepodites.

Lance (1964) explains the pattern of distribution through the salinity tolerances of the species. *A. clausi* is least tolerant of dilution and cannot comfortably propagate into the lower salinity waters of Southampton Water for any great length of time. Indeed, it is least tolerant of all the species, with order of increasing tolerance *A. clausi, A. discaudata, A. bifilosa* with *A. tonsa* the most tolerant. Adult males are less tolerant of dilution than female, as are copepodites (Lance 1964). Interestingly, if the change in environment, (salinity or temperature) is gradual, giving *Acartia* copepods time to acclimatise, their tolerance range may exceed the norm, allowing them to survive unusual or harsher conditions (Lance 1963). Since young or spawning stages of *Acartia* species are more sensitive and have narrower survival limits than non-spawning adults (Lance 1964), Tester and Turner (1991) investigated whether the salinity tolerance of nauplii was a major factor in restricting *A. tonsa* to near shore and estuarine habitats. They found that there was a significantly greater naupliar survival at salinities less than full strength seawater. It was observed that the ideal conditions for *A. tonsa* nauplii were

<25 and >15°C. So, even though adult *A. tonsa* have good osmoregulatory abilities and can tolerate a wide range in salinity, they are probably restricted to their habitat by the physiology of the nauplii. This may also be true for other species of *Acartia.*

1.2 Aims of the Study

Recent work has quantitatively investigated the distribution of the copepod genus *Acartia* in the Southampton Water-Solent estuarine system, with particular reference to the ability of adults to survive, and lay eggs, in certain salinities and temperatures (Castro-Longoria 1998). The current study is intended to further examine this and will also look at aspects of the potential biological constraints which ultimately influence the spatial and temporal distribution of *Acartia* spp. and their ability to breed successfully in Southampton Water. Part of the work will focus on *Acartia* naupliar response to a variety of temperatures and salinities, as these are factors known to affect population dynamics and spatial distribution (Tester and Turner 1991).

Besides spatial distribution of the *Acartia* species, seasonal occurrence will also be examined. *Acartia bifilosa* is numerically the most successful congener over winter, but diapauses through summer. This is an unusual strategy as it removes the species from the water column during the most productive part of the year. Other than the timing, very little is known about *A. bifilosa* diapause, neither the proximate cues nor the ultimate cause of this unusual tactic. Previous studies have shown that the trigger for *Labidocera aestiva* diapause is temperature-mediated photoperiodism (Marcus 1982, 1984, 1986) and so *A. bifilosa* diapause behaviour with respect to temperature and daylength will be investigated. The ultimate reason behind over-summering in the egg phase could be to avoid a food bottle-neck (Santer and Lampert 1995), predators

(Slusarczyk 1995) or higher temperatures. A scope for growth assay will be carried out on adult female *A. bifilosa* and, for comparison, *A. discaudata* (a congener very similar to *A. bifilosa* in spatial distribution but does not diapause) to determine if there are differences in performance at the various temperatures, which may explain the diapause strategy. *Acartia bifilosa*'s success in winter could also be a result of it switching its diet focus successfully, or that it has a superior ability to form essential fatty acids from its food and its monthly fatty acid composition will be looked at to determine which is the case.

Feeding preference also relates to the seasonal distribution of the *Acartia* genus as the suite of phytoplankton species in Southampton Water changes throughout the year (Kifle 1992). Given the rapid reproduction response of *Acartia* spp. to food (Tester and Turner 1990), egg production is primarily a reflection of the immediate past diet. The congeners that feed best on a certain food type will produce more eggs and will therefore, maintain a population in the water column. So, *Acartia* spp. feeding rate on a flagellate (*Isochrysis galbana*), a diatom (*Phaeodactylum tricornutum*), a chain-forming diatom (*Skeletonema costatum*) and a dinoflagellate (*Scrippsiella trochoidea*) will be examined, as will their fecundity when fed these food types. Egg production is a more sensitive assay of the species preference and performance on the different food types. The quality of the food will also be assessed with respect to its fatty acid content as it is this component that is known to affect the fecundity and viability of copepod eggs (Anderson and Pond 2000; Ban *et al.* 1997; Ianora *et al.* 1995, 1999; Jonasdottir 1994, Laabir *et al.* 1999; Pond *et al* 1996; Stottrup and Jensen 1990).

It is intended that the patterns seen in this work will go towards explaining the temporal and spatial distribution of the genus *Acartia* in Southampton Water. In particular the

unusual over summer diapause strategy of *A. bifilosa* as opposed to the more usual overwintering of *A. tonsa,* or the continual presence in the water column of *A. clausi* and *A. discaudata.* Results will also help to clarify the reasons behind *A. clausi* and *A. tonsa* being restricted to the mouth and the head of Southampton Water respectively, whereas *A. bifilosa* and *A. discaudata* are found along the length of the estuary.

Chapter 2:
The effects of temperature and salinity on *Acartia* nauplii survival

2.1 Introduction

Copepod development is characterised by 13 stages; egg, 6 naupliar and 6 copepodite stages, with CVI being the adult, (Ozaki and Ikeda 1997). Information on the development time and growth rate of life history stages is essential to assess the population dynamics and production rates of copepods, (Uye 1991). There have been previous investigations into the effect of temperature and salinity on the survival of adult copepods (Lance 1963, 1964; Bhattacharya 1986; Castro-Longoria 1998) and their influences on species dispersal (Collins and Williams 1981). To date however, little attention has been paid to the responses of the nauplii, even though this stage is perhaps the most sensitive to environmental parameters (Templeman 1936; Tester and Turner 1991). Ultimately, it is not important whether developmental stages are long or short if the animal does not survive or cannot breed and so maintain a population in the area.

It is universally accepted that there is an increase in copepod development rate with temperature, (Kimoto *et al.* 1986; Munro 1975; Nagaraj 1988; Tester 1985; and Uye 1991), in contrast, Uye (1981) found that salinity had very little influence on *A. clausi* development. Another contributing factor in determining distribution is that certain young or spawning stages, may be more sensitive and consequently have narrower survival limits than non-spawning adults. Work on *Eurytemora velox* (Nagaraj 1988) has shown that nauplii suffered relatively high mortalities under certain environmental conditions, indicating the relative sensitivity of this stage to variations in temperature and salinity.

Although adult *E. velox* coped with freshwater conditions for a long time, it failed to maintain a population as the nauplii were incapable of completing development; this was also the case in hypersaline water of 40. Lance (1964) reported that *Acartia* copepodites did not thrive in full strength seawater and those which attempted to moult failed to complete development, whereas adults survived well. Heavy naupliar mortality also occurred in *Acartia californiensis* following hatch at low salinities, (Johnson 1980).

Of the Acartiidae in Southampton Water, it might be expected that *A. tonsa* nauplii have the greater physiological plasticity, with *A. clausi* nauplii the least tolerant of dilution, as this would mirror the salinity tolerances of the adults (Lance 1963). Despite its widespread abundance, *A. tonsa* is generally absent from continental shelf water and restricted to estuarine and coastal habitats. This may be due to insufficient food or predation, but Tester and Turner (1991) argue that it is the salinity tolerance of the naupliar stage that plays a major part in restricting its seaward distribution. Significantly greater naupliar survival was recorded at salinities less than full strength seawater, and the optimum salinity and temperature conditions for *A. tonsa* nauplii in this case were <25 and >15°C respectively. Early naupliar stages of *A. tonsa* do show some osmoregulatory ability and even the non-feeding NI can drink. By comparison adults are good osmoregulators and consequently can tolerate wider ranges in salinity (Tester and Turner 1991). So, the restriction of this species to estuarine and coastal habitats may be a function of the physiology of the nauplii, rather than the adult.

The naupliar survival rates of four *Acartia* congeners; *bifilosa, discaudata, clausi* and *tonsa* were examined over a variety of temperatures at a fixed salinity, and at a range of salinities

at a fixed temperature, in an attempt to characterise the individual and combined roles of temperature and salinity as restricting factors in the species spatial and seasonal distributions.

2.2 Method

For each of the 4 *Acartia* congeners, adult females were taken directly from Southampton Water and 50 were placed in each of four egg production chambers (see Appendix 7); plastic cylinders with a 200μm mesh on one end held supported in a 1l beaker filled with 600ml of seawater from the SOES aquarium (30-32 salinity). Food was provided in excess concentrations of 1×10^5 cell ml^{-1} of *Isochrysis* and the chambers were kept under conditions previously identified as suitable for high egg production of 15°C and a light period of 12L:12D for *A. bifilosa* (to prevent the production of diapause eggs) and *A. clausi* and *A. discaudata*. *Acartia tonsa*, however, was kept under longer daylength conditions (14L:10D) and at a higher temperature of 20°C to simulate summer conditions, in order to prevent it laying diapause eggs.

After two days, the subitaneous eggs were collected and placed in incubation chambers; large petri dishes containing ~25ml of water and 1ml *Isochrysis*, equivalent to 10^5 cells ml^{-1}, and incubated under specific temperature and salinity conditions, (3 replicates in each case) and a photoperiod of 12L:12D. For the salinity investigation, the range was 33.3, 25.1, 20.6 and 15.5 (100%, 75%, 62% and 47% of full strength sea water respectively), all held at 20°C. Water of 33.3 salinity was used for all the temperature experiments at 5°C, 10°C and 20°C. Hatch success, naupliar survival and the time taken for the first CI to appear was monitored in each experiment. No arbitrary cut-off time was given to the experiment, instead observation continued until the maximum number of nauplii had hatched and developed to copepodite I (CI). As a result, there is a range in duration time of the various investigations.

Initially, the aim was to have 50 eggs per dish, but given the less fecund nature of *A. bifilosa* and the low numbers of adult *A. tonsa* found, this was not always possible. Consequently, there was a great range in the number of eggs at the start of each replicate (13-54). Egg development was monitored every two days using a binocular microscope, and the eggs and nauplii were carefully transferred by pipette into fresh incubation chambers every four days and supplied with fresh food. This infrequent replacing of the rearing medium was to minimise handling of the animals in an attempt to decrease mortality (Landry 1975). Hatch success was measured for each replicate and once the maximum number of nauplii had appeared, 20 (or all the nauplii, depending on which was lower) were placed into a fresh chamber and their continuing development to the CI stage observed.

The mean percent hatch success, the percent naupliar survival and time taken for the first CI to appear were calculated for each experimental regime and one-way ANOVA and, where possible, the Tukey Test (Fowler and Cohen 1990) was performed to see if there was a statistically significant difference (at the 0.05% level) between the means both intraspecifically and between the four congeners. When ANOVA indicates that there is a significant difference between the means of the groups, the more sensitive Tukey Test will distinguish which specific means are significantly different from each other.

2.3 Results

2.3.1 Hatch Success – Temperature

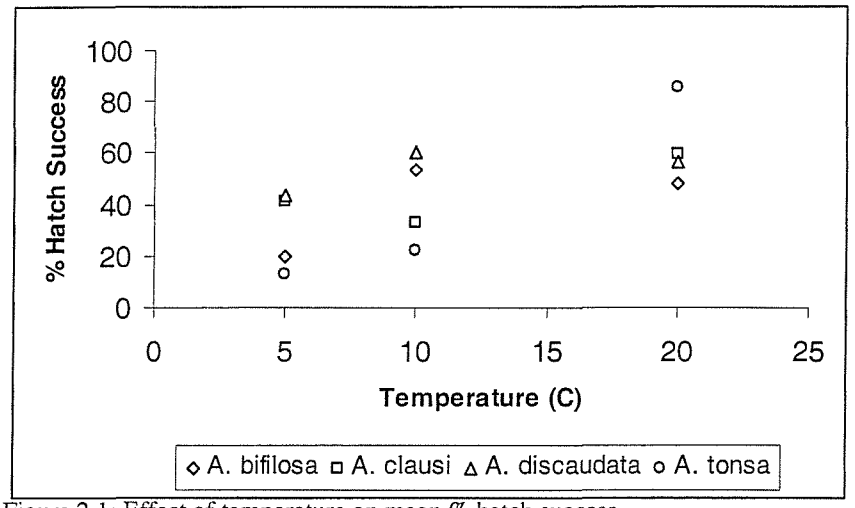


Figure 2.1: Effect of temperature on mean % hatch success.

Table 2.1: Effect of temperature on mean % hatch success.

Temperature	**Mean % hatch success ± 1 SD**			
(°C)	*A. bifilosa*	*A. clausi*	*A. discaudata*	*A. tonsa*
5	19.5±5.23	41.6±33.60	42.6±10.70	13.1±7.09
10	53.4±24.80	33.1±13.30	60.3±4.37	21.9±11.90
20	48.0±5.67	59.9±3.35	56.7±8.21	85.4±2.38

SD=standard deviation

Table 2.2: Time taken until % hatch success readings taken.

Temperature	**Mean time until max number of nauplii seen (days) ± 1 SD**			
(°C)	*A. bifilosa*	*A. clausi*	*A. discaudata*	*A. tonsa*
5	9.0±0.0	8.7±1.2	7.0±0.0	7.0±0.0
10	5.7±3.5	3.0±0.0	3.0±0.0	7.0±0.0
20	3.3±2.3	4.0±1.7	3.0±0.0	2.0±1.7

SD=standard deviation

The current investigation found that the mean hatching success of all *Acartia* spp. generally increased with increasing temperature (Fig.2.1). There was no arbitrary cut-off point for the experiment instead, observations continued until the maximum number of nauplii were seen in each replicate (Table 2.2) before % hatch success was calculated (Table 2.1). It is

obvious that hatching generally occurred more quickly at the higher temperatures for all species, indicating swifter pre-hatch development. *Acartia bifilosa* nauplii appeared in the 5°C experiment, but at 19.5%, the hatch success rate was significantly lower (P=0.05) than at the other two higher temperatures. Castro-Longoria (1996) obtained the highest *A. bifilosa* egg hatch at 15°C, although that temperature was not tested here. Current results showed a temperature sequence, in terms of % hatching success of 10°C>20°C>5°C, with 53.4%, 48.0% and 19.5% eggs hatching respectively. *Acartia discaudata* also performed best at 10°C in terms of hatch success, but the difference between the three temperatures (42-60% hatch success) was not significant. *Acartia discaudata* is present in the water column throughout the year and so must be able to successfully reproduce over a range of temperatures. The hatching success of both *A. tonsa* and *A. clausi* eggs incubated at 20°C was greater than at the other temperatures, significantly so (P=0.05) in the case of *A. tonsa.* This reflects the seasonal distribution of *A. tonsa, which* could be considered a summer species because it enters egg diapause stage over the winter months. Although it does not diapause, *A. clausi* may also be considered a summer species as it is numerically superior to other *Acartia* sp. during the summer. Although there was no interspecific difference in egg hatch at 5°C and 10°C, *A. tonsa* significantly (P=0.05) outperformed all the other species at 20°C, confirming this summer species' preference for higher temperatures.

2.3.2 Hatch success – Salinity

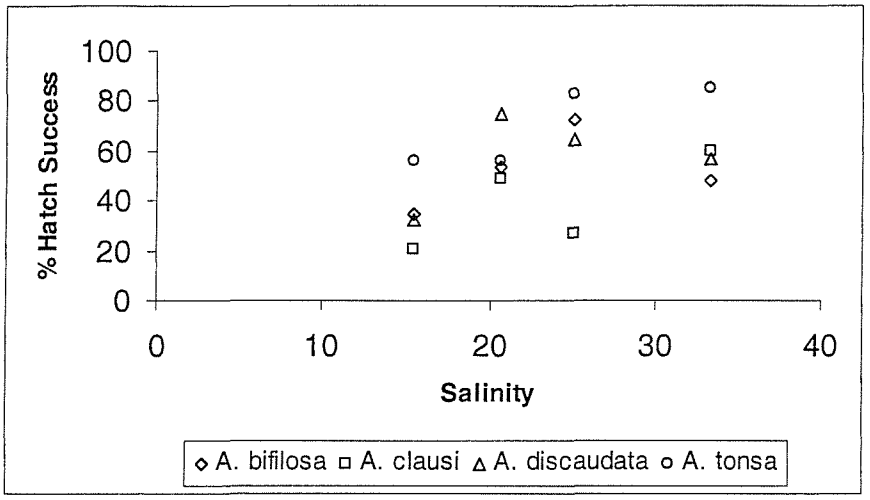


Figure 2.2: Effect of salinity on mean % hatch success.

Table 2.3: Effect of salinity on mean % hatch success.

Salinity	**Mean % hatch success ± 1 SD**			
	A. bifilosa	*A. clausi*	*A. discaudata*	*A. tonsa*
33.3	48.0±5.67	59.9±3.35	56.7±8.21	85.4±2.38
25.1	72.5±4.73	27.1±11.20	64.8±7.77	82.9±1.90
20.6	53.2±26.30	49.2±14.90	74.5±15.10	55.6±9.47
15.5	34.3±5.72	20.1±15.70	32.4±9.17	55.9±13.90

SD=standard deviation

Table 2.4: Time taken until % hatch success readings taken.

Salinity	**Mean time until max number of nauplii seen (days) ± 1 SD**			
	A. bifilosa	*A. clausi*	*A. discaudata*	*A. tonsa*
33.3	3.3±2.3	6.0±0.0	3.0±0.0	5.0±1.7
25.1	3.3±2.3	1.7±1.2	3.0±0.0	5.0±1.7
20.6	2.0±0.0	2.3±1.2	7.0±0.0	4.0±0.0
15.5	2.0±0.0	2.3±1.2	1.7±1.2	5.0±1.7

SD=standard deviation

Hatch success of the *Acartia* congeners over a range of salinities did not give as clear a pattern as temperature (Fig. 2.2) possibly because the eggs would not naturally encounter some of the lower experimental salinities in Southampton Water, and so are not adapted to cope with them. In Southampton Water salinities range between 28.0-34.3 at the head of the estuary, where the water column is stratified, through a typical longitudinal salinity gradient to the mouth where salinity values peak at 35 (Lucas 1995). Lance (1963) found

adult *Acartia* tolerance for dilution to range from *A. tonsa* > *A. bifilosa* > *A. discaudata* > *A. clausi*. This pattern was also observed for egg hatch (Table 2.3) for, although *A. tonsa* hatch success was highest at the higher salinities, it also had the highest hatch success at the lowest salinity, suggesting that it is the most tolerant of the four species. Relative hatch success at the lower salinities, especially 15.5, was significantly reduced for *A. clausi, A. discaudata* and *A. tonsa,* and *A. clausi* showed the lowest hatch success at the lowest salinity, indicating it is the least tolerant of the four congeners. *Acartia bifilosa* and *A. discaudata* have similar hatch success percentages at the lowest salinity and are intermediate between *A. tonsa* and *A. clausi.* Salinity had no statistically significant effect on egg hatch of *A. bifilosa*, but this species performed better at salinities less than full seawater; 25.1>20.6>33.3>15.5. *Acartia clausi* had a very low hatch success at 25.1 salinit, this result did not fit with the trend evident in figure 2.2. Ignoring this data point, *A. clausi*'s salinity preferences were 33.3>20.6>15.5 which agrees with it's known distribution pattern in Southampton Water – a higher population density at the mouth in the more saline regions of the estuary. The hatch success of *A. discaudata* with respect to salinity was similar to that of *A. bifilosa*; both showed a larger % egg hatch at the intermediate salinities. *Acartia tonsa* had the highest hatch success for 3 out of the 4 salinities indicating that it had the physiological plasticity to cope with a range of salinities and was clearly the most tolerant to dilution. Table 2.4 gives an indication of the duration of the experiment and the maximum number of *A. clausi* nauplii at 33.3 salinity were seen after 6 days, but after only 2.3 days at 15.5 salinity. 33.3 salinity is a better environment for *A. clausi* nauplii in terms of hatch success (Table 2.3) so nauplii numbers increase with time. However, there is reduced hatch success at 15.5 and also some naupliar mortality (not measured) hence maximum nauplii numbers seen after such a short time.

2.3.3 Naupliar survival – Temperature

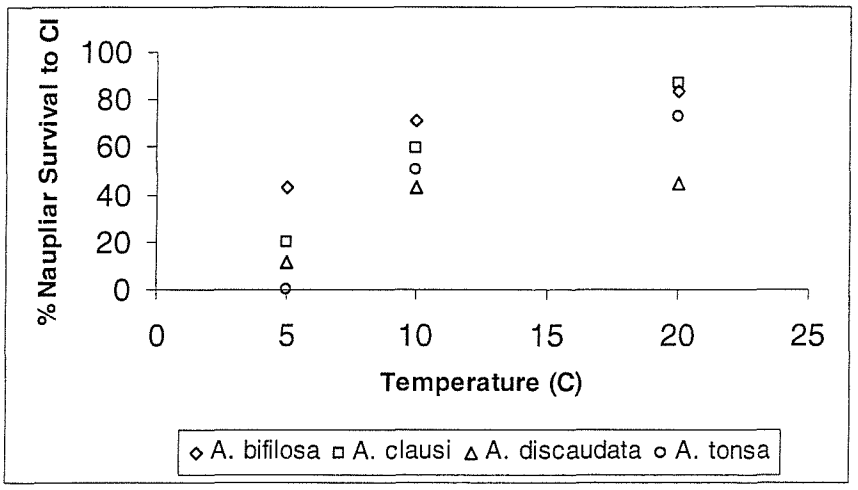


Figure 2.3: Effect of temperature on mean % naupliar survival.

Table 2.5: Effect of temperature on mean % naupliar survival.

Temperature	**Mean % naupliar survival ± 1 SD**			
(°C)	*A. bifilosa*	*A. clausi*	*A. discaudata*	*A. tonsa*
5	42.8±29.50	20.0±0.00	11.9±4.13	0.0±0.00
10	70.9±5.24	59.6±13.60	43.3±31.80	50.5±14.90
20	83.3±28.90	86.5±7.31	45.0±18.00	72.9±25.30

SD=standard deviation

Table 2.6: Time until % Copepodite I (CI) survival readings taken.

Temperature	**Mean time until max number of CI seen (days) ± 1 SD**			
(°C)	*A. bifilosa*	*A. clausi*	*A. discaudata*	*A. tonsa*
5	20.0±0.0	16.0±1.7	14.7±1.2	/
10	11.3±2.9	17.0±0.0	14.0±0.0	13.0±5.6
20	10.0±0.0	14.0±0.0	13.0±1.7	18.0±0.0

SD=standard deviation

As with hatch success, there is an increase in naupliar survival with temperature with all the congeners showing a higher relative survival rate at 20°C>10°C>5°C (Fig. 2.3, Table 2.5). Temperature had no statistically significant effect on the survival of *A. bifilosa* and *A. discaudata* nauplii. This was unexpected for the former species given that it diapauses over the summer, possibly to avoid higher temperatures. In contrast, temperature influences the survival of *A. discaudata* which is found in the water column during the whole year and so would be expected to present the physiological plasticity to cope with the range of temperatures in Southampton Water. Unsurprisingly, *A. tonsa* naupliar survival was

statistically significantly lower at 5°C than at the other two temperatures, given its preference for higher temperatures. The only interspecific difference in performance with respect to temperature occurred at 5°C where *A. bifilosa* (the "winter" species) naupliar survival was significantly higher than that of *A. clausi* and *A. tonsa* (the "summer" species). Table 2.6 may be a little misleading as it simply shows the time until the maximum number of CI appeared, i.e. experiment duration. However, this is not a good indication of development time because the number of CIs increase in the better environments over time, but mortality occurs in the poorer salinity regimes and so the numbers decrease with time and so the maximum numbers are seen earlier on in the investigation. The time until the first CI appears is a better measure of development time.

2.3.4 Naupliar survival – Salinity

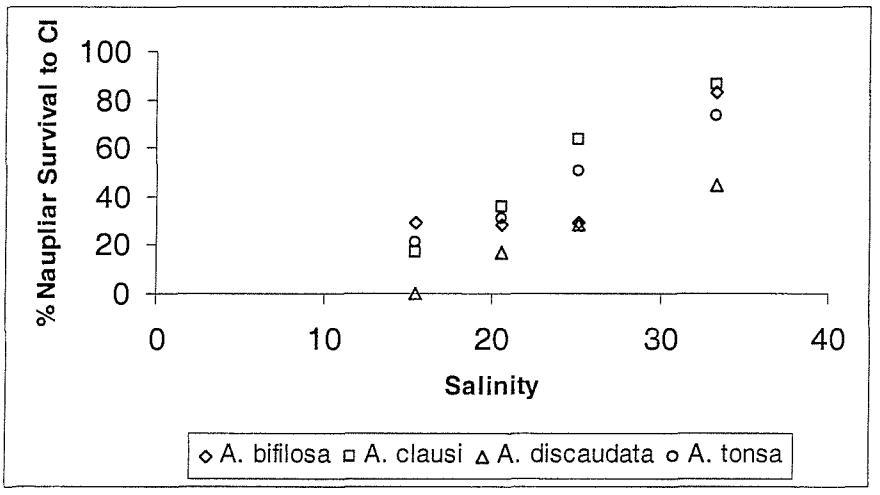


Figure 2.4: Effect of salinity on mean % naupliar survival.

Table 2.7: Effect of salinity on mean % naupliar survival.

Salinity	Mean % naupliar survival ± 1 SD			
	A. bifilosa	*A. clausi*	*A. discaudata*	*A. tonsa*
33.3	83.3±28.9	86.5±7.31	45.0±18.0	72.9±25.3
25.1	29.0±24.0	63.1±28.6	28.3±16.1	50.7±8.97
20.6	28.2±11.6	36.1±12.7	16.7±12.6	30.6±17.3
15.5	29.3±12.0	16.7±28.9	0.00±0.00	20.9±8.80

SD=standard deviation

Table 2.8: Time until % Copepodite I (CI) survival readings taken.

Salinity	Mean time until max number of CI seen (days) ± 1 SD			
	A. bifilosa	*A. clausi*	*A. discaudata*	*A. tonsa*
33.3	11.0±0.0	13.0±0.0	11.7±0.6	11.0±0.0
25.1	10.3±1.2	9.0±1.4	10.3±2.1	8.3±1.2
20.6	7.5±2.1	6.7±1.2	10.7±1.8	11.0±0.0
15.5	11.0±0.0	10.0±0.0	/	11.0±0.0

SD=standard deviation

Unlike the hatch success results, all the congeners showed greatest naupliar survival at full strength seawater (Fig. 2.4) and survival generally decreased with reduced salinity. Nauplii are comparatively poor swimmers and so can not easily maintain station and are likely to be swept towards the mouth and into more saline regions, of the estuary. Early naupliar stages do exhibit some osmoregulatory ability, and even the non-feeding NI stage can drink (Tester and Turner 1991). This ability to osmoregulate may enable the nauplii to survive in higher salinities before developing further, when the more advanced stages propagate back up the estuary to regions of their preferred salinity and the adults take up their more familiar spatial distribution.

2.3.5 Time taken to reach CI

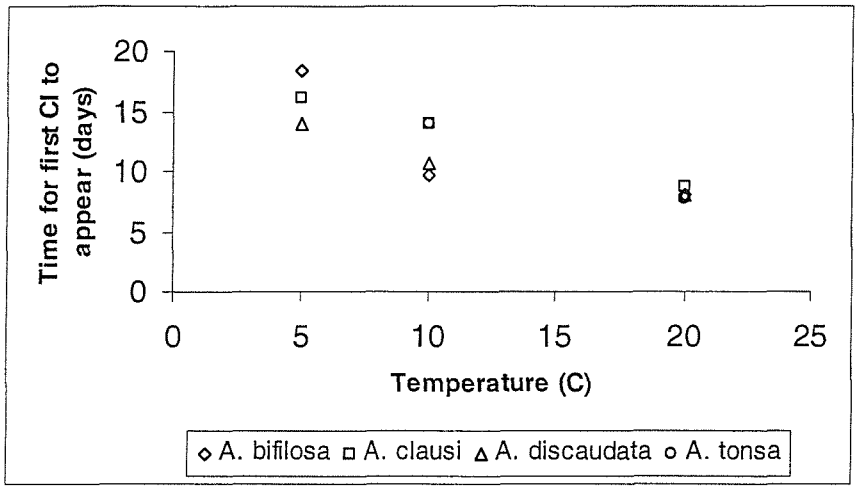


Figure 2.5: Temperature effect on mean development time to CI.

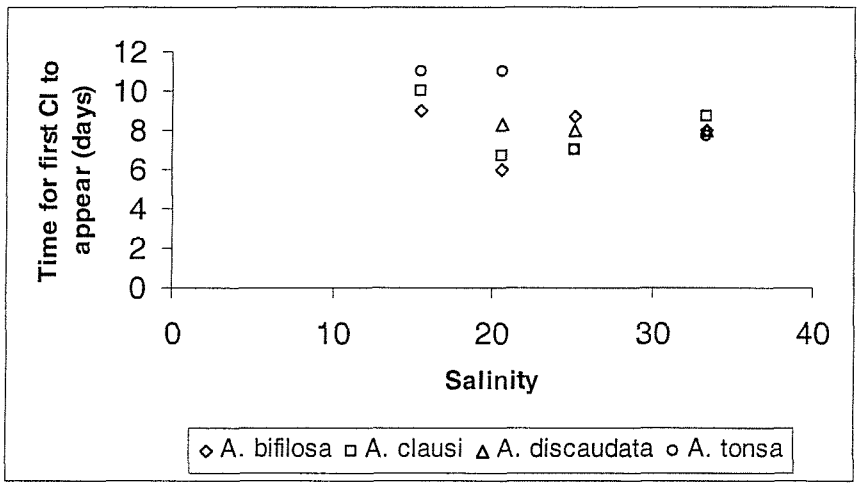


Figure 2.6: Effect of salinity on mean development time to CI.

Table 2.9: Effect of temperature and salinity on mean naupliar development time.

Temperature (°C)	**Mean time for first CI to appear (days) ± 1 SD**			
	A. bifilosa	*A. clausi*	*A. discaudata*	*A. tonsa*
5	18.3±2.89	16.0±1.73	14.0±0.00	/
10	9.67±0.58	14.0±0.00	10.7±2.89	14.0±0.00
20	8.0±1.73	8.7±1.41	8.0±0.00	7.7±1.15
Salinity				
33.3	8.0±1.73	8.7±1.15	8.0±0.00	7.7±1.15
25.1	8.67±2.52	7.0±1.41	8.0±0.00	7.0±0.00
20.6	6.0±0.00	6.7±1.15	8.3±1.15	11.0±0.00
15.5	9.0±0.00	10.0±0.00	/	11.0±0.00

SD=standard deviation

There was a statistically significant difference ($P<0.05$) between the time taken for the nauplii from all four *Acartia* congeners to develop to CI at the three test temperatures and development time lengthened as the temperature decreased; 20°C<10°C<5°C (Fig. 2.5, Table 2.9). The time taken for nauplii to reach CI was also affected by salinity (Table 2.9), with salinity lower than that of seawater producing quicker development times; 25.1<20.6<33.3<15.5, but this difference was not statistically significant. There was no apparent consistency in the development, i.e. no one species developed quickest or slowest throughout. However, all four congeners developed at almost exactly the same time under conditions of 33.3 salinity, and also at 20°C (Table 2.9).

2.4 Discussion

Lance (1963) considers *Acartia* spp. to be marine organisms, even though some species do migrate into brackish waters. The salinity limits of estuarine species cannot be sharply defined and adult *Acartia* tolerate dilution to a varying degree where *A. tonsa* is the most tolerant, then *A. bifilosa* followed by *A. discaudata*, with *A. clausi* surviving best at higher salinities (Lance 1963). This argument is reflected in the animals' distribution in Southampton Water, with adult *A. clausi* being most abundant at the mouth while *A. tonsa* is most successful in lower salinities nearer the head of the estuary, with *A. bifilosa* and *A. discaudata* classed as intermediate species (Castro-Longoria 1998).

Copepod egg development and vitality are known to be affected by temperature (Ban 1994) and changes in temperature can increase or decrease egg hatch time (Castro-Longoria 1998). Under appropriate conditions though, *Acartia* eggs typically hatch within a few hours (Castro-Longoria 1998) and, with the exception of *A. tonsa* eggs at 5°C, hatching of eggs from all species at all temperatures in the present study started after one day although, highest nauplii numbers were seen after 7-9 days at the lowest temperature. While it is considered a winter species, hatching success of *A. bifilosa* was greatly reduced at 5°C, and it performed best at 10°C. Castro-Longoria (1998) reported a similar pattern and stated that this would explain the species' low abundance in winter and sudden increase in numbers in the spring. There was no statistically significant difference in percent egg hatch for *A. discaudata* at each of the three temperatures, and this would explain why this species is present in the water column throughout the year. Uye and Fleminger (1976) reported that *A. tonsa* and *A. clausi* eggs from southern Californian coastal waters developed into nauplii at

all temperatures in the range 5-25°C. The eggs of these two species in the current study did hatch over the range of experimental temperatures, but success was higher at 20°C, significantly so for *A. tonsa.* In terms of adult growth, measured as egg production, *A. tonsa* also performs better at higher temperatures with daily egg production rate (EPR) increasing with temperature as 20°C>15°C>10°C>5°C, with an EPR of <1 egg female^{-1} day^{-1} at 5°C and a mean EPR of 17.6 eggs female^{-1} day^{-1} at 20°C (Castro-Longoria 1996). It was not possible to determine EPR for the species in this investigation as, although the females were left for 2 days and then ~50 eggs per replicate were collected, the total number of eggs produced was not counted.

Castro-Longoria (1998) reported delayed-hatching of *A. bifilosa* eggs that produced nauplii after 13, 48 and 81 days when kept at 28-30°C, 25°C and 20°C respectively. No extended development times were seen in this investigation as all the eggs for all the species under all conditions began hatching after two days, with the exception of *A. tonsa* eggs kept at 5°C which required 6-7 days to produce nauplii. Photoperiods of 12L:12D for *A. bifilosa* and 14L:10D for *A. tonsa* were deliberately used during adult incubation to ensure that the females produced only subitaneous eggs. This was confirmed by checking the eggs themselves and all were smooth indicating that they were not diapause eggs which would have resulted in a prolonged hatch time.

Salinity is another major environmental factor influencing the hatching of copepod eggs (Uye and Fleminger 1976). The egg hatch results with respect to salinity paralleled the adult preferences as observed by Lance (1963). *Acartia tonsa* showed comparatively high hatch success percentages at all salinities; indeed it was most successful in the more saline

waters. However, it also outperformed the other congeners in the water of lowest salinity indicating that this species is most tolerant to dilution and it would also seem that salinity, in terms of egg hatch, is not a factor that limits *A. tonsa* distribution. The order of salinity preference for *A. clausi* was 33.3>20.6>15.5, confirming that it is the most marine of the four species. *Acartia bifilosa* and *A. discaudata* showed higher hatch success at the intermediate salinities, 25.1 for *A. bifilosa,* this supports Castro-Longora's (1998) findings and 20.6 for *A. discaudata.*

Though adult copepods can tolerate a relatively wide range in salinity (Lance 1963; 1964), this may not necessarily be true of the juveniles. Adult *Eurytemora velox* can endure freshwater and a salinity of 40, but a population could not be maintained because the nauplii did not complete development (Nagaraj 1988). Similarly, Lance (1964) reported that *Acartia* spp. copepodites did not thrive in full strength seawater and even though the adults, in general, survived well, those copepodites that attempted to moult failed to complete development. Regardless of the success of adults, if nauplii cannot survive in certain conditions, then a self-sustaining population cannot be established and so species dispersal is limited. Supporting this logic, Tester and Turner (1991) reported a significant increase in *A. tonsa* naupliar survival at salinities less than the strength of seawater, optimal conditions for *A. tonsa* naupliar survival were found to be <25 salinity and >15°C, and they concluded that it is the salinity tolerance of nauplii that restricts *A. tonsa* to estuarine and near shore waters.

Nauplii are considered to be the most vulnerable life stage of an organism and consequently suffer the greatest amount of mortality (Templeman 1936). Investigations into the effect of

temperature and salinity on larval survival have been carried out on a range of crustaceans, the lobster *Homarus americanus* (Templeman 1936), the crabs *Callinectes sapidus* (Sandoz and Rodgers 1944), *Sesarma cinereus* (Costlow *et al.* 1960) and *Panopeus herbstii* (Costlow *et al.* 1962), the barnacles *Balanus balanus, Balanus balanoides* and *Balanus crenatus* (Barnes 1953), and also the copepods *Sinocalanus tellus* (Kimoto *et al.* 1986), *Eurytemora velox* (Nagaraj 1988), *Acartia tonsa* (Tester and Turner 1991) and *Paraeuchaeta elongata* (Ozaki and Ikeda 1997).

Temperature is considered the most important factor affecting development and growth rate, Sandoz and Rodgers (1944) observed that none of the *C. sapidus* first zoeae completed the moult into the second stage at temperatures below 15°C or above 28°C, instead they become inactive and cease feeding. However, temperature generally has a more definite effect on the length of larval development rather than on mortality. The duration of individual larval stages at 30°C was approximately half the time for comparable development at 20°C for both *S. cinereus* zoeae (Costlow *et al.* 1960) and *P. herbstii* larvae (Costlow *et al.* 1962). Similarly in copepods, the development time of juvenile *P. elongata* (Ozaki and Ikeda 1997) and *S. tenellus* nauplii (Kimoto *et al.* 1986) decreased with increasing temperature, although very few *S. tenellus* individuals reached maturity at the extremely high temperature of 34.9°C. In the present study, the time taken at 20°C for the *Acartia* congeners to develop to CI was 7.7-8.7 days, and this compares favourably with previous work where Yoon *et al.* (1998) reported that *A. bifilosa* took 7.5 days to complete naupliar development at 17°C ± 1°C and 12L:12D. Similarly, Landry (1975) found that *A. clausi* kept at 20°C ± 1°C under 16L:8D took 180.4 hours or 7.5 days to develop to CI. Landry (1975) also noted that *A. clausi* eggs from winter acclimated females (10°C)

developed faster at 20°C (summer conditions) than those from summer acclimated females, and called this the "enhancement effect". Although not tested for here, parental acclimation temperature had a major effect on egg hatch time of *A. tonsa* as well as egg incubation time, indicating that *A. tonsa* are sensitive to temperature and changes in temperature during development (*in vivo)* and after they are laid (*in vitro*) and up to the time of hatching, but no evidence of the "enhancement effect" was seen (Tester 1985). For the *Acartia* congeners in the present study development time decreased as the temperature increased, however this effect is known to diminish as the temperature rises (Munro 1974). This was seen for *A. bifilosa and A. discaudata* because the difference in time for the egg to reach CI between 5°C and 10°C is larger than between 10°C and 20°C. Landry (1975) explained this by assuming that the development of *A. clausi* was controlled by a series of biochemical reactions which are entirely rate regulated by temperature effects, providing that the other factors are optimal temperature can therefore be responsible for larval mortality, but it more usually governs the length of development time from egg to adult.

Variations in salinity also affect growth and development time; moulting of larval lobsters occurred earlier in waters of lower salinity, though Templeman (1936) is the only worker to have observed this response. By contrast, in the crabs *S. cinereus* (Costlow *et al.* 1960) and *P. herbstii* (Costlow *et al.* 1962) the duration of the individual zoeal stages, as well as the total period of larval development, is affected by water salinity with an increase in salinity resulting in a decrease in development time. Kimoto *et al.* (1986) found that post-embryonic development of the calanoid copepod *S. tenellus* was successful in salinities of 5-30, but both the rate of growth and overall body size decreased at the lowest and highest salinities. They concluded that unsuitable salinities might cause the animal some

physiological stress due to the additional osmoregulation and/or respiration required at these salinities, resulting in a suppression of growth rate. Conversely, salinity had no effect on the development time on the copepods *S. tenellus* (Kimoto *et al.* 1986). Naupliar development time of the four *Acartia* congeners was not statistically significantly affected by salinity, even in the lowest saline water (15.5). There was no consistency in the development, one species did not develop faster/slower than the others at all of the salinities tested, and at 33.3 development time for all 4 congeners was practically the same. Salinity appears to have a greater effect on juvenile survival rather than on development time, as reported for the lobster *H. americanus* (Templeman 1936) and barnacles *B. balanoides, B. crenatus* and *B. balanus* (Barnes 1953). Sandoz and Rodgers (1944) observed that zoea of the blue crab (*C. sapidus*) hatched in a salinity of 14, but while they did not die, they were sluggish and did not respond to light, however, zoea hatched in 18 salinity were active and positively phototrophic and those in 24 salinity were very active. In contrast, Costlow *et al.* (1960 and 1962) working on the crabs *S. cinereus* and *P. herbstii* respectively, found a 100% hatch success rate over a range of salinities (12.5-31.1), and reported the first stage zoea of both species were positively phototrophic and active in all salinities. Optimal salinities were found for each *S. cinereus* larval stage (Costlow *et al.* 1960) but not for *P. herbstii* (Costlow *et al.* 1962), but in the most saline conditions, 31.1, the highest *S. cinereus* mortality occurred when the final stage zoea attempted to moult into the megalopa. So, although the initial juvenile stages did well at higher salinities, extremes killed the more advanced juvenile stages and the adults. Costlow *et al.* (1960) concluded that salinity was the chief physical factor that confined *S. cinereus* to an estuarine environment. Similarly, this could explain *Acartia* naupliar survival results as the highest salinity did not have a detrimental effect on any of the *Acartia* species, indeed they all

performed best in full strength seawater and survival of all species generally decreased with reduced salinity. This was not expected as Tester and Turner (1991) found that *Acartia* nauplii survive better in salinities less than full seawater. Villante *et al.* (1993) observed that adult *A. bifilosa* maximum density in the Mundaka estuary (Spain) occurred at 30 salinity, but nauplii peaked numerically in water of >30 salinity. Given that weight and swimming ability increase with development stage, both passive and active transport could account for the differential distribution of the nauplii as a result of size-dependent differential transport and retention of individuals. It is known that earlier naupliar stages of *A. tonsa* can osmoregulate and even though the NI can not feed it does drink (Tester and Turner 1991), and this ability allows the nauplius to survive in higher salinities before the more advanced, better swimming stages can move back up the estuary into regions of their preferred salinity.

Temperature had a less pronounced effect on naupliar survival than salinity. In general, *Acartia* survival to CI was highest at 20°C>10°C>5°C, but this effect was not significant in *A. bifilosa* or *A. discaudata. Acartia discaudata* is found in Southampton Water throughout the year and so was expected to be able to cope with a range of temperatures. However, *A. bifilosa* diapauses over summer, and so it was surprising that the higher temperature had no appreciable effect on naupliar survival, if they do indeed diapause to avoid higher temperatures. The only significant difference in naupliar survival between the species with respect to temperature occurred at 5°C where *A. bifilosa* which is generally numerically dominant in winter outperformed *A. clausi* and *A. tonsa,* the "summer/autumn" species that diapauses overwinter to avoid the lower temperatures.

In conclusion, temperature usually affects the duration of developmental stage of the Acartiidae, with increasing temperatures resulting in reduced development time for all of the species and is responsible for their temporal distribution. *Acartia bifilosa* oversummers in the diapause egg phase and so is considered a winter species. Although its hatching success was reduced at 5°C, just 19.5%, it performed best at 10°C, results also reported by Castro-Longoria (1998) and this would explain its low abundance in Southampton Water in winter and its sudden increase in numbers in the spring. The impression that this is a cold water species is further enhanced because the survival rate of the nauplii of this species is significantly higher at 5°C than those of *A. clausi* or *A. tonsa* nauplii, the summer species. *Acartia discaudata* is present in the water column all year round and this is reflected in the fact that temperature had no statistically significant effect on it hatch success, although naupliar survival for this species is higher at 10°and 20°. Since it diapauses over winter, *A. tonsa* is a summer species and its percentage hatch success and naupliar survival are significantly higher at 20°C, indeed it statistically significantly outperforms all of the other species at this temperature in terms of egg hatch. Although present in Southampton Water all year round like *A. discaudata*, *A. clausi* numbers increase in the water column over summer and this species was seen to perform best at 20° both in terms of egg hatch and naupliar survival.

Salinity has an obvious effect on egg hatch. Both *A. bifilosa* and *A. discaudata* had higher percentage hatch success at salinities lower than full strength seawater, mirroring the adults' salinity preferences and classification as intermediate species (Castro-Longoria 1998). Though *A. tonsa* had a higher percent egg hatch in the more saline waters, it also performed best of the four congeners at the lower salinities, confirming Lance's (1963)

definition that it is the most tolerant to dilution of the *Acartia* species in Southampton Water and explaining why it is found towards the head of the estuary in fresher water. *Acartia clausi* is usually found in greater numbers nearer the mouth of Southampton Water, and its preference for more a marine environment is echoed in its higher hatch success in full strength seawater as opposed to the lower salinities. In contrast to the pattern of egg hatch with respect to salinity, the nauplii of all four species survived best in full strength seawater. From this, one could assume that the adults remain in regions of their preferred salinity and lay eggs there, which hatch well. However, because the nauplii are not good swimmers (Villate *et al.* 1993) they are swept towards the mouth of Southampton Water and into areas of higher salinity where they remain and develop into more advanced stages before propagating back up the estuary to take up their adult distribution pattern. It may be possibly to test this theory by taking zooplankton samples from the mouth of Southampton water and rearing the nauplii taken from there to adulthood to see if indeed all of the species are present.

2.5 Summary

Hatch successs, naupliar survival and time to first CI were tested with respect to seasonal temperature and salinity spectrums of Southampton Water.

1) Hatch success (HS) of the *Acartia* congeners with respect to temperature mirrored the known temporal distribution patterns of the adults.

A. bifilosa	Highest hatch success at 10°C agrees with Castro-Longoria
10>20>5°C	(1998), and even though *A. bifilosa* is numerically dominant
53.4>48.0>19.5 %HS	over winter these results explain the sharp increase in *A. bifilosa* numbers during spring.
A. clausi	Summer species, therefore performed best at higher
20>5>10°C	temperatures.
59.9>41.6>33.1 %HS	
A. discaudata	No statistically significant differences in hatch success
10>20>5°C	results. Not surprising as this species is present in
60.3>56.7>42.6 %HS	Southampton Water all year round.
A. tonsa	Hatch success was significantly higher (P=0.05) at 20°C than
20>10>5°C	at the other two temperatures. This is also a summer species.
85.4>21.9>13.1 %HS	

2) Hatch success of the *Acartia* congeners with respect to salinity agreed with the known spatial distribution of the adults.

A. bifilosa	No statistically significant differences. This is an
25.1>20.6>33.3>15.5	intermediate species, in terms of spatial distribution,
72.5>53.2>48.0>34.3 %HS	and can compete equally well over a range of salinities
A. clausi	Performed significantly better at full strength
33.3>20.6>25.1>15.5	seawater (P=0.05). Of the four congeners tested, this
59.9>49.2>27.1>20.1 %HS	species preferred the more saline waters, as seen by its distribution in Southampton Water.
A. discaudata	Also an intermediate species and performed equally
20.6>25.1>33.3>15.5	well at the higher three salinities investigated.
74.5>64.8>56.7>32.4 %HS	
A. tonsa	Highest hatch success at 33.3, but of the four
33.3>25.1>15.5>20.6	congeners it also had the highest hatch success at 15.5
85.4>82.9>55.9>55.6 %HS	which agrees with its preferred distribution at the head of the estuary.

3) Although naupliar survival (NS) with respect to temperature was highest at 20°C for all four congeners, finer analysis of the results gave evidence of their temperature preferences, which in turn reflected their temporal presence in Southampton Water.

A. bifilosa	This species performed significantly better (P=0.05) at 5°C
20>10>5°C	than the two summer species, *A. clausi* and *A. tonsa.*
83.3>70.9>42.8 %NS	
A. clausi	Naupliar survival of this summer species at 20°C
20>10>5°C	significantly higher (P=0.05) than at 5°C.
86.5>59.6>20.0 %NS	
A. discaudata	No significant differences, this species is present in the water
20>10>5°C	column all year round.
45.0>43.3>11.9 %NS	
A. tonsa	Naupliar survival at 5°C significantly lower (P=0.05) than the
20>10>5°C	other two temperatures for this summer species.
72.9>50.5>0.0 %NS	

4) Naupliar survival with respect to salinity did not appear to be a regulating factor in spatial distribution.

A. bifilosa	33.3 significantly different (P=0.05) to all other
33.3>15.5>25.1>20.6	salinities.
83.3>29.3>29.0>28.2 %NS	

A. clausi 33.3 significantly different (P=0.05) to 15.5.

33.3>25.1>20.6>15.5

86.5>63.1>36.1>16.7 %NS

A. discaudata 33.3 significantly different (P=0.05) to 15.5.

33.3>25.1>20.6>15.5

45.0>28.3>16.7>0.0 %NS

A. tonsa 33.3 significantly different (P=0.05) to 15.5.

33.3>25.1>20.6>15.5

72.9>50.7>30.6>20.9 %NS

All species showed highest naupliar survival at 33.3, probably because nauplii are poor swimmers and so are swept towards the mouth of the estuary into more saline waters until they develop further. Once they improve their swimming ability they can then return to regions of their preferred salinity.

5) Time to first CI (days) with respect to temperature indicated that temperature was the most important factor affecting development time and growth rate, but not survival as all congeners developed significantly faster (P=0.05) at 20°C, but this was an expected physiological response.

A. *bifilosa*	20<10<5°C	8.0<9.67<18.3 days
A. clausi	20<10<5°C	8.7<14.0<16.0 days
A. discaudata	20<10<5°C	8.0<10.7<14.0 days
A. tonsa	20<10°C, none survived at 5°C	7.7<14.0 days

6) Time to first CI was not statistically significantly influenced by salinity.

A. bifilosa 20.6<33.3<25.1<15.5 6.0<8.0<8.67<9.0 days

A. clausi 20.6<25.1<33.3<15.5 6.7<7.0<8.7<10.0 days

A. discaudata 33.3=25.1<20.6, none survived at 15.5 8.0=8.0<8.3 days

A. tonsa 25.1<33.3<20.6=15.5 7.0<7.7<11.0=11.0 days

Generally, the *Acartia* congeners responded best to salinities less than full strength seawater, as supported by the mean of the results for the four species: 25.1<20.6<33.3<15.5, but salinity does not appear play a large role in affecting development time or spatial distribution of the species.

Chapter 3:
Photoperiod and temperature regulation of diapause egg production in *Acartia bifilosa*

3.1 Introduction

The abundance of most copepod species varies on a seasonal basis, and this pattern is most typical of temperate coastal waters in which environmental fluctuations are greater than in tropical or open ocean waters. During periods of environmental adversity some species disappear from the plankton entirely, (Grice and Marcus 1981). Hairston and Munns (1984) noted that organisms living in an environment that undergoes periodic natural, but detrimental variations in temperature and salinity either must move by migration or dispersal to a more favourable habitat, or become metabolically quiescent or dormant during the inhospitable period. For estuarine *Acartia bifilosa* migration is not a viable option, and a dormant stage in the life cycle of this species, (which is better equipped to withstand harsh environmental conditions than the active individuals) is critical for its continued existence, as it usually ensures survival during periods that are unfavourable for development, growth and reproduction.

Diapause is a complex, genetic, adaptive response expressed as arrested development. Characteristically, the stimulus that triggers the expression of diapause acts prior to the onset of environmental adversity, thereby providing an individual with sufficient time to adapt. The diapause response may involve biochemical, physiological and endocrinal adjustments, (Grice and Marcus 1981). Diapause itself consists of two consecutive phases; refractory and competent. In the first phase, development of an individual can not resume even if the environmental conditions are favourable. In the latter phase, the individual is capable of resuming development when the conditions permit it, (Grice and Marcus 1981). Diapause is significant because it ensures long-term viability

through adverse conditions and acts to synchronise a species' life cycle with the environment's seasonal rhythm, (Alekseev and Starobogatov 1996).

It is important not to confuse diapause with quiescence. Quiescence is a state of retarded development that is also induced by hostile environmental conditions, (e.g. extremes in temperature, salinity, pH, oxygen concentration etc.). Individuals that become quiescent undergo no prior acclimation and resume development immediately without a lag phase when the habitat becomes favourable once again (Grice and Marcus 1981).

Diapause typically occurs when living conditions deteriorate so that species overwinter to avoid unfavourable conditions, or oversummer to avoid predators or a food bottleneck (Santer and Lampert 1995). Anticipation of these conditions by the adult is achieved by responding to a range of token environmental stimuli that do not in themselves necessarily influence fitness, but are reliable predictors of environmental change (Slusarczyk 1995). Slusarczyk (1995) found that exudates released into the water column by fish induce diapause in the planktonic cladoceran *Daphnia manga*. The calanoid copepod *Eurytemora affinsis* produces diapause eggs under crowded conditions, and Ban and Minoda (1994) hypothesise the accumulation of unidentified metabolic products in the medium to be responsible for this reaction. In contrast, Ban and Minoda (1994) reported that while food shortage did not induce diapause egg production in *E. affinsis*, photoperiod did.

Photoperiod does appear to be the primary cue for diapause egg production, with many workers reporting its effect (Marcus 1982; Uye 1985; Viitasalo 1992; Ban and Minoda 1994). However, Marcus (1982) found that the response is mediated by ambient temperature. In the lab, short days (8L:16D) resulted in *L. aestiva* diapause egg

production at all temperatures, but the number of diapause eggs decreased as the time of experimental daylength increased. Conversely, the trend was reversed for conventional subitaneous eggs. Marcus (1982) also reported that if water temperatures were unusually warm during autumn, (25°C or higher) then the decrease in subitaneous egg production would be postponed or more gradual, as the current adult population would take advantage of the longer growing season and attempt to maximise the number of adults in that year's population. If the temperature was unusually cold, Marcus (1982) proposed that the effect would be reversed with earlier diapause.

In addition to both the smooth, normal-hatch subitaneous eggs and the spiny diapause eggs, *Acartia* species can also produce quiescent, development retarded, eggs (Chen and Marcus 1997) which are morphologically identical to subitaneous eggs (Uye 1985). In these eggs hatching is delayed until short-term unfavourable conditions have passed. Unlike diapause eggs, this refractory phase is not pre-programmed and quiescent eggs are merely a "stopgap" measure responding to immediate environmental conditions Diapause eggs, given their genetically pre-programmed nature, survive in the sediment for longer than quiescent subitaneous ones, and have a higher hatch percentage.

Acartia bifilosa appears in Southampton Water from November/December to June, (Conover 1957; Raymont and Carrie 1964) and is common in many European estuaries, (Sautour and Castel 1995; Irigoien and Castel 1995) despite these studies, little is known about its biology and even less about its diapause behaviour. In Southampton Water, Castro-Longoria (1998) reported that *Acartia bifilosa* produced two morphologically distinct eggs-types, a smooth subitaneous egg and a diapause egg that is characteristically covered in spines. Subitaneous eggs are laid during the first months, with diapause eggs produced only in the two-month period prior to *A. bifilosa*

disappearing from the water column. It is unusual for a species to oversummer because it is a costly strategy as it reduces the number of generations per year and removes the organism from the water column at a time which is generally considered the most advantageous for it in terms of food availability. The ultimate cause of this seasonality is unknown, but *A. bifilosa* diapause does coincide with the maximum population density of gelatinous predators *Aurelia* and Ctenophores in Southampton Water (Lucas 1993), so its diapause could be an attempt at predator avoidance. However, real-time cues, like the presence of the predators in the water column, would come too late for *A. bifilosa* to avoid this threat and so the animal must rely on proximate cues that provide a reliable forecast of future conditions. This study will focus on the effects of photoperiod and temperature on the diapause egg production of *A. bifilosa*.

3.2 Method

Acartia bifilosa samples were taken directly from Calshot (33 salinity), during March where temperature and light conditions were 6°C and 12L:12D respectively, using a 200μm mesh net. Females in the field were not yet producing diapause eggs. In each case, 20 females, which were not left to acclimatise, were introduced directly into individual incubation chambers (see Appendix 7). These were plastic cylinders with a 200μm mesh on one end held supported in a 1l beaker filled with 600ml of seawater from the SOES aquarium (30-32 salinity) and provided with excess concentrations (1×10^5 cells ml^{-1}) of *Isochrysis* for food. The effect of temperature and photoperiod on *A. bifilosa* diapause egg production was investigated using combinations of 5, 10, 14 and 18°C and 12L:12D, 13L:11D and 14L:10D photoperiod. Three replicates were conducted for each regime. Eggs were collected every 2 days over an 8 day period and the diapause:subitaneous egg ratio produced at each stage was recorded. Diapause eggs are spiny and are easily distinguished from smooth subitaneous eggs.

One-way ANOVA (Fowler and Cohen 1992) was performed on the results of each experimental regime Once collected, the eggs were stored in large petri dishes in 20ml of seawater (30-32 salinity) at 18°C for 8 days and then checked to monitor % hatch success. Castro-Longoria (1999) reported that the vast majority of subitaneous *A. bifilosa* eggs hatched within 5 days under good temperature and salinity conditions (>10°C and >25 salinity). This then enabled an investigation of whether *A. bifilosa* produces an intermediate spiny subitaneous egg.

3.3 Results

The mean percentage of diapause eggs produced under each experimental regime are shown in Table 3.1, and these data represented graphically in Figures 3.1-3.6, shown in terms of photoperiod and temperature effects.

Table 3.1: Mean percentage of diapause eggs produced (± 1 S.D.). Data are not cumulative.

Photoperiod	Day	Temperature (°C)			
		5	*10*	*14*	*18*
12L:12D	**2**	-	-	-	-
	4	-	0 n=30	0 n=72	0 n=44
	6	-	0 n=63	1.1 ± 1.91 n=72	1.43 ± 2.48 n=53
	8	-	0 n=30	23.0 ± 39.8 n=50	14.4 ± 24.8 n=30
13L:11D	**2**	31.9 ±18.0 n=28	43.9 ± 2.84 n=36	41.2 ± 5.12 n=49	32.7 ± 18.9 n=30
	4	-	-	-	-
	6	93.3 ± 11.5 n=14	77.6 ± 25.4 n=39	61.1 ± 9.64 n=11	61.1 ± 9.64 n=9
	8	93.3 ± 11.5 n=10	100 ± 0.00 n=22	83.3 ± 28.9 n=6	91.7 ± 14.4 n=6
14L:10D	**2**	-	13.7 ± 20.7 n=68	3.7 ± 6.4 n=63	10.6 ± 15.7 n=125
	4	-	41.3 ± 11.5 n=76	40.6 ± 4.9 n=55	46.3 ± 11.6 n=22
	6	-	60.2 ± 18.7 n=84	63.9 ± 25.5 n=48	54.9 ± 4.6 n=39
	8	-	65.5 ± 14.2 n=158	67.3 ± 19.9 n=54	84.1 ± 9.3 n=43

- = no readings taken.
n = total number of eggs produced from all replicates those 2 days.

3.3.1 Effect of temperature

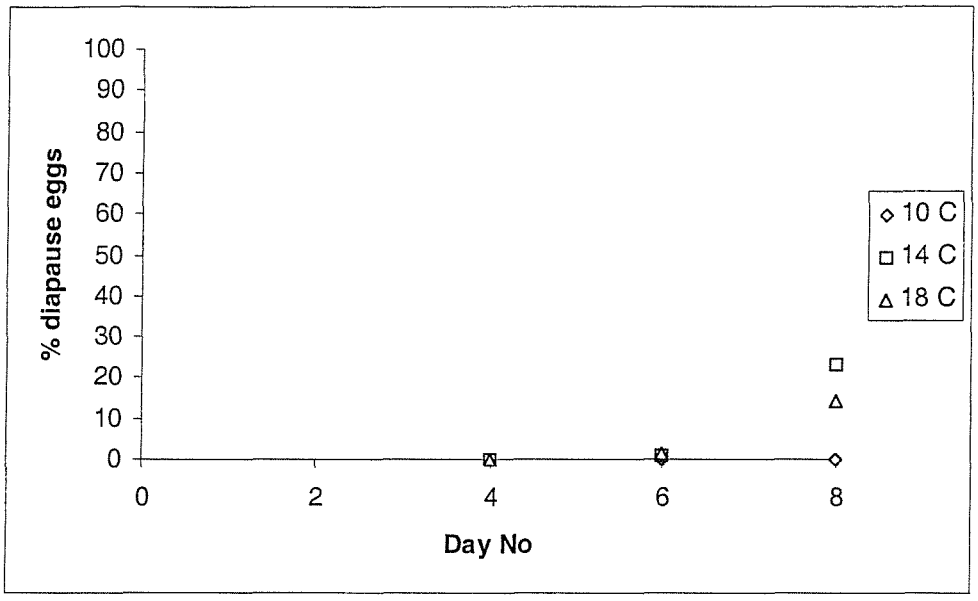


Figure 3.1: Diapause egg production under 12L:12D photoperiod

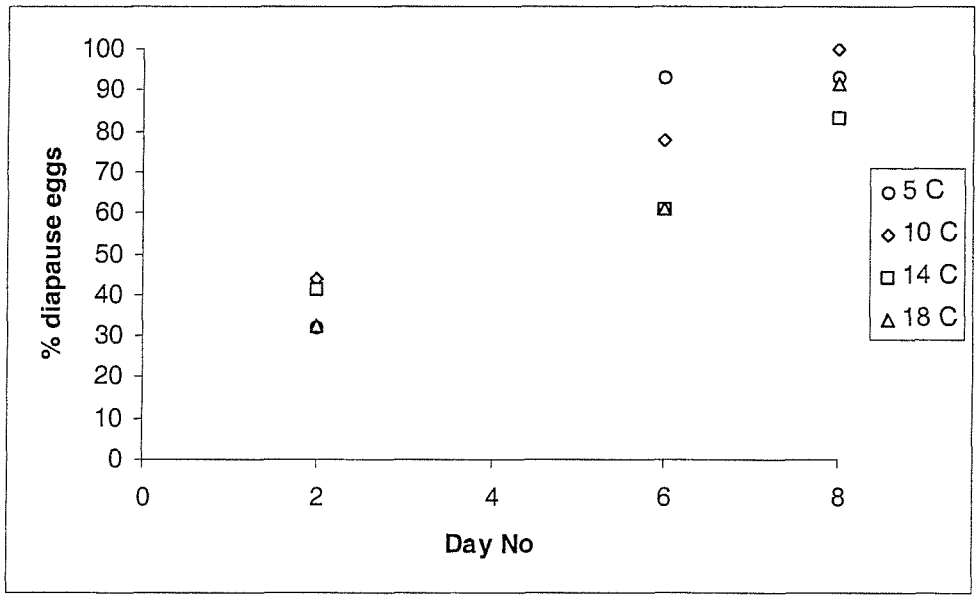


Figure 3.2: Diapause egg production under 13L:11D photoperiod.

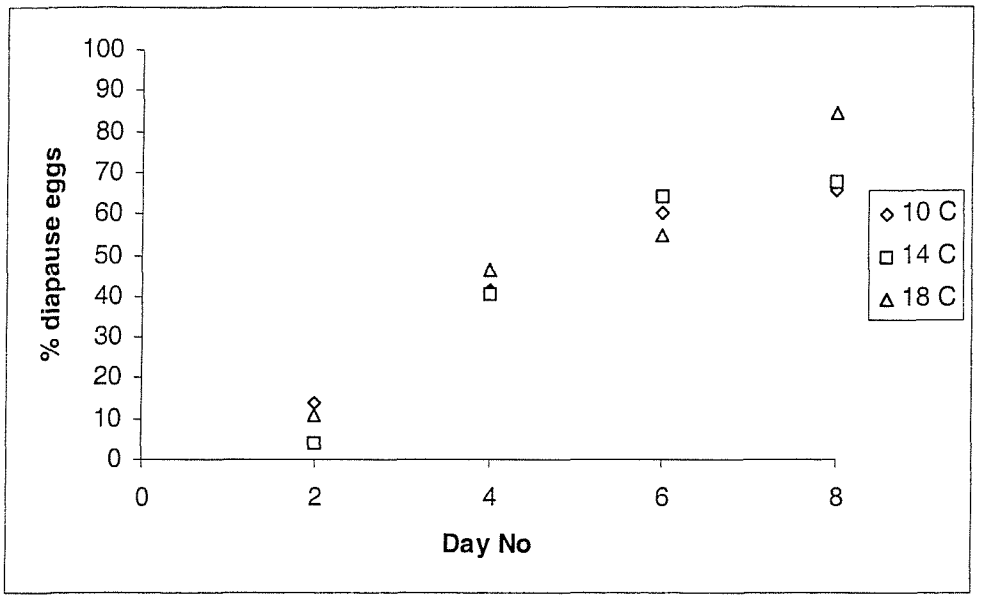


Figure 3.3: Diapause egg production under 14L:10D photoperiod.

3.3.2 Effect of photoperiod

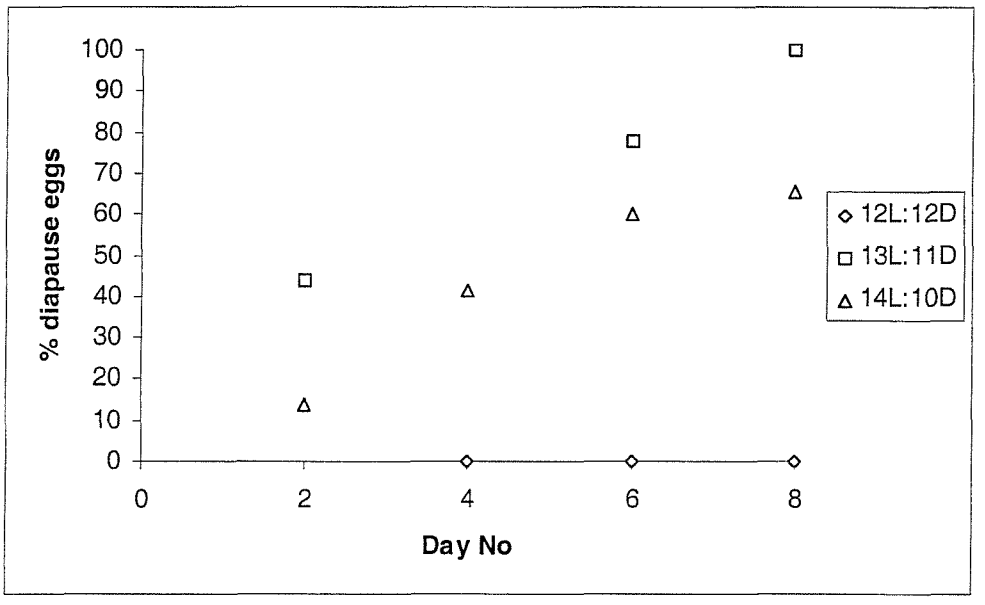


Figure 3.4: Diapause egg production at 10°C.

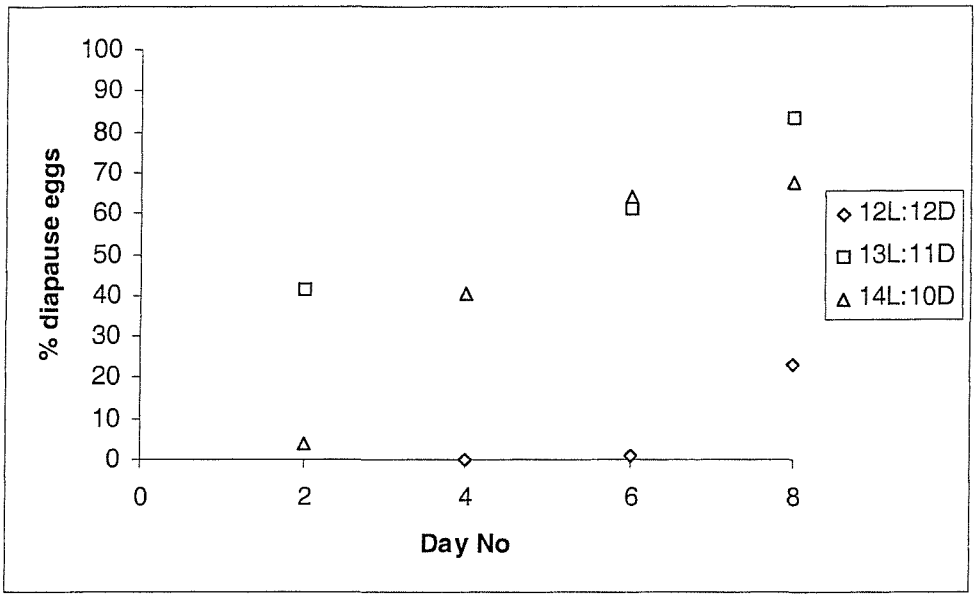


Figure 3.5: Diapause egg production at 14°C.

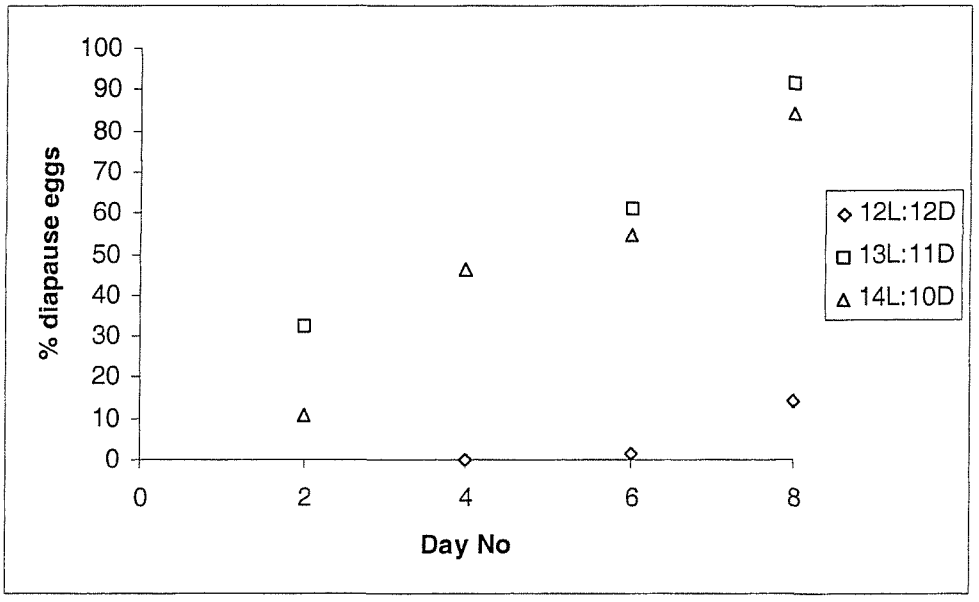


Figure 3.6: Diapause egg production at 18°C.

Adult mortality was negligible (<5%), but *A. bifilosa* egg production rates in all of the replicates were very low, ranging from 0.15 eggs female^{-1} day^{-1} to 3.95 eggs female^{-1} day^{-1} (Table 3.1). Despite this, it can be seen that there is an immediate shift in egg-type production between the 12 hour and 13 hour daylengths, as after only 2 days there is a statistically significant difference between the mean percentage of diapause eggs produced by the *A. bifilosa* females under the light regimes of 12L:12D and 13L:11D at all temperatures (Figures 2, 3) (ANOVA, $P<0.05$). Temperature does play a small part in the response as, even under 12 hours of light, at the higher temperatures (14°C and 18°C), a small number of diapause eggs were produced after 8 days, although the mean percentage produced at this photoperiod is significantly less than that produced under 13L:11D and 14L:10D ($P<0.05$). The percentage of diapause eggs produced by the animals each day at 13L:11D and 14L:10D increased over the period of investigation and after 8 days, diapause egg production was 100% in some replicates.

Table 3.2: The effect of photoperiod on mean percentage of diapause egg production after 8 days (temperature-collated data).

Day	**Mean % of diapause eggs**		
	12L:12D	13L:11D	14L:10D
8	12.4 ± 25.5	92.1 ± 15.9	72.3 ± 15.8

The difference in the mean percentage of diapause egg production of 3 experimental regimes, where n=9 in each case is statistically significant, ($F_{2,24} = 5.61$, $P<0.05$). The Tukey test (Fowler and Cohen 1992) was then used to determine that the means were all significantly different from each other, ($P<0.05$). It is clear that 13L:11D is the threshold photoperiod, with the highest percentage of diapause eggs produced after 8 days, results were similar for the 14L:10D experiment. However, the diapause response was not temperature-mediated at this sensitive photoperiod because spiny eggs were produced even at temperatures as low as 5°C.

Table 3.3: The effect of temperature on mean percentage of diapause egg production after 8 days at 13L:11D.

Day	Mean % of diapause eggs			
	5°C	10°C	14°C	18°C
8	90 ± 14.1	100 ± 0	83.3 ± 28.9	91.7 ± 14.4

The difference in the mean percentage of diapause egg production of 3 experimental regimes, where n=3 in each case is not statistically significant, ($F_{3,8} < 1$).

Table 3.4: The effect of temperature on mean percentage of diapause egg production after 8 days (photoperiod-collated data).

Day	Mean % of diapause eggs		
	10°C	14°C	18°C
8	55.2 ± 44.6	57.9 ± 37.9	63.3 ± 39.9

The difference in the mean percentage of diapause egg production of 3 experimental regimes, where n=9 in each case is not statistically significant, ($F_{2,24} < 1$). The types of egg produced are affected primarily by photoperiod, as there is no significant temperature effect (Table 4), even at the threshold daylength of 13L:11D (Figures 3.4-3.6). The temperature data were collated (Table 3.5, Figure 3.7) to better see the significance of photoperiod.

Table 3.5: Temperature collated data. (Data are not cumulative).

Day	12L:12D		13L:11D		14L:10D	
	Total n° eggs	Mean % diapause eggs	Total n° eggs	Mean % diapause eggs	Total n° eggs	Mean % diapause eggs
2	110	0	143	37.4±12.7	256	9.3±14.1
4	146	0	-	-	153	42.7±8.9
6	188	0.84±1.7	73	73.3±19.3	171	59.7±16.4
8	110	12.4±25.5	44	92.1±15.9	255	72.3±15.8

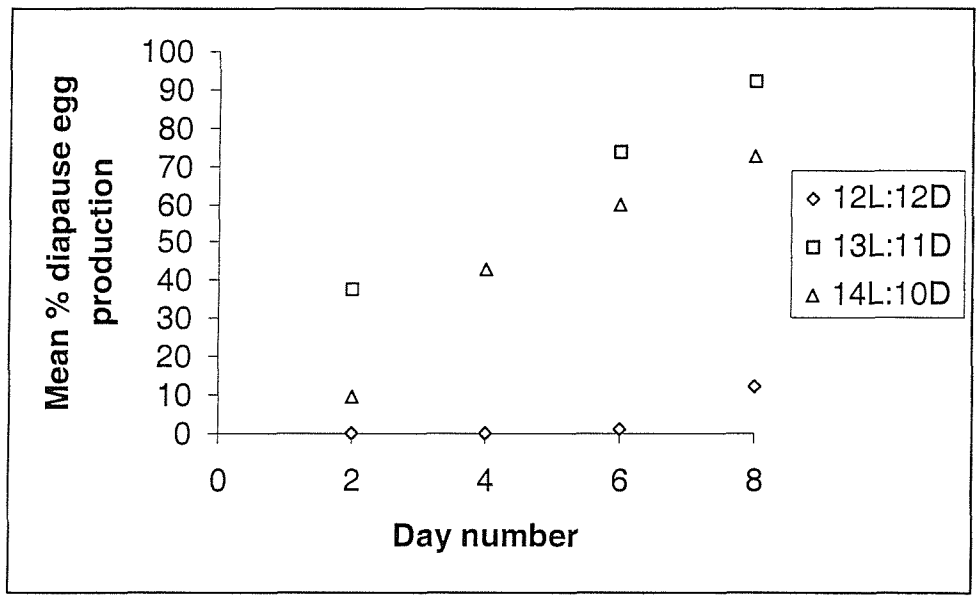


Figure 3.7: Diapause egg production (temperature-collated data).

Although a mixture of subitaneous and diapause eggs was collected from the incubation chambers, it is not clear if, like *Labidocera aestiva* females (Marcus 1982), one *A. bifilosa* female can produce both smooth and spiny eggs at the same time, or if the switch is a complete one, with some females producing the diapause eggs only and others continuing to lay subitaneous eggs. There was no evidence of production of a spiny subitaneous egg. None of the 'spiny' eggs collected hatched within the 8-day observation period, suggesting the absence of the spiny-subitaneous egg-type.

3.4 Discussion

Species living in environments that undergo periodic natural, but detrimental variations in temperature and salinity must either move by migration or dispersal to a more favourable habitat, or must become metabolically quiescent or dormant during the inhospitable period (Hairston and Munns 1984). For estuarine *Acartia* species, migration is not a viable option, whereas dormant stages, which are better equipped to withstand harsh environmental conditions than active individuals (Slusarczyk 1995), are common. Eggs appear to be the preferred resting stage of copepods because if later stages become dormant it may be difficult to protect such a large mass from attack by predators, microbes and physical processes (Hairston and Caceres 1996). An extension of an environmentally-regulated dormancy strategy is diapause, which defines a state of arrested development that is genetically controlled (Uye 1985).

Diapause may be induced by a number of cues, like temperature (Hairston *et al.* 1990), overcrowding (Ban and Minoda 1994; Stross and Hill 1968; Stross 1969) and food limitation (Santer and Lampert 1995) may also play a part. Slusarczyk (1995) observed that the cladoceran, *Daphnia magna,* diapause was triggered by exudates released by predatory fish so, predation may also be a cause of diapause. It is important to know the life cycle of a copepod when attempting to determine the reason for diapause (Grice and Marcus 1981). Real-time cues to environmental change may come too late to permit the optimum fitness response (Hairston *et al.* 1990). For this reason, many animals begin diapause in response to token stimuli that do not directly influence fitness, but are reliable predictors of future conditions. Alekseev and Starobogatov (1996) maintain that the evolutionary transmission of diapause is mainly a photoperiodic response because of the constancy of environmental conditions, such as light and temperature, maintained by

the rotation of the globe around the sun from the early days of life on the planet. Early ancestors of today's organisms adapted to the periodicity of these important factors, and the most successful inventions in this direction were fixed genetically and supported by stabilising selection related to the acting factor. These were then the general base from which specific development and specialisation in the different groups led to diverse forms of diapause.

Marcus (1980, 1982[a], 1982[b]), Norrbin (1996), Viitasalo (1992), Stross and Hill (1968) and Stross (1969) found that photoperiod was responsible for inducing diapause in a variety of animals. This investigation has shown that the diapause response of Southampton Water *A. bifilosa* was triggered by a photoperiod of 13L:11D, or even longer daylengths and this is comparable to the light regime *A. bifilosa* would experience over the summer. *A. bifilosa* produce diapause eggs from May to July before disappearing from the water column until November (Castro-Longoria 1998). Unlike *Labidocera aestiva* which took 1-2 weeks to fully adjust from subitaneous to diapause egg production (Marcus 1982), *A. bifilosa* diapause response was rapid. The animals were taken directly from the field and given no time to acclimatise to laboratory conditions. Yet above the threshold daylength they began producing spiny eggs within 2 days (37% diapause eggs at 13L:11D), and % diapause egg production continued to increase dramatically over the period of the experiment, reaching 100% after only 8 days in some replicates. So, some, if not all, of the *A. bifilosa* females can switch the types of egg they produce very quickly. However, egg production was not monitored for individual females, and although the eggs collected were a mixture of subitaneous and diapause types it is unclear if, like *L. aestiva* (Marcus 1982), *A. bifilosa* has a transitional phase during the switch where both egg types are produced, or if this animal has an all-or-nothing response where the switch is instantaneous. The latter is unlikely

because diapause-induction was determined by the cumulative input of long daylength light-dark cycles, hence the increase in the number of diapause eggs over time. If females have different response thresholds, i.e. it takes different numbers of cycles to trigger diapause, due to genetic variation there will be a gradual change in egg-type production followed by stabilisation. The duration of the complete subitaneous to diapause switch may be initiated by a certain photoperiod, but it will ultimately be determined be the female's own biochemistry. The animals can not switch instantaneously because of the time needed for the physiological changes necessary for diapause egg production to occur. This conversion time does appear to be faster for a smaller copepod, (8 days for *A. bifilosa*) than for a larger one, (up to 2 weeks for *L. aestiva* (Marcus 1982)). Hormonal control of diapause has been observed in the silkworm, *Bombyx mori* (Sato *et al.* 1997; Shiomi *et al.* 1998 and Xu *et al.* 1995), the mosquito, *Culex pipiens* (Spielman 1974) and the copepods *Calanus finmarchicus* and *Calanus helgolandicus* (Carlisle and Pitman 1961). In the silkworm, diapause is a temperature controlled expression, the temperature-independent production of DH (diapause hormone) is a stage-dependent expression related to pheromone production (Xu *et al.* 1995). Diapause is triggered by a particular set of environmental stimuli, these signals are transduced into endogenous chemical messengers, neurohormones and hormones in the endocrine organs. Neurohormones bring about the phase change from development to diapause, or vice versa, through the metabolic shift of the target organs (Xu *et al.* 1995). There is also evidence for the hormonal control of diapause in copepods (Carlisle and Pitman 1961). Endocrine structures of the cerebral region of *Calanus finmarchicus* include a pair of lateral groups of neurosecretory cells at the front edge of the brain. From these, neurosecretory fibres pass forward and outward, skirting around the anterior blood sinus to reach the frontal organ. In adults and copepodites taken during summer months, these neurosecretory cells contain abundant granular

secretions. In overwintering larvae, however, secretion is grossly depleted or even absent. It was concluded that the secretion of these cells is playing some part in the control of diapause, moulting and the overwintering metabolism and behaviour. The hormonal control of diapause in *A. bifilosa* clearly needs to be investigated.

In species that diapause as eggs, the subitaneous and diapause eggs are morphologically distinct, except in *Acartia tonsa* (Zillioux and Gonzalez 1972). *A. bifilosa* produces dark, spiny diapause eggs that are easily distinguished from the dark, smooth subitaneous ones, however, little is known about the internal structure of the diapause eggs. The "shell" of the *Labidocera aestiva* summer egg (subitaneous) is much thinner than that of the diapause/winter egg (Marcus 1996). This is also the case for the subitaneous and diapause eggs of *Pontella mediterranea* (Santella and Ianora 1990), but this animal also produces spiny subitaneous as well as spiny diapause eggs that represent the transition phase as females switch from producing summer eggs to winter eggs. Since the oocytes that gave rise to the two types of egg were similar in structure, it was concluded that the layers were assembled after spawning. There was no evidence of the spiny-subitaneous egg-type *A. bifilosa* can produce in this investigation. In the case of *Acartia latisetosa* and *Anomalocera patersoni*, the subitaneous and diapause eggs are similar in size, and are both smooth and dark in appearance. However, the subitaneous egg envelope is thin and the diapause multi-layered (Ianora and Santella 1991). The multi-layered, tough outer covering of diapause eggs is believed to protect the embryo from destruction by predators, since diapause eggs show greater survival after passage through the gut than summer eggs, it may also play a part in maintaining diapause (Ianora and Santella 1991). Eggs are known to enter diapause at the multi-celled stage. Santella and Ianora (1990) investigated the TEM sections of *P. mediterranea* eggs and concluded that the diapause eggs had undergone cleavage. Examination of the embryos

of *A. patersoni* revealed that development had ceased at the 32-cell stage. Subsequent examination at 2 weeks and periodically up to 4 months indicated that the development of the embryos did not progress beyond this stage. Only after 6 months were embryos observed at the blastula stage, and hatching occurred at 7 months (Ianora and Santella 1991). Further work on *A. bifilosa* diapause is needed in order to better understand the internal structure of the diapause eggs, the cell-stage at which development ceases during diapause, and the metabolic activity of the diapausing embryo.

The diapause response of *A. bifilosa* may vary with location. *Diaptomus sanguineus* collected at northern latitudes initiate diapause earlier in the autumn, or at longer daylengths, than those collected further south (Hairston and Olds 1986). Such variation is genetically based, indicating that populations are specifically adapted to the latitudes where they reside. *L. aestiva* is not genetically homogenous throughout its range either (Marcus 1984). In populations used to pronounced seasonality, (Virginian region) diapause eggs are produced at cool temperatures and short daylength periods. In the Carolinian province, however, where the planktonic stages occur all year round, diapause eggs are rarely produced by either field-collected individuals or their lab-reared offspring. This suggests that these animals lack the genetic capacity to produce diapause eggs (Marcus 1984). So, the expression of diapause, though dependent on environmental conditions, is fundamentally a genetically determined trait and it may provide a meaningful measure of intra-population variability and interpopulation differentiation. Regional adaptations may lead to reproductive isolation and ultimately to the formation of distinct species (Grice and Marcus 1981). Comparisons of the diapause behaviour of *A. bifilosa* from other regions, if indeed it does diapause elsewhere, would be valuable in understanding the ultimate reason behind its behaviour in Southampton Water. Here, the cause for its diapause is unlikely to be food scarcity

because the phytoplankton bloom in Southampton Water starts in March/April and extends until autumn (Kifle 1992). Given that *A. bifilosa* also produces diapause eggs before the maximum adult density, crowding is also unlikely to be the cause. The ultimate reason for diapause might be predation pressure because maximum gelatinous predator activity from *Aurelia* and Ctenophores does coincide with maximum *A. bifilosa* density (Lucas 1993). *A. bifilosa* is less fecund than other members of the genus, and egg-hatching times are also longer (Castro-Longoria 1998) so the *A. bifilosa* population has a reduced capacity for recovering from a greater attack by predators.

Photoperiod is often accompanied by another secondary factor when governing diapause and temperature generally plays that part in diapause induction in terrestrial insects, but its role for zooplankton is less consistent (Hairston *et al.* 1990). Marcus (1979) found a strong correlation between temperature and the type of egg produced by *L. aestiva*, but the compensatory role of temperature was not so evident in this current investigation as even at the threshold daylength (13L:11D), very low temperatures (5°C) did not inhibit diapause egg production. However, at the 12L:12D photoperiod, a very low number of diapause eggs were produced after 8 days at the higher temperatures (14°C and 18°C), but not in a statistically significant number ($P<0.05$). This indicates that if summer, and the associated change in the biological system, (phytoplankton bloom, increase in zooplankton numbers, increase in the number of gelatinous predators) comes early to Southampton Water, *A. bifilosa* will begin producing diapause eggs in low numbers as insurance. This flexible diapause response should help to ensure the maximum numbers of animals in next year's population.The females produce subitaneous eggs as long as there is time for those eggs to hatch, mature and reproduce themselves safely. When a certain photoperiod indicates the onset of environmental adversity, (increased numbers of predators) the females switch to

diapause egg production because energy spent on producing subitaneous eggs would be wasted, (those animals would likely be eaten before being able to reproduce themselves). Photoperiod is the main cue as it is an excellent long-term predictor of season. Temperature, though less reliable than photoperiod, does have a minor secondary role, and this is reflected in the weaker effect it has on *A. bifilosa* diapause response.

3.5 Summary

1) Diapause egg production in *Acartia bifilosa* is triggered by a photoperiod of 13L:11D.
2) It took a minimum of two days for diapause eggs to start being produced at this daylength.
3) Temperature plays a small role as after 8 days at 14°C and 18°C diapause eggs were produced under a 12L:12D photoperiod. However, significantly ($P<0.05$) higher numbers of diapause eggs were produced under 13L:11D and 14L:10D.
4) Diapause egg production reached 100% in some replicates after 8 days under 13L:11D and 14L:10D.
5) The highest mean number of diapause eggs after 8 days was reached under 13L:11D. This response was not temperature-mediated as diapause eggs were produced even at 5°C.
6) There was no evidence of spiny subitaneous eggs.
7) *Acartia bifilosa* probably has a transitional phase during the switch from subitaneous to diapause egg production where both types of eggs are produced, but this was not tested for in the present work.

Chapter 4:
The influence of temperature stress on the scope for growth of *A. bifilosa* and *A. discaudata*

4.1 Introduction

Castro-Longoria (1998) observed that *Acartia bifilosa* occurred throughout the Southampton Water system, though in low numbers during the winter and reached seasonal dominance in April-May with a maximum density of 4791 individuals m^{-3}. There was a marked fall in numbers in June to less than 5 individuals m^{-3} and *A. bifilosa* disappeared from the water column come July, reappearing in November. This disappearance is due to *A. bifilosa* entering diapause and oversummering in the egg stage. Photoperiod appears to be the primary cue for diapause egg production in *A. bifilosa* (Chapter 3) as well as in other species (Marcus 1982; Uye 1985; Viitasalo 1992; and Ban and Minoda 1994) and it is sometimes mediated by temperature (Marcus 1982). However, the ultimate cause of diapause in *A. bifilosa* is unknown. It is rare for a species to oversummer as this reduces the number of generations per year, and removes the animal from the water column during the time which, in terms of food availability, is considered to be the most advantageous. Diapause in *A. bifilosa* may be a strategy to evade predators as the maximum abundance of this species coincides with the highest numbers of the gelatinous predators *Aurelia aurita* and *Pleurobranchia pileus* in Southampton Water (Lucas 1993), or as an attempt to avoid the higher temperatures during the summer months. Generally, an increase in temperature raises the rate of development but *A. bifilosa* is known to produce more eggs per day at 10°C than at 20°C (Castro-Longoria 1998). Although a temperature of 20°C is not extremely damaging to *A. bifilosa* eggs, indeed Castro-Longoria (1998) reported that egg-hatch was high with a mean of 66.6% over 10 days, adult intolerance of higher temperatures may be the cause of the unusual reproductive strategy. In order to try to explain *A.*

bifilosa summer diapause this present study looks at the ability of *A. bifilosa* to cope with temperature stress, and compares it to that of *A. discaudata*, a copepod with a very similar spatial distribution to that of *A. bifilosa* but which does not diapause in either summer or winter, and so is present in Southampton Water all the year round (Castro-Longoria 1998). The "scope for growth" (SfG) assay will be used to determine the optimum temperature for *A. bifilosa* and *A. discaudata*. Ideally, the SfG of *A. bifilosa* and *A. discaudata* should be looked at regularly over the course of the year to get a clearer picture of the energy available to individuals in the field, and to determine if the species are under some physiological stress induced by temperature at any point in the year. However, the SfG assays in the present investigation were conducted during April and May at temperatures that artificially reproduced the conditions of winter (5°C), spring/autumn (10°C) and summer (20°C).

In theory, with equitable food input, the closer experimental conditions are to optimal, the more energy an organism has available for production (Bayne *et al.* 1985). Physiological responses are useful when assessing the condition of an individual because they represent the integration of the many cellular and biochemical processes that can alter in response to a change, both natural (Lampitt and Gamble 1982; Abou Debs 1984; Kiorboe *et al.* 1985) and anthropogenic (Naylor *et al.* 1989; Maltby *et al.* 1990) in the environment. They are often stressed more easily and precisely than population or community reactions i.e. a physiological response in an individual will reflect any deterioration in the environment before the effects are manifested in the population or community (Bayne *et al.* 1985). The SfG measure of an organism is a comprehensive stress indicator that includes all the major components of the bioenergetic equation, and so offers a sensitive assay of the energy balance between the food absorbed (A) and the metabolic output (R) of an animal. Ultimately, it is an

indicator of how much energy an organism has available for net production (growth and reproduction). It therefore gives a good physiological measure of stress that is assumed to relate closely to population and community dynamics (Bayne *et al.* 1985; Naylor *et al.* 1989).

SfG can be calculated from the balanced energy equation of Winberg (1960):

$$\mathbf{C - F = A = R + U + P} \quad \text{.....(1)}$$

or,

$$\mathbf{P = A - (R + U)} \quad \text{.....(2)}$$

Where,
C = food energy consumed
F = energy lost as faeces
R = energy respired
A = energy absorbed from food
U = energy excreted
P = energy available for somatic growth and reproduction (SfG)

SfG is not measured directly but derived, as all of the energy components on the right-hand side of equation 2 are physiological processes that can be measured and converted into their energy equivalents in Joules mg dry wt^{-1} d^{-1}. SfG can be positive, indicating that energy is available for growth, (somatic, gametic or both); zero when energy input simply balances the energy expended; or negative when an individual must metabolise essential body reserves in order to survive (Bayne *et al.* 1985; Naylor *et al.* 1989). The main advantages of SfG over actual measurements of growth and fecundity are that results can be obtained relatively quickly, in hours or days rather than in weeks (Naylor *et al.* 1989). The sensitivity of the procedure means that it can distinguish the more subtle influences of a change in environment that a direct measure of growth would not detect (Bayne *et al.* 1985; Maltby *et al.*1990). SfG also provides an insight into which particular components of the energy budget are altered by stress. Reduced feeding rates, a fall in food availability or reduced food digestion efficiency could cause a drop in

energy intake. A rise in energy loss could result from an increase in the respiration rate or excretion rate and a change in any one component can therefore have a great influence on the energy equation, and hence SfG. When investigated individually, interpretation can be difficult because each physiological response alone does not have a simple, quantitative relationship with stress. For example, an increase in respiration rate could, on its own, indicate a loss of energy, but this may just be a response to a rise in feeding rate, so that SfG will remain the same because the energy input compensates for the energy loss (Bayne *et al.* 1985).

4.2 Method

After collection from the field, where the temperature was 15°C, the *Acartia* individuals were identified and separated in to *A. bifilosa* and *A. discaudata* sub-samples and placed in feeding chambers (see Appendix 7) at 15°C. They were then conditioned to an *Isochrysis* food source at similar concentrations to those which would be used in the SfG investigations (~1×10^5 cells ml^{-1}). Individuals were taken from these sub-samples to determine the dry weight measurements of a single adult female *A. bifilosa* and *A. discaudata* (Appendix 2).

Following from equations (1) and (2) four parameters of the energy equation must be calculated to derive SfG.

4.2.1 Feeding (C)

Feeding rate is related to the amount of energy ingested and sets the upper limit of an organism's productivity. The "cell count method" (Gauld 1951; Frost 1972) was used to compare the number of food cells in the algal culture before and after a period during which the *Acartia* fed.

$$\mathbf{f = \frac{V(C_o - C_t)}{T * wt}}$$

f = feeding rate (cells mg dry wt^{-1} hr^{-1})
V = volume of water per copepod (ml)
C_o = initial food concentration (cells ml^{-1})
C_t = food concentration at the end of the experiment (cells ml^{-1})
T = duration of the experiment (hrs)
wt = weight of one *Acartia* female

Five replicates for *A. bifilosa* and *A. discaudata* at each of three experimental temperatures, 5, 10 and 20°C, were conducted. 500ml vials were filled with 26μm

filtered seawater at 32 salinity each containing 5 mature females with 8ml of *Isochrysis* culture added to give an initial food concentration of ~1×10^5 cells ml^{-1}. The vials were then left at the appropriate experimental temperatures for 24hrs on a plankton wheel set at ½ a revolution min^{-1} to allow the copepods to acclimatise. After 24hrs, the copepods were carefully pipetted into fresh vials containing water at the experimental temperature and with the same initial food concentration. A running (Cr) and standard (C_o) control was also set up for each temperature. The C_o was fixed with Lugol's iodine and the food concentration determined using a haemocytometer. The vials were then replaced on the plankton wheel in the dark, to prevent the algae from multiplying, at the experimental temperature for 6 hours. After this, Lugol's iodine was added to fix the algal cells, and the concentration of algal cells in Cr and the experimental vials were determined. Feeding rate was multiplied by the derived calorific value of the algae (1.09×10^{-6} J $cell^{-1}$) to determine the amount of energy taken in during feeding as J mg dry wt^{-1} d^{-1}.

4.2.2 Assimilation (A)

Assimilation efficiency was calculated using the Conover (1966) equation:

$$\mathbf{e = ((F - E)/((1-E) \times F) \times 100}$$

F = ash-free dry weight:dry weight ratio of the food
E = ash-free dry weight:dry weight ratio of the faeces
e = assimilation efficiency (%)

This method assumes that only the organic component of the food is significantly affected and so no knowledge of the amount of food eaten or quantitative recovery of the faeces is needed. Instead, only the fraction of organic matter, the ratio of ash-free dry weight to dry weight, for a sample of food and faeces is necessary.

10mm× 10mm aluminium capsules were ashed at 500°C for 24 hrs. For 5 replicates, 1ml of *Isochrysis* culture was placed into each capsule, this was then dried at 60°C for

24 hours (dry weight) and then ashed at 500°C for 3 hours (ash weight) to obtain the ash free dry weight (dry weight-ash weight) of the food.

Three experimental populations of either 25 *A. discaudata* or 25 *A. bifilosa* were placed in individual feeding chambers (see Appendix 8). The copepods were given *Isochrysis* at a concentration of 1×10^5 cells ml^{-1} and left for 24 hours to acclimate at either 5, 10 or 20°C. They were then placed in fresh chambers with water at the appropriate temperature and given fresh food. After a further 24 hours the faecal pellets were picked out, placed onto pre-ashed aluminium capsules and dried at 60°C for 24 hours and then ashed at 500°C for three hours to determine the ash-free dry weight of the faeces. Assimilation efficiency was calculated from the Conover (1966) equation.

The mean f value determined for each temperature for both congeners (4.2.1) was multiplied by the appropriate assimilation efficiency to give the amount of energy absorbed by each *Acartia* spp.

4.2.3 Respiration (R)

Respiration rates were determined using the basic Winkler procedure and the formula:

$$\mathbf{C = \frac{n \times F \times 1000 \times v^1}{4 \times v\,(v^{-1} - 0.4)}}$$

C = concentration of oxygen (m mol l^{-1})
n = volume of thiosulphate (ml)
F = normality of thiosulphate
v = volume of the respiratory chamber
v^{-1} = volume of the aliquot (ml)

The amount of oxygen consumed by the copepods was determined in ml O_2 l^{-1} mg dry wt^{-1} d^{-1}. This value was then multiplied by 20.33 (Crisp 1971; Bayne *et al.* 1985; Lucas 1996) to give the values in J mg dry wt^{-1} d^{-1}.

Experimental populations of *Acartia bifilosa* and *A. discaudata* were acclimated to the three temperatures, 5, 10 or 20°C, in feeding chambers (see Appendix 7) for 24 hours during which time they were fed *Isochrysis* at a concentration of 1×10^5 cells ml^{-1}. The respiration chambers used were small glass vials of ~ 3.5ml volume with an appropriate stopper, exact volumes were calculated for each experiment. Five replicates were conducted for each species at each of the three experimental temperatures, as well as a three standard (C_o) and running (Cr) controls. For both species, five adult female copepods were pipetted into each vial, which was then topped up with water before replacing the stopper. The experimental and Cr chambers were stored under water in a water bath or in CT rooms at the required temperatures. The copepods were given no food during the 6 hour experiment as preliminary experiments had established that this procedure did not influence respiration rate. The concentration of oxygen in each vial was determined by Winkler titration, (200µl manganous sulphate and 200µl alkaline iodide) using a microburette.

4.2.4 Excretion (U)

This analysis used the Solorozano (1969) method for ammonia to determine the concentration of ammonia in the water. The ammonia is chlorinated and the product reacted with phenol to form indol blue and measured by spectrophotometric assay.

Experimental populations of *Acartia bifilosa* and *A. discaudata* were left for 24 hours to acclimate to the three temperatures, 5, 10 and 20°C. For each species 5 replicates were conducted at each temperature, as well as two running (Cr) and two standard controls (C_o). 25ml vials were filled with 10ml seawater of 32 salinity at the appropriate temperature and 10 mature females were placed into each chamber. Water baths were used to maintain the experimental temperature over the 6 hour experimental period. At the end of this period, the ammonia content of the water in each bottle was analysed using the basic Solorozano (1969) technique (400µl phenol, 400µl sodium nitroprusside and 1000µl oxidising solution). The absorbency values obtained by spectrophotometer were converted into micrograms of ammoniacal nitrogen per litre (µg NH_4-N l^{-1}) using a pre-constructed calibration curve (Appendix 8). The amount of ammonia is then expressed as µg NH_4-N mg dry wt^{-1} d^{-1} and multiplied by 0.0249 (Elliott and Davison 1975; Bayne *et al.* 1985; Lucas 1996) to provide the excretion equivalent as Joules mg dry wt^{-1} d^{-1}.

4.2.5 Scope for Growth (P) calculation

The objective of the experimental programme was to determine, and compare, the scope for growth of mature female *A. bifilosa* and *A. discaudata* at 5, 10 and 20°C. The mean results from the feeding, assimilation, respiration and excretion replicates in J mg dry wt^{-1} d^{-1} were used in the modified energy equation of Winberg (1960):

$$\mathbf{P = A - (R + U)} \quad \text{.....(2)}$$

4.2.6 Statistical analyses

The significance for the SfG, feeding, amount of energy assimilated, respiration and excretion results was taken at the $P<0.05$ level using ANOVA (Fowler and Cohen 1990). The assimilation efficiency was analysed using the t-test (Fowler and Cohen 1990) and significance was taken at the $P<0.01$ level.

4.3 Results

4.3.1 Feeding (C)

From functional response studies (Chapter 6) it is clear that a food concentration of ~1×10^5 cells ml^{-1} was appropriate for an investigation of feeding rate in as much as the copepods were not food limited, and this would also give a detectable difference between the initial and final food concentrations. For the current series of experiments the starting food concentrations ranged from 1.7×10^5 to 1.875×10^5 cells ml^{-1}.

Table 4.1: The effect of temperature on feeding rate (f) of *A. bifilosa* and *A. discaudata* (± 1 S.D).

Species	Temperature (°C)	f (J mg dry wt^{-1} d^{-1})
A. bifilosa	5	30.01 ± 9.27
	10	38.76 ± 6.36
	20	35.01 ± 12.7
A. discaudata	5	27.85 ± 9.55
	10	31.50 ± 7.26
	20	42.50 ± 6.78

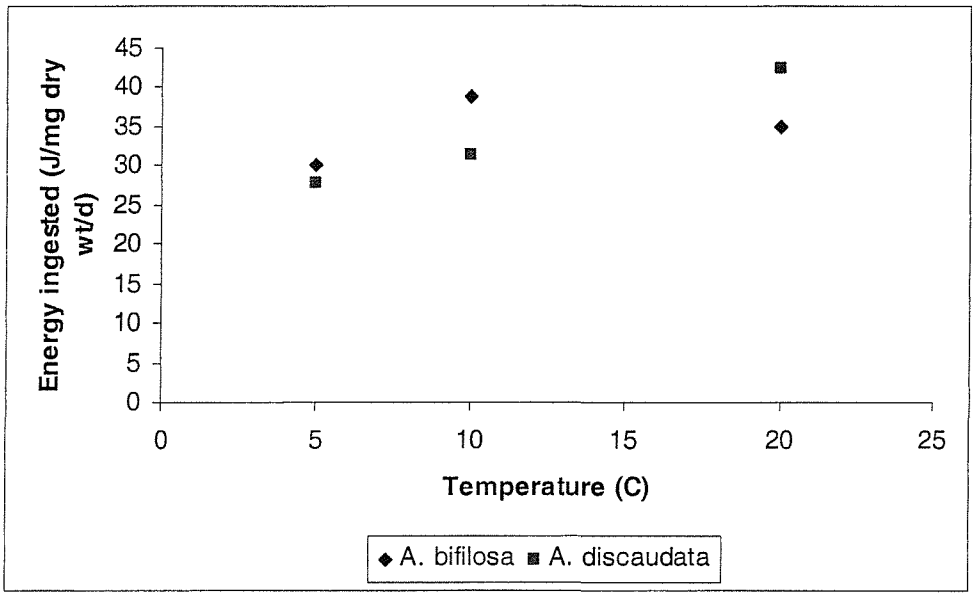


Figure 4.1: The effect of temperature on the energy ingested by *A. bifilosa* and *A. discaudata*.

From the results, it can be seen that *A. discaudata* ingests more food with increasing temperature, suggesting that it is more active at higher temperatures. Its feeding rate at 20°C is significantly greater ($P<0.05$) than that at 5°C. In contrast, *A. bifilosa* ingests more food at 10°C, but the difference in feeding rate recorded for the three temperatures is not significant. Comparing the two congeners there is no difference in feeding rate at 5°C, but *A. bifilosa* has a significantly ($P<0.01$) higher feeding rate at 10°C whereas *A. discaudata* ingests significantly ($P<0.01$) more food at 20°C.

4.3.2 Assimilation (A)

Table 4.2: Effect of temperature on the assimilation efficiency of *A. bifilosa* and *A. discaudata* (± 1 S.D).

Species	Temperature (°C)	Assimilation efficiency (%)
A. bifilosa	5	79.31 ± 4.20
	10	87.20 ± 5.09
	20	64.87 ± 2.84
A. discaudata	5	73.39 ± 3.80
	10	80.81 ± 2.85
	20	82.51 ± 7.61

There is a clear difference in the % assimilation of for *A. bifilosa* at the three temperatures, with a significantly ($P<0.01$) lower assimilation percentage at 20°C and the highest assimilation efficiency recorded at 10°C. Despite the % assimilation of *A. discaudata* consistently increasing with temperature there was no significant difference between the results. The only significant difference between the two species was observed at 20°C where *A. discaudata* had a higher ($P<0.01$) assimilation efficiency than *A. bifilosa.* Assimilation is an important parameter of the Scope for Growth equation and consequently is a good indicator of stress (Maltby *et al.* 1990; Naylor *et al.* 1989).

Using the feeding data in the previous section it is possible to determine the mean amount of energy assimilated by the two congeners at each temperature.

Table 4.3 The effect of temperature on the energy assimilated by *A. bifilosa* and *A. discaudata.*

Species	Temperature (°C)	Energy ingested (J mg dry wt^{-1} d^{-1})	Assimilation efficiency (%)	Energy assimilated (J mg dry wt^{-1} d^{-1})
A. bifilosa	5	30.01	79.31	23.80
	10	38.76	87.20	33.80
	20	35.01	64.87	22.72
A. discaudata	5	27.85	73.39	20.44
	10	31.50	80.81	25.45
	20	42.50	82.51	35.06

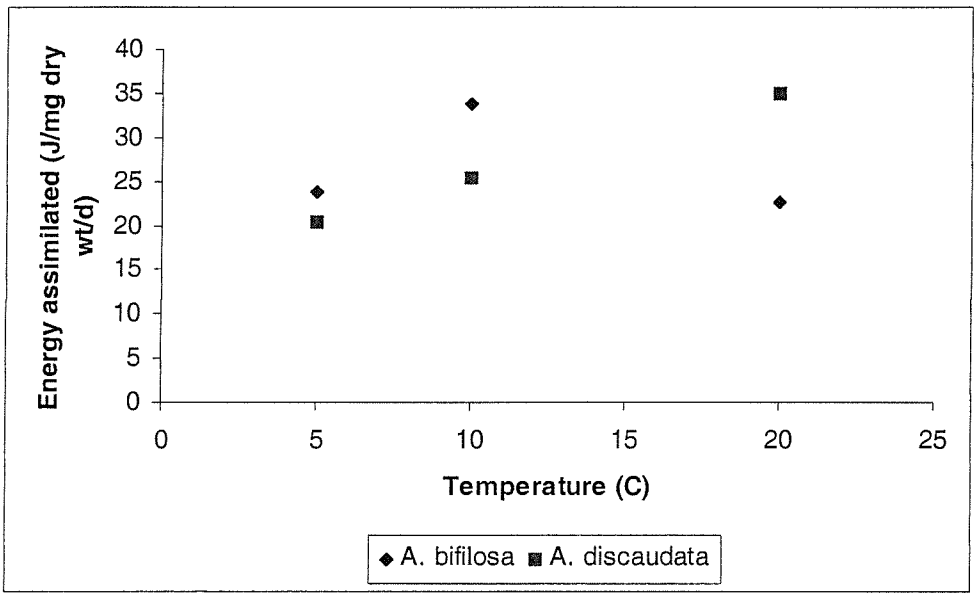


Figure 4.2: The effect of temperature on the energy assimilated by *A. bifilosa* and *A. discaudata.*

The amount of energy the copepods assimilated increases at their optimum temperature, which for *A. bifilosa* was 10°C, while in contrast, *A. discaudata* assimilated a significantly ($P<0.05$) greater amount of energy at 20°C. The amount of energy assimilated (digested) by an individual is arguably the most important part of the Scope for Growth equation (Maltby *et al.* 1990; Naylor *et al.* 1989).

4.3.3 Respiration (R)

Table 4.4: The effect of temperature on the respiration rate of *A. bifilosa* and *A. discaudata* (± 1 S.D.)

Species	Temperature (°C)	Respiration rate (J mg dry wt^{-1} d^{-1})
A. bifilosa	5	17.93 ± 8.18
	10	18.04 ± 7.31
	20	15.39 ± 6.48
A. discaudata	5	15.61 ± 1.38
	10	17.27 ± 4.26
	20	14.21 ± 8.10

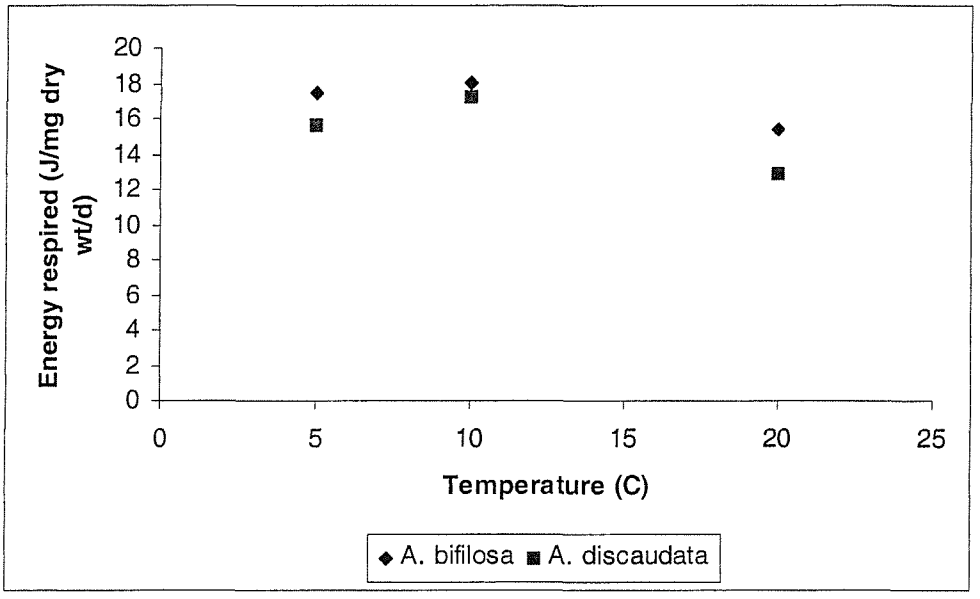


Figure 4.3: The effect of temperature on the respiration rate of *A. bifilosa* and *A. discaudata.*

The present results are an order of magnitude higher than those of Mills (1997) and respiration was responsible for the majority of the energy used within the Scope for Growth definition of both species, 53.5-73.4% for *A. bifilosa* and 37.0-76.6% for *A. discaudata.* Unusually, both congeners lost most energy through respiration at 10°C and the least energy at 20°C. This is a different response for *A. discaudata*, although *A. bifilosa* follows the pattern set in its feeding and assimilation results. There were no statistically significant differences between the intra- or interspecific respiration rates recorded at the experimental temperatures.

4.3.4 Excretion (U)

Table 4.5: The effect of temperature on the excretion rate of *A. bifilosa* and *A. discaudata* (± 1S.D).

Species	Temperature (°C)	Excretion rate (J mg dry wt^{-1} d^{-1})
A. bifilosa	5	0.0434 ± 0.00313
	10	0.0442 ± 0.00303
	20	0.0586 ±0.00777
A. discaudata	5	0.0366 ± 0.00134
	10	0.0454 ± 0.01130
	20	0.0478 ± 0.00356

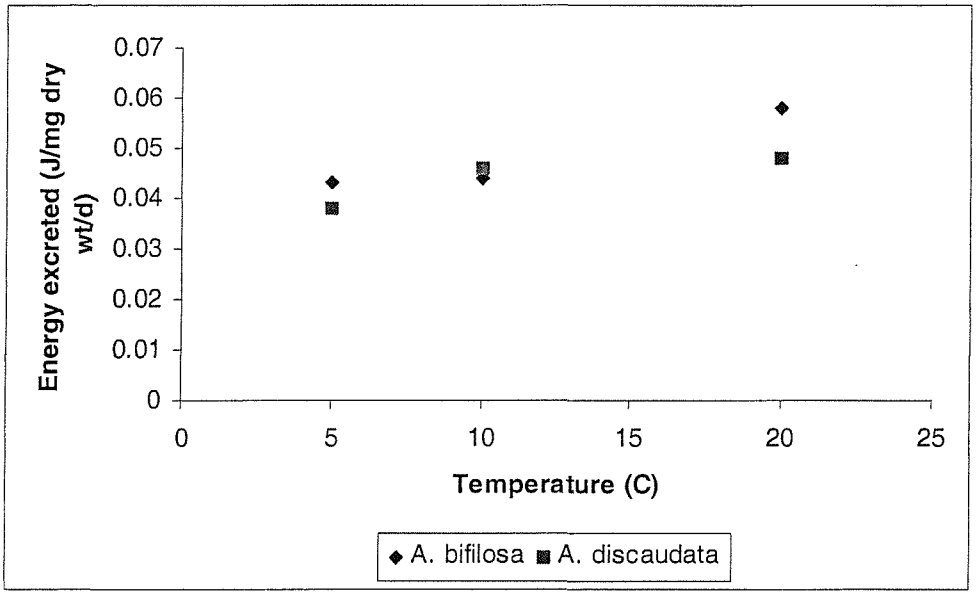


Figure 4.4: The effect of temperature on the excretion rate of *A. bifilosa* and *A. discaudata*.

As temperature increased, so did the excretion rate of both *A. bifilosa* and *A. discaudata*. However there were no inter- or intraspecific significant differences in the excretion rate measured at the three temperatures.

4.3.5 Scope for Growth - SfG (P)

The mean results for each of the parameters were used to determine the SfG of *A. bifilosa* and *A. discaudata* at the three test temperatures.

$$P = A - (R + U)$$

P = Scope for Growth (SfG)
A = Amount of energy assimilated from food
R = Amount of Energy lost through respiration
U = Amount of energy lost through excretion

Table 4.6: The effect of temperature on the Scope for Growth of *A. bifilosa* and *A. discaudata.*

Species	T (°C)	F	A (%)	A	R	U	P
A. bifilosa	5	30.01	79.3	23.80	17.47	0.043	6.28
	10	38.76	87.2	33.80	18.08	0.044	15.67
	20	35.01	64.9	22.72	15.42	0.058	7.24
A. discaudata	5	27.85	73.4	20.44	15.66	0.038	4.74
	10	31.50	80.8	25.45	17.28	0.046	8.13
	20	42.50	82.5	35.06	12.97	0.048	22.04

T = Temperature
F = Energy ingested (J mg dry wt^{-1} d^{-1})
A(%) = Assimilation efficiency
A = Energy assimilated (J mg dry wt^{-1} d^{-1})
R = Energy respired (J mg dry wt^{-1} d^{-1})
U = Energy excreted (J mg dry wt^{-1} d^{-1})
P = Scope for Growth (J mg dry wt^{-1} d^{-1})

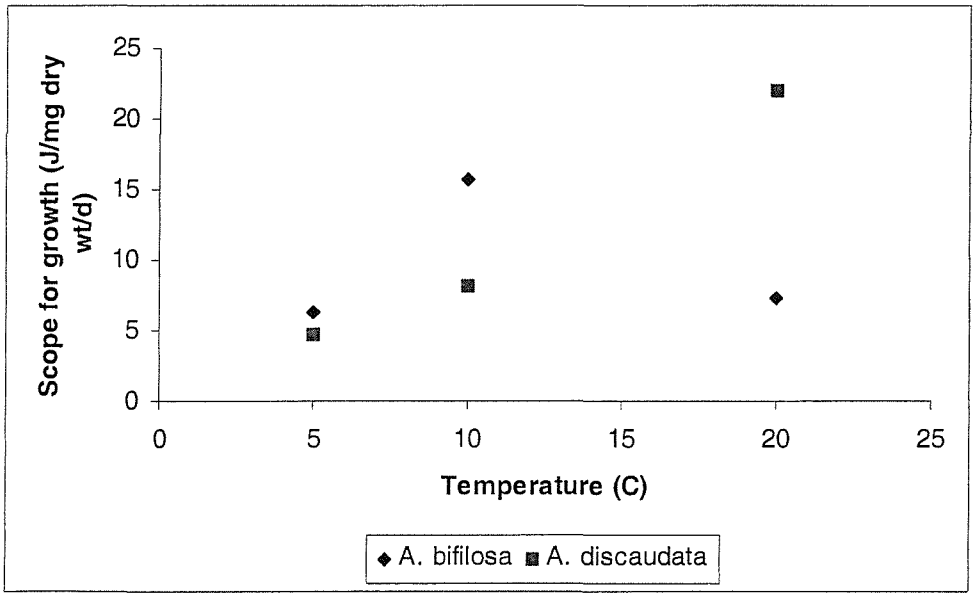


Figure 4.5: The effect of temperature on the Scope for Growth of *A. bifilosa* and *A. discaudata.*

Considered as a single parameter the SfG of an individual will increase as the stressor approaches optimum. Although it was not possible to test the results statistically, it is clear that *A. bifilosa* has a much higher SfG at 10°C than at the other two test temperatures while *A. discaudata*'s SfG at 20°C is substantially higher than at 5°C and 10°C. There was no significant differences in the excretion (Fig. 4.4) or respiration (Fig

4.3) results which suggests that SfG is more dependent on the amount of food ingested, the assimilation efficiency and ultimately, the amount of energy assimilated by the individual. Indeed, the temperature-induced pattern of SfG results (Fig. 4.5) most closely mirror the pattern of the amount of energy assimilated (Fig. 4.2) indicating that of all the energy-parameters measured, assimilation is the best indicator of stress.

4.4 Discussion

4.4.1 Feeding (C)

Functional response measurements in Chapter 6 have shown that the amount of phytoplankton consumed increases linearly with an increase in initial food concentration. Although not seen in the Chapter 6 results, consumption rates generally plateau once food is in excess (Durbin and Durbin 1992; Lampitt and Gamble 1982; Mills 1997). Kiorboe *et al.* (1985) reported on the specific dynamic action (SDA) or "heat increment of feeding" for *Acartia tonsa.* This is the energy cost involved in capturing a food cell, intestinal work, digestion, absorption and transport, amino acid oxidation and urea synthesis, and biomass formation/growth. So, there must also be a minimum food concentration at which no feeding occurs because the energy expended in taking and processing the food is greater than that obtained from the food (Mills 1997). To avoid this in the present work food was given at an excess concentration, identified in Chapter 6, of ~1×10^5 cell ml^{-1}.

It is accepted that feeding experiments that use a single prey species can skew results and may not reflect the functional response of a predator to that food in the water column where it is found in a mixture of food sources (Lampitt and Gamble 1982). Although, the current investigation was conducted in the dark to prevent the algae from reproducing Irigoien *et al.* (1993) and Irigoien and Castel (1995) found no significant differences in gut content of *Acartia bifilosa* in day and night samples. In contrast, Checkley *et al.* (1992) reported significant ($P<0.05$) diel variations in the feeding rate of *Acartia* spp. with gut pigment levels 29% higher at night. So, the present results may give an artificially high daily ingestion rate.

The current results show that *A. bifilosa* ingests more food at 10°C than at the other two temperatures, but that this result is not statistically significant. It does however, take significantly more food at 10°C than *A. discaudata*. In contrast, *A. discaudata* ingests significantly more food at 20°C and its feeding rate is significantly greater than that of *A. bifilosa* at this higher temperature. These results correspond well with Mills (1997) who found feeding rates of ~30-45 J mg dry wt^{-1} d^{-1} for *A. bifilosa* over a range of temperatures. In contrast, in terms of cells eaten mg dry wt^{-1} d^{-1} the results from the present work are two or even three orders of magnitude larger than previous investigations for *A. clausi* (Ayukai 1987), *A. tonsa* (Cowles *et al.* 1988) and *A. hudsonsica* (Durbin and Durbin 1992). However, in all of these cases the initial food concentration was several orders of magnitude smaller and *Acartia* ingestion has been seen to increase linearly with food concentration (Chapter 6).

4.4.2 Assimilation (A)

This parameter was assessed using the Conover equation and the range in assimilation efficiency for *A. bifilosa* was 64.87-87.20% with the highest value at 10°C and the lowest at 20°C. For *A. discaudata* efficiency increased with temperature from 73.39% at 5°C to 82.51% at 20°C. These results are relatively high when compared to Abou Debs (1984) who obtained an assimilation efficiency of 70% for *Temora stylifera* when fed on *Phaeodactylum tricornutum* and *Hymenomonas elongata*. Gaudy (1974) reported a mean assimilation efficiency of 55.41% for *Temora stylifera* when fed *Phaeodactylum*, and *Calanus hyperboreus* has a range of assimilation efficiency of 45.6-69.9 on *Skeletonema costatum* (Conover 1966). There was no significant difference in the

assimilation efficiencies at the three temperatures for *A. discaudata* and so its energy intake depends solely on the amount of food it ingests. In contrast, *A. bifilosa* had a significantly reduced efficiency at 20°C, which further reduces its energy intake at the higher temperature. Its highest percentage assimilation was at 10°C and so it maximises its energy intake at this temperature. For both congeners, the maximum and minimum assimilation efficiencies occurred at the same temperatures as their maximum and minimum rates of ingestion. Assimilation efficiency is not related to the amount of food offered or ingested, instead food items with low ash content are more completely assimilated than those with a high ash content (Conover 1966). The amount of energy assimilated is the most sensitive component of the SfG assay to stress (Naylor *et al.* 1989; Maltby *et al.* 1990). Therefore, *A. bifilosa* is clearly reflecting some stress at 20°C whereas *A. discaudata* was not stressed over the range of temperatures. This observation may go some way to explaining why *A. discaudata* is present in Southampton Water all year round and why *A. bifilosa* diapauses over summer.

4.4.3 Respiration (R)

Although Hirche (1987) observed fluctuations in respiration rate with temperature for *Calanus glacialis, Metridia longa, Calanus hyperboreus* and *Calanus finmarchicus*, Lampitt and Gamble (1982) found that respiration in *Oithona nana* is actually temperature-independent. For both congeners in the current investigation, respiration rate was lowest at 20°C and highest at 10°C, but that these results were not significant. At 10°C the respiration rate for *A. bifilosa* was 18.04 J mg dry wt^{-1} d^{-1} and 17.27 J mg dry wt^{-1} d^{-1} for *A. discaudata*. These results are much higher than those reported by Mills (1997) who noted respiration rates of ~2.5 J mg dry wt^{-1} d^{-1} for *A. bifilosa;* Abou Debs (1984) who reported a range in respiration rate of 0.203-4.54 J mg dry wt^{-1} d^{-1} for

T. stylifera; and Lampitt and Gamble (1982) who observed a respiration rate range of 0.325-0.81 J mg dry wt^{-1} d^{-1} for *O. nana*. These studies in turn were far higher than the respiration rate of 0.0039 J mg dry wt^{-1} d^{-1} for *A. tonsa* reported by Kiorboe *et al.* (1985). The copepods were not provided with food during the experiment, but well-fed individuals should not be unduly stressed by a lack of food for this length of time. Kiorboe *et al.* (1985) assessed the effect of starvation on *A. tonsa* over 4 days and found that respiration rates remained constant over the first 8 hours but then fell dramatically. Before the experimental procedure, the copepods were exposed to a food concentration well in excess of that found in the field for 24 hours while they were acclimated to the required temperatures. Kiorboe *et al.* (1985) found that respiration rates for *A. tonsa* fed at saturation point were 4 times higher than for starved animals. Further, the respiration rate for *T. stylifera* fed at excess food concentrations was 20 times higher than the standard respiration rate in the field (Abou Debs 1984). The respiratory needs of *O. nana* can account for up to 40% of the food ingested (Lampitt and Gamble 1982). In the current investigation, respiration is responsible for the majority of the energy lost, 53.3-83.7% for *A. bifilosa* and 40.5-76.4% for *A. discaudata.* If this is due to stress, then both species were stressed to the same degree at all the temperatures, which is unlikely and would suggest a problem with the procedure. However, since respiration rate increases with food intake (Abou Debs 1984) the very high respiration rates are more likely a reflection of the fact that the copepods were fed to satiation for 24 hours before the start of the experiment.

4.4.4 Excretion (U)

Copepods are assumed to be ammonotelic i.e. the losses of organic matter through metabolism of protein can be estimated from the rate of ammonium excretion (Abou

Debs 1984; Checkley *et al.* 1992). The experiments were conducted in the light and copepod ammonium excretion is known to be at maximum during the daytime (Checkley *et al.* 1992). The effects of starvation were negligible as before the experiment the copepods were fed at concentrations much greater than field levels which has been seen to have a positive correlation with excretion rates in *A. tonsa* (Kiorboe *et al.* 1985). The present results show that as temperature increased so too did the excretion rates of *A. bifilosa* and *A. discaudata*, but none of the results were significantly different from the others. The energy lost through this component is almost negligible when compared to the other factors of the SfG assay, only ~ 0.05 J mg dry wt^{-1} d^{-1} for both species. This compares favourably with results for *A. bifilosa (*Mills 1997), *A. clausi* (Harris 1959) and *T. stylifera* (Abou Debs 1984) but is 5 times lower than results for *A. tonsa* (Kiorboe *et al.* 1985).

4.4.5 Scope for Growth (P)

The rationale of the SfG as an indicator of stress is that the SfG is strongly correlated with growth and reproduction – key components of fitness and very influential on population dynamics (Maltby 1992). Stress can induce a fall in SfG by an increase in energy expenditure or, more commonly, a decrease in energy intake (Maltby 1992). It is known that the main limiting factor of SfG in excess food conditions is the amount of energy absorbed from the food ingested (Bayne *et al.* 1985; Naylor *et al.* 1989; Maltby *et al.* 1990). This was also seen in the current results so this assay can therefore be made even less demanding in time and effort by eliminating the need to determine respiration and excretion rates (Naylor *et al.* 1989).

The present investigation concluded that the SfG for both temperatures was affected by temperature:

A. bifilosa 10 > 20 = 5°C

A. discaudata 20 > 10 > 5°C

The SfG of *A. discaudata* increases twofold with a doubling of temperature, whereas *A. bifilosa* SfG at 20°C is virtually equal to that at 5°C but both are only half the SfG at 10°C. At the optimum temperature, (10°C for *A. bifilosa* and 20°C for *A. discaudata*) the maximum amount of energy is available to the individual for gametic and somatic growth. Viitasalo *et al.* (1995) noted that the largest *A. bifilosa* adults in the Bothnian Sea were found in June when the water temperature was ~8°C. Once adult, all of the available energy goes into reproductive effort and *A. bifilosa* produces more eggs at 10°C than 20°C (Castro-Longoria 1998). A photoperiod of 13L:11D triggers diapause in Southampton Water *A. bifilosa* (Chapter 3) and given *A. bifilosa*'s greatly reduced SfG at 20°C, the ultimate reason behind its oversummering strategy is likely to be an attempt to avoid the higher temperatures of this season. Although *A. bifilosa* had an equally low SfG at 5°C it is tactically unsound, if not impossible, for it to diapause twice in one year. This species probably diapauses over summer as competition is greatly reduced during the winter months and so it would have a better chance of survival even with its poor SfG.

4.5 Summary

Acartia bifilosa and *A. discaudata* are considered intermediate species in terms of their spatial distribution in that they are both found throughout Southampton Water. Despite this, they have very different temporal distributions. *Acartia discaudata* is present in the water column all year round, albeit at very low numbers during winter and reaches its maximum density in summer. In contrast, *A. bifilosa* is the numerically dominant *Acartia* congener over winter, but diapauses in the egg stage over summer. The SfG assay tested to see if temperature was responsible for any physiological stress in these two species, which in turn would explain the timing of their presence in the water column.

4.5.1 Feeding (C)

During the feeding investigations *Isochrysis* was offered to the congeners at a concentration of 1×10^5 cells ml^{-1}.

A. bifilosa 10 > 20 > 5°C 38.76>35.01>30.01 J mg dry wt^{-1} d^{-1}

A. discaudata 20 > 10 > 5°C 42.50>31.50>27.85 J mg dry wt^{-1} d^{-1}

A. discaudata feeding rate at 20°C was significantly higher ($P<0.05$) than at 5°C. *A. bifilosa* ingests most food at 10°C, but the differences at the various temperatures are not significant. There is no difference in the feeding rate of the two species at 5°C, but *A. bifilosa* has a significantly higher ($P<0.01$) feeding rate at 10°C than *A. discaudata*, but *A. discaudata* ingests significantly ($P<0.01$) more food at 20°C. These results reflect their seasonal distribution patterns. Food was given in excess and the copepods were kept in the dark to prevent the algae from reproducing. This may have given artificially high feeding rates (Checkley *et al.* 1992).

4.5.2 Assimilation (A)

Efficiency: *A. bifilosa* 10 > 5 > 20°C 87.20>79.31>64.87 %

A. discaudata 20 > 10 >5°C 82.51>80.81>73.39 %

A. bifilosa had a significantly lower ($P<0.01$) assimilation efficiency at 20°C than the other two temperatures. There was no difference between the *A. discaudata* results even though efficiency increases with a rise in temperature. At 20°C, *A. discaudata* assimilation efficiency was significantly higher ($P<0.01$) than *A. bifilosa.*

Energy assimilated from food: *A. bifilosa* 10 > 5 > 20°C

33.80>23.80>22.72 J mg dry wt^{-1} d^{-1}

A. discaudata 20 > 10 > 5°C

35.06>25.45>20.44 J mg dry wt^{-1} d^{-1}

A. bifilosa assimilates a significantly greater ($P<0.05$) amount of energy at 10°C whereas *A. discaudata* assimilates most energy ($P<0.05$) at 20°C.

This component is the most sensitive component to stress of the SfG assay (Naylor *et al.* 1989; Maltby *et al.* 1990) so *A. bifilosa* is stressed at 20°C but *A. discaudata* is not stressed at any of the temperatures.

4.5.3 Respiration (R)

A. bifilosa 10 > 5 > 20°C 18.08>17.47>15.42 J mg dry wt^{-1} d^{-1}

A. discaudata 10 > 5 > 20°C 17.28>15.66>12.97 J mg dry wt^{-1} d^{-1}

There are no statistically significant differences in the results which suggests that respiration is not responsible for any stress that the copepods may experience at the

various temperatures. The very high figures are probably due to the copepods being fed to satiation for 24 hours before the experiment (Abou Debs 1984).

4.5.4 Excretion (U)

A. bifilosa 20 > 10 > 5°C 0.058>0.044>0.043 J mg dry wt^{-1} d^{-1}

A. discaudata 20 > 10 > 5°C 0.048>0.046>0.038 J mg dry wt^{-1} d^{-1}

As temperature increased so too did the excretion rate of both congeners, but the results were not significant. The amount of energy lost through this component is negligible when compared to the other factors.

4.5.5 Scope for Growth (P)

A. bifilosa 10 > 20 ≈ 5°C 15.67>7.24≈6.28 J mg dry wt^{-1} d^{-1}

A. discaudata 20 > 10 > 5°C 22.04>8.13>4.74 J mg dry wt^{-1} d^{-1}

Since there were no significant differences in the respiration or excretion rates, the SfG is dependent on the amount of food ingested and ultimately, the amount of energy assimilated. So, the assay can be made less demanding in time and effort by eliminating the respiration and excretion investigations. The SfG of *A. discaudata* increased twofold with a doubling of temperature. The SfG of *A. bifilosa* at 10°C is twice that at 20°C and 5°C. Since there is less energy available to *A. bifilosa* at 20°C it is reasonable to assume that it diapauses over summer to avoid the higher temperatures which reduces its SfG and therefore decrease its competitive ability. Also, although its SfG at 5°C is equally low it is unfeasible to diapause twice in a year and competition is reduced during the winter months.

Chapter 5:
Analysis of seasonal fatty acid in A. *bifilosa*

5.1 Introduction

Lipids are significant components of the pelagic marine food web and serve two crucial functions in that they are an efficient source of metabolic energy, and the essential polyunsaturated fatty acids (PUFAs) have vital structural and functional roles in cell membranes and therefore are required for growth and reproduction (Sargent and Henderson 1986). Fatty acids are used as static and dynamic biomarkers in marine organisms like algae (Mayzaud *et al.* 1989; Volkman *et al.* 1989; Dunstan *et al.* 1992 and Cheucas and Riley 1969) and zooplankton (Pond *et al.* 1993; 1995[a]; 1995[b]) including copepods (Norrbin *et al.* 1990; Kattner and Krause 1989; Anderson and Pond 2000), and, as such, fatty acids can also be used to study trophic interactions between marine consumers and their food supply (Lee *et al.* 1971; Fraser *et al* 1989 and Ederington *et al.* 1995). Grazers and carnivores generally derive all of their lipid requirement from their food (Sargent and Henderson 1986), and well-nourished copepods incorporate dietary fatty acids without modifications (Lee *et al.* 1971). During spring, the fatty acid composition of *Calanus finmarchicus, Calanus helgolandicus* and *Pseudocalanus elongatus* will reflect their diatom food source, with increased levels of the 20:5(n-3)[1] acid. Conversely, when food becomes scarce, e.g. during winter, the fatty acid compositions of grazers are much more simple and are nearly reduced to the three main fatty acids; 16:0, 20:5(n-3) and 22:6(n-3) (Kattner and Krause 1989).

[1]Fatty acids are described as numbers separated by a colon *x:y(n-w)*. Where *x* specifies the carbon chain length and *y* indicates the number of double bonds. The position of the double bond is defined by *(n-w)* where *n* is the total number of carbon atoms and *w* is the number of carbon atoms between the terminal methyl group and the first double bond encountered. Unless otherwise indicated, double bonds in biological polyunsaturated fatty acids (PUFAs) are invariably separated by one $-CH_2$ group so that only the first double bond nearest the methyl terminus need be designated. For example, palmitoleic acid 16:1(n-7) is $CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$.

This pattern may also be the case in Southampton Water, as the potentially most varied diet for copepods occurs in summer, whereas food availably in winter is very poor (Kifle 1992).

As copepods must accommodate energy expenditure through the year by food input, temporal differences in lipid storage and utilisation act as important clues to a species' life history and reproductive strategy. Wax esters are the main storage-lipid class (Kattner and Krause 1989), and are considered good long-term energy reserves for copepods as they are both incorporated and utilised at a slower rate than triglycerols (Lee *et al.* 1971). Alternatives exist, and *Acartia longiremis* uses a store of triglycerols over winter as a short-term buffer against starvation, (Norrbin *et al.* 1990). In northern norwegian fjords copepods present a range of overwintering survival strategies. *Calanus finmarchicus* stores large amounts of wax esters and spends the winter in diapause as copepodite V, while *Metridia longa* is primarily dependent on continuous feeding. Alternatively, *Pseudocalanus acuspes* and *A. longiremis* both store lipid in an oil sac (Norrbin *et al.* 1990). The inverse relationship between basal metabolism and size means that small copepods have a higher metabolic rate per unit weight. There is also limited room for lipid storage in small copepods, and such stores would soon be depleted when food availability and quality become low (Kattner and Krause 1989), it can be argued that feeding must supplement the stored energy reserves in species of *Acartia* which are therefore, dependent on a constant food supply (Dagg 1977).

In Southampton Water, *Acartia bifilosa* is very much a winter species as, although its maximum numbers occur in May before it disappears from the water column (Castro-Longoria 1998), in terms of *Acartia* species it is numerically dominant over winter.

Temporal changes in the fatty acid composition of the species are investigated to determine whether season and hence main food source in Southampton Water is reflected in their composition. In winter, flagellates (e.g. *Isochrysis galbana*) and detritus are the principle components in the water (Kifle 1992) and so the potential food sources, and therefore high concentrations of the 18:1(n-7) fatty acid would be indicative of bacteria in the copepods' diet. Bacteria are also low in 20:5(n-3) and 22:6(n-3) as long-chain PUFAs are considered absent from prokaryotes, and are therefore characteristics of eukaryotes (Sargent *et al.* 1987). Alternatively, high levels of 16:0, 18:4(n-3) 22:6(n-3) and the C_{18} PUFAs would indicate *Isochrysis* (Cheucas and Riley 1969; Volkman *et al.* 1989 and Ben-Amotz and Tornabene 1985). In contrast, diatoms (e.g. *Skeletonema costatum*) contain greater amounts of 16:1(n-7), 20:5 (n-3) (EPA – eicosapentaenoic acid) and C_{16} PUFAs (poly-unsaturated fatty acids) and this may be reflected in copepod fatty acid composition during the spring bloom. C_{18} PUFAs and the 22:6(n-3) fatty acid (DHA - docosahexaenoic acid) in the diet would also correspond to the dinoflagellate bloom (e.g. *Scrippsiella trochoidea)* later in the year.

Sargent and Henderson (1986) noted that the calanoid copepod *Calanus finmarchicus* was able to synthesise monounsaturated fatty acids from precursors in its diet.

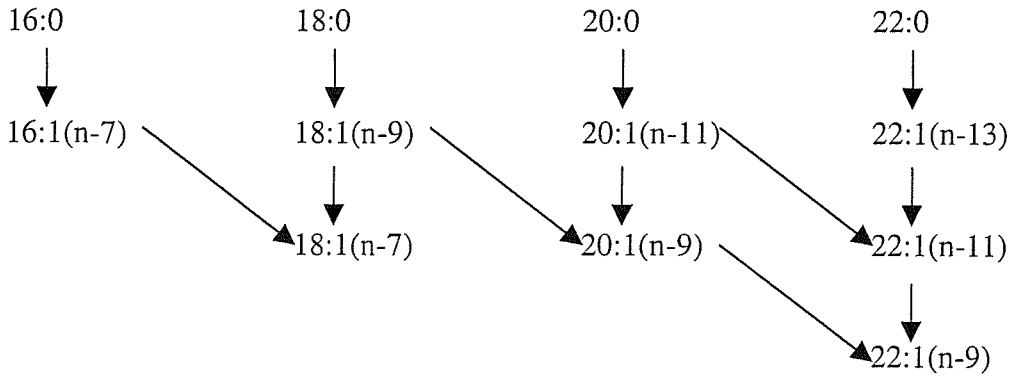


Figure 5.1: Possible pathways of biosynthesis of monounsaturated fatty acids in calanoid copepods, assuming the vertical operation of a Δ9 desaturase and chain elongation reactions (2C addition) operating horizontally. (After Sargent and Henderson 1986).

Of the above fatty acids, 18:1(n-9) would give the most noticeable reading in an *A. bifilosa* fatty acid trace. *Acartia bifilosa* does not utilise a storage mechanism over winter, so the question is raised as to whether *A. bifilosa* is most successful in winter because, like *C. finmarchicus* (Sargent and Henderson 1986), it has a physiologically superior ability to form essential fatty acids from its food, or whether it is simply better at switching its diet focus than other *Acartia* species. Along with the adult analyses, the fatty acid composition of seasonal water samples and diapause and subitaneous eggs will also be looked at. The analysis of different tissue levels may show the flow of essential fatty acids through the system.

5.2 Method

The intention is to measure the seasonal percentage composition of total fatty acid in *A. bifilosa* using methods based on Folch *et al.* (1957) and Olsen and Henderson (1989). To extract the lipid, every month five replicates of four females were placed in 500µl chloroform:methanol (2:1), 125µl KCl (0.88%) was added, the sample was whirlimixed, centrifuged and the upper layer discarded. The samples were then blown down under nitrogen and analysis continued from the "alkaline hydrolysis" stage.

For monthly subitaneous egg analysis 5 replicates of 4 *A. bifilosa* females were placed in an egg production chamber, (a plastic cylinder with a 200µm mesh on one end which was supported in a 1l beaker filled with 600ml of 26µm filtered seawater from the SOES aquarium (30-32 salinity), and initially provided with an excess concentration (10^5 cells ml^{-1}) of *Isochrysis* for food. The eggs were collected after 2 days (minimum 30 eggs) and then placed into a small pointed vial and 500µl chloroform:methanol (2:1) added. Diapause eggs were collected by a similar protocol after the adult females were held at temperature conditions of 18°C and a photoperiod of 14L:10D for a week. To extract the lipid from the eggs, 125µl KCl (0.88%) was added to the sample, whirlimixed, centrifuged and the upper layer discarded. The samples were then blown down under nitrogen and analysis continued from the "alkaline hydrolysis" stage.

The fatty acid content of the water column was also examined. A 60ml sample of seawater from 8m depth at Calshot was passed through a syringe attached to a GF/F 25mm ∅ filter. The residue was stored in 6ml of chloroform:methanol (2:1) in an 8ml

tube and sonicated for 30 seconds, and then filtered through GF/F filter paper into another 8ml tube and 1.5ml KCl (0.88%) added. The resulting sample was then whirlimixed, centrifuged and the upper layer discarded before being blown down under nitrogen. The procedure then continued from the "alkaline hydrolysis" stage.

Alkaline hydrolysis

- Add 100µl 1M KOH in 95% ethanol
- Heat for 1 hour at 78^0C
- Cool samples down
- Add 250µl H_2O per sample
- Acidify with 2 drops of 0.6M HCl (check with pH paper)
- Extract twice with 250µl diethylether (N.B. fatty acids are in the ether in the upper layer): Add the ether to the first tube, whirlimix, centrifuge, transfer upper organic layer into second tube, repeat.
- Blow samples down under N_2

Preparation of pentafluorobenzyl (PFB) esters

- Add 30µl acetonitrile (agitate to dissolve sample)
- Add 100µl PFB bromide (3.5% in acetonitrile)
- Add 100µl triethylamine
- Agitate to dissolve sample
- Leave for 15 mins at room temperature (agitate occasionally)
- Add 500µl isooctane
- Whirlimix, centrifuge, transfer upper layer into second tube (PFB esters are in upper layer), repeat.
- Blow samples down under N_2.

Purification

- Add 100µl isooctane
- HPTLC (high performance thin-layer chromatography) with 50µl PFB standard in small tank. (Solvent = 18ml hexane: 2ml diethylether: 0.2ml acetic acid), apply samples far apart in small spots on 10×10×0.25cm silica gel plates.
- Dry plates briefly, spray standards only with DFC (dissolved in methanol), dry under vacuum.
- Scrape upper band off plates into 8ml vials containing 2ml isooctane (or hexane:diethylether (1:1)).
- Add 1ml $NaHCO_3$ (2%)
- Whirlimix
- Centrifuge
- Freeze

Injection

- Decant upper layer (solvent) off of the frozen sample into a new 8ml vial.
- Blow down under N_2 until about 1ml is left, transfer this into a small, pointed vial.
- Blow down entirely
- Dry in desiccator
- Add 100µl isooctane (for the copepod and water samples), or 50µl of isooctane for the egg samples.
- Ready to inject into the GC to obtain fatty acid trace.

For reasons of assay sensitivity and biological relevance, only a fixed range of fatty acids was looked at and the percentages of these were summed to a total of 100%. These results were then analysed using non-metric MDS (multi-dimensional scaling), hierarchical cluster analysis and SIMPER (similarity percentages) in Primer-E (Clarke and Warrick 1994) to compare the monthly similarity readings with each other. All data were $\sqrt{\sqrt{}}$-transformed.

5.3 Results

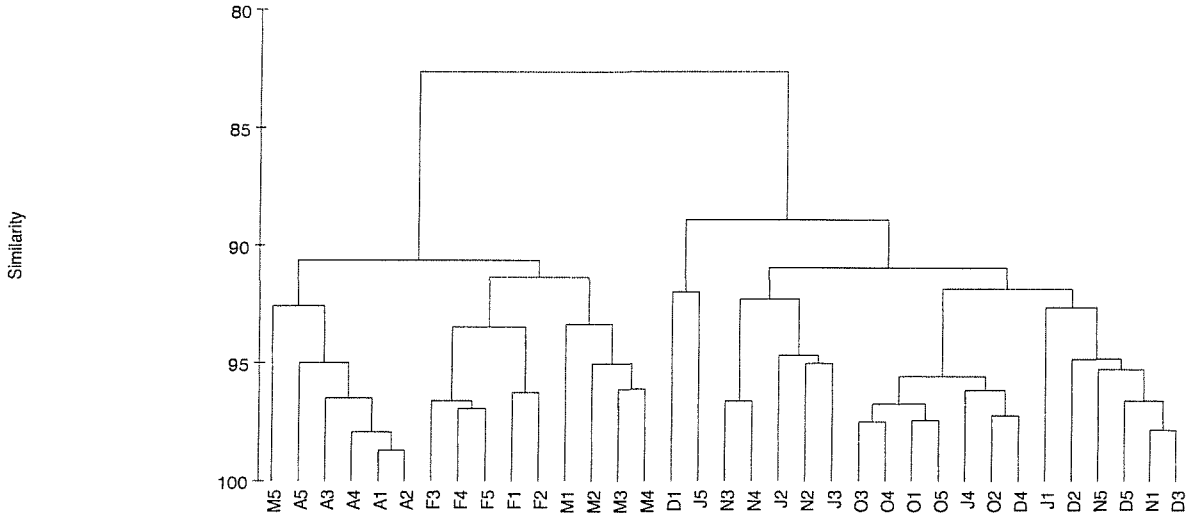


Figure 5.2: Cluster analysis of monthly fatty acid content of *A. bifilosa*.
J = January, F = February, M = March, A = April, O = October, N = November, D = December

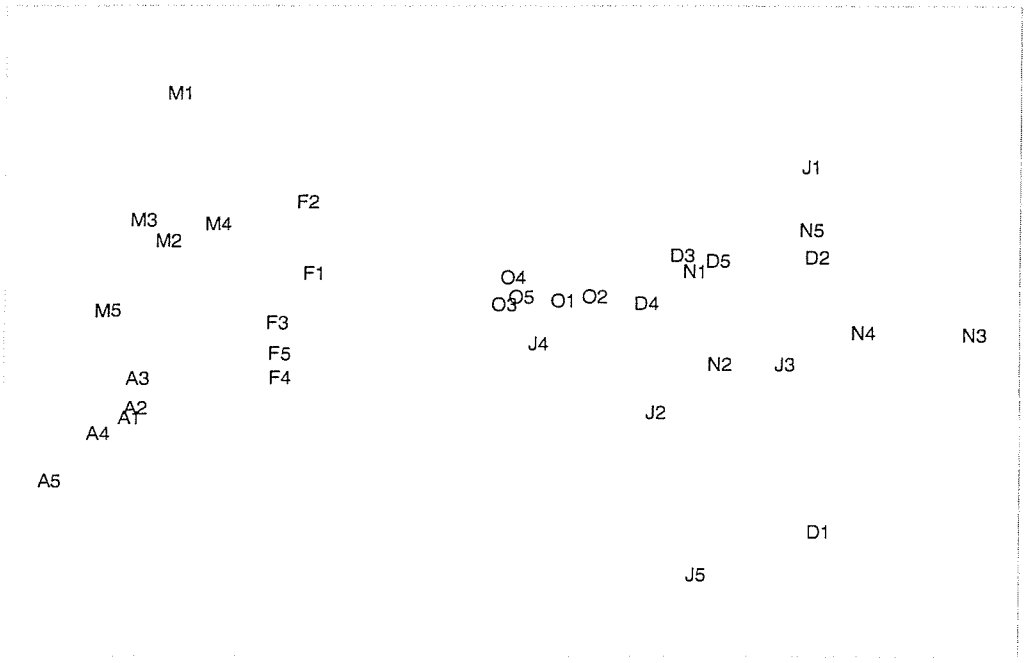


Figure 5.3: MDS analysis of monthly fatty acid content of *A. bifilosa*.
J = January, F = February, M = March, A = April, O = October, N = November, D = December

Table 5.1: Mean percentage fatty acid content of *A. bifilosa* and eggs (± 1 SD).

Fatty acid	Feb	Mar	Apr		Oct	Nov	Dec	Jan	S. egg (Mar)	D. egg (Mar)
14:0	13.9 (4.03)	15.6 (4.39)	24.7 (3.47)		4.30 (0.77)	1.78 (0.64)	2.01 (0.75)	3.60 (1.72)	32.4 (8.49)	18.7 (5.40)
16:0	44.6 (3.42)	33.6 (3.25)	31.2 (2.82)	D	17.5 (0.82)	14.3 (1.37)	16.1 (0.86)	22.0 (1.86)	34.4 (1.56)	35.0 (15.7)
16:1(n-7)	2.30 (1.10)	9.68 (3.17)	13.0 (3.21)	I	2.69 (1.31)	0.95 (0.53)	1.52 (1.41)	1.14 (0.59)	6.14 (1.82)	4.59 (2.40)
16:2(n-3)	1.68 (0.46)	2.19 (1.56)	2.52 (0.40)	A	1.17 (0.49)	0.60 (0.29)	0.44 (0.16)	0.85 (0.72)	1.35 (0.14)	1.75 (0.37)
16:3(n-3)	2.27 (1.72)	1.72 (1.48)	2.41 (0.67)	P	0.90 (0.05)	0.38 (0.12)	0.38 (0.13)	0.78 (0.44)	2.17 (2.24)	1.42 (1.64)
18:0	19.0 (3.03)	13.4 (2.78)	6.86 (1.81)	A	48.1 (3.76)	62.7 (2.31)	56.6 (1.47)	51.8 (3.67)	6.55 (5.33)	22.8 (6.2)
18:1(n-9)	2.50 (0.42)	10.6 (3.63)	1.95 (1.00)	U	2.35 (0.62)	1.14 (0.29)	1.35 (1.06)	2.39 (1.53)	4.27 (1.11)	9.18 (9.9)
18:1(n-7)	3.29 (0.52)	2.71 (0.50)	3.26 (1.36)	S	12.9 (0.82)	14.9 (1.18)	17.3 (2.75)	14.8 (3.47)	5.89 (6.23)	1.32 (0.95)
18:3(n-3)	1.18 (1.08)	1.43 (0.89)	0.41 (0.12)	E	1.18 (0.28)	1.56 (0.98)	1.17 (0.30)	0.60 (0.28)	2.49 (0.22)	1.50 (1.74)
18:4(n-3)	0.74 (0.68	1.39 (1.29)	2.51 (0.47)		0.80 (0.61)	0.30 (0.19)	0.47 (0.22)	0.48 (0.18)	2.38 (0.30)	0.72 (0.01)
20:5(n-3)	4.73 (1.56)	4.07 (3.61)	5.97 (1.04)		3.05 (0.96)	0.64 (0.60)	1.19 (0.78)	0.44 (0.65)	0.86 (0.12)	0.69 (0.10)
22:6(n-3)	3.91 (1.54)	3.60 (2.64)	1.46 (0.34)		5.08 (1.35)	0.78 (1.09)	1.44 (1.03)	1.12 (1.18)	1.15 (0.30)	0.61 (0.85)

S. egg = subitaneous egg
D. egg = diapause egg

Both the cluster analysis (Fig. 2) and MDS (Fig. 3) show a very clear delineation between the percentage fatty acid content of *A. bifilosa* before and after diapause, which occurs towards the end of May and lasts until October (Castro-Longoria 1998). February, March, April and October samples separate relatively clearly into groups between 90 and 95% similarity, although this is not seen for November, December or January (Fig. 5.3). This was not unexpected because the data were arbitrarily divided into months but a copepod does not know the date, it simply feeds on what is available. So, the cluster analysis would suggest that the food available to *A. bifilosa* over the winter did not vary much from November to January. SIMPER results indicated that all of the month groups had an average intra-group similarity of 85% or more. Although SIMPER is not a statistical test, just an exploratory analysis (Clarke and Warrick 1994),

it did give an indication as to which fatty acid differences were responsible for any dissimilarity between the months.

Table 5.2: Sequential percentage dissimilarity in *A. bifilosa* fatty acid composition. SIMPER results (see Appendix 6).

Months	Average dissimilarity (%)
February and March	24.60
March and April	23.86
April and October	55.06*
October and November	17.31
November and December	8.49
December and January	11.63

The sequential changes in fatty acid composition were looked at in order to determine if the copepod's diet focus was indeed shifting, or if it was manufacturing its own monounsaturated fatty acids to compensate for a poor diet (Table 5.2). The most noticeable difference (*) between the months was between the pre- and post-diapause months (April and October). SIMPER results indicate that the fatty acids most responsible for the difference are the saturates 18:0, 14:0 and 16:0, contributing 38.21%, 18.89% and 12.64% respectively. Although the fatty acid percentage data were √√-transformed prior to analysis to give increased weighting to the rarer species (i.e. the PUFAs) the saturates still have a comparatively large influence on results. Ignoring these, the PUFAs 16:1(n-7), 18:1(n-7) and 22:6(n-3) are important contributors to the percent dissimilarity between April and October, responsible for 9.52%, 8.88% and 3.35% respectively. In terms of diet, these results are of consequence because 16:1(n-7) is indicative of diatoms and April copepods contained much higher levels of this fatty acid that October *A. bifilosa* (Table 5.1). 18:1(n-7) signifies bacteria in the diet and 22:6(n-3) reflects dinoflagellate grazing. The October copepods contained much higher levels of both of these fatty acids (Table 5.1) suggesting that in the autumn *A. bifilosa* was feeding on the end of the dinoflagellate bloom, not diatoms, and was supplementing

its diet with bacteria from detritus. From the increased reliance on bacteria as a food source, it can be concluded that the autumn food supply is not as good as the spring one.

Table 5.3: % dissimilarity in monthly *A. bifilosa* fatty acid composition. SIMPER results (see Appendix 6).

Months	Average dissimilarity (%)
November and April	68.28*
December and April	64.63*
November and March	62.97
December and March	59.18
January and April	57.28*
November and February	56.11
October and April	55.06
December and February	52.62
January and March	51.93
October and March	48.17
January and February	45.33
October and February	42.34
February and April	29.64
February and March	24.60
March and April	23.86
October and November	17.31
November and January	14.98
October and December	13.57
October and January	12.72
December and January	11.63
November and December	8.49

Other noteworthy high % dissimilarities are between April and November, December and January (* Table 5.3) and from the MDS (Fig. 5.2) it can be seen that the most distant groups are April and the loose November/December/January cluster. Again, the fatty acids contributing most to the dissimilarity are the saturates. However, ignoring these, 16:1(n-7), 18:1(n-7) and 20:5(n-3) are also responsible for the dissimilarities (SIMPER results – Appendix 5). Clearly, *Acartia bifilosa*'s diet is poorer in winter than in spring because it is forced to supplement its diet with bacteria, probably from detritus, as it cannot meet its dietary requirements feeding on algae alone. 18:1(n-7) is responsibly for 8.69%, 11.10% and 10.31% dissimilarity between April and November,

December and January (SIMPER results). 16:1(n-7) and 20:5(n-3) are biomarkers of diatoms and percentages of these fatty acids are much higher in April, as are levels of the C_{16} PUFAs, signifying that *A. bifilosa* is feeding well on diatoms and has no need to augment its diet with detritus/bacteria as suggested by the low levels of 18:1(n-7) (Table 5.1). These results imply that *A. bifilosa* is switching its diet focus as the year progresses, in response to the algae in Southampton Water. In short, in spring (April) the copepods are feeding well on diatoms, in autumn (October) they graze on dinoflagellates and augment their diet with detritus, and overwinter rely heavily on bacteria as food source.

A *Phaeocystis pouchetti* bloom occurred in late April and through May in Southampton Water, coinciding with the last collection date. The presence of *Phaeocystis* in the water column was coincident with a demise in *Acartia tonsa, A. clausi* and *A. discaudata* adults and nauplii in the northern Wadden Sea of Sylt (Martens 1981) and there is an open argument about the quality of *Phaeocystis* as food for *Acartia.* One reason behind this may be that, at 3μm, single cells of *Phaeocystis* are too small for copepods to take, but by the same token, colonies are too large for *Acartia* to consume. In addition, the gelatinous matrix exuded by *Phaeocystis* causes the alga to adhere to the feeding appendages of copepods, making it even more difficult for them to graze easily on this species (Bautista *et. al.* 1992). Claustre *et al.* (1990) noted that *Phaeocystis* is also a poor food in terms of its biochemistry. Owing to the potential importance of this alga to the April feeding preferences, and hence fatty acid make-up of *A. bifilosa*, water samples were taken and compared to similar data for *Phaeocystis.*

Table 5.4: Comparison of the fatty acid composition of the April water sample with that of adjusted *Phaeocystis* data from Claustre *et al.*(1990).

Fatty acid	Water sample-Apr (± 1 SD)	*Phaeocystis* <22μm	*Phaeocystis* 22-100μm	*Phaeocystis* 100-200μm
14:0	21.0 (4.73)	20.8	15.2	18.4
16:0	32.2 (5.70	32.8	42.2	41.8
16:1(n-7)	9.60 (2.12)	4.31	13.0	16.0
16:2(n-3)	2.81 (1.52)	0.00	0.00	0.00
16:3(n-3)	0.54 (0.17)	0.11	0.00	0.00
18:0	15.0 (2.40)	3.83	9.52	10.6
18:1(n-9)	11.06 (1.25)	34.5	19.6	11.0
18:1(n-7)	1.91 (0.20)	1.48	0.00	1.10
18:3(n-3)	0.96 (0.79)	1.27	0.00	0.00
18:4(n-3)	2.42 (0.48)	0.69	0.00	0.00
20:5(n-3)	1.98 (1.57)	0.17	0.48	0.51
22:6(n-3)	0.51 (0.11)	0.08	0.00	2.09

It would seem that the April data do not show dominance of *Phaeocystis* over other algae, possibly the bloom was not fully formed at the time of sampling, or the *Phaeocystis* patch was missed when the water sample was taken. Samples came from 8m deep at Calshot, whereas *Phaeocystis* seemed to dominate nearer the surface.

The percentage fatty acid content of subitaneous and diapause eggs was also investigated. The subitaneous eggs were taken from experimental females in March. *Acartia bifilosa* subitaneous eggs can hatch within 48hrs after being laid and this swift development means that the fatty acid content of the eggs changes rapidly. Also, as *A. bifilosa* females lay few eggs per day and at least 30 were needed for suitable analysis, eggs were not always analysed directly after laying as time was needed to accumulate a suitable sample size. Diapause eggs are usually produced in the field in late April and May, this production is triggered by longer day-lengths. For this study, diapause egg-laying was induced in March by exposing the females to 14L:10D photoperiods for a week, the spiny eggs were then collected. SIMPER analyses showed that in March the saturates again accounted for the main dissimilarities between copepods and subitaneous eggs, and between subitaneous and diapause eggs. One would expect the

egg fatty acid content to reflect that of the parent, and since the oocytes that give rise to both types of eggs are the same it can be argued that a diapause egg is simply a subitaneous one with extra protective layering, but no fundamental biochemical differences (Ianora and Santella 1991).

5.4 Discussion

Like many small copepods, *Acartia bifilosa* does not lay down lipid reserves over winter and instead has to rely on continual feeding to survive the pronounced seasonality in Southampton Water. Present results indicate that its fatty acid content reflects this seasonality, mirroring the changing food environment the copepod is exposed to over the months. In February/March, *A. bifilosa* contained relatively high percentages of 16:1(n-7), C_{16} PUFAs and 20:5(n-3), indicative of a diatom diet. These levels increased in April as *A. bifilosa* continued to graze heavily on diatoms in the spring bloom. One of these would have been *Skeletonema costatum* (Kifle 1992) a species known to be C_{16} PUFA abundant compared to other species of diatom (Volkman *et al.* 1989). During these months, comparatively low levels of the 18:1(n-7) fatty acid were recorded, so *A. bifilosa* was not supplementing its diet with bacteria to any great extent and was instead able to feed well on the algae in the water column.

A *Phaeocystis pouchetii* bloom occurred in Southampton Water at the time of the April sampling run and was expected to be the main food source for *A. bifilosa*, but no evidence was seen of this in either the *A. bifilosa* or water sample fatty acid compositions. There is an open argument about the quality of *Phaeocystis* as a food source for copepods. Copepods will feed on it (Claustre *et al.* 1990), but there have also been reports that it is a poor food source (Martens 1981; Estep *et al.* 1990; Frangoulis *et al.* 2001). Verity and Smayda (1989) reported a lack of *Phaeocystis* ingestion by *Acartia* sp., which they attributed to the size of the colonies, rather than chemical undesirability. *Phaeocystis pouchetii* is a euryhaline and eurythermal prymnesiophyte and is one of the few phytoplankton species to alternate between free-living flagellated

cells of 3-9 μm diameter, and palmelloid colonies bound by a gelatinous matrix, that range in size from tens of microns to 10 mm (Lancelot *et al.* 1994; Tande and Bamstedt 1987; Rousseau *et al.* 1994). However, feeding is not just a mechanical response and the biochemical nature of *Phaeocystis* is also important as it is believed to be of poor nutritional value because it contains low levels of polyunsaturated fatty acids (PUFAs) and vitamin C (Claustre *et al.* 1990), it also has a low nitrogen content because *Phaeocystis* blooms occur during times of low nutrient levels in the water column (Bautista *et al.* 1992). Additionally, *Phaeocystis* produces an extra-cellular, (or slime-embedded) compound. *Phaeocystis* cells contain chrysolaminarin vesicles that are extruded through the plasmalemma. Upon contact with seawater, they solidify into the mucilaginous envelope that forms the colony (Estep *et al.* 1990). This process provides an excellent mechanism for the release of anti-predation and anti-microbial substances. *Phaeocystis* produces acrylic acid and dimethylsulphide (DMS) (Bautista *et al.* 1992). The acrylic acid makes grazing on *Phaeocystis* similar to eating hot plastic, and the dimethylsulphide is related to the alkyl sulphides of the onion and garlic families (Estep *et al.* 1990) and so are very effective deterrents. As one would expect, colonies produce much more of these substances that the single cells (Estep *et al.* 1990). The discrepancies in the results may also be due to the fact that the *Phaeocystis* colonies appeared to congregate near the surface, but the water sample was taken from a depth of 8m at Calshot. Also, copepod abundance tends to correlate to areas where food has a high lipid content (Attwood and Peterson 1989), and owing to the poor fatty acid content of *Phaeocystis*, chemoreception may have warned *A. bifilosa* off of this food source. Given the more varied diet available to copepods in summer (Kifle 1992), the animals may have been preferentially feeding on anything else, consequently, no trace of *Phaeocystis* was seen in their fatty acid make-up.

After the summer diapause, *A. bifilosa* returned to the water column in October and its fatty acid composition indicated that it was now feeding on dinoflagellates, possibly *Scrippsiella trochoidea* (Kifle 1992), suggested by the increased levels of 18:0 and 22:6(n-3) fatty acids. It may also have been augmenting its diet with some detritus as higher percentages of 18:1(n-7) were recorded. This level rose over the winter months (November/December/January) as *A. bifilosa* reliance on this food source increased because algae became scarce in the water column. During the winter months, the fatty acid composition of *A. bifilosa* was much more simple and, besides 18:1(n-7), was reduced to the main unsaturated fatty acids 16:0 and 18:0. The levels of both C_{16} and C_{18} PUFAs were decreased, as were the levels of 20:5(n-3) and 22:6(n-3), but for all winter months (Oct-Jan) 22:6(n-3) was present at a higher percentage than 20:5(n-3) suggesting that flagellates are a preferred food source to diatoms. Kifle (1992) reported that flagellates, including *Isochrysis galbana*, were present in Southampton Water over winter. Although *Isochrysis* is rich in C_{18} PUFAs which were not reflected in *A. bifilosa*'s fatty acid make-up, it does also contain high levels of 16:0 and 22:6(n-3) fatty acids (Cheucas and Riley 1969; Volkman *et al.* 1989; Ben-Amotz and Tornabene 1985) and reduced levels of 20:5(n-3) (Dunstan *et al.* 1992) which would help explain the November-January results.

(n-3) PUFAs are essential for growth in copepods but, unlike the production of monounsaturated fatty acids, *de novo* synthesis of (n-3) PUFAs is generally considered to only occur in phytoplankton, with higher trophic organisms able to undertake further chain-elongation and limited desaturation (Fraser *et al.* 1989). So, although *A. bifilosa* may be able to manufacture some monounsaturated fatty acids itself from precursors in

its diet during winter, the healthiest animals, in terms of PUFA composition, will be those that can assimilate their fatty acids directly from the environment without having to expend energy adjusting their fatty acid content (Lee *et al.* 1974) i.e. spring animals.

Ronnestadt *et al.* (1994) observed a marked positive relationship between gilthead seabream egg development and total PUFA concentration. Neutral fatty acids were used as the main metabolic fuel after hatching and those catabolised were the most abundant ones, regardless of the degree of saturation (Ronnestadt *et al.* 1994). The polar fatty acids are closely associated with biomembranes and eicosanoids, a class of compound involved in a wide range of physiological processes (Sargent *et al.* 1989). While carbon, phosphorus and nitrogen are immutable and must be of dietary origin, PUFAs can be synthesised by elongating and desaturating dietary precursors. Although, unlike those which manufacture monounsaturated fatty acids, the enzymes that do this in zooplankton are thought to be slow and inefficient. Therefore it is argued (Anderson and Pond 2000) that high zooplankton growth rates are only observed when their diet is rich in essential fatty acids which cannot be synthesised in sufficient quantities to maintain good growth and survival (Dunstan *et al.* 1992). Storage reserves of PUFAs are not believed to significantly contribute to sustained provision of fatty acids for egg production in natural populations (Anderson and Pond 2000). Therefore, the PUFA composition of eggs does not necessarily reflect the composition of the diet, but rather, it sets the parameters of the materials copepods need to ingest to successfully reproduce. So, although *A. bifilosa* subitaneous and diapause eggs were taken in March, their fatty acid content did not closely reflect that of the adults, and while both types of eggs were notably low in 20:5(n-3) and 22:6(n-3) fatty acids they did contain much higher levels of the C_{16} and C_{18} PUFAs. *Calanus helgolandicus* eggs are known to vary in PUFA

composition with adult diet (Pond 1996), although a strong correlation was not clear. Hatching viability of *C. helgolandicus* was closely correlated with 18:2 (n-6) and 20:4 (n-6) fatty acids, but not with diatom concentration. PUFAs 20:5(n-3) and 22:6(n-3) were good indicators of food availability, but not of egg viability or naupliar survival (Pond 1996) and hatching success and naupliar survival in *C. helgolandicus* is known to depend on total organic content of the eggs (Guisande and Harris 1995). Jonasdottir *et al.* (1995) found that the fatty acid (n-3):(n-6) ratio was an important indicator in metabolic growth and reproduction processes in crustaceans. It also has a highly significant positive correlation with egg production in *Acartia hudsonica.* However, predictions of egg production in the field are not truly dependent on one or a few components, but are a combination of direct and indirect causal paths which all affect the ability of the female copepods to produce eggs (Jonasdottir *et al.* 1995).

In conclusion, high zooplankton growth rates are observed when their diet is rich in essential fatty acids (Anderson and Pond 2000). This explains the *A. bifilosa* abundance maximum in April/May when it is feeding well on diatoms, but its numerical dominance in winter is more likely due to its ability to successfully switch its diet focus to feed on detritus, (as seen by the increased levels of 18:1(n-7)), rather than an ability to generate PUFAs itself from a poor food source, as PUFA levels in winter were very low.

5.5 Summary

1) *Acartia bifilosa* diapauses from May/June to late September/October and there was a clear delineation between the percentage fatty acid content of *A. bifilosa* before and after diapause, the greatest dissimilarity was between April and October.
2) February, March, April and October samples separate relatively clearly into groups of 90-95% similarity. This was not seen for November, December and January.
3) The saturates (18:0, 16:0 and 14:0) were most responsible for the dissimilarities. The PUFAs 16:1(n-7), 18:1(n-7) and 22:6(n-3) were also important contributors to the dissimilarity.
4) A diatom diet was indicated by increased levels of C_{16} PUFAs, 16:1(n-7) and 20:5(n-3). A dinoflagellate diet was indicated by C_{18} PUFAs and 22:6(n-3). A flagellate diet was indicated by 16:0, C_{18} PUFAs particularly 18:4(n-3) and 22:6(n-3). Elevated levels of 18:1(n-7) suggested bacteria in the diet.
5) April showed high levels of 16:1(n-7), increased levels of C_{16} PUFA and 20:5(n-3) suggesting that *A. bifilosa* was feeding well on diatoms with no need to supplement its diet with bacteria. October samples were high in 22:6(n-3) and 18:1(n-7) indicating dinoflagellates and bacteria respectively. The winter samples (November-January) showed the highest levels of 18:1(n-7) suggesting that *A. bifilosa* is forced to supplement its diet with bacteria, probably from detritus, during this period of poor food availability.
6) *Acartia bifilosa* switches its diet focus throughout the year.
7) The diapause and subitaneous eggs were similar in fatty acid composition.
8) The fatty acid content of the eggs did not reflect the adult make-up.

Chapter 6:
Acartia feeding rates on a variety of food types.

6.1 Introduction

Calanoid copepods are arguably the most abundant and ecologically significant marine group, as they are a fundamental part of the food chain and link all of the trophic levels above. Knowledge of their feeding behaviour, as it is related to the amount of energy taken in by an individual, which in turn sets an upper limit to the organism's productivity, (Gauld 1951; Frost 1972), is therefore essential as their feeding rate also sets up the dynamic equilibrium between the consumer, its food and the remainder of the food web.

Feeding rate is influenced by a number of factors. It is known that grazing rate increases with copepod stage and weight, (Berggreen *et al.* 1988). Allan *et al.* (1977) found a linear relationship between feeding rate and weight on a log-log scale for *Calanus helgolandicus* and *Acartia tonsa.* Nejstgaard *et al.* (1995) observed that cell size is the main regulatory factor for copepod predation in natural food suspensions, and reported a strong general relationship between phytoplankton cell size and copepod clearance rate for algae in the range 3-17μm ESD (equivalent spherical diameter). The optimal cell size for *A. tonsa* increases with developmental stage, and the lower size limit for particle capture at all stages is 2-4μm and the adults can take cells up to 250μm (Allan *et al.* 1977). It was also recorded that the optimum food size (ESD) for *A. tonsa* is 2-5% of the prosome length (Allan *et al.* 1977). The Southampton Water community of *A. clausi* retains particles >20μm with close to 100% efficiency (Barlow and Monteiro 1979).

Mouthpart morphology and size play a vital part in dictating the size of particles that copepod can take. There is no fundamental modification of the structure of *Acartia* feeding appendages during development, except that of growth. Morphological features, (mesh size, surface area etc.) are directly related to cephalothorax length, and so increase in size with age (Nival and Nival 1976). Boyd (1976) describes *Acartia* mouthparts as "leaky sieves" created by a mesh of setules on the various appendages that is not rigid and will distend. *Acartia clausi* can also alter the effective spacing themselves to optimise feeding behaviour (Donaghay and Small 1979). Calanoid copepods filter through their maxillary setae, or use the maxillae more actively to sweep and scoop particles, with the setae spreading downward and sideways to form a filtering funnel (Nival and Nival 1976). *A. clausi* moves its maxillae through the water like a seine net (Donaghay and Small 1979). In most copepods, the mesh size on the maxilliped is larger than those on the second maxilla. In *Acartia*, however, the second maxilliped is reduced. This anatomical peculiarity gives it a larger efficiency per unit effort for the retention of small particles (Nival and Nival 1976).

The type of food also affects what copepods eat, and food is often rejected on "taste" (i.e. quality). Traditionally, diatoms are associated with a productive food web. However, in terms of egg production, diatoms are now considered an inferior food source for copepods (Ban *et al.* 1997). Ianora *et al.* (1996) found that the diatom *Thalassiosira rotula* decreased faecal pellet production, suggesting that the copepods were not feeding, possibly due to inhibitors produced by the diatom, this argument is still open, however. One of the diatom species present in Southampton Water is the chain-forming *Skeletonema costatum,* which Conover (1966) found was assimilated at a relatively low rate by copepods. In contrast, Martin (1970) observed selective grazing on *Skeletonema* by natural populations of zooplankton dominated by *A. tonsa* and *A.*

clausi in Narragansett Bay, and that the organisms preferentially selected the larger chains.

As might be expected from the temporal succession of algae within estuarine blooms, the timing of any laboratory-based feeding experiments may also be important as there is evidence of seasonal physiological adaptation of copepods to certain food types. Donaghay and Small (1979) found evidence of pre-conditioning in *A. clausi* that affected its subsequent feeding. Nejstgaard *et al.* (1995) witnessed a pronounced seasonal shift in the feeding rate of freshly-caught *C. finmarchicus* on *E. huxleyi,* with the highest grazing rates coinciding with the maximum abundance of *E. huxleyi* (May – June) which may be caused by the seasonal adaptation of the copepod. This seasonal physiological adaptation of copepods collected from the wild could potentially bias laboratory-estimated feeding rates.

In this study, the feeding rates of the *Acartia* congeners *bifilosa, discaudata, clausi* and *tonsa* on *Isochrysis galbana* (flagellate), *Phaeodactylum tricornutum* (diatom) and *Skeletonema costatum* (chain-forming diatom) are investigated, and their performance examined with reference to the various characteristics of the food type.

6.2 Method

6.2.1 Feeding rate determination

125ml vials were filled with 26μm filtered seawater at 15°C and 30-32 salinity. For each species, 10 *Acartia* females were placed in individual vials and a different volume of one of the chosen food types, (thus giving different concentrations ranging from 1×10^4 to 1×10^5 cell ml^{-1}) was added to each of the vials. The experiment was left in the dark (to prevent the algae from reproducing) at 10°C for 24 hours at ½ revolution of the plankton wheel min^{-1} to allow the *Acartia* to acclimatise. After 24 hours, the copepods were carefully pipetted into fresh vials containing the original concentration of the algal food source to which they had been acclimated. A 1ml sub-sample was taken from each vial prior to the beginning of each experiment and fixed with Lugol's iodine. A haemocytometer was used to determine this initial food concentration (C_o). The *Acartia* were then left to feed under experimental conditions for a further 24 hours after which Lugol's iodine was added to stain the algal cells. This process killed the copepods and so they could not be used for dry weight determination (instead other females were used for this purpose, see Appendix 2). The number of algal cells ml^{-1} (C_t) was counted and the feeding rate calculated using the Cell Count Method after Frost (1972) and Gauld (1951), and the data corrected to a weight-specific unit (cells hr^{-1} mg dry wt^{-1}).

$$\mathbf{f = \frac{V(C_o - C_t)}{T*wt}}$$

f = feeding rate (cells hr^{-1} mg dry wt^{-1})
V = volume of water (ml)
C_o = initial food concentration (cells ml^{-1})
C_t = food concentration at the end of the experiment (cells ml^{-1})
T = duration of the experiment (hours)
Wt = weight of one *Acartia* female (mg)

Owing to the large variety of C_o concentrations used in the experiments, it was not possible to run C_r vials for them. The procedure was conducted for *A. bifilosa, A. discaudata, A. clausi* and *A. tonsa* with the food types *Isochrysis galbana* (flagellate), *Phaeodactylum tricornutum* (diatom) and *Skeletonema costatum* (chain-forming diatom).

6.2.2 Statistical analysis

Figures of the initial food concentration versus feeding rate were produced for each animal and each food type. Statistical comparisons of the trend line of the three different food types for each species were carried out using the Analysis of Regression Line method from Fowler and Cohen (1990). Significance level was set at P=0.05 or lower.

6.3 Results

A.bifilosa

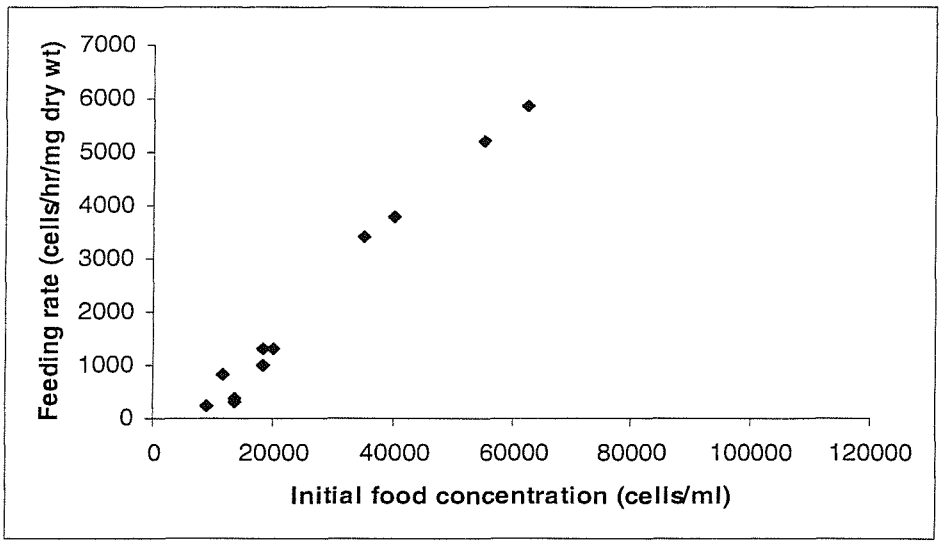


Figure 6.1:The feeding rate of *A. bifilosa* on *Isochrysis.*

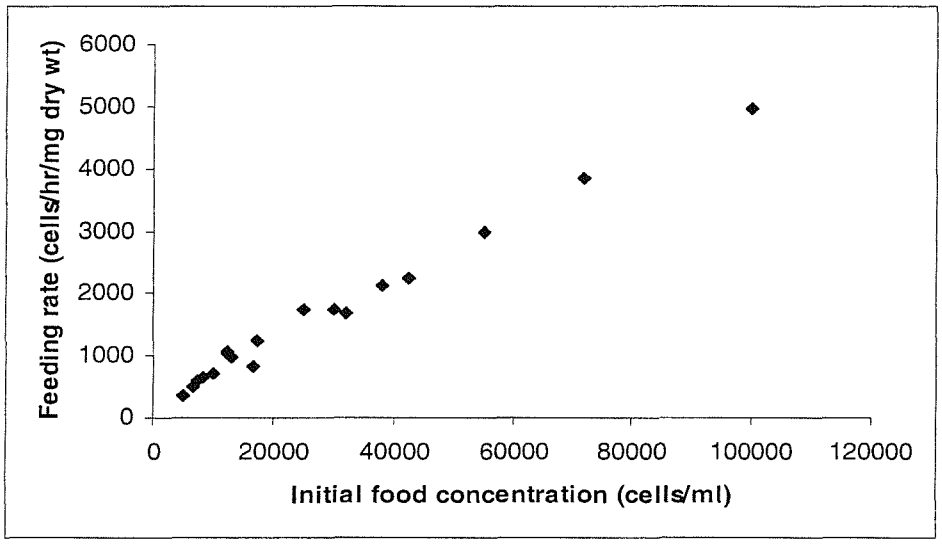


Figure 6.2: The feeding rate of *A. bifilosa* on *Phaeodactyulm.*

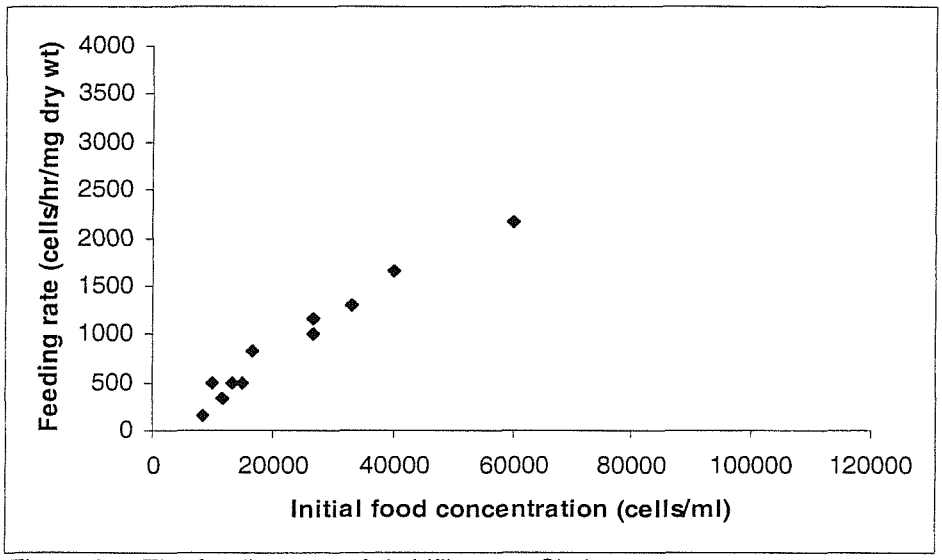


Figure 6.3: The feeding rate of *A. bifilosa* on *Skeletonema.*

Table 6.1: Summary of *A. bifilosa* feeding results.

	Isochrysis	*Phaeodactylum*
Phaeodactylum	P=0.02 Significant	
Skeletonema	P=0.01 Significant	Not significant

Low food concentrations (1×10^4 to 1×10^5 cells ml^{-1}) were deliberately used to mimic conditions the copepods would be exposed to in the field. This should give a clearer idea of feeding rates in Southampton Water with respect to food concentration as well as food type.

The relationship between feeding rate and initial food concentration was a good linear correlation for each food type. Table 6.1 shows the significant differences between the overall trend in feeding rates on the three algae and it can be seen that *A. bifilosa* feeds at statistically significantly (P=0.02) different rates on *Phaeodactylum* and *Isochrysis*, and *Isochrysis* and *Skeletonema* (P=0.01), but the difference between *Phaeodactylum* and *Skeletonema* is not significant. A more revealing picture can be seen by looking at the gradients of figures 6.1-6.3. Readings were taken across from initial food concentrations of 20000, 40000 and 60000 cells ml^{-1} and in each case the highest feeding rate was on *Isochrysis*. So, *A. bifilosa* preferred the flagellate to either of the two diatoms and the results can be summarised: I > S = P, where > indicates a significantly higher feeding rate and = represents no significant difference in the feeding rates.

A.clausi

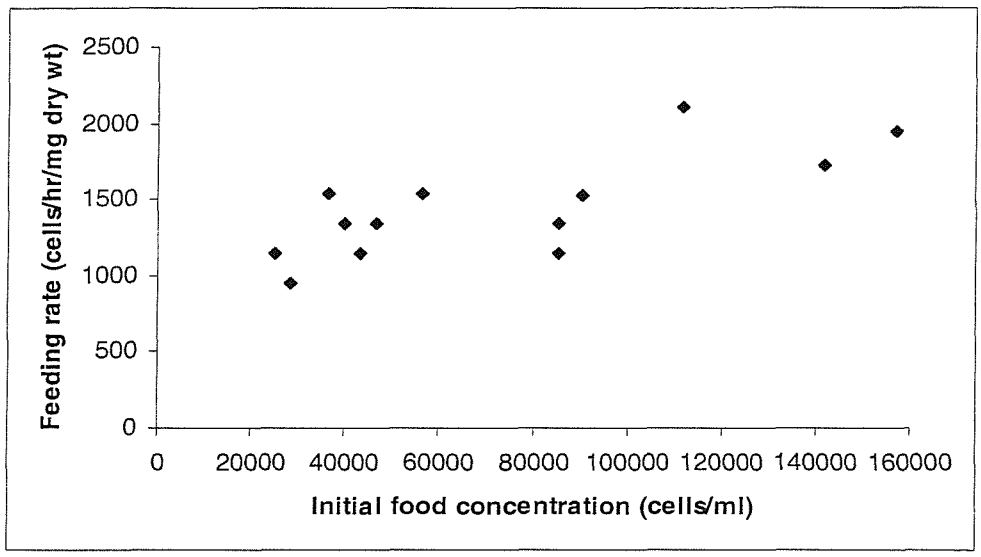


Figure 6.4: The feeding rate of *A. clausi* on *Isochrysis.*

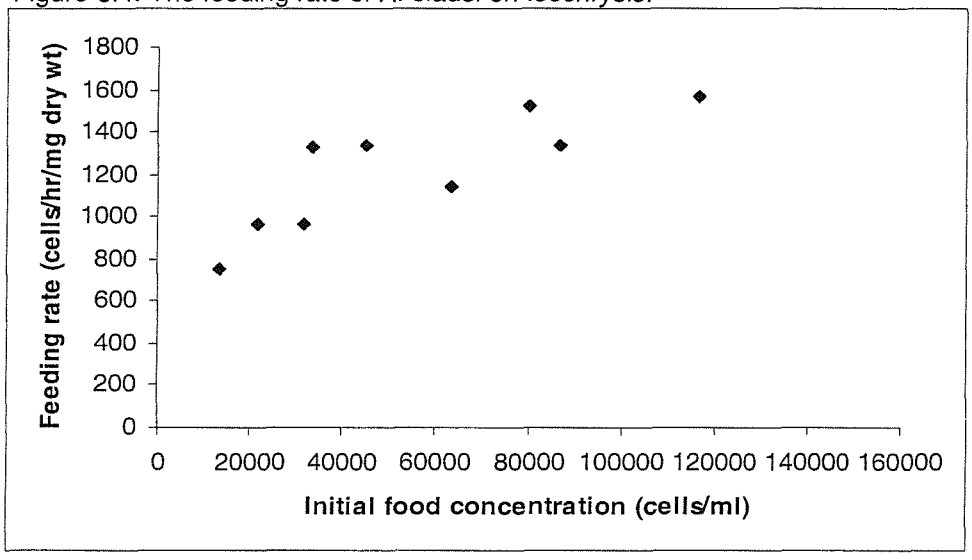


Figure 6.5: The feeding rate of *A. clausi* on *Phaeodactylum.*

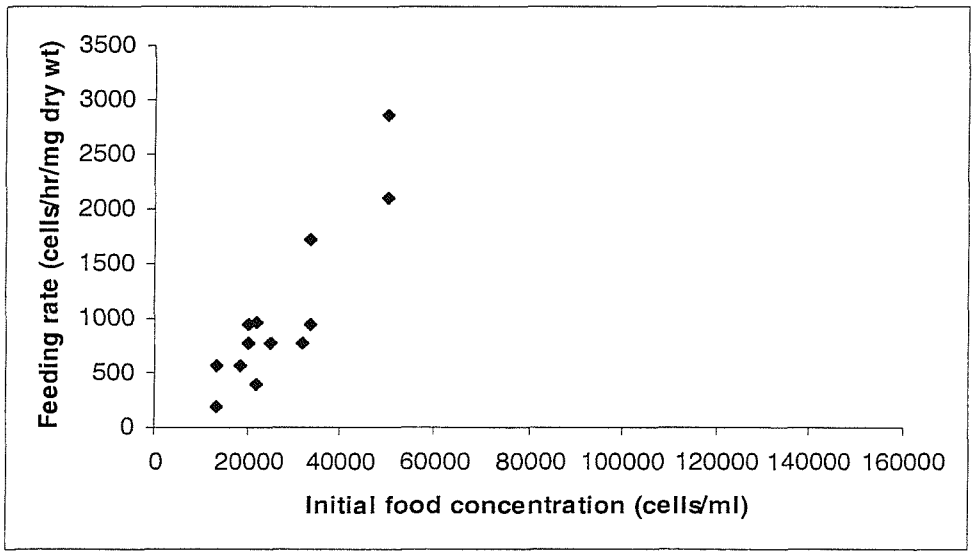


Figure 6.6: The feeding rate of *A. clausi* on *Skeletonema.*

Table 6.2: Summary of *A. clausi* feeding results.

	Isochrysis	*Phaeodactylum*
Phaeodactylum	Not significant	
Skeletonema	P=0.01 Significant	P=0.01 Significant

The overall feeding behaviour of *A. clausi* on the three food types was different to *A. bifilosa*, in that there was no significant differences between the feeding rates on *Isochrysis* and *Phaeodactylum*. However, there was a significant difference (P=0.01) between the results for *Isochrysis* and *Skeletonema,* and *Skeletonema* and *Phaeodactylum*. Looking at the gradients in figures 6.4-6.6 at the 20000, 40000 and 60000 cell/ml initial food concentration levels, *Skeletonema* is the preferred food source at the higher two concentrations, but looking across at 20000 cells ml^{-1}, the feeding rates for *Isochrysis* are the highest and lowest were for *Skeletonema*. Generally, S > I = P, although at the lowest concentrations as experienced in the field, *A. clausi* may feed best on *Isochrysis*.

Again, all the feeding relationships were linear, although problems did occur with the *Skeletonema* culture during this experiment, and it was not possible to use initial food concentrations greater that ~65 000 cells ml^{-1}.

A.discaudata

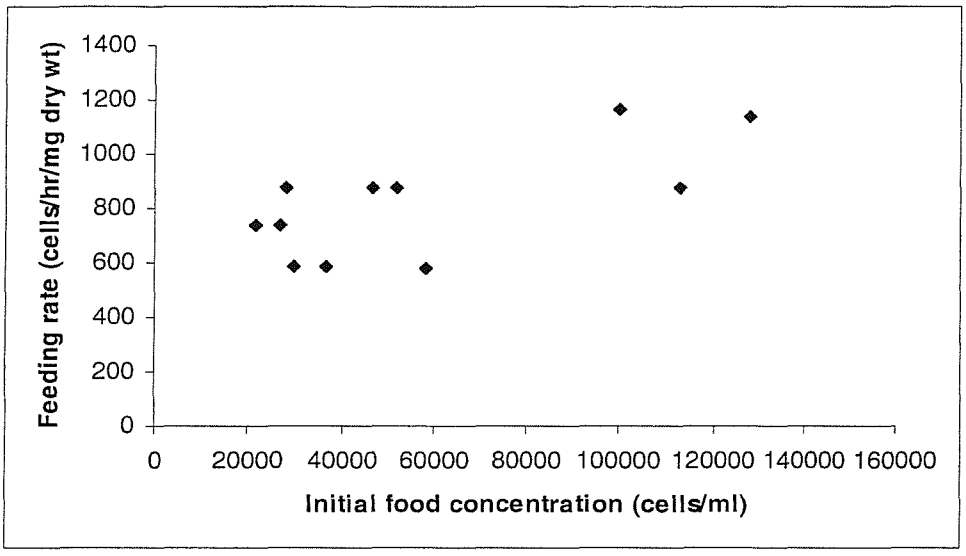


Figure 6.7:The feeding rate of *A. discaudata* on *Isochrysis.*

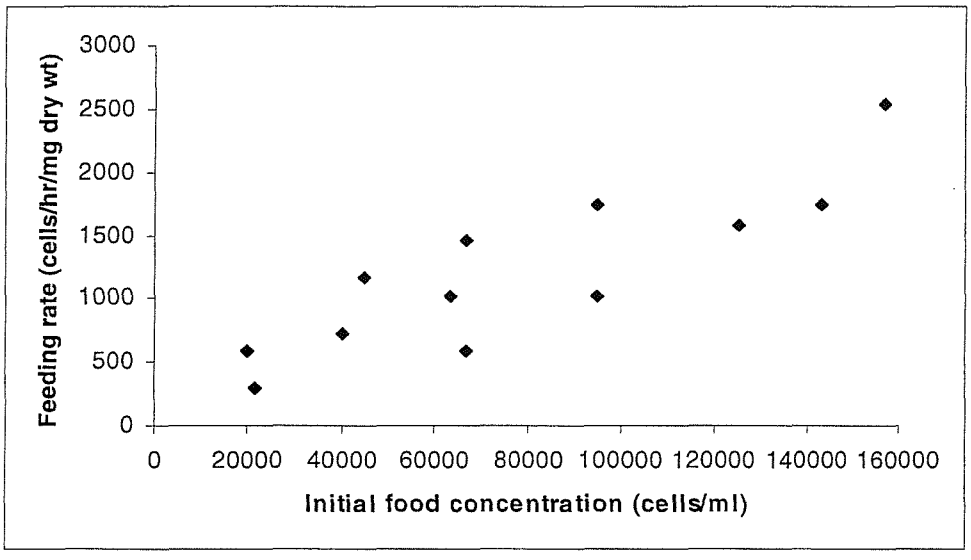


Figure 6.8: The feeding rate of *A. discaudata* on *Phaeodactylum.*

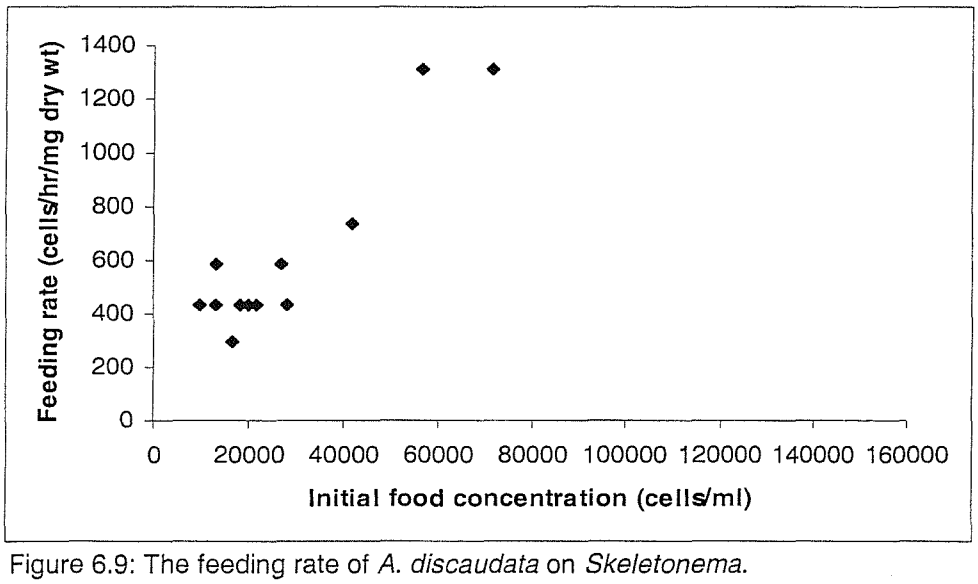


Figure 6.9: The feeding rate of *A. discaudata* on *Skeletonema.*

Table 6.3: Summary of *A. discaudata* feeding results.

	Isochrysis	*Phaeodactylum*
Phaeodactylum	P=0.01 Significant	
Skeletonema	Not significant	P=0.01 Significant

Figures 6.7-6.9 show linear relationships again, but the three different gradients make comparisons difficult. At 20000 cells ml^{-1}, *Isochrysis* is taken up at a much faster rate than the two diatoms. However, as with *A. clausi*, at the higher two food concentrations, *A. discaudata* will take up *Skeletonema* most rapidly. Overall, there was a statistically significant difference between the feeding rate on *Phaeodactylum* and the other two algae (Table 6.3) and at the higher initial food concentrations, S > P > I.

A.tonsa

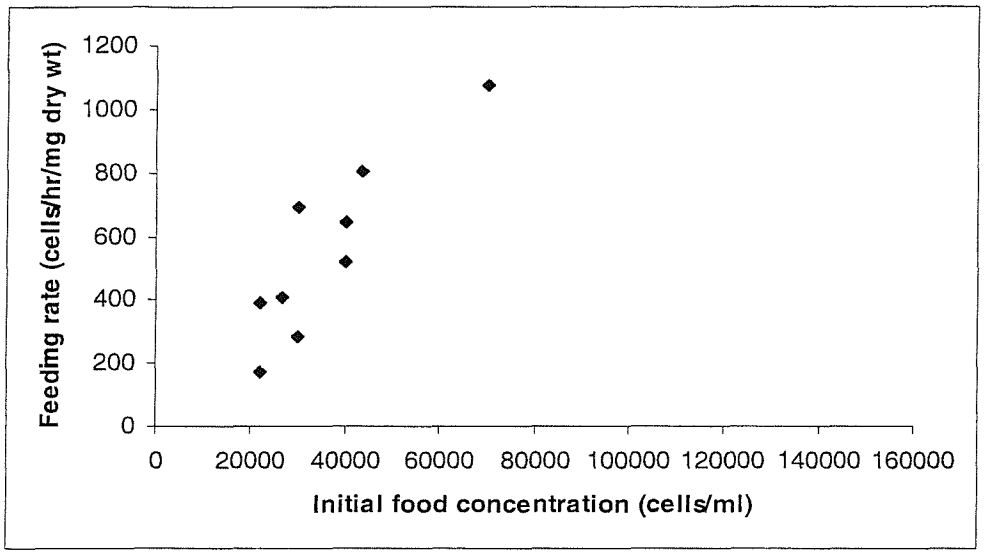


Figure 6.10: The feeding rate of *A. tonsa* on *Isochrysis.*

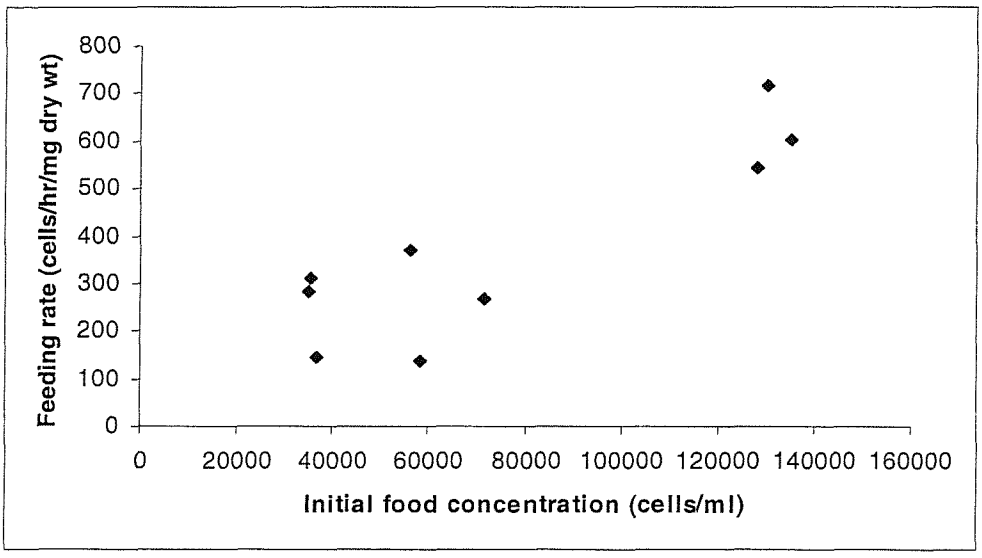


Figure 6.11: The feeding rate of *A. tonsa* on *Phaeodactylum.*

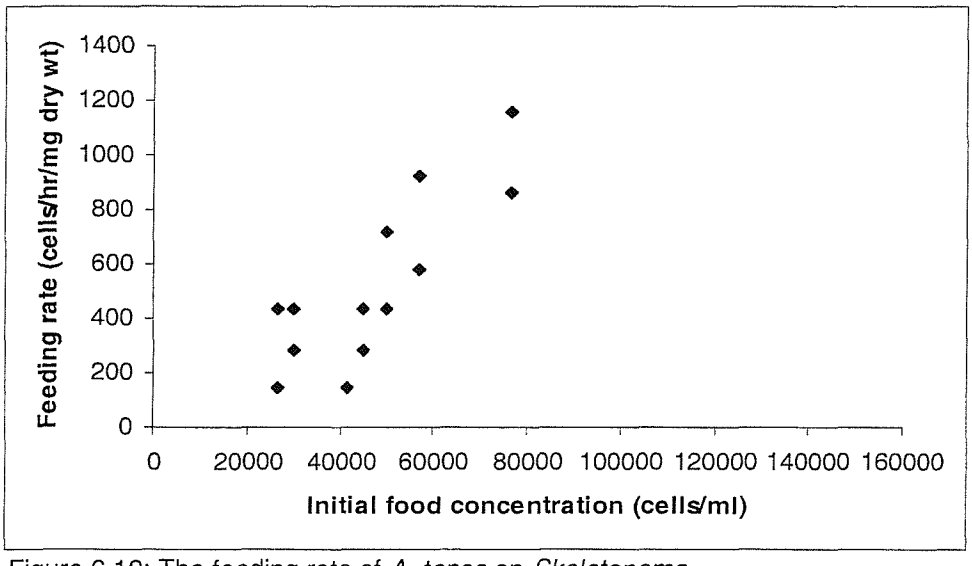


Figure 6.12: The feeding rate of *A. tonsa* on *Skeletonema.*

Table 6.4: Summary of *A. tonsa* feeding results.

	Isochrysis	*Phaeodactylum*
Phaeodactylum	P=0.01	
Skeletonema	P=0.01 Significant	Not significant

Acartia tonsa mirrors *A. bifilosa* and in its feeding preferences. There is a statistically significant difference between the feeding rates of *Isochrysis* and the two diatoms. Graphical evidence from figures 6.10-6.12 indicates that the order of preference for feeding by *A. tonsa* was I > P = S at the three initial food concentrations of 40000, 60000 and 80000 cells ml^{-1}.

6.4 Discussion

From the functional response patterns, it can be seen that *A. clausi* and *A. discaudata* consume *Skeletonema* cells at a greater rate than flagellate (*Isochrysis*) and single-cell diatom (*Phaeodactylum*) cells at the higher food concentrations. However, *A. bifilosa* and *A. tonsa* take *Isochrysis* up fastest of the three food sources, as do the other two congeners at lower initial food concentrations (20000 cells ml^{-1}). So, under conditions similar to those that the copepods experience in the field, all of the congeners feed best on *Isochrysis*. However, beyond this it is difficult to draw any clear conclusions about the feeding preferences of the *Acartia* species. For each food source, *A. bifilosa* shows the highest feeding rates, with *A. tonsa* generally presenting the lowest rates.

The variations in the copepod feeding rates on the range of food sources can be the result of a number of factors. Cell size is the main regulatory factor for copepod predation in natural food suspensions, (Nejstgaard *et al.* 1995). The lower limit for particle capture by adult *A. tonsa* is 2-4 μm, and the optimum particle size range for adults is 14-70 μm (max 250 μm) (Berggreen *et al.* 1988). These figures correspond to 2-5% of the copepod prosome length (Allan *et al.* 1977). Few calanoid species can filter particles smaller that 5μm or larger than 100 μm (Boyd 1976) and most grazers filter in a much narrower size range. In the current study, two of the food types used are classified as "small", (*Isochrysis* = 4.5μm, *Phaeodactylum* = 3.5μm) and but the *Acartia* spp. seemed to have had little difficulty in feeding on them. In contrast, *Skeletonema* at 14-16μm, lies well within the optimum size range for *Acartia* feeding.

Boyd (1976) studied the feeding apparatus of adult *Acartia,* and indicated that the lower limit of particle acceptability is established by the intersetule distance, while the upper limit is set by screening setae on the maxillae that reject large particles from the filtering process. In short, copepod mouthparts are leaky sieves (Boyd 1976). Escapement, the leakage through the setule mesh that results from distension as particles impinge on it, is a function of the distensibility of both the mesh and the object passing through it. Motions of the mouthparts as they beat back and forth in their filtering action will alter the mesh distance of the setules, thereby increasing the variance of the intersetule distances. This process strengthens the perception that the apparatus for filterfeeding is basically a sieve with a wide range of pore sizes. It would be expected that escapement is less for rigid diatoms (*Phaeodactylum*) than for naked flagellates (*Isochrysis*), consequently *Phaeodactylum* should be taken with greater success than *Isochrysis*, but this was clearly not seen in the current results.

A. clausi has two methods of feeding; active and passive (Ayukai 1987). This copepod captures individually perceived cells in active mode, and poorly perceived ones by passive means. In the active mode, copepods bring individual cells to their mouthparts by the manoeuvring movements of feeding appendages. They then capture the water parcel with the cell inside and remove the water by squeezing it out. In the passive mode, copepods accumulate cells near the mouthparts by continuous low-amplitude vibration of the second maxillae, and then ingest these cells somewhat inefficiently. In contrast, *A. tonsa* mouth morphology differs from that of most calanoid copepods, having a reduced maxilliped and using its second maxillae as a "scoop" much like the cirral net of a barnacle (Allan *et al.* 1977). Irigoien and Castel (1991) state that *A. tonsa* cannot feed effectively on small particles since it does not create low amplitude mandible II motions, and consequently it actively selects large, good quality particles,

and so its ingestion rate should increase with particle diameter, (Roman 1984). However, Berggreen *et al.* (1988) maintain that *A. tonsa* can take particles as small as 2-4μm and this was seen in the current work as *A. tonsa* fed most successfully on *Isochrysis* (4.5μm), and worst on the two diatoms, *Skeletonema* (14-16μm) and *Phaeodactylum* (3.5μm). Copepods can also sense the nutritional value of food, therefore, small particles of good quality are taken in preference to larger, poor quality cells, (Ambler 1986, Ayukai 1987). Acartiidae have fewer chemoreceptors on their first antennae (A1) than other copepods like *Calanus* spp., but they do have a pronounced ability to sense hydrodynamic signals, (Parrish and Wilson 1978). Stimulation of both the chemo-, and especially the mechanoreceptors, increases ingestion rate, (Roman 1984).

Food quality, (type/ "taste") is another major determinant of what copepods will take. Diatoms have been considered a good food source, but in terms of egg production they are an inferior food for copepods (Ban *et al.* 1997; Ianora *et al.* 1995; Ianora etal 1996; Ianora *et al.* 1999). However, this did not stop the *Acartia* congeners in the present study from feeding on diatoms and *Skeletonema* was one of the more popular food sources over all. The faecal pellets produced were not investigated however, so it is not known how well the algal species were assimilated.

Another potential influence on copepod feeding rate is food pre-conditioning (Donaghay and Small 1979). Nejstgaard *et al.* (1995) found distinct evidence for seasonal physiological adaptation of freshly caught *C. finmarchicus* feeding on *E. huxleyi,* with the highest feeding rates in the laboratory coinciding with the maximum abundance of *E. huxleyi* in the water column. There was some evidence of this in the current feeding study as all of the experiments were performed in February and March

when *Isochrysis* and *Skeletonema* were present in the water column (Kifle 1992), and all of the congeners fed best on one of these two food types. Many calanoids are omnivorous and will take, in addition to algae, nauplii, rotifers, ciliates and detritus in their diet, (Stoecker and Egloff 1987; Irigoien and Castel 1995) and although grazing predominantly on microplankton, (2-200μm) some species take detritus of a similar size (Stoecker and Egloff 1987).

In short, copepod feeding *in situ* is much more complex than simply feeding on the resident phytoplankton. *Acartia* spp. are opportunistic feeders, and will simply take whatever food is available (Jonasdottir 1994 and Koski and Kuosa 1999). They are not adapted to low food concentrations, (Parrish and Wilson 1978, Uye 1981) and are food limited in nature, (Irigoien and Castel 1996; Checkley *et al.* 1992) this in turn limits their distribution. Paffenhofer and Stearns (1988) concluded that *A. tonsa* is restricted to estuaries and areas of upwelling where it encounters high food concentrations, because it cannot obtain enough food offshore to sustain its production. In feeding experiments, there is a simple, positively linear relationship between food concentration and ingestion rate, which is in part, a function of food particle size (Nejstgaard *et al.* 1995). Food quality also has a great influence, but "quality" itself is more than a result of food particle diameter and quantity, (Cahoon 1981). Requirements for successful reproduction in *Acartia* are complex and are not satisfied by one food type alone, a varied diet is best as it supplies a balance of nutrients. Literature, and the results generated by the current study suggest that one will not see marked differences in feeding rate in terms of food "quality" because this is not a sensitive enough measure. Instead, it is more likely any effect will be seen in the egg production rates and egg viability, and this can likely be explained in terms of FAA and PUFA content of the food.

6.5 Summary

Acartia females from each species were fed a variety of food types (*Isochrysis, Phaeodactylum* and *Skeletonema*) at varying concentrations (1×10^4 to 1×10^5 cells ml^{-1}). Feeding rate was determined using the Cell Count Method of Gauld (1951) and Frost (1972) to see if food type as well as concentration affected feeding rate. The low food concentrations were deliberately used to reflect conditions in the field.

1) *Acartia bifilosa*

The relationship between feeding rate and initial food concentration was linear for each food type.

This congener feeds at a significantly higher rate on *Isochrysis* (I) than on the two diatoms *Phaeodactylum* (P) and *Skeletonema* (S), (P=0.02 and P=0.01 respectively) at the initial food concentrations 20000, 40000 and 60000 cells ml^{-1}.

So, **I > S = P**

This congener also had the highest feeding rates overall.

2) *Acartia clausi*

Again, there was a linear relationship between the initial food concentration of each food type and feeding rate.

No significant differences between the feeding rates on *Isochrysis* and *Phaeodactylum*, but there were significant differences (P=0.01) between the feeding rates on *Skeletonema* and the other two diets.

At higher initial food concentrations (40000 and 60000 cells ml^{-1}) *Skeletonema* was the preferred food source, but at 20000 cells ml^{-1} *Isochrysis* was fed on at a higher rate.

Generally, **S > I = P**

3) *Acartia discaudata*

There was a linear relationship between initial food concentration of the three food types and feeding rate.

At the lower initial food concentrations, this congener preferred *Isochrysis*. However at higher concentrations it fed at a higher rate on *Skeletonema*.

The only significant differences in feeding rate were between *Phaeodactylum* and the other two diets.

At higher food concentrations, **S > P > I**

4) *Acartia tonsa*

Mirrors *A. bifilosa* in its diet preferences.

Once again, there is a linear relationship between feeding rate and initial food concentration.

Isochrysis is fed on at a significantly different rate to the other two food types (P=0.01).

At all initial food concentrations, **I > S = P**

This congener had the lowest feeding rates overall.

5) *Acartia* are opportunistic feeders and will feed on whatever is available. They have no storage abilities.

Chapter 7:
The effect of food type on *Acartia* fecundity

7.1 Introduction

Egg production and viability in marine copepods are known to be influenced by a range of circumstances. Important extrinsic factors are temperature (Ambler 1985, 1986; Ban 1994; Castro-Longoria 1998; Deason 1980; Durbin *et al.* 1983; Kiorboe *et al.* 1988; Koski and Kuosa 1999; and Hassett *et al.* 1993), salinity (Ambler 1986; Bhattacharya 1986; and Castro-Longoria 1998) and dissolved oxygen concentration of the water (Lutz *et al.* 1992). All these parameters have been studied extensively, especially in *Acartia* congeners (Castro-Longoria 1998). The present study will investigate the effect of food, the major extrinsic or "biological" variable, on fecundity, more specifically food composition as opposed to concentration which has already been examined (Marshall and Orr 1972).

Fecundity is defined as the total number of viable eggs, i.e. those which hatch successfully, produced by a copepod in its lifetime, (Ambler 1985). While the quality of the diet may overcome a limited food supply to support egg production, egg viability, however, cannot be maximised under poorer food conditions (Ianora and Poulet 1993). Feeding history is known to have a strong effect on the egg production rate (total EPR/day) of *Calanus finmarchicus* (Hirche *et al.* 1997). There is a time lag before copepods produce eggs, ~10-90 hrs depending on the copepod species (Tester and Turner 1990). Before this phase, the new food is not incorporated into the egg and therefore, fecundity is still influenced by the past feeding history of the females. After this time, freshly ingested food is used as nutrition for oocyte production. Consequently, it is necessary to incubate the females for 24hrs before beginning any experiment.

It is not well established which food "properties" are responsible for variations in a copepod's fecundity response. Ambler (1985) found that the type of diet did not affect *A. tonsa* fecundity. Similarly, *C. helgolandicus* egg production and hatch success, (in the short term of <24 hr) was not affected by food quantity or type (Laabir *et al.* 1995). In contrast, Cahoon (1981) reported significant food quality effects on the reproduction of *A. tonsa*, as did Kleppel and Burkart (1995). Both studies found reduced fecundity and viability with a unialgal diet because it did not offer a nutritionally balanced ration. Recent literature suggests that diatoms are a poor food source (Ban *et al.* 1997; Kleppel and Burkart 1995). In the field, *C. finmarchicus* spawned at a greatly reduced rate during the spring (diatom) bloom (Hirche *et al.* 1997) and observations made in the lab indicated that the diatoms *S. costatum* and *Phaeodactylum* sp. similarly greatly reduced both fecundity and hatch success of *A. clausi* (Ban *et al.* 1997).

In further studies on the effect of food type, Ianora (1993) reported that although *Prorocentrum minimum* (dinoflagellate) and the *Thalassiosira rotula* (diatom) diets caused *Temora stylifera* to lay a similar number of eggs per day, there was a great discrepancy in the hatch success, with greater success with *P. minimum* . This could be accounted for by the fact that dinoflagellates have more lipids, carbohydrates and proteins per cell volume than diatoms (Gill and Harris 1987; Kleppel *et al.* 1991). Copepods fed *T. rotula* also produced more faecal pellets, indicating poor ingestion, possibly owing to the silica frustules of the diatom making the cells difficult to digest. When the *Temora stylifera* was fed *Isochrysis galbana* (flagellate) a high EPR was observed, with the vast majority of eggs developing successfully (Ianora *et al.* 1995). In contrast, while *Phaeodactylum* produced a poor EPR with as low as 20% hatch success, the effect of *Skeletonema* was even worse, with eggs produced for only 3-4 days before

the females became sterile or died (Ianora *et al.* 1995). This low viability was blamed on the deficiency of some essential nutrient, or the presence of an unidentified inhibitor in the diatom species. Continuing this work, Ianora *et al.* (1996) examined the effect of the diatom *T. rotula* on freshly-caught *A. clausi* reproduction, and once again found poor faecal pellet production and reduced EPR with a very low percentage hatch success. It was concluded that inhibitors produced by the diatom, rather than bacteria associated with the diatom, blocked embryogenesis in the copepod. Contrary to this, Jonasdottir and Kiorboe (1996) working on *A. tonsa* in the laboratory concluded that there were no inhibitory or toxic effects of diatom cell components on hatching, although there is still much debate over the matter. Also, the results of Ianora *et al.* (1996) do not relate to *in situ* conditions where the food concentration is much lower.

It is generally accepted that food quality, in terms of fatty acid composition and controlled largely by maternal nutrition, does affect copepod egg production (Jonasdottir 1994; Jonasdottir and Kiorboe 1996) but not hatching success (Ban *et al.* 1997). The EPR of *Acartia* spp. show a marked correlation with the PUFAs (polyunsaturated fatty acids) 20:5(n-3) (eicosapentaenoic acid; EPA) and 22:6(n-3) (docosahexaenoic acid; DHA) (Jonasdottir 1994; Anderson and Pond 2000). However, Pond *et al.* (1996) found that *C. helgolandicus* viability correlated best with the concentrations of 18:2(n-6) and 20:4(n-6) fatty acids in the environment and eggs. EPA and DHA (of which there are high concentrations in both flagellates and dinoflagellates) showed no relationship with egg viability or naupliar survival. C_{16} acids are the most common PUFAs in diatoms, with C_{18} acids being prevalent in dinoflagellates (Mayzaud *et al.* 1989). In this study, the PUFA content of the variety of food types used in the fecundity experiments will be investigated and used to determine how, or if, the

chemical composition of diet influences *Acartia* reproduction, and if this can be used to define a "good" food source.

7.2 Method

7.2.1 Egg production

Egg production was measured for all 4 *Acartia* congeners, fed with three food types; *Isochrysis galbana, Phaeodactylum tricornutum* and *Skeletonema costatum* at 18°C, and at 25 salinity. It was not possible to bulk culture the dinoflagellate *Scrippsiella trochoidea* and so this alga was not offered as a food source.

It has long been considered that remating is necessary for *Acartia* to produce eggs under field conditions (Wilson and Parrish 1971, Uye 1981), however, Ianora *et al.* (1996) found no evidence of this for *A. clausi*. Laabir *et al.* (1995) also had some concerns about crowding, and determined that experimental densities of less than 10 females per litre are necessary as, above this there is a significant reduction in daily egg production rate. For this experimental programme, 3 replicates of 3 females and 1 male were placed in an egg separation tube, (a plastic tube with a 200μm mesh on one end), which was then held supported within a 1l beaker filled with 650ml of 26μm mesh size filtered seawater from the SOES aquarium, (30-32 salinity) to which distilled water was added to obtain the desired salinities).

Ban (1994) observed *Eurytemora affinis* food limitation at 10^3 cells ml^{-1} when fed on a 1:1 mixture of *Chlamydomonas reinhardii* and *Cryptomonas tetrapyrenoidosa*. To avoid this effect, excess concentrations (10^5 cells ml^{-1}) of each food type were initially added to the egg separation chamber, and the set-up placed at the experimental temperature and left for 24 hours for the copepods to acclimatise to the experimental conditions. Unlike Castro-Longoria (1998), the copepods were not gradually adjusted to

the salinities, as no evidence of impairment of copepod performance (i.e. egg-laying) was found when they were placed directly into the experimental salinity. The egg production chambers were subjected to a 12L:12D photoperiod as there is evidence for a diel variation in egg production rates (Checkley *et al.* 1992), as well as evidence for nocturnal spawning in copepods (Marcus 1985).

The egg production rate for each experimental regime was determined daily (i.e. every 24 hours) over a continuous 5 day observational period. Each morning, individual pots containing the *Acartia* were carefully removed and the water filtered through a 45μm mesh to collect the eggs. Fresh water, at the correct temperature and salinity, and food was then placed in the 1l beaker and the pot replaced. Eggs were counted with a binocular microscope. The egg production rates were recorded as the number of eggs produced per female per day, using the equation from Bautista *et al.* (1994). No nauplii were seen in any of the replicates.

$$\mathbf{EPR = [(E + N) / F] \times [24/ T]}$$

EPR = egg production rate (eggs female^{-1} day^{-1})
E = number of eggs
N = number of nauplii
F = living females
T = incubation time in hours

The results are reported as the mean and standard deviation (± 1SD) for each experimental situation.

7.2.2 Fatty acid analysis of algae

The percentage composition of the total fatty acid content of four algal food sources was determined using a method based on Folch *et al.* (1957) and Olsen and Henderson (1989). The algal cultures were filtered onto ashed filter paper, then soaked in 8ml chloroform:methanol (2:1) to obtain a concentrated sample for fatty acid analysis. This was then sonicated for 30 seconds and two 500µl sub-samples for each algal species were taken for the analysis procedure, as detailed.

To the 500µl sub-sample:

Lipid extraction

- Add 125µl KCl
- Whirlimix
- Centrifuge
- Discard upper layer
- Blow samples down under N_2

Alkaline hydrolysis

- Add 100µl 1M KOH in 95% ethanol
- Heat for 1 hour at 78^0C
- Cool samples down
- Add 250µl H_2O per sample
- Acidify with 2 drops of 0.6M HCl (check with pH paper)
- Extract twice with 250µl diethylether (N.B. fatty acids are in the ether in the upper layer): Add the ether to the first tube, whirlimix, centrifuge, transfer upper organic layer into second tube, repeat.
- Blow samples down under N_2

Preparation of PFB esters

- Add 30µl acetonitrile (agitate to dissolve sample)
- Add 100µl pentafuorobenzyl (PFB) bromide (3.5% in acetonitrile)
- Add 100µl triethylamine
- Agitate to dissolve sample
- Leave for 15 mins at room temperature (agitate occasionally)
- Add 500µl isooctane
- Whirlimix, centrifuge, transfer upper layer into second tube (PFB esters are in upper layer), repeat.
- Blow samples down under N_2.

Purification

- Add 100µl isooctane
- High performance thin-layer chromatography (HPTLC) with 50µl PFB standard in small tank. (Solvent = 18ml hexane: 2ml diethylether: 0.2ml acetic acid), apply samples far apart in small spots on 10×10×0.25cm silica gel plates.
- Dry plates briefly, spray standards only with DFC (dissolved in methanol), dry under vacuum.
- Scrape upper band off plates into 8ml vials containing 2ml isooctane (or hexane:diethylether (1:1)).
- Add 1ml $NaHCO_3$ (2%)
- Whirlimix
- Centrifuge
- Freeze

Injection

- Decant upper layer (solvent) off of the frozen sample into a new 8ml vial.
- Blow down under N_2 until about 1ml is left, transfer this into a small, pointed vial.
- Blow down entirely
- Dry in desiccator
- Add 50µl isooctane
- Ready to inject into the GC to obtain a fatty acid trace.

For reasons of assay sensitivity and biological relevance, only a fixed range of fatty acids was looked at, and the percentages of these were summed to a total of 100%.

7.2.3 Statistical Analysis

In the case of egg production results, ANOVA (Fowler and Cohen 1992) was used to determine if there was a statistically significant variation in the data sets. All significant differences were taken at the $P<0.01$ level.

7.3 Results

It was not possible to bulk culture the dinoflagellate *Scrippsiella trochoidea* so no fecundity data was obtained, although its fatty acid composition was determined for comparison with the three species offered as experimental diets.

7.3.1 Egg production

Table 7.1: Mean number of eggs laid female^{-1} day^{-1} (± 1 SD).

Congener	Food type			ANOVA result
	Isochrysis	*Phaeodactylum*	*Skeletonema*	
A. bifilosa	9.3(5.68)	4.6(4.02)	3.5(1.37)	P<0.01
A. clausi	15.2(3.18)	4.0(3.07)	4.0(1.46)	P<0.01
A. discaudata	15.7(6.04)	6.8(2.31)	3.9(0.74)	P<0.01
A. tonsa	12.4(3.68)	4.0(2.54)	1.6(1.40)	P<0.01

ANOVA was used to compare the means of the egg production results for the three algae for each copepod species. In each case, the results are the same, with females of each congener producing the most eggs when fed on *Isochrysis*. The next most successful diet, in terms of fecundity is *Phaeodactylum*, followed by *Skeletonema*. This corresponded to the feeding experiments, described in chapter 6 where, generally, *Isochrysis* was the preferred food source at lower concentrations (20000 cells/ml). However, at higher concentrations similar to those used in this investigation, *Acartia clausi* and *A. discaudata* showed high feeding rates on *Skeletonema*. This may be due to its size as *Skeletonema* has a diameter of 14-16μm and forms chains and so is much larger than either *Isochrysis* or *Phaeodactylum* (4.5μm and 3.5μm diameter respectively). So, although *Skeletonema* is possibly easier for *Acartia* spp. to handle

physically and thus is fed on more readily, it is presumably low in terms of quality, as all four congeners produce the least number of eggs when fed this diet.

All of the fecundity experiments were carried out in winter when flagellates are at high concentrations in Southampton Water. Preconditioning could possibly explain the higher fecundity when the animals were fed *Isochrysis*, although *Skeletonema* is also present in the water column at this time (Kifle 1992), but the 24hr acclimation time should have been sufficient to prevent this.

Although the mean number of eggs laid for each species are low compared to earlier studies, the data are still complementary (Castro-Longoria 1998). The differences may be explained by the season, as some *Acartia* are considered winter species and some summer, and might be expected to perform better in their preferred season.

Table 7.2: Comparison of *Acartia* fecundity with a previous study.

Congener	**Castro-Longoria (1998) Max. n° eggs female^{-1} d^{-1}**	**Castro-Longoria (1998) Mean n° eggs female^{-1} d^{-1}**	**This study (*Isochrysis*) Mean n° eggs female^{-1} d^{-1}**
A. bifilosa	15	9.5 (Mar, 15°C, 30)	9.3 (Nov)
A. clausi	37	9.9 (Oct, 17°C, 32)	15.2 (Oct)
A. discaudata	15	6.7 (Sept, 15°C, 32)	15.7 ((Nov)
A. tonsa	29	11.2 (Oct, 15°C, 32)	12.4 (Jan)

The quality of the diet was also assessed in terms of fatty acid content.

7.3.2 Fatty acid analyses

Table 7.3: Percent fatty acid composition of *Phaeodactylum* (single-celled diatom), *Isochrysis* (flagellate), *Scrippsiella* (dinoflagellate) and *Skeletonema* (chain-forming diatom).

Fatty acid	*Phaeodactylum*	*Isochrysis*	*Scrippsiella*	*Skeletonema*
14:0	11.25	28.48	21.93	24.34
16:0	16.24	22.23	30.22	18.53
16:1(n-9)	3.06	2.68	5.05	1.46
16:1(n-7)	18.35	7.18	5.98	20.58
16:1(n-5)	3.49	0.44	0.11	1.84
16:2(n-3)	5.93	0.00	0.00	8.61
16:3(n-3)	8.97	0.00	0.00	9.56
18:0	5.68	4.04	6.53	3.27
18:1(n-9)	6.96	9.45	7.70	1.51
18:1(n-7)	1.49	3.59	1.43	2.93
18:2(n-6)	3.29	5.15	5.94	1.31
18:3(n-3)	1.02	8.35	5.67	0.51
18:4(n-3)	0.66	6.80	6.38	0.96
20:5(n-3)	11.72	0.36	0.49	4.17
22:6(n-3)	1.91	1.22	2.56	0.40

As seen from Table 3, the principle fatty acids of diatoms are16:1(n-7) and 20:5(n-3), with the C_{16} PUFAs predominant. In contrast, the major fatty acids of dinoflagellates are 18:4(n-3) and 22:6(n-3) and the C_{18} polyunsaturates (Mayzaud *et al.* 1989). The fatty acid composition of the flagellate *Isochrysis* more closely resembles that of *Scrippsiella* than the two diatoms.

7.4 Discussion

Calanoid copepod feeding and egg production rates have been investigated by a number of workers. The species studied include *Calanus finmarchicus* , notably by Hirche *et al.* (1997), Frost (1972, 1977) and Marshall and Orr (1952; *Centropages furcatus* (Checkley *et al.* 1992); *Paracalanus* spp. (Checkley 1980) and *Eurytemora affinis* (Heinle *et al.* 1977, Ban 1994). In-depth studies have been conducted on *Acartia* spp., most commonly on *Acartia tonsa* (Allan *et al.* 1977; Ambler, 1985, 1986; Beckman and Peterson 1986; Berggreen *et al.*1988; Cahoon 1981; Castro-Longoria 1998; Dagg 1977; Durbin *et al.* 1983; Gifford and Dagg 1988; Jonasdottir 1994; Jonasdottir and Kiorboe 1996; Kleppel and Burkart 1995; Lutz *et al.* 1992; Parrish and Wilson 1978; Roman 1984; Stoecker and Egloff 1987; Stottrup and Jensen 1990 and White and Roman 1992) and *Acartia clausi* (Ayukai 1987; Castro-Longoria 1998; Donaghay and Small 1979; Ianora *et al.* 1996; Tiselius 1989; Uye 1981). *Acartia bifilosa* behaviour has also been studied intensively, (Castro-Longoria 1998; Irigoien and Castel 1995; Koski and Kuosa 1999 and Uriarte *et al.* 1998).

Fecundity is generally governed by a number of parameters, including; temperature, (Ambler 1985, 1986; Koski and Kuosa 1999; Uriarte *et al.* 1998 and White and Roman 1992), salinity, (Ambler 1985, 1986), food concentration/quantity, (Ambler, 1985, 1986; Cahoon, 1981; Hirche *et al.* 1997; Jonasdottir 1994; Kleppel and Burkart 1995; Koski and Kuosa 1999 and Marshall and Orr 1952) food quality, (Ambler 1986; Cahoon 1981; Heinle *et al.* 1997; Irigoien and Castel 1993; Jonasdottir 1994; Kleppel and Burkart 1995; Marshall and Orr 1952; Roman 1984; Stoecker and Egloff 1987 and Stottrup and Jensen 1990), feeding history, (Beckman and Peterson 1986; Edmondson

1962 and Hirche *et al.* 1997) and ingestion rate, (Cahoon 1981; Kiorboe *et al.* 1985 and Stottrup and Jensen 1990).

Temperature and food availability are generally considered the most important factors influencing post-embryonic development time, (clutch size/egg production and body size/growth). Ban (1994) found that *Eurytemora affinis* individuals reared under food limited conditions (i.e. 10^3 cells ml^{-1}) in the laboratory needed a longer period to mature and produced fewer eggs than well-fed ones reared at a lower temperature. This suggests that food concentration is a more influential factor than temperature. Adult body size of *E. affinis* was also more affected by food shortage than temperature. Prosome length of malnourished females was 75% of those of well-fed ones. However, egg production is a more sensitive parameter to food shortage than somatic growth (Ban 1994) and feeding rate. The excess food concentrations used in many laboratory experiments do not always assure adequate food quality and food limitation for egg production may occur, even if the animals are not limited in their somatic growth by food shortage.

So, food quality/type is another factor which influences copepod ingestion rates and hence fecundity. In the current study, all of the *Acartia* congeners showed a statistically significant greater egg production when fed on the flagellate *Isochrysis* compared to the other two algal diets. However, it is difficult to quantify food quality, as copepods appear to respond positively to a number of different components of their food. Although a good correlation was seen between the nitrogen/protein content of the food and the number of eggs laid by *A. tonsa* per day, Jonasdottir (1994) found that the strongest relationship was with the lipid fraction of the phytoplankton, specifically the (n-3):(n-6) fatty acid ratio, and concluded that the lipid content of the food was

important for egg production, whereas the protein was responsible for the somatic growth of nauplii and copepodites. Jonasdottir and Kiorboe (1996), and Stottrup and Jensen (1990) support this view in their work on *A. tonsa.*

Analyses were conducted to determine the fatty acid composition of the marine algae used in the egg production investigations (Table 7.3). The polar lipid composition of glycolipids and phospholipids in *Isochrysis* is typical of a photosynthetic eukaryotic algae (Ben-Amotz and Tornabene 1985). *Isochrysis* is rich in PUFA (Cheucas and Riley 1969). Its main synthesised fatty acids are 14:0, 16:0, 18:1(n-9), 18:3(n-3), 16:1(n-7) and 18:4(n-3) (Table 7.3). These results are similar to the findings of Cheucas and Riley (1969), Volkman *et al.* (1989) and Ben-Amotz and Tornabene (1985). However, unlike the results of the latter two references, *Isochrysis* was seen to contain comparatively low levels of 22:6(n-3) indeed, only *Skeletonema* contained less. Although not investigated here, *Isochrysis* also lacks the fatty acid 16:1(n-13) which is present in almost all other classes of microalgae (Volkman *et al.* 1989). *Isochrysis* also contains low levels of 20:5(n-3) (Table 7.3, Volkman *et al.* 1989; Dunstan *et al.* 1992). Dunstan *et al.* (1992) found that *Isochrysis* also produces the unusual 18:5(n-3) fatty acid. However, it is not known whether 18:5 (n-3) is formed by further desaturation of 18:4(n-3) or from chain-forming of 20:5(n-3) (Dunstan *et al.* 1992).

Dinoflagellates are low in long chain PUFAs whereas diatoms have a comparatively high concentration of PUFAs, and hence play a significant role in supplying the marine food chain with these molecules (Collier 1965). Consequently, limitation by PUFAs is most prevalent when dinoflagellates dominate the diet (Anderson and Pond 2000). The most common PUFAs in dinoflagellates are the C_{18} acids (Mayzaud *et al.* 1989). More specifically, *Scrippsiella* was seen to contain a high proportion of the PUFAs 18:1(n-9),

18:3(n-3), 18:2(n-6), 18:4(n-3) and 22:6(n-3) and the saturates 14:0 and 16:0 (Table 7.3). It is believed that the C_{22} PUFA could result from elongation by four carbon atoms of the 18:2(n-6) to 22:5(n-6) and 18:3(n-3) to 22:6(n-3). A *Scrippsiella* diet did result in suppressed egg production in *Calanus helgolandicus* and *Temora longicornis* (Gill and Harris 1987). Unfortuantely, it was not possible to bulk culture the dinoflagellate so it is not known what effect, if any, this diet would have had on *Acartia* egg production.

PUFA analyses have been carried out on the diatom *Skeletonema costatum* (Chuecas and Riley 1969; Ackman *et al.* 1968; and Volkman *et al.* 1989). Volkman *et al.* (1989) found *S. costatum* to be C_{16} PUFA abundant when compared with other species. Its major components were 14:0, 16:1(n-7), 16:0, 16:3(n-3), 16:2(n-3), 20:5(n-3) (Table 7.3). Volkman *et al.* (1989) also report that diatoms also have high concentrations of 16:1(n-7) and 16:0, and variable concentrations of 14:0 and 20:5(n-3) fatty acids, and these four acids alone can represent 62-70% of the total fatty acid concentration in a diatom cell. This was observed in current results with these four acids responsible for 67.62% of total fatty acid content of *Skeletonema.* C_{18} and C_{22} PUFAs were minor constituents (Table 7.3, Volkman *et al.* 1989). Morris (1984) found that 20:5(n-3) represented <1.5%, and 22:6(n-3) <0.5% of the total fatty acid concentration in diatoms i.e. they are minor constituents. In contrast, Volkman *et al.* (1989) and current results show there to be much higher quantities of 20:5(n-3) present in the diatom *Skeletonema* indeed, it represented 4.17% of the total amount of fatty acid.

Kates and Volcani (1966) performed an extensive examination of the lipid content of *Phaeodactylum tricornutum* and their analysis agreed with current results (Table 7.3)). Chief fatty acids of this alga were palmitoleic (16:1(n-7)), palmitic (16:0) and eicosapentaenoic acid (20:5(n-3)) and its composition is similar to that of *S. costatum.*

The lipid content of diatoms resembles that of green algae and higher plants. However, linolenic (18:3(n-3)) acid, a major acid in green algae and higher plants, was only a comparatively minor constituent in diatom lipids. In contrast, higher plants are low in C_{16} acids and C_{20} acids, which are the main components of the lipid in diatoms (Kates and Volcani 1966). In another study, Parish and Wangersky (1987) found that lipid made up 2-34% of the dry weight of *Phaeodactylum* (very variable). Two of the main lipid types in *Phaeodactylum* are triglycerides and phospholiopids. Triglycerides are a storage class, whereas phospholipids are incorporated into cell membranes. Parrish and Wangersky (1987) found there to be a strong negative correlation between the two. It was observed that intracellular synthesis of triglyceride was clearly triggered by nitrogen-stress, while synthesis of the membrane-associated polar lipid class was reduced under these conditions. This is because lecithins, the major phospholipids in *Phaeodactylum* contain equimolar proportions of nitrogen and phosphorus and so are likely to be nitrogen-limited in the marine environment. Phospholipids and triglycerides have common precursors, so common intermediates will be transferred to triglycerides if phospholipid synthesis is hindered by the lack of nitrogen. Although there is a switching between the two types of lipid produce during times of stress, the overall total of lipids produced is not affected (Parrish and Wangersky 1987).

Attwood and Peterson (1989) demonstrated a clear link between food lipid content and fecundity. It is known that lipids are a source of metabolic energy and that essential PUFAs have a vital structure needed for functional roles in cell membranes and hence, are required for growth and reproduction. Consumers acquire lipids from their diet, or by endogenous lipogenesis from dietary protein and carbohydrate precursors (Sargent *et al.* 1987). Polar lipid content of an organism is much less variable than neutral lipid content, which tends to fluctuate considerably with dietary input (Pond *et al.* 1995 [a]).

Fatty acids of the (n-3) series represent, by far, the most abundant PUFAs in planktonic marine ecosystems (Fraser *et al.* 1989). *De novo* synthesis of (n-3) PUFAs is generally considered to only occur in phytoplankton, with higher trophic organisms able to undertake further chain elongation and limited desaturation. (n-3) PUFAs are, therefore, essential dietary factors for the above trophic level (Dunstan *et al.* 1992).

Lipids provide vital materials and energy that support the early stages of reproduction and development, Ronnestadt *et al.* (1994) observed a marked positive relationship between gilthead seabream egg development and total PUFA concentration. Neutral fatty acids from the oil globule provided the aerobic energy from hatching until the onset of first feeding. High zooplankton growth rates are only observed when their diet is rich in essential fatty acids (Anderson and Pond 2000). Storage reserves of PUFAs are not believed to significantly contribute to sustained provision of these fatty acids for egg production in natural populations (Anderson and Pond 2000) and PUFAs in eggs originate from recently ingested food (Pond 1996). *Acartia* spp. are opportunistic feeders, (Jonasdottir 1994 and Koski and Kuosa 1999). *Acartia tonsa* has no energy reserves, and functions mainly on its present supply of food (Durbin *et al.* 1983), its entire metabolic product goes into egg production. Indeed, it spawns just 9.5 hours after ingestion, and *A. hudsonica* is not much slower, laying its eggs a mere 24 hours after feeding, (Jonasdottir 1994). *Acartia bifilosa* also lacks all storage elements and responds to changes in food availability with only a short time lag, (Koski and Kuosa 1999). So, for a good EPR *Acartia* spp. need plenty of (n-3) PUFA from their diet as they can not store it. Hatching success and naupliar survival in *C. helgolandicus* was seen to depend on total organic content of the eggs (Guisande and Harris 1995). The current results would therefore suggest that *Isochrysis* contains the highest levels of total PUFA.

A minimum level of PUFA per egg is also needed in order to sustain production, and hatching viability of *C. helgolandicus* was closely correlated with 18:2 (n-6) and 20:4 (n-6) fatty acids. The current results show that *Skeletonema* contained a much lower percentage of 18:2(n-6) than any of the other algae and *Isochrysis* the most. This could explain the low *Acartia* fecundity when fed a *Skeletonema* diet and the statistically significant higher egg production rates when fed on *Isochrysis*. Although PUFAs 20:5(n-3) and 22:6(n-3) are good gauges of food availability, they are not indicators of egg viability or naupliar survival (Pond 1996).

The classic pelagic food web links diatom blooms to a rapid increase in copepod production, however, the quality of diatoms as a food source for copepods has recently been questioned as they have been seen to inhibit copepod production. This inhibition could occur in two ways; either they lack essential nutrients, like fatty acids, or they contain inhibitory compounds. Diatoms, as a group, do contain less lipids, carbohydrates and proteins per cell than dinoflagellates (Bàn *et al.* 1997), but a diet "to excess" in any experiment undertaken should compensate for this. It is the current belief that some diatoms contain a toxic compound that blocks copepod embryogenesis (Ianora *et al.* 1995). All *Acartia* species in the current study produced the highest number of eggs when fed on *Isochrysis* (flagellate) and the least when given a *Skeletonema* (diatom) diet. These findings echo previous results as Ianora *et al.* (1995) found that an *Isochrysis* diet induced the production of good quality eggs by *A. clausi* that developed successfully to hatching, as did a dinoflagellate (*Prorocentrum minimum*) diet. In contrast, the experiments with copepods fed on four diatom diets, (*Thalassiosira rotula, Chaetoceros curvisetum, Phaeodactylum tricornutum* and *Skeletonema costatum*) resulted in poor egg quality, with hatching success as low as 20% of total egg production. In the case of *S. costatum*, females produced eggs for only

3-4 days before either becoming sterile or dying. Fecundity and hatch success decreased with female age, but female longevity was not affected by food type, other than *S. costatum*. A. *clausi*'s low egg viability when fed *T. rotula* is due to inhibitory compounds blocking cell division during early copepod embryogenesis and not lack of nutrients or remating, and blocking occurred at extracts of less than 10^5 cells ml^{-1} (Ianora *et al.* 1996). This is a higher concentration than naturally occurs during blooms. The blockage of egg development occurred in axenic and non-axenic cultures thus proving that the inhibitors are produced intracellularly by the diatom and not by a bacteria or virus associated with the diatom (Ianora *et al.* 1996). The toxic compound responsible for inhibition of cell division during copepod embryogenesis are 3-aldehydes of low molecular weight (Ianora *et al.* 1999). These arrest cell division and incite apoptosis, programmed cell death, and have been isolated from *T. rotula*. The mechanism by which they affect copepods is unknown, but Ianora *et al.* (1999) believe that they are transferred to the gonads after feeding by diffusion through gut epithelium, and then accumulate in the developing oocytes from oogenesis. This inhibition was not species specific, and the aldehydes proved not to be noxious for adult copepods, but lethal for the eggs. The high egg mortality goes some way to explain why the seasonal copepod cycle lags behind the spring diatom bloom by as much as three months in many coastal areas. Indeed, most diatoms sediment out of the water column rather than being ingested (Ianora *et al.* 1996).

Egg production is not dependent on one or a few components, but is a combination of direct and indirect causal paths which all affect the ability of the female copepods to produce eggs (Jonasdottir *et al.* 1995). Many workers have observed chemoreception in copepods and while it is possibly that copepods may detect toxins released by certain algae, it is also possible for them to detect the nutritional content of their food. Gill and

Harris (1987) reported that detection of particle-free dinoflagellate homogenates resulted in elevated beating of *T. longicornis* first maxilla (M1). Thus, copepods feed to maximise their nutritional intake, especially in relation to the ingestion of lipid/fatty acid. *Acartia* fecundity, with respect to diet, is most likely linked to total fatty acid content, rather than with one, or a few specific fatty acids.

7.5 Summary

1) Egg production rate (EPR) is a more sensitive parameter of food suitability than feeding rate.
2) All four species showed a statistically higher mean EPR ($P<0.01$) when fed *Isochrysis* than *Phaeodactylum* or *Skeletonema*. *Skeletonema* was the least suitable diet.
3) This corresponded to the feeding experiments where *Isochrysis* was the preferred food source at the lower food concentrations.
4) The results complement those of Castro-Longoria (1998).
5) Major fatty acid components of the diets:

   Isochrysis: 14:0, 16:0, 18:1(n-9), 18:3(n-3), 16:1(n-7), 18:4(n-3) but low in 22:6(n-3). Rich in PUFA (Cheucas and Riley 1969).

   Phaeodactylum: 16:1(n-7), 16:0, 20:5(n-3) and 14:0.

   Skeletonema: 14:0, 16:1(n-7) 16:0, 16:3(n-3), 16:2(n-3) and 20:5(n-3).

   Scrippsiella: 18:1(n-9), 18:3(n-3), 18:2(n-6), 18:4(n-3), 22:6(n-3), 14:0 and 16:0. Low in PUFA (Collier 1965)
6) There is a link between food lipid content and fecundity (Attwood and Peterson 1989) as lipids are a good source of metabolic energy.
7) Fecundity is linked to total fatty acid content (Guisande and Harris 1995), rather than one or a few specific fatty acids. This suggests that *Isochrysis* contains the most fatty acid of the three food types used in the egg production experiments.

Chapter 8: General Discussion

Salinity (Greenwood 1981), temperature (Conover 1957; Greenwood 1981) and food availability (Conover 1956; Paffenhofer and Stearns 1988) are the major regulators which usually govern the spatial and temporal distribution and abundance of adult estuarine copepods. The spatial distribution of adult *Acartia* congeners in Southampton Water reflect the adults' tolerance of salinity (Lance 1963, 1964) and the species' egg production rates and hatch success (Castro-Longoria 1998). *Acartia tonsa* is known to be the most tolerant and so survives at the head of the estuary, *A. clausi* is the least tolerant and so fills the niche at the mouth of the system (Lance 1963). *Acartia bifilosa* and *A. discaudata* are considered intermediate species and are found along the length of Southampton Water (Castro-Longoria 1998).

Previous work indicates salinity also influences fecundity in all *Acartia* species and highest egg production rates (EPR) obtained for *A. bifilosa* and *A. discaudata* matched the salinity of their typical spatial distribution in Southampton Water (Castro-Longoria 1998). EPRs of *A. clausi* were similar at salinities of 35, 30 and 25 but are dramatically reduced at lower salinities, especially at 15 where egg production fell below 1 egg female^{-1} d^{-1}, and were completely suppressed after 4 days (Castro-Longoria 1998). This again agrees with the basic distribution pattern of this species in Southampton Water. However, Castro-Longoria (1998) observed that the EPR of *A. tonsa* indicated that this species can reproduce at similar rates over the entire range of salinity tested (35-15), with fecundity only decreasing in the least saline water. This finding is surprising as it disagrees with the known distribution pattern of *A. tonsa*, which is typically restricted to the inner, reduced salinity region of Southampton Water (Raymont and Carrie 1964).

Looking beyond basic egg production it is important to also look at hatch success and naupliar survival as this gives a more appropriate measure of actual recruitment to the population (Ambler 1985; Ianora *et al.* 1992). The current study established that egg hatch results for the four species paralleled the adult salinity preferences put forward by Lance (1963). *Acartia tonsa* showed comparatively high hatch success at all salinities. It was most successful in the most saline water (33.3) and outperformed the other congeners at the lowest salinity (15.5). So, this species is the most tolerant to dilution and egg hatch with respect to salinity is not the factor responsible for its limited distribution. It has been suggested that *A. tonsa* is restricted to nearshore environments because of insufficient food in the open sea (Paffenhofer and Stearns 1988). Egg production in *Acartia* species falls dramatically at low levels of food (Dagg 1977; Parrish and Wilson 1978; Uye 1981; Durbin and Durbin1992). The response time of *A. tonsa* is especially short, with a fall in egg production after only 6 hours of starvation (Dagg 1977). For the remaining three species, hatch success in *A. clausi* decreased with salinity reinforcing its preference for more saline waters. As expected, *A. bifilosa* and *A. discaudata* showed higher hatch success at the intermediate salinities tested, 25.1 and 20.6 respectively.

The nauplius is the most sensitive life stage of an organism (Templeman 1936) and despite the salinity tolerances of the adults, if the nauplii do not survive then a population can not be maintained. Adults and juvenile stages of some estuarine and neritic copepods show different salinity tolerances (Lance 1964; Bhattacharya 1986). Young stages of *Eurytemora velox* (Nagaraj 1988) and *A. tonsa* (Tester and Turner 1991) show optimum survival under a restricted range of salinity. The current results indicated that salinity did not have a detrimental effect on *Acartia* naupliar survival

indeed, highest survival for all species was at 33.3 salinity. These results agree with Villante *et al.* (1993) who observed that the maximum adult *A. bifilosa* density in the Mundaka estuary (Spain) occurred at a salinity of 30, but nauplii numbers peaked in waters of >30 salinity. This was explained by the lack of swimming ability of the nauplii. Unable to maintain station, the juveniles are swept towards the mouth of the estuary until they mature when the later stages are then able to return to the upper areas of the estuary and to regions of preferred salinity.

The seasonal occurrence of *Acartia* congeners in Southampton Water is also governed by temperature. Castro-Longoria (1998) reported that *A. discaudata* and *A. clausi* were present in the estuary throughout the year and both showed maximum numbers in the summer. In contrast, *A. bifilosa* and *A. tonsa* disappear from the water column during summer and winter respectively. Life histories of the congeners explain this temporal distribution. Castro-Longoria (1998) observed that egg production rates of all four species were affected by temperature and a positive relationship between temperature and egg production was seen. In terms of hatch success, the current study showed that *A. bifilosa* and *A. discaudata* performed best at 10°C, although the differences for *A. discaudata* were not significant. This was not unexpected as the species is present in Southampton Water all year round. *Acartia tonsa* and *A. clausi* showed a higher hatch success at 20°C, significantly so in the case of *A. tonsa* reinforcing the reasoning that it is a summer species. Temperature is the most important factor affecting the development time and growth rate of crustaceans (Templeman 1936; Sandoz and Rodgers 1944; Costlow *et al.* 1960; Costlow *et al.* 1962) and it generally has a more definite effect on the length of larval development time rather than mortality as the highest naupliar survival, and shortest time for the first copepodite I (CI) to appear, was at 20°C for all four *Acartia* species.

A basic principle of population ecology is that all species have the potential for exponential growth, but in the field maximum reproduction rates are seldom achieved. Many variables, both intrinsic and extrinsic factors, regulate and limit copepod reproduction. One method copepods have of enhancing their reproductive fitness is their reproductive strategy. As mentioned, *A. bifilosa* and *A. tonsa* both diapause in the egg phase. *Acartia bifilosa* oversummers which is unusual as it removes the species from the water column during the most productive time of year. Real-time cues to environmental change may come too late to allow an optimum fitness response (Hairston *et al.* 1990) and the proximate reason behind this diapause tactic in other species is photoperiod (Marcus 1980, 1982[1], 1982[2]; Norrbin 1996; Stross and Hill 1968; Stross 1969) as it is a good predictor of future conditions, but does not influence fitness itself. The present work found that a daylength of 13L:11D triggered diapause in *A. bifilosa* at all temperatures tested (5-18°C) after only 2 days. A low number of diapause eggs were produced after 6 days at a 12L:12D daylength at the higher temperatures, so the response is marginally temperature-mediated. However, it is unlikely that the higher temperatures would induce diapause at a 10L:14D photoperiod for example. The ultimate reason behind the diapause strategy is still unknown. Workers have hypothesised that diapause in various species is a result of predator avoidance (Slusarczyck 1995), avoiding food limited conditions (Santer and Lampert 1995) or to avoid crowding (Ban and Minoda 1994). Although maximum predation pressure from *Aurelia* and *Pleurobranchia* coincides with maximum *A. bifilosa* abundance (just before the species diapauses), the current investigation theorised that *A. bifilosa* diapauses primarily to avoid the higher temperatures in summer. A scope for growth (SfG) assay was conducted to test this assumption and it was seen that *A. bifilosa*'s SfG at 10°C was twice that at 20°C. A SfG assay was also carried out on *A.*

discaudata for comparison and this species showed a doubling of SfG with every twofold increase in temperature.

Acartia bifilosa does not lay down lipid reserves over winter and given its restriction to the colder months when the food environment is poor, the question arises as to whether *A. bifilosa* is successful because it can manufacture essential nutrients, in this case fatty acids, from precursors in its diet, or whether it simply switches its diet focus more effectively than the other species. Polyunsaturated fatty acid (PUFA) analyses were conducted on adult females of this species in 2001, from February to April and, after its period of diapause, from October 2001 to January 2002 to determine their percentage PUFA composition. It was found that *A. bifilosa*'s fatty acid content reflected the season, with the PUFA content of the females mirroring that of the phytoplankton present in Southampton Water. During spring the individuals were feeding on diatoms, then diapaused over summer, and took dinoflagellates in autumn. During winter increased levels of 18:1(n-7) fatty acid suggested that the diet was heavily supplemented with bacteria from detritus. There was some evidence of food selection as a *Phaeocystis pouchetii* bloom occurred in Southampton Water in April, but no evidence of this was seen in the copepods. *Phaeocystis* is known to be difficult for *Acartia* to handle physically (Verity and Smayda 1989) and it is also undesirable in terms of its chemical composition (Martens 1981; Estep *et al.* 1990; Frangoulis *et al.* 2001; Bautista *et al.* 1992; Claustre *et al.* 1990).

Knowledge of the food assimilated by a species is important because food supply is one of the main variables controlling copepod fecundity and viability of the eggs (Ianora *et al.* 1992; Ianora and Poulet 1993; Jonasdottir 1994; Laabir *et al.* 1999). In the laboratory, various herbivorous copepods exhibit an EPR that is positively correlated

with food concentration (Marshall and Orr 1977; Kiorboe *et al.* 1985; Verity and Smayda 1989; Durbin *et al.* 1992). Positive correlations between EPR and chlorophyll-a concentration have also been seen in the field (Beckman and Peterson 1986; Tiselius *et al.* 1991; Bautista *et al.* 1994). In contrast other cases indicate high variability in EPR and poor correlation with various measurements of food availability (Ambler 1985; Ayukai 1988; Ianora and Poulet 1993; Hay 1995), especially with chlorophyll-a which gives no indication of composition or properties of the food. Food quality in terms of type of algae, chemical composition and size are key factors determining production and viability of copepod eggs (Kiorboe 1989; Stottrup and Jensen 1990; Ianora and Poulet 1993; Jonasdottir 1995; Kleppel and Burkart 1995). Despite contradictory evidence feeding is an important parameter governing copepod survival. *Acartia* species are opportunistic feeders and take whatever food is available (Jonasdottir 1994; Koski and Kuosa 1999). They are not adapted to low food concentrations (Parrish and Wilson 1978; Uye 1981) and are food limited in nature (Checkley *et al.* 1992; Irigoien and Castel 1996). Experiments were conducted to determine the functional feeding response of the congeners. Generally, feeding rate increased with initial food concentration for all food types (*Isochrysis, Phaeodactylum* and *Skeletonema*) and all congeners. Clearer results were seen looking at EPR on the same food types as this is a much more sensitive parameter. The best diet for all four species in terms of EPR was the flagellate *Isochrysis*, followed by the single-celled diatom *Phaeodactylum.* The lowest EPRs were seen for all species when fed on the chain-forming diatom *Skeletonema.* It is not well established which properties of the food are responsible for the variations as copepods respond positively to a number of different components of their food. Decreased fecundity may be due to a fall in the level of a limiting nutrient (Kiorboe 1989) or to an increase in an inhibitor (Ianora *et al.* 1996, 1999). The current study concentrated on the PUFA content of the food as it complemented the previous

PUFA analysis of *A. bifilosa* and it is known that lipids are vital materials for reproduction and development (Ronnestadt *et al.* 1994). *Isochrysis* is rich in PUFA (Cheucas and Riley 1969). Despite appearing to be a poor food source, *Skeletonema* was also seen to be high in PUFAs, especially the C_{16} species (Volkman *et al.* 1989). Unsurprisingly, since it is also a diatom, the PUFA make-up of *Phaeodactylum* as seen in the present results and determined by Kates and Volcani (1966) resembled that of *Skeletonema.* Egg viability of *Calanus helgolandicus* was closely correlated with the 18:2(n-6) and 20:4(n-6) fatty acids (Guisande and Harris 1995). Current results show that *Skeletonema* contained a much lower percentage of 18:2(n-6) than the other algae, and *Isochrysis* the highest percentage. Data obtained from the current investigation was only percentage composition of the PUFAs and not actual weights so it is difficult to compare results and determine if it is the PUFA content of the diet alone which is affecting *Acartia* EPR. Nutrition and production are clearly related. It is evident that within the parameters set by other intrinsic and extrinsic variables, food concentration and composition can greatly affect copepod EPR and ultimately a population's survival.

In conclusion, the Acartidae congeners in Southampton Water have a very definite spatial distribution which is primarily governed by the effect salinity has on certain stages of their lifecycles. The adults in particular are segregated into separate salinity niches to reduce interspecies competition since they are all similar in size, feed on the same food and have a large degree of temporal overlap. Adult *A. tonsa* are the most tolerant to dilution of the four species (Lance 1963) and are found at the head of Southampton Water where the surface salinity is approximately 28 and rises to 34 nearer the bottom of the water column (Lucas 1993). Lance (1963) classified *A. bifilosa* and *A. discaudata* as intermediate species in terms of salinity tolerance and distribution and both species are found along the length of the estuary. *Acartia clausi* is the most

marine of the four species and the highest numbers are seen at Calshot where salinity values peak at 35. Highest egg production rates (EPR) and hatch success for all species were at the salinities preferred by the adults, however the greatest percentage of naupliar survival for all congeners was at full strength seawater. A critical question concerns whether the *Acartia* nauplii are retained in the estuary or whether they are initially passively transported seawards and are subsequently re-recruited. *Callinectes sapidus* early zoea stages are transported to the mouth of Delaware Bay by tidal flushing. It re-colonises the estuary through rhythmic swimming off the bottom on flood tides but not on ebb tides and avoids swimming in the upper seaward flowing layers of the water column (Epifanio *et al.* 1984). The recruitment of penaeid post-larvae to estuarine areas also exhibits rhythmic vertical migratory behaviour in relation to tidal water movements and generally the larvae traverse estuaries on the flood tide (Naylor 1988). Villante *et al.* (1993) observed that *A. bifilosa* nauplii are poor swimmers and so are swept to the mouth of the estuary by water circulation until they develop further and can swim back up the estuary to their regions of preferred salinity. So, salinity does influence *Acartia* distribution, but it is the adults that are the sensitive stage. EPR and hatch success mirror adult salinity preferences to maximise the number of individuals in the next generation but the resultant nauplii exhibit a higher degree of physiological plasticity to survive their net lagrangian transport downstream to the mouth.

Interspecific competition is further reduced by seasonal segregation. *Acartia tonsa* is clearly a summer species since its highest EPR, hatch success and naupliar survival are all at 20°C. Highest adult density is during summer and this species diapauses over winter to avoid harsher seasonal conditions. *Acartia discaudata* and *A. clausi* are present in Southampton Water all year round but at different locations. There is a

distinct *A. clausi* maximum in summer since its EPR, hatch success and naupliar survival are highest at 20°C. Whereas *A. discaudata* has a stronger presence throughout the year because there are no significant differences in its hatch success or naupliar survival over 5-20°C, even though its maximum numbers are seen in summer. *Acartia bifilosa*'s highest EPR, hatch success and scope for growth is at 10°C and it diapauses over summer in an attempt to avoid higher temperatures. This diapause strategy also reduces competition since there is a large degree of spatial overlap between *A. bifilosa* and *A. discaudata*. *Acartia bifilosa* dominates in winter/spring when *A. discaudata* numbers are low, but it is absent during summer when *A. discaudata* density is at its maximum. *Acartia bifilosa* is also able to survive winter because it is an efficient feeder since it showed the highest feeding rates on *Isochrysis, Phaeodactylum* and *Skeletonema* and also switches its diet focus successfully throughout the year as well as supplementing its diet in winter with bacteria.

Future work

In order to complete to picture of the Acartidae distribution in Southampton Water, the copepodite tolerance to temperature and salinity needs to be investigated. Nauplii and copepodite spatial distribution also needs to be looked into to determine if the nauplii are transported downstream before the copepodite/young adults return to the upper regions of the estuary.

Further work on *A. bifilosa* diapause behaviour is needed because so little is known about its overwintering strategy, including:

- An investigation into the internal morphology of the diapause eggs
- Determining if subitaneous and diapause eggs are produced simultaneously
- Investigating what breaks diapause once the eggs are in the refractory phase
- Establishing if *A. bifilosa* from other geographical locations diapause and if so at what time of year and what photoperiod is the cue. If this varies it may be leading to speciation as seen by Marcus (1987) for *Labidocera aestiva*. Genetic studies would be necessary to confirm this.

Work has already been done on *A. tonsa* diapause (Zillioux and Gonzalez 1972) but it needs to be taken further, as above for *A. bifilosa*, for comparison since these are two closely related species in the same region but with very different life history strategies.

References

Abou Debs, C. (1984). Carbon and nitrogen budget of the calanoid copepod *Temora stylifera*: effect of concentration and composition of food. *Mar. Ecol. Prog. Ser.* **15,** 213-223.

Ackman, R. G., Tocher, C.S. and McLachlan, J. (1964). Marine phytoplankter fatty acids. *Fish. Res. B. Can.* **21**, 747-756.

Aksnes, D. L. (1996). Natural mortality, fecundity and development time in marine planktonic copepods - implications of behaviour. *Mar. Ecol. Prog. Ser.* **131**, 315-316.

Alcaraz, M. (1983). Coexistence and segregation of congeneric pelagic copepods: spatial distribution of the *Acartia* complex in the ria of Vigo (NW of Spain). *J. Plank. Res.* **5**, 891-900.

Alekseev, V. R. and Starobogatov, Y.I. (1996). Types of diapause in Crustacea: definitions, distribution, evolution. *Hydrobiologia* **320**, 15-26.

Allan, J. D., Richman, S., Heinle, D.R. and Huff, R. (1977). Grazing in juvenile stages of some estuarine calanoid copepods. *Mar. Biol.* **43**, 317-331.

Ambler, J. W. (1985). Seasonal factors affecting egg production and viability of eggs of *Acartia tonsa* Dana from East Lagoon, Galveston, Texas. *Est. Coast. Shelf Sea Sci.* **20**, 743-760.

Ambler, J. W. (1986). Effect of food quantity and quality on egg production of *Acartia tonsa* Dana from East Lagoon, Galveston, Texas. *Est. Coast. Shelf sea Sci.* **23**, 183-196.

Andersen, T. and Hessen, D.O. (1991). Carbon, nitrogen and phosphorus content of freshwater zooplankton. *Limnol. Oceanogr.* **36**, 807-815.

Anderson, T. R. and Hessen, D.O. (1995). Carbon or nitrogen limitation in marine copepods? *J. Plank. Res.* **17**, 317-334.

Anderson, T. R. and Pond, D.W. (2000). Stoichiometric theory extended to micronutrients: Comparison of the roles of essential fatty acids, carbon and nitrogen in the nutrition of marine copepods. *Limnol. Oceanogr.* **45:** 1162-1167.

Ansell, A. D. and Sivadas, P. (1973). Some effects of temperature and starvation on the bivalve *Donax vittatus* (da Costa) in experimental laboratory populations. *J. Exp. Mar. Biol. Ecol.* **13**, 229-262.

Antai, E. E. (1989). Seasonal trophodynamics of bacterioplankton and heterotrophic microflagellates in Southampton Water. : PhD thesis, University of Southampton. 384pp.

Atkinson, A. (1995). Omnivory and feeding selectivity in five copepod species during spring in the Bellingshausen Sea, Antarctica. *ICES J. mar. sci.* **5**, 385-396.

Attwood, C. G. and Peterson, W.T. (1989). Reduction in fecundity and lipids of the copepod *Calanus australis* (Brodskii) by strongly pulsed upwelling. *J. Exp. Mar. Biol. Ecol.* **129**, 121-131.

Ayukai, T. (1987). Discriminate feeding of the calanoid copepod *Acartia clausi* in mixtures of phytoplankton and inert particles. *Mar. Biol.* **94**, 579-587.

Bainbridge, R. (1953). Studies on the interrelationships of zooplankton and phytoplankton. *J. Mar. Biol. Ass. UK* **32**, 385-447.

Bamstedt, U. and Tande, K.S. (1985). Respiration and excretion rates of *Calanus glacialis* in arctic waters of the Barents Sea. *Mar. Biol.* **87**, 259-266.

Ban, S. (1994). Effect of temperature and food concentration on post-embryonic development, egg production and adult body size of calanoid copepod *Eurytemora affinsis*. *J. Plank. Res.* **16**, 721-735.

Ban, S., Burns, C., Castel, J., Chaudron, Y., Christou, E., Escribano, R., Umani, S.F., Gasparini, S., Guerrero Ruiz, F., Hoffmeyer, M., Ianora, A., Kang, H-K., Laabir, M., Lacoste, A., Miralto, A., Ning, X., Poulet, S., Rodriguez, V., Runge, J., Shi, J., Starr, M (1997). The paradox of diatom-copepod interactions. *Mar. Ecol. Prog. Ser.* **157**, 287-293.

Ban, S. and Minoda, T. (1994). Induction of diapause egg production in *Eurytemora affinsis* by their own metabolites. In *Ecology and Morphology of Copepods*, pp. 185-189. Edited by F. D. a. B. Ferrari, B.P. Belgium: Kluwer Academic Publishers.

Barlow, J. P. and Monteiro, J.D.C. (1979). Selective grazing by zooplankton populations in Southampton Water. *Mar. Biol.* **53**, 335-344.

Barnes, H. (1953). The effect of lowered salinity on some barnacle nauplii. *J. Animal Ecol.* **22**, 328-330.

Bartram, W. C. (1981). Experimental development of a model for the feeding of neritic copepods on phytoplankton. *J. Plank. Res.* **3**, 25-51.

Bautista, B., Harris, R.P., Tranter, P.R.G. and Harbour, D. (1992). *In situ* copepod feeding and grazing rates during a spring bloom dominated by *Phaeocystis* sp. in the English Channel. *J. Plank. Res.* **14**, 691-703.

Bautista, B., Harris, R.P., Rodriguez, V. and Guerrero, F. (1994). Temporal variability in copepod fecundity during two different spring bloom periods in coastal waters off Plymouth (SW England). *J. Plank. Res.* **16**, 1367-1377.

Bayne, B. L., Gabbott, P.A. and Widdows, J. (1975). Some effects of stress in the adult on the eggs and larvae of *Mytilus edulis* L. *J. Mar. Biol.. Ass. UK* **55**, 675-689.

Bayne, B. L., Brown, D.A., Burns, K., Dixon, D.R., Ivanovici, A., Livingstone, D.R., Lowe, D.M., Moore, M.N., Stebbing, A.R.D. and Widdows, J. (1985). *The effects of stress and pollution on marine animals.* Preager publishers. 384pp.

Baynes, S. M., Emerson, L. and Scott A.P. (1979). Production of algae for use in the rearing of larval fish. In *Fisheries Research Technical Report*: Ministry of Agriculture, Fisheries and Food, Directorate of Fisheries Research. 247pp.

Beckman, B. R. and Peterson, W.T. (1986). Egg production by *Acartia tonsa* in Long Island Sound. *J. Plank. Res.* **8**, 917-925.

Behrends, G., Korshenko, A. and Viitasalo, M. (1997). Morphological aberrations in females of the genus *Acartia* (Copepoda, Calanoida) in the Baltic Sea. *Crustaceana* **70**, 594-607.

Bell, M. V., Henderson, R.J. and Sargent, J.R. (1986). The role of polyunsaturated fatty acids in fish. *Comparative biochemistry and physiology* **83B**, 711-719.

Bellantoni, D. C. and Peterson, W.T. (1987). Temporal variability in egg production rates of *Acartia tonsa* Dana in Long Island Sound. *J. Exp. Mar. Biol. Ecol.* **107**, 199-208.

Belmonte, G. (1997). Resting eggs in the life cycle of *Acartia italica* and *A. adriatica. Crustaceana* **70**, 114-117.

Belmonte, G. and Mazzocchi, M.G. (1997). Records of *Acartia margalefi* from the Norwegian and Black Seas. *Crustaceana* **70**, 252-256.

Ben-Amotz, A., Tornabene, T.G. and Thomas, W.H. (1985). Chemical profile of selected species of microalgae with emphasis on lipids. *J. Phycol.* **21**, 72-81.

Berggreen, U., Hansen, B. and Kiorboe,T. (1988). Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. *Mar. Biol.* **99**, 341-352.

Bhattacharya, S. S. (1986). Individual and combined effects of salinity and temperature on the calanoid copepod *Paracalanus aculeatus* Giesbrecht. In *Proceedings of the 2nd international conference on Copepoda. Canada. 13-17th Aug. 1984. Nat. Museum Can.*, pp. 220-228. Edited by S. a. S. Scriever: Syllogeous 58.

Boaden, P. J. S. and Seed, R. (1985). *An Introduction to Coastal Ecology.*: Blackie and Son Ltd. 218pp.

Boyd, C. M. (1976). Selection of particle sizes by filter-feeding copepods: A plea for reason. *Limnol. Oceanogr.* **21**, 175-180.

Bradford-Greive, J. M. (1999). *ICES identification leaflets for plankton (181).* Copenhagen, Denmark.

Brylinski, J. M. (1981). Report on the presence of *Acartia tonsa* Dana (Copepoda) in the harbour of Dunkirk (France) and its geographical distribution in Europe. *J. Plank. Res.* **3**, 255-260.

Buskey, E. J. (1984). Swimming pattern as an indicator of the roles of copepod sensory systems in the recognition of food. *Mar. Biol.* **79**, 165-175.

Cahoon, L. (1981). Reproductive response of *Acartia tonsa* to variations in food ration and quality. *Deep Sea Res.* **28**, 1215-1221.

Calow, P. (1979). The cost of reproduction - a physiological approach. *Biol. Rev.* **54**, 23-40.

Carlisle, D. B. and Pitman, W.J. (1961). Diapause, neurosecretion and hormones in Copepoda. *Nature* **190**, 827-828.

Carr, J. F., de Turville, C.M., Jarman, R.J. and Spencer, J.F. (1980). Water temperatures in the Solent estuarine system. In *The Solent Estuarine System: An Assessment of Present Knowledge*, pp. 36-44. Edited by R. D. Burton. Southampton: NERC.

Castro-Longoria, E. (1998). Seasonal and spatial distribution patterns of the congeneric group *Acartia* in the Solent-Southampton Water estuarine system, with special reference to aspects of their fecundity. PhD thesis, University of Southampton.

Castro-Longoria, E. and Williams, J.A. (1996). First report of the presence of *Acartia margalefi* (copepoda:Calenoida) in Southampton Water and Horsea Lake, UK. *J. Plank. Res.* **18**, 567-575.

Castro-Longoria, E. and Williams, J.A. (1999). The production of subitaneous and diapause eggs: a reproductive stratergy for *Acartia bifilosa* (Copepoda:Calenoida) in Southampton Water, UK. *J. Plank. Res.* **21**, 65-84.

Cataletto, B. and Fonda Umani, S. (1994). Seasonal variations in carbon and nitrogen content of *Acartia clausi* (Copepoda, Calanoida) in the gulf of Trieste (Northern Adriatic Sea). *Hydrobiologia* **292/293**, 283-288.

Cervelli, M., Battaglia, B., Bisol, P.M., Comaschi Scaramuzza, A. and Meneghetti, F. (1995). Genetic differentiation in the genus *Acartia* from the lagoon of Venice. *Vie Milieu* **45**, 117-122.

Checkley Jr, D. M. (1980). The egg production of a marine planktonic copepod in relation to its food supply: lab studies. *Limnol. Oceanogr.* **25**, 430-446.

Checkley Jr, D. M., Dagg, M.J. and Uye, S. (1992). Feeding, excretion and egg production by individuals and populations of the marine planktonic copepods, *Acartia* spp *and Centropages furcatus. J. Plank. Res.* **14**, 71-96.

Chen, F. and Marcus, N.H. (1997). Subitaneous, diapause, and delayed-hatching eggs of planktonic copepods from the northern Gulf of Mexico: morphology and hatching success. *Mar. Biol.* **127**, 587-597.

Christie, W. W. (1982). *Lipid analysis*, 2nd edn. Oxford: Pergamon Press. 338pp.

Christou, E. D.and Verriopoulos, G. C. (1993). Analysis of the biological cycle of *A.clausi* (Copepoda) in a meso-oligotrophic coastal area of the eastern Mediterranean Sea using time-series analysis. *Mar. Biol.* **115**, 643-651.

Christou, E. D. and Verriopoulous, G.C. (1996). Length, weight and condition factor of *Acartia clausi* (Copepoda) in the Eastern Mediterranean. *J. Mar. Biol. Ass. UK* **73**, 343-353.

Chuecas, L. and Riley, J.P. (1969). Component fatty acids of the total lipid of some marine phytoplankton. *J. Mar. Biol. Ass. UK* **49**, 97-116.

Clarke, K. R. and Warrick, R.M. (1994). *Change in marine communities: An approach to statistical analysis and interpretation.* Plymouth Marine Laboratory. 144pp.

Claustre, H., Poulet, S.A., Williams, R., Marty, J-C., Coombs, S., Ben Mlih, F., Hapette, A.M. and Martin-Jezequel, V. (1990). A biochemical investigation of a *Phaeocystis* sp. bloom in the Irish Sea. *J. Mar. BIiol. Ass. UK* **70**, 197-207.

Collier, A. (1965). Fatty acids in certain plankton organisms. In *Estuaries: Nutrients and biological production*, pp. 353-360.

Collins, N. R. and Williams, R. (1981). Zooplankton of the Bristol Channel and Severn Estuary. The distribution of four copepods in relation to salinity. *Mar. Biol.* **64**, 273-283.

Conover, R. J. (1957). Notes on the seasonal distribution of zooplankton in Southampton Water, with special reference to the genus *Acartia. Ann. Mag. Nat. Hist.* **12**, 63-67.

Conover, R. J. (1966). Factors affecting the assimilation of organic matter by zooplankton and the question of superfluous feeding. *Limnol. Oceanogr.* **11**, 346-354.

Corkett, C. J. (1967). Technique for rearing marine calanoid copepods in laboratory conditions. *Nature Lond.* **216**, 58-59.

Corkett, C. J. (1984). Observations on the development of copepods. *Crustaceana (suppl.)* **7**, 150-153.

Corner, E. D. S. and O'Hara, S.C.M (eds) (1986). *The biological chemistry of marine copepods.* Oxford: Clarendon Press. 349pp.

Costa, H. H. (1966). Responses of *Gammarus pulex* (L.) to a modified environment I. Reactions to toxic substances. *Crustaceana* **11**, 245-256.

Costlow, J. D., Rees, G.H. and Bookhout, C.G. (1959). Preliminary note on the complete larval development of *Callinectes sapidus* Rathbun under laboratory conditions. *Limnol. Oceanogr.* **4**, 222-223.

Costlow, J. D., Bookhout, C.G. and Monroe, R. (1960). The effect of temperature and salinity on larval development of *Sesarma cinereum* (Bosc) reared in the laboratory. *Biol. Bull.* **118**, 183-202.

Costlow, J. D., Bookhout, C.G. and Monroe, R. (1962). Salinity-temperature effects on the larval development of the crab, *Panopeus herbstii* Milne-Edwards, reared in the laboratory. *Physiol. Zool.* **35**, 79-93.

Cowles, T. J., Olson, R.J. and Chisholm, S.W. (1988). Food selection by copepods: discrimination on the basis of food quality. *Mar. Biol.* **100**, 41-49.

Crisp, D.J. (1971). Energy flow measurements. In: *Methods for the study of the marine benthos.* N.A. Holme and A.D. McIntyre (eds). Blackwell Scientific Publications. Oxford. pp. 197-279.

Dagg, M. (1977). Some effects of patchy food environments on copepods. *Limnol. Oceanogr.* **22**, 99-107.

Deason, E. E. (1980). Grazing of *Acartia hudsonica* (*A. clausi*) on *Skeletonema costatum* in Narragansett Bay (USA): Influence of food concentration and temperature. *Mar. Biol.* **60**, 101-113.

Donaghay, P. C. and Small, L.F. (1979). Food selection capabilities of the estuarine copepod *Acartia clausi. Mar. Biol.* **52**, 137-146.

Dunstan, G. A., Volkman, J.K., Jeffrey, S.W. and Barrett, S.M. (1992). Biochemical composition of microalgae from the green algal classes Chlorophyceae and Prasinophyceae. 2. Lipid classes and fatty acids. *J. Exp. Mar. Biol. Ecol.* **161**, 115-134.

Durbin, E. G., Durbin, A.G., Smayda, T.J. and Verity, P.G. (1983). Food limitation of production by adult *Acartia tonsa* in Narragansett Bay, Rhode Island. *Limnol. Oceanogr.* **28**, 1199-1213.

Durbin, E. G. and Durbin, A.G. (1992). Effects of temperature and food abundance on grazing and short-term weight change in the marine copepod *Acartia hudsonica. Limnol. Oceanogr.* **37**, 361-378.

Dyer, K. R. (1973). *Estuaries:A physical introduction*: Wiley and Sons. 195pp.

Ederington, M. C., McManus, G.B. and Harvey, H.R. (1995). Trophic transfer of fatty acids, sterols, and a triterpenoid alcohol between bacteria, a ciliate, and the copepod *Acartia tonsa*. *Limnol. Oceanogr.* **40**, 860-867.

Edmondson, W. T., Comita, G.W. and Anderson, G.C. (1962). Reproductive rate of copepods in nature and its relation to phytoplankton population. *Ecology* **43**, 625-634.

Elliott, J.M. and Davison, W. (1975). Energy equivalents of oxygen consumption in animal energetics. *Oecologia* **19,** 195-201.

Epifanio, C.E., Valenti, C.C. and Pembroke, A.E. (1984). Dispersal and recruitment of blue crab larvae in Delaware Bay, USA. *Estuar. Coastal Shelf Sci.* **18,** 1-12

Escaravage, V. and Soetaert, K. (1993). Estimating secondary production for the brackish Westerschelde copepod population *Eurytemora affinis* (Poppe) combining experimantal data and field observaions. *Cahiers de biologie marine* **34**, 201-214.

Estep, K. W., Nejstgaard, J., Skjoldal, H.R. and Rey, F. (1990). Predation by copepods upon natural populations of *Phaeocystis pouchetii* as a function of the physiological state of the prey. *Mar. Ecol. Prog. Ser.* **67**, 235-249.

Falkowski, L., Pazdro, K., Lewandowska, J., Osterroht, C. and Slebioda, M. (1993). A method for determining free fatty acids (FAA) in marine plankton and sediments by means of high performance liquid chromatographic analysis of their p-nitrobenzyl esters. *Oceanologia* **34**, 57-68.

Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipid. *J. Biol. Chem.* **226**, 497-509.

Fowler, J. and Cohen, L. (1990). *Practical statistics for field biology.* John Wiley and Sons. Chichester. 227pp.

Frangoulis, C., Belkhiria, S., Goffart, A. and Hecq, J-H. (2001). Dynamics of copepod fecal pellets in relation to a *Phaeocystis* dominated phytoplankton bloom: characteristics, production and flux. *J. Plank. Res.* **23**, 75-88.

Fraser, A. J. and Sargent, J.R. (1989). Formation and transfer of fatty acids in an enclosed marine food chain comprising phytoplankton, zooplankton and herring (*Clupea harengus* L.) larvae. *Mar. Chem.* **27**, 1-18.

Frost, B. W. (1972). Effects of size and concentration of food particles on the feeding behaviour of the marine planktonic copepod *Calanus pacificus*. *Limnol. Oceanogr.* **17**, 805-815.

Frost, B. W. (1977). Feeding behaviour of *Calanus pacificus* in mixtures of food particles. *Limnol. Oceanogr.* **22**, 472-491.

Fulton III, R. S. (1983). Interactive effects of temperature and predation on an estuarine zooplankton community. *J. Exp. Mar. Biol. Ecol.* **72**, 67-81.

Gabbott, P. A. and Bayne, B.L. (1973). Biochemical effects of temperature and nutritive stress on *Mytilus edulis* L. *J. Mar. Biol. Ass. UK* **53**, 269-286.

Gamble, J. C. (1978). Copepod grazing during a declining spring phytoplankton bloom in the northern North Sea. *Mar. Biol.* **49**, 303-315.

Gatten, R. R., Corner, E.D.S., Kilvington, C.C. and Sargent, J.R. (1979). A seasonal survey of the lipids in *Calanus helgolandicus* Claus from the English Channel. In *Cyclic phenomena in marine plants and animals.*, pp. 275-284. Edited by R. G. Hartnol. Oxford: Pergamon Press.

Gatten, R. R., Sargent, J.R., Forsberg, T.E.V., O'Hara, S.C.M. and Corner, E.D.S. (1980). On the nutrition and metabolism of zooplankton. *J. Mar. Biol. Ass. UK* **60**, 391-399.

Gaudy, R. (1974). Feeding four species of pelagic copepods under experimental conditions. *Mar. Biol.* **25**, 125-141.

Gauld, D. T. (1951). The grazing rates of planktonic copepods. *J. Mar. Biol. Ass. UK* **29**, 695-706.

George, C. L. and Lindley, J.A. (1997). Hatching nauplii of planktonic calanoid copepods from intertidal estuarine sediments. *J. Mar. Biol. Ass. UK* **77**, 899-902.

Gifford, D. J. and Dagg, M.J. (1988). Feeding of the estuarine copepod *Acartia tonsa* Dana: Carnivory vs. herbivory in natural microplankton assemblages. *Bull. Mar. Sci.* **43**, 458-468.

Gill, C. W. and Harris, R.P. (1987). Behavioural responses of the copepods *Calanus helgolandicus* and *Temora longicornis* to dinoflagellate diets. *J. Mar. Biol. Ass. UK* **67**, 785-801.

Goutx, M. and Saliot, A. (1980). Relationship between dissolved and particulate fatty acids and hydrocarbons, chlorophyll a and zooplankton biomass in Villefranche Bay, Mediterranean Sea. *Mar. Chem.* **8**, 299-318.

Graeve, M., Hagen, W. and Kattner, G. (1994). Herbivorous or omnivorous? On the significance of lipid compositions as trophic markers in Antarctic copepods. *Deep Sea Res. I* **5/6**, 915-924.

Greenwood, J. G. (1981). Occurences of congeneric pairs of *Acartia* and *Pseudodiaptomus* species (Copepoda:Calenoida) in Moreton Bay, Queensland. *Est. Coast. Shelf Sea Sci.* **13**, 591-596.

Grice, G. D. and Gibson, V.R. (1975). Occurence, viability and significance of resting eggs of the calanoid copepod *Labidocera aestiva*. *Mar. Biol.* **31**, 335-337.

Grice, G. D. and Gibson, V.R. (1977). Resting eggs in *Pontella meadi* (Copepoda:Calanoida). *J. Fish. Res. Board Can.* **34**, 410-412.

Grice, G. D. and Lawson, T.J. (1976). Resting eggs in the marine calanoid copepod *Labidocera aestiva* Wheeler. *Crustaceana* **30**, 9-13.

Grice, G. D. and Marcus, N.H. (1981). Dormant eggs of marine copepods. *Oc. Mar. Biol. Ann. Rev.* **19**, 125-140.

Guezennec, J. G., Dussauze, J., Bian, M., Rocchiccioli, F., Ringleberg, D., Hedrick, D.B. and White, D.C. (1996). Bacterial community structure in sediments from Guaymas basin, Gulf of California, as determined by analysis of phospholipid ester-linked fatty acids. *J. Mar. Biotechnol.* **4**, 165-175.

Guisande, C. and Harris, R. (1995). Effect of total organic content of eggs on hatching success and naupliar survival in the copepod *Calanus helgolandicus*. *Limnol. Oceanogr.* **40**, 476-482.

Hagen, W. and Schnack-Schiel, S.B. (1996). Seasonal lipid dynamics in dominant Antarctic copepods: Energy for overwintering or reproduction? *Deep Sea Res. I* **43**, 139-158.

Hairston Jr, N. G. and Munns Jnr, W.R. (1984). The timing of copepod diapause as an evolutionary stable stratergy. *Am. Nat.* **123**, 733-751.

Hairston, N. G., Dillon, T.A. and De Stasio, B.T. (1990). A field test for the cues of diapause in a freshwater copepod. *Ecology* **71**, 2218-2223.

Hairston, N. G. and Caceres, C.E. (1996). Distribution of crustacean diapause: micro- and macroevolutionary pattern and process. *Hydrobiologia* **320**, 27-44.

Hairston, N. G. and Olds, E.J. (1986). Partial photoperiodic control of diapause in three populations of the freshwater copepod *Diaptomus sanguineus*. *Biol. Bull.* **171**, 135-142.

Hansen, S., Verity, P., Falkenberg, T., Tande, K.S. and Norrbin, F. (1994). On the trophic fates of *Phaeocystis pouchetti* (Harriot). V. Trophic relationships between *Phaeocystis* and zooplankton: an assessment of methods and size dependence. *J. Plank. Res.* **16**, 487-511.

Harris, E. (1959). The nitrogen cycle in Long Island Sound. *Bull. Bingham oceanogr. Coll.* **17**, 31-65.

Hassett, R. P., Duggins, D.O. and Simenstad, C.A. (1993). Egg production rates of the neritic marine copepod *Acartia tumida* Willey in the Aleutian Archipelago. *Polar Biol.* **13**, 515-523.

Hay, S. (1995). Egg production and secondary production of common North Sea copepods, field estimates with regional and seasonal comparisons. *ICES J. mar. sci.* **52**, 315-327.

Heinle, D. R., Harris, R.P., Ustach, J.F. and Flemer, D.A. (1977). Detritus as food for estuarine copepods. *Mar. Biol.* **40**, 341-353.

Hessen, D. O. (1993). The role of mineral nutrients for zooplankton nutrition: Reply to the comment by Brett. *Limnol. Oceanogr.* **38**, 1340-1343.

Hirche, H.-J. (1987). Temperature and plankton II Effect on respiration and swimming activity in copepods from the Greenland Sea. *Mar. Biol.* **94**, 347-356.

Hirche, H. J., Meyer, U. and Niehoff, B. (1997). Egg production of *Calanus finmarchicus*: effect of temperature, food and season. *Mar. Biol.* **127**, 609-620.

Hirst, A. (1996). Zooplankton production and energy flow towards a biological model of Southampton water. PhD thesis, University of Southampton. 374pp.

Hirst, A. G., Sheader, M. and Williams, J.A. (1999). Annual pattern of calanoid copepod abundance, prosome length and their role in pelagic carbon flux in the Solent, UK. *Mar. Ecol. Prog. Ser.* **177**, 133-146.

Hodgkin, E. P. and Rippingale, R.J. (1971). Interspecies conflict in estuarine copepods. *Limnol. Oceanogr.* **16**, 573-576.

Huntley, M., Sykes, P., Rohan, S. and Martin, V. (1986). Chemically-mediated rejection of dinoflagellate prey by copepods *Calanus pacificus* and *Paracalanus parvus*: mechanism, occurence and significance. *Mar. Ecol. Prog. Ser.* **28**, 105-120.

Huntley, M. E., Barthel, K-G. and Star, J.L. (1983). Particle rejection by *Calanus pacificus*: discrimination between similarly sized particles. *Mar. Biol.* **74**, 151-160.

Ianora, A., Mazzocchi, M.G. and Grottoli, R. (1992). Seasonal fluctuations in fecundity and hatching success in the planktonic copepod *Centropages typicus*. *J. Plank. Res.* **14**, 1483-1494.

Ianora, A., Poulet, S.A. and Miralto, A. (1995). A comparative study of the inhibitory effect of diatoms on the reproductive biology of the copepod *Temora stylifera*. *Mar. Biol.* **121**, 533-539.

Ianora, A., Poulet, S.A., Miralto, A. and Grottoli, R. (1996). The diatom *Thalassiosira rotula* affects reproductive success in the copepod *Acartia clausi*. *Mar. Biol.* **125**, 279-286.

Ianora, A., Miralto, A. and Poulet, S.A. (1999). Are diatoms good or toxic for copepods? Reply to comment by Jonasdottir *et al. Mar. Ecol. Prog. Ser.* **177**, 305-308.

Ianora, A. and Poulet, S.A. (1993). Egg viability in the copepod *Temora stylifera*. *Limnol. Oceanogr.* **38**, 1615-1626.

Ianora, A. and Santella, L. (1991). Diapause embryos in the neustonic copepod *Anomalocera patersoni*. *Mar. Biol.* **108**, 387-394.

Ianora, A. and Scotto di Carlo, B. (1988). Observations on egg production rates and seasonal changes in the internal morphology of Mediterranean populations of *Acartia clausi* and *Centropages typicus*. *Hydrobiologia* **167/168**, 247-253.

Irigoien, X., Castel, J. and Sautour, B. (1993). In situ grazing activity of planktonic copepods in the Gironde estuary. *Cah. Biol. Mar.* **34**, 225-237.

Irigoien, X., Castel, J. and Gasparini, S. (1996). Gut clearance rate as a predictor of food limitation situations. Application to two estuarine copepods: *Acartia bifilosa* and *Eurytemora affinis*. *Mar. Ecol. Prog. Ser.* **131**, 159-163.

Irigoien, X., Harris, R.P., Head, R.N. and Harbour, D. (2000). The influence of diatom abundance on the egg production rate of *Calanus helgolandicus* in the English Channel. *Limnol. Oceanogr.* **45**, 1433-1439.

Irigoien, X. and Castel, J. (1995). Feeding rates and productivity of the copepod *Acartia bifilosa* in a highly turbid estuary; The Gironde (SW France). *Hydrobiologia* **311**, 115-125.

Jacobs, J. (1968). Animal behaviour and water movement as co-determinants of plankton distribution in a tidal system. *Sarsia* **34**, 355-370.

Jahnke, J. (1989). The light and temperature dependence of growth rate and elemental composition of *Phaeocystis globosa* Scherffel and *P. pouchetii* (Har.) Lagerh. in batch cultures. *Neth. J. sea Res.* **23**, 15-21.

Jawed, M. (1973). Ammonia excretion by zooplankton and its significance to primary productivity during summer. *Mar. Biol.* **23,** 115-120.

Jeffries, H. P. (1962). Succession of two *Acartia* species in estuaries. *Limnol. Oceanogr.* **7**, 354-364.

Jeffries, H. P. (1967). Saturation of estuarine zooplankton by congeneric associates. In *Estuaries*, pp. 500-508. Edited by G. H. lauff. Washington: Amer. Ass. Advan. Sci.

Jeffries, H. P. (1970). Seasonal composition of temperate plankton communities: fatty acids. *Limnol. Oceanogr.* **15**, 419-426.

Jespersen, P. and Russell, F.S. (eds) (1939). Fiches d'Identification du Zooplancton. *Cons. int. Explor. Mer.* , 12.

Johnson, J. K. (1980). Effects of temperature and salinity on production and hatching of dormant eggs of *Acartia californiensis* (Copepoda) in an Oregon estuary. *Fish. Bull. US* **77**, 567-584.

Jonasdottir, S. H. (1994). Effects of food quality on the reproductive success of *A. tonsa* and *A. hudsonica*: laboratory observations. *Mar. Biol.* **121**, 67-81.

Jonasdottir, S. H., Fields, D. and Pantoja, S. (1995). Copepod egg production in Long Island Sound, USA, as a function on the chemical composition of seston. *Mar. Ecol. Prog. Ser.* **119**, 87-98.

Jonasdottir, S. H. and Kiorboe, T. (1996). Copepod recruitment and food composition: do diatoms affect hatching success? *Mar. Biol.* **125**, 743-750.

Kasahara, S. and Uye, S. (1979). Calanoid copepod eggs in sea-bottom muds. V Seasonal changes in hatching of subitaneous and diapause eggs of *Tortanus forcipatus*. *Mar. Biol.* **55**, 63-68.

Kates, M. and Volcani, B.E. (1966). Lipid components of diatoms. *Biochim. Biophys. Acta* **116**, 264-278.

Kattner, G., Gercken, G. and Eberlein, K. (1983). Development of lipids during a spring plankton bloom in the northern North Sea. *Mar. Chem.* **14**, 149-162.

Kattner, G. and Krause, M. (1989). Seasonal variations of lipids (wax esters, fatty acids and alcohols) in calanoid copepods from the North Sea. *Mar. Chem.* **26**, 261-275.

Ketchum, B. H. (1954). Relation between circulation and planktonic populations in estuaries. *Ecology* **35**, 191-200.

Kifle, D. (1992). Seasonal and spatial variations in species composition, abundance, biomass and primary production of phytoplankton in Southampton Water. PhD thesis, University of Southampton. 293pp.

Kimoto, K., Uye, S. and Onbe, T. (1986). Growth characteristics of a brakish-water calanoid copepod *Sinocalanus tenellus* in relation to temperature and salinity. *Bull. Plank. Soc. Jap.* **33**, 43-57.

Kinne, O. (1967). Physiology of estuarine organisms with special reference to salinity and temperature: general aspects. In: *Estuaries* Lauff, G.H. (ed). American association for advancement of science, Washington. p 525-540.

Kiorboe, T., Mohlenberg, F. and Hamburger, K. (1985). Bioenergetics of the plankton copepod *A.tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. *Mar. Ecol. Prog. Ser.* **26**, 85-97.

Kiorboe, T. (1989). Phytoplankton growth rate and nitrogen content: implications for feeding and fecundity in a herbivorous copepod. *Mar. Ecol. Prog. Ser.* **55**.

Kiorboe, T. (1994). Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. *Limnol. Oceanogr.* **39**, 493-507.

Kiorboe, T. M., F. and Tiselius, P. (1988). Propagation of planktonic copepods: Production and mortality of eggs. *Hydrobiologia* **167/168**, 219-225.

Klein Breteler, W. C. M., Franz, H.G. and Gonzalez, S.R. (1982). Growth and development of four calenoid copepod spp under experimental and natural conditions. *Neth. J. Sea. Res.* **16**, 195-207.

Klein Breteler, W. C. M., Schogt, N. and van der Meer, J. (1994). The duration of copepod life stages estimated from stage-frequency data. *J. Plank. Res.* **16**, 1039-1057.

Klein Breteler, W. C. M. and Schogt, N. (1994). Development of *Acartia clausi* (Copepoda, Calanoida) cultured at different conditions of temperature and food. *Hydrobiologia* **292/293**, 469-479.

Kleppel, G. S., Holliday, D.V. and Pieper, R.E. (1991). Trophic interactions between copepods and microplankton: A question about the role of diatoms. *Limnol. Oceanogr.* **36**, 172-178.

Kleppel, G. S. (1992). Environmental regulation of feeding and egg production by *Acartia tonsa* off southern California. *Mar. Biol.* **112**, 57-65.

Kleppel, G. S., Burkart, C.A. and Houchin, L. (1998). Nutrition and regulation of egg production in the calanoid copepod *Acartia tonsa*. *Limnol. Oceanogr.* **43**, 1000-1007.

Kleppel, G. S. and Burkart, C.A. (1995). Egg production and the nutritional environment of *A.tonsa*: the role of food quality in copepod nutrition. *ICES J. Mar. Sci.* **52**, 297-305.

Koski, M. and Kuosa, H. (1999). The effect of temperature and food concentration and female size of the egg production of the planktonic copepod *Acartia bifilosa*. *J. Plank. Res.* **21**, 1779-1789.

Kouwenberg, J. H. M. (1993). Sex ratio of calanoid copepods in relation to population composition in the NE Mediterranean. *Crustaceana* **64**, 281-299.

Kustenko, N. G. (1987). Dynamics of the size structure in a population of the diatomic algae *Skeletonema costatum* (Grev.) CL. in plankton. *Oceanology* **27**, 79-82.

Laabir, M., Poulet, S.A. and Ianora, A. (1995). Measuring production and viability of eggs in *Calanus helgolandicus*. *J. Plank. Res.* **17**, 1125-1142.

Laabir, M., Poulet, R.P., Pond, D.W., Cueff, A., Head, R.N. and Ianora, A. (1998). Comparative study of the reproduction of *Calanus helgolandicus* in a well-mixed and seasonally stratified coastal waters of the western English Channel. *J. Plank. Res.* **20**, 407-421.

Laabir, M., Poulet, S.A., Cueff, A. and Ianora, A. (1999). Effect of diet on levels of amino acids during embryonic and naupliar development of the copepod *Calanus helgolandicus*. *Mar. Biol.* **134**, 89-98.

Lakkis, S. (1994). Coexistence and competition within *Acartia* (Copepoda, Calanoida) congeners from Lebanese coastal water: niche overlap measurements. In *Ecology and Morphology of Copepods*, pp. 481-490. Edited by F. D. Bradley and B.P. Ferrari. Belgium: Kluwer Academic Publishers.

Lalli, C. M. and Parsons, T.R. (1997). *Biological oceanography:An introduction*, 2nd edn: Butterworth Heinemann.

Lampitt, R.S. and Gamble, J.C. (1982). Diet and respiration on the small planktonic marine copepod *Oithona nana*. *Mar. Biol.* **66,** 185-190.

Lance, J. (1963). The salinity tolerance of some estuarine planktonic copepods. *Limnol. Oceanogr.* **8**, 440-449.

Lance, J. (1964). The salinity tolerances of some estuarine planktonic crustaceans. *Biol. Bull.* **127**, 108-118.

Lancelot, C., Wassmann, P. and Barth, H. (1994). Ecology of *Phaeocystis*-dominated ecosystems. *J. Mar. Sys.* **5**, 1-4.

Landry, M. R. (1975). Dark inhibition of egg hatching of the marine copepod *Acartia clausi* Giesbr. *J. Exp. Mar. Biol. Ecol.* **20**, 43-47.

Landry, M. R. (1975). The relationship between temperature and the development of the life stages of the marine copepod *Acartia clausi* (Giesbr.). *Limnol. Oceanogr.* **20**, 854-857.

Landry, M. R. (1978). Population dynamics and production of a planktonic marine copepod, *Acartia clausi,* in a small temperate lagoon on San Huan Island, Washington. *Int. Rev. ges. Hydrobiol.* .

Laurence, G. C. (1976). Calorific values of some North Atlantic calanoid copepods. *Fish. Bull.* **74**, 218-220.

Leakey, R. J. G., Burkill, P.H. and Sleigh, M.A. (1992). Planktonic cilliates in Southampton Water: abundance, biomass, production, and role in pelagic carbon flow. *Mar. Biol.* **114**, 67-83.

Lee, R. F., Hirota, J. and Barnett, A.M. (1974). Distribution and importance of wax esters in marine copepods and other zooplankton. *Deep Sea Res.* **18**, 1147-1165.

Lee, R. F., Nevenzel, J.C. and Paffenhoffer, G.A. (1971). Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. *Mar. Biol.* **9**, 99-108.

Lindley, J. A., John, A.W.G. and Robins, D.B. (1997). Dry weight, carbon and nitrogen content of some calanoid copepods from the seas around southern Britain in winter. *J. Mar. Biol. Ass. UK* **77**, 249-252.

Lucas, C. (1993). The biology of gelatinous predators and their impact on the mesozooplankton community of Southampton Water. PhD thesis, University of Southampton. 312pp.

Lucas, A. (1996). *Bioenergetics of aquatic animals.* Taylor and Francis. London. pp 169.

Lutz, R. V., Marcus, N.H. and Chanton, J.P. (1992). Effect of low oxygen concentrations on the hatching and viability of eggs of marine calanoid copepods. *Mar. Biol.* **114**, 241-247.

Maltby, L., Naylor, C. and Calow, P. (1990). Effect of stress on a freshwater benthic detritivore: Scope for growth in *Gammarus pulex. Ecotoxicology and environmental safety* **19**, 285-291.

Maltby, L. (1992). The use of the physical energetics of *Gammarus pulex* to assess toxicity: A study using artificial streams. *Env. Tox. And Chem.* **11,** 79-85.

Marcus, N. H. (1979). On the population biology and nature of diapause of *Labidocera aestiva* (Copepoda:Calanoida). *Biol. Bull.* **157**, 297-305.

Marcus, N. H. (1980). Photoperiodic control of diapause in the marine calanoid copepod *Labidocera aestiva. Biol. Bull.* **159**, 311-318.

Marcus, N. H. (1982[a]). Photoperiodic and temperature regulation of diapause in *Labidocera aestiva* (Copepoda:Calenoida). *Biol. Bull.* **162**, 45-52.

Marcus, N. H. (1982[b]). The reversibility of subitaneous and diapause egg production by individual females of *Labidocera aestiva* (Copepoda: Calanoida). *Biol. Bull.* **162**, 39-44.

Marcus, N. H. (1984[a]). Recruitment of copepod nauplii into the plankton: importance of diapause eggs and benthic processes. *Mar. Ecol. Prog. Ser.* **15**, 47-54.

Marcus, N. H. (1984[b]). Variation in the diapause response of *Labidocera aestiva* (Copepoda:Calanoida) from different latitudes and its importance in the evolutionary process. *Biol. Bull.* **166**, 127-139.

Marcus, N. H. (1986). Population dynamics of marine copepods - the importance of photoperiodism. *Am. Zool.* **26**, 469-477.

Marcus, N. H. (1987). Differences in the duration of egg diapause of *Labidocera aestiva* (Copepoda:Calanoida) from the Woods Hole, Massachusetts, Region. *Biol. Bull.* **173**, 169-177.

Marcus, N. H. (1996). Ecological and evolutionary significance of resting eggs in marine copepods: past, present and future. *Hydrobiologia* **320**, 141-152.

Marshall, S. M. and Orr, A.P. (1952). On the biology of *Calanus finmarchicus* VII. Factors affecting egg production. *J. Mar. Biol. Ass. UK* **30**, 527-547.

Martens, P. (1978). Faecal pellets. *Fiches d'Identification du zooplankton* **162.**

Martens, P. (1981). On the *Acartia* species of the northern wadden sea of Sylt. *Kieler Meeresforsch. Sonderh.* **5**, 153-163.

Martin, J. H. (1970). Phytoplankton-zooplankton relationships in Narragansett Bay. IV. The seasonal importance of grazing. *Limnol. Oceanogr.* **15**, 413-418.

Mayzaud, P., Chanut, J.P. and Ackman, R.G. (1989). Seasonal changes of the biochemical composition of marine particulate matter with special reference to fatty acids and sterols. *Mar. Ecol. Prog. Ser.* **56**, 189-204.

Mayzaud, P. and Martin, J-L. M. (1975). Some aspects of the biochemical and mineral composition of marine plankton. *J. Exp. Mar. Biol. Ecol.* **17**, 297-310.

McClaren, I. A. (1963). Effects of temperature on the growth of zooplankton, and the adaptive value of vertical migration. *J. Fish. Res. Bd. Canada* **20**, 685-722.

McClaren, I. A. (1965). Some relationships between temperature and egg size, body size, development rate, and fecundity of the copepod *Pseudocalanus. Lim. Oc* **10**, 528-538.

McKinnon, A. D., Kimmerer, W.J. and Benzie, J.A.H. (1992). Sympatric sibling spp within the genus *Acartia.* A case study from Westernport and Port Phillip Bays, Australia. *J. Crus. Biol.* **12**, 239-259.

McLaren, I. A. and Leonard, A. (1995). Assessing the equivalence of growth and egg production of copepods. *ICES J. mar. sci.* **52**, 397-408.

Miller, C. B., Johnson, J.K. and Heinle, D.R. (1977). Growth rules in the marine copepod genus *Acartia. Limnol. Oceanogr.* **22**, 326-335.

Mills, J. O. R. (1997). The influence of temperature and salinity stress on the scope for growth of a Calanoid copepod, *Acartia bifilosa* in Southampton Water. BSc dissertation, 74pp..

Miralto, A., Ianora, A. and Poulet, S.A. (1995). Food type induces different reproductive responses in the copepod *Centropages typicus. J. Plank. Res.* **17**, 1521-1534.

Morris, R. J. (1984). Studies of a spring phytoplankton bloom in an enclosed experimental ecosystem. II. Changes in the component fatty acids and sterols. *J. Exp. Mar. Biol. Ecol.* **75**, 59-70.

Morris, R. J., McCartney, M.J., Joint, I.R. and Robinson, G.A. (1985). Further studies of a spring phytoplankton bloom in an enclosed experimental ecosystem. *J. Exp. Mar. Biol. Ecol.* **86**, 151-170.

Muller-Navarra, D. C., Brett, M.T., Liston, A.M. and Goldman, C.R. (2000). A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature* **403**, 74-77.

Mullin, M. M. (1988). Production and distribution of nauplii and recruitment variability - putting the pieces together. In *Towards a theory on biological-physical interactions in the world ocean.*, pp. 297-320. Edited by B. J. Rothschild. New York: Kluwer Academic Publishers.

Mullin, M. M. and Brooks, E.R. (1967). Laboratory culture, growth rate, and feeding behaviour of a planktonic marine copepod. *Limnol. Oceanogr.* **7**, 657-666.

Munro, I. G. (1974). The effect of temperature on the development of egg, naupliar and copepodite stages of two species of copepods, *Cyclops vicinus* Uljanin and *Eudiaptomus gracilis* Sars. *Oecologia* **16**, 355-367.

Nagaraj, M. (1988). Combined effects of temperature and salinity on the complete development of *Eurytemora velox* (Crustacea: Calanoida). *Mar. Biol.* **99**, 353-358.

Nagasawa, H. (1993). Mini Review: Recent advances in insect neuropeptides. *Comp. Biochem. Physiol.* **106C**, 295-300.

Naylor, E. (1988). Rhythmic behaviour of decapod crustaceans. In: *Aspects of decapod crustacean biology*. A.A. Fricham and P.S. Rainbow (eds). Clarendon Press. Oxford. pp 375.

Naylor, C., Maltby and Calow, P. (1989). Scope for growth in *Gammarus pulex*, a freshwater benthic detritivore. *Hydrobiologia* **188/189**, 517-523.

Nejstgaard, J. C., Bamstedt, U., Bagoien, E. and Solberg, P.T. (1995). Algal constraints on copepod grazing. Growth rate, toxicity, cell size, and season as regulating factors. *ICES J. Mar. Sci.* **52**, 347-357.

Neumann, D. and Kruger, M. (1985). Combined effects of photoperiod and temperature on the diapause of an intertidal chronomid. *Oecologia* **67**, 154-156.

Newell, G. E. and Newell, R.G. (1977). *Marine plankton: a practical guide*, 5th edn: Hutchinson and Co.

Nival, P. and Nival, S. (1976). Particle retention efficiencies of an herbivorous copepod, *Acartia clausi* (adult and copepodite stages): Effects on grazing. *Limnol. Oceanogr.* **21**, 24-38.

Norrbin, M. F., Olsen, R-E. and Tande, K.S. (1990). Seasonal variation in lipid class and fatty acid composition of two small copepods in Balsfjorden, northern Norway. *Mar. Biol.* **105**, 205-211.

Norrbin, M. F. (1996). Timing of diapause in relation to the onset of winter in the high latitude copepods *Pseudocalanus acuspes* and *Acartia longiremis*. *Mar. Ecol. Prog. Ser.* **142**, 99-109.

Ohman, M. D., Aksnes, D.L. and Runge, J.A. (1996). The interrelationship of copepod fecundity and mortality. *Limnol. Oceanogr.* **41**, 1470-1477.

Olsen, R. E. and Henderson, R.H. (1989). The rapid analysis of neutral and polar marine lipids using double-development HPTLC and scanning densitometry. *J. Exp. Mar. Biol. Ecol.* **129**, 189-197.

Omori, M. and Ikeda, T. (1992). *Methods in marine zooplankton ecology.*: Krieger publishing company.

Ozaki, K. and Ikeda, T. (1997). The effect of temperature on the development of eggs and nauplii of the mesopelagic copepod *Paraeuchaeta elongata*. *Plankton Biol. Ecol.* **44**, 91-95.

Paffenhofer, G.-A. (1984[a]). Does *Paracalanus* feed with a leaky sieve? *Limnol. Oceanogr.* **29**, 155-160.

Paffenhofer, G.-A. (1984[b]). Food ingestion by the marine planktonic copepod *Paracalanus* in relation to abundance and size distribution of food. *Mar. Biol.* **80**, 323-333.

Paffenhofer, G.-A. and Knowles, S.C. (1978). Feeding of marine planktonic copepods on mixed phytoplankton. *Mar. Biol.* **48**, 143-152.

Paffenhofer, G.-A. and Knowles, S.C. (1980). Omnivorousness in marine planktonic copepods. *J. PLank. Res.* **2**, 355-365.

Paffenhofer, G.-A. and Stearns, D.E. (1988). Why is *Acartia tonsa* (Copepoda:Calenoida) restricted to nearshore environments? *Mar. Ecol. Prog. Ser.* **42**, 33-38.

Parrish, C. C. and Wilson, P.J. (1987). Particulate and dissolved lipid classes in cultures pf *Phaeodactylum tricornutum* grown in cage culture turbidostats with a range of nitrogen supply rates. *Mar. Ecol. Prog. Ser.* **35**, 119-128.

Parrish, K. K. and Wilson, D.F. (1978). Fecundity studies in *Acartia tonsa* (Copepoda:Calanoida) in standardised culture. *Mar. Biol.* **46**, 65-81.

Parsons, T. R., Stephens, K. and Strickland, J.D.H. (1961). On the chemical composition of eleven species of marine phytoplankters. *J. Fish. Res. Bd. Can.* **18**, 1001-1016.

Pequeux, A. (1995). Osmotic regulation. *J. Crust. biol.* **15**, 1-60.

Perkins, E. J. (1974). *The Biology of Estuaries and Coastal Waters.* London: Acardemic Press Inc.

Phillips, A. J. (1980). Distribution of chemical species. In *The Solent Estuarine System: An Assessment of Present Knowledge*, pp. 44-62. Edited by J. D. Burton. Southampton: NERC.

Pond, D. W., Priddle, J., Sargent, J. and Watkins, J.L. (1993). Lipid composition of Antarctic microplankton in relation to the nutrition of krill. *Antarctic special topic* , 133-139.

Pond, D. W., Priddle, J., Sargent, J.R. and Watkins, J.L. (1995[a]). Laboratory studies of assimilation and egestion of algal lipid by Antarctic krill - methods and initial results. *J. Exp. Mar. Biol. Ecol.* **187**, 253-268.

Pond, D. W., Watkins, J., Priddle, J. and Sargent, J. (1995[b]). Variation in the lipid content and composition of Antarctic krill *Euphausia superba* at South Georgia. *Mar. Ecol. Prog. Ser.* **117**, 49-57.

Pond, D. W., Harris, R., Head, R. and Harbour, D. (1996). Environmental and nutritional factors determining seasonal variability in the fecundity and egg viability of *Calanus helgolandicus* in coastal waters off Plymouth, UK. *Mar. Ecol. Prog. Ser.* **143**, 45-63.

Pond, D. W., Bell, M.V., Harris, R.P. and Sargent, J.R. (1998). Microplanktonic polyunsaturated fatty acid markers: a mesocosm trial. *Est. Coast. Shelf Sci.* **46**, 61-67.

Poulet, S. A., Ianora, A., Miralto, A. and Meijer, L. (1994). Do diatoms arrest embryonic development in copepods? *Mar. Ecol. Prog. Ser.* **111**, 79-86.

Price, H. J. and Paffenhofer, G-A. (1983). Modes of cell capture in calanoid copepods. *Limnol. Oceanogr.* **28**, 116-123.

Purcell, J. E., White, J.R. and Roman, M.R. (1994). Predation by gelatinous zooplankton and resource limitation as potential controls of *A.tonsa* copepod populations in Chesapeake Bay. *Limnol. Oceanogr.* **39**, 263-278.

Raymont, J. E. G., Austin, J. and Linford, E. (1961). Biochemical studies on marine zooplankton. *J. Mar. Biol. Ass. UK* **6**, 154-164.

Raymont, J. E. G. and Krishnaswamy, S. (1960). Carbohydrates in some marine planktonic animals. *J. Mar. Biol. Ass. UK* **39**, 239-246.

Raymont, J. E. G. and Conover, R.J. (1961). Further investigations on the carbohydrate content of marine zooplankton. *Limnol. Oceanogr.* **6**, 154-164.

Raymont, J. E. G. and Carrie, B.G.A. (1964). The production of zooplankton in Southampton Water. *Int. Revue ges. Hydrobiol.* **49**, 185-232.

Raymont, J.E.G. (1983). Plankton and productivity in the oceans. Vol II Zooplankton. Pergammon Press, Oxford. 824pp.

Reeve, M. R. and Walter, M.A. (1977). Observations on the existence of lower threshold and upper critical food concentrations for the copepod *Acartia tonsa* Dana. *J. Exp. Mar. Biol. Ecol.* **29**, 211-221.

Riebesell, U. (1993). Aggregation of *Phaeocystis* during phytoplankton spring blooms in the southern North Sea. *Mar. Ecol. Prog. Ser.* **96**, 281-289.

Riley, G. A. (1967). The plankton of estuaries. In *Estuaries*, pp. 757. Edited by G. H. Lauff. Washington DC: American Association for the Advancement of Science.

Rippingale, R. J. and Hodgkin, E.P. (1974). Predation effects on the distribution of a copepod. *Aust. J. mar. Freshwat. Res.* **25**, 81-91.

Robertson, J. R. (1983). Predation by estuarine zooplankton on tinntinid ciliates. *Est. Coast. Shelf Sci.* **16**, 27-36.

Roman, M. R. (1977). Feeding of the copepod *Acartia tonsa* on the diatom *Nitzschia closterium* and brown algae, (Fucus vesiculosus) detritus. *Mar. Biol.* **42**, 149-155.

Roman, M. R. (1983). Nitrogenous nutrition of marine invertebrates. In *Nitrogen in the Marine Environment.*, pp. 347-383. Edited by D. G. Capone. New York: Academic Press.

Roman, M. R. (1984). Utilisation of detritus by the copepod, *Acartia tonsa. Limnol. Oceanogr.* **29**, 949-959.

Roman, M. R. (1991). Pathways of carbon incorporation in marine copepods: Effects of developmental stage and food quantity. *Limnol. Oceanogr.* **36**, 796-807.

Ronnestadt, I., Koven, W.M., Tandler, A., Harel, M and Fyhn, H.J. (1994). Energy metabolism during development of eggs and larvae of gilthead sea bream (*Sparus aurata*). *Mar. Biol.* **120**, 187-196.

Rose, M. (1933). *Copepods Pelagiques (Faune de France 26).* Paris: Paul Lechevalier.

Rousseau, V., Vaulot, D., Casotti, R., Cariou, V., Lenz, J., Gunkel, J. and Baumann, M. (1994). The life cycle of *Phaeocystis* (Prymnesiophyceae): Evidence and hypotheses. *J. Mar. Sys.* **5**, 23-39.

Roy, S. and Poulet, S.A. (1990). Laboratory study of the chemical composition of ageing copepod fecal material. *J. Exp. Mar. Biol. Ecol.* **135**, 3-18.

Saiz, E., Calbert, A., Trepat, I., Irigoien, X. and Alcaraz, M. (1997). Food availability as a potential source of bias in the egg production method for copepods. *J. Plank. Res.* **19**, 1-14.

Sandoz, M. and Rodgers, R. (1944). Zoea larvae of the blue crab. *Ecology* **25**, 216-228.

Santer, B. and Lampert, W. (1995). Summer diapause in cyclopoid copepods - adaptive responses to a food bottle neck. *J. An. Ecol.* **64**, 600-613.

Sargent, J. R., Parkes, R.J., Mueller-Harvey, I. and Henderson, R.J. (1987). Lipid biomarkers in marine ecology. In *Microbes in the sea.*, pp. 119-138. Edited by M. A. Sleigh: Ellis Horwood Ltd.

Sargent, J. R. and Henderson, R.J. (1986). Lipids. In *Biological Chemistry of Marine Copepods*, pp. 59-108. Edited by E. D. S. C. a. S. C. M. O'Hara. Oxford: Clarendon Press.

Sato, Y., Shiomi, K., Saito, H., Imai, K. and Yamashita, O. (1998). Phe-X-Pro-Arg-Leu-NH2 peptide producing cells in the central nervous system of the silkworm, *Bombyx mori*. *J. Insect Phys.* **44**, 333-342.

Sautour, B. and Castel, J. (1995). Comparative spring distribution of zooplankton in three macrotidal European estuaries. *Hydrobiologia* **311**, 139-151.

Savage, P. D. V. (1965). Preliminary observations of the phytoplankton of Southampton Water. *Br. Phycol. Bull.* **2**, 515-516.

Sciandra, A., Gouze, J-L. and Nival, P. (1990). Modelling the reproduction of *Centropages typicus* (Copepoda: Calanoida) in a fluctuating food supply: effect of adaptation. *J. Plank. Res.* **12**, 549-572.

Seckiguchi, H., McLaren, I.A. and Corkett, C.J. (1980). Relationship between growth rate and egg production in the copepod *Acartia clausi hudsonica*. *Mar. Biol.* **58**, 133-138.

Shiomi, K., Sato, Y., Imai, K. and Yamashita, O. (1998). A hydrophobic peptide (VAP-peptide) of the silk worm *Bombyx mori*: Structure, expression and an enhancing function of diapause hormone activity. *Insect Biochem. Molec. Biol.* **28**, 75-82.

Slusarczyk, M. (1995). Predator induced diapause in Daphnia. *Ecology* **76**, 1008-1013.

Solorozano, L. (1969). Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnol. Oceanogr.* **14,** 799-801

Spielman, A. (1974). Effect of synthetic juvenile hormone on ovarian diapause of *Culex pipiens* mosquitoes. *J. Med. Ent.* **11**, 223-225.

Stearns, D. E., Tester, P.A. and Walker, R.L. (1989). Diel changes in the egg production rate of *Acartia tonsa* (Copepoda, Calanoida) and related environmental factors in two estuaries. *Mar. Ecol. Prog. Ser.* **52**, 7-16.

Stoecker, D. K. and Egloff, D.A. (1987). Predation by *Acartia tonsa* Dana on planktonic ciliates and rotifers. *J. Exp. Mar. Biol. Ecol.* **110**, 53-68.

Stoecker, D. K. and McDowell Capuzzo, J. (1990). Predation on protozoans importance to zooplankton. *J. Plank. Res.* **12**, 891-908.

Stottrup, J. G. and Jensen, J. (1990). Influence of algal diet on feeding and egg production of the calanoid copepod *A.tonsa* (Dana). *J. Exp. Mar. Biol. Ecol.* **141**, 87-105.

Stross, R. G. (1969). Photoperiod control of diapause in *Daphnia* II. Induction of winter diapause in the arctic. *Biol. Bull.* **136**, 264-273.

Stross, R. G. (1969). Photoperiod control of diapause in *Daphnia* III. Two-stimulus control of long-day, short-day induction. *Biol. Bull.* **137**, 359-374.

Stross, R. G. and Hill, J.C. (1965). Photoperiod control of winter diapause in the fresh-water crustacean, *Daphnia. Biol. Bull.* **134**, 176-198.

Tande, K. S. and Bamstedt, U. (1987). On the trophic fate of *Phaeocystis pouchetii* I. copepod feeding rates on solitary cells and colonies of *P. pouchetii. Sarsia* **72**, 313-320.

Tanskanen, S. (1994). Seasonal variability in the individual carbon content of the calanoid copepod *Acartia bifilosa* from the northern Baltic Sea. *Hydrobiologia* **292/293**, 397-403.

Taylor, F. (1980). Optimal switching to diapause in relation to the onset of winter. *Theor. Pop. Biol.* **18**, 125-133.

Templeman, W. (1936). The influence of temperature, salinity, light and food conditions on the survival and growth of the larvae of the lobster (*Homarus americanus*). *J. Biol. Bd. Can.* 2, 485-497.

Tester, P. A. (1985). Effects of parental acclimation temperature and egg-incubation temperature on egg-hatching time in *Acartia tonsa* (Copepoda: Calanoida). *Mar. Biol.* **89**, 45-53.

Tester, P. A. and Turner, J.T. (1990). How long does it take copepods to make eggs? *J. Exp. Mar. Biol. Ecol.* **141**, 169-182.

Tester, P. A. and Turner, J.T. (1991). Why is *A.tonsa* restricted to estuarine habitats? *Bull. Plank. Soc. Japan* **Spec. Vol.**, 603-611.

Tiselius, P. (1989). Contribution of aloricate ciliates to the diet of *Acartia clausi* and *Centropages hamatus* in coastal waters. *Mar. Ecol. Prog. Ser.* **56**, 49-56.

Tiselius, P. (1992). Behaviour of *A.tonsa* in patchy food environments. *Limnol. Oceanogr.* **37**, 1640-1651.

Tiselius, P., Hansen, B., Jonsson, P., Kiorboe, T., Nielsen, T.G., Piontkovski, S and Saiz E. (1995). Can we use laboratory-reared copepods for experiments? A comparison of feeding behaviour and reproduction between a field and a laboratory population of *Acartia tonsa*. *ICES J. Mar. Sci.* **52**, 369-376.

Todd, C. D. and Laverack, M.S. (1991). *Coastal marine zooplankton: a practical manual for students*: Cambridge Uni Press.

Touratier, F., Legendre, L. and Vezina, A. (1999). Model of copepod growth influenced by the food carbon:nitrogen ratio and concentration, under the hypothesis of strict homeostasis. *J. Plank. Res.* **21**, 1111-1132.

Trinast, E. M. (1975). Tidal currents and *Acartia* distribution in Newport Bay, California. *Est. Coast. Mar. Sci.* **3**, 165-176.

Urban-Rich, J., Hansell, D.A. and Roman, M.R.R. (1998). Analysis of copepod fecal pellet carbon using a high temperature combustion method. *Mar. Ecol. Prog. Ser.* **171**, 199-208.

Uriarte, I., Lotano, U. and Villate, F. (1998). Egg production of *A. bifilosa* in the small temperate estuary of Mundaka, Spain. *Mar. Ecol. Prog. Ser.* **166**, 197-205.

Uye, S. (1981). Fecundity studies of neritic calanoid copepods *Acartia clausi* (Giesbrecht) and *S. steueri* (Smirnov): A simple empirical model of daily egg production. *J. Exp. Mar. Biol. Ecol.* **50**, 255-271.

Uye, S. (1985). Resting egg production as a life history strategy of marine planktonic copepods. *Bull. Mar. Sci.* **37**, 440-449.

Uye, S. (1991). Temperature-dependent development and growth of the planktonic copepod *Paracalanus* sp. in the laboratory. *Bull. Plank. Soc. Jap. Spec. Vol.* , 627-636.

Uye, S. and Fleminger, A. (1976). Effects of various environmental factors on egg development of several species of *Acartia* in Southern California. *Mar. Biol.* **38**, 253-262.

Uye, S. and Liang, D. (1998). Copepods attain high abundance, biomass and production in the absence of large predators but suffer cannibalistic loss. *J. Mar. Syst.* **15**, 495-501.

Veldhuis, M. J. W., Admiraal, W. and Colijn, F. (1986). Chemical and physiological changes of phytoplankton during the spring bloom, dominated by *Phaeocystis pouchetii* (Haptophyceae): Observations in Dutch coastal waters of the North Sea. *Neth. J. Sea Res.* **20**, 49-60.

Verity, P. G. and Smayda, T.J. (1989). Nutritional value of *Phaeocystis poucheti* (Prymnesiophyceae) and other phytoplankton for *Acartia* spp. (Copepod): Ingestion, egg production and growth of nauplii. *Mar. Biol.* **100**, 161-171.

Viitasalo, M. (1992). Calanoid resting eggs in the Baltic Sea: Implications for the population dynamics of *A.bifilosa* (Copepoda). *Mar. Biol.* **114**, 397-405.

Viitasalo, M. and Katajisto, T. (1994). Mesozooplankton resting eggs in the Baltic Sea: identification and vertical distribution in laminated and mixed sediments. *Mar. Biol.* **120**, 455-465.

Viitasalo, M., Koski, M., Pellikka, K. and Johnson, J. (1995). Seasonal and long term variations in the body size of planktonic copepods in the Northern Baltic Sea. *Mar. Biol.* **123,** 241-250.

Villante, F., Ruiz, A. and Franco, J. (1993). Summer zonation and development of zooplankton populations within a shallow mesotidal system: the estuary of Mundaka. *Cah. Biol. Mar.* **34**, 131-143.

Volkman, J. K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I. and Garland, C.D. (1989). Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol.* **128**, 219-240.

Walve, J. and Larsson, U. (1999). Carbon, nitrogen and phosphorus stoichiometry of crustacean zooplankton in the Baltic Sea: implications for nutrient recycling. *J. Plank. Res.* **21**, 2309-2321.

Webber, N. B. (1980). Hydrology and water circulation in the Solent. In *The Solent Estuarine System: An Assessment of Present Knowledge.*, pp. 25-36. Edited by J. D. Burton. Southampton: NERC.

Weisse, T. (1983). Feeding of calanoid copepods in relation to *Phaeocystis pouchetii* blooms in the German Wadden Sea off Sylt. *Mar. Biol.* **74**, 87-94.

White, J. R. and Roman, M.R. (1992). Egg production by the calanoid copepod *A.tonsa* in the mesohaline Chesapeake Bay: the importance of food resources and temperature. *Mar. Ecol. Prog. Ser.* **86**, 239-249.

Widdows, J. and Bayne, B.L. (1971). Temperature acclimation of *Mytilus edulis* with reference to its energy budget. *J. Mar. Biol. Ass. UK* **51**, 827-843.

Williams, J. A. (1996). Blooms of *Mesodinium rubrum* in Southampton Water - do they shape mesozooplankton distribution? *J. Plank. Res.* **18**, 1685-1697.

Williams, P. J. L. (1980). Phytoplankton in Southampton Water. In *The Solent Estuarine System:an assessment of present knowedge.* Edited by J. D. Burton.

Williams, R., Conway, D.V.P. and Hunt, H.G. (1994). The role of copepods in the planktonic ecosystems of mixed and stratified waters of the European shelf seas. *Hydrobiologia* **292/293**, 521-530.

Williams-Howze, J. and Coull, B.S. (1992). Are temperature and photoperiod necessary cues for encystment in the marine benthic harpacticoid copepod *Heterosyllus nunni* (Coull)? *Biol. Bull.* **182**, 109-116.

Williamson, C. E. and Butler, N.M. (1987). Temperature, food and mate limitation of copepod reproductive rates: separating the effects of multiple hypotheses. *J. Plank. Res.* **9**, 821-836.

Wilson, D. S. (1972). Food size selection among copepods. *Ecology* **54**, 909-914.

Winberg, G. G. (1960). Rate of metabolism and food requirements of fishes. *Fish. Res. Bd. Can. Trans. Ser.* **194**, 202.

Wooldridge, T. and Erasmus, T. (1980). Utilisation of tidal currents by estuarine zooplankton. *Est. Coast. Mar. Sci.* **11**, 107-114.

Wooldridge, T. and Melville-Smith, R. (1979). Copepod succession in two South African estuaries. *J. Plank. Res.* **1**, 329-341.

Xu, W., Sato, Y., Ikeda, M. and Yamashita, O. (1995). Stage-dependent and temperature-controlled expression of the gene encoding the precursor protein of diapause hormone and pheremone biosynthesis activating neuropeptide in the silkworm, *Bombyx mori. Am. Soc. Biochem. Mol. Biol.* **270**, 3804-3808.

Yoon, W. D., Shim, M.B. and Choi, J.K. (1998). Description of the developmental stages in *Acartia bifilosa* Giesbrecht (Copepoda:Calanoida). *J. Plank. Res.* **20**, 923-942.

Zillioux, E. J. and Gonzalez, J.G. (1970). Egg dormancy in a neritic calanoid copepod and its implications to overwintering in boreal estuaries. In *5th European Marine Biology Symposium*, pp. 217-230. Edited by B. Battaglia.

Zinger, I. (1989). Zooplankton community structure in Southampton Water and its potential response to estuary chronic oil pollution. PhD thesis, University of Southampton. 365pp.

Zmijewska, M. I., Yen, J., Bielecka, L. and Blachowiak-Samolyk, K. (1997). The effect of sex ratio on the population pattern and abundance of the predominant antarctic copepod in Croker Passage. *Oc. Stud. Gdansk* **26**, 127-149.

Appendix 1: Reagents/solutions for algal cultures

Reagents	Ingredients	Reference
Solution A	2.60g $FeCl_3.6H_2O$	Baynes *et al.* 1979
Nutrient enrichment solution	0.72g $MnCl_2.4H_2O$	
	67.20g H_3BO_4	
	90.00g EDTA (Na salt)	
	40.00g $NaH_4PO_4.2H_2O$	
	200g $NaNO_3$	
	2.0 ml of Solution B made up to 2l with distilled water.	
Solution B	2.10g $ZnCl_2$	
Trace metal solution	2.0g $CoCl_2.6H_2O$	
	0.9g $(NH_4)_6Mo_7O_{24}.4H_2O$	
	2.0g $CuO_4.5H_2O$	
	made up to 100l with distilled water.	
Solution C	10 mg Vitamin B_{12}	
Vitamin stock solution	200mg Vitamin B_1	
	made up to 200ml with distilled water.	
Silicate solution	20g $Va_2SiO_3.5H_2O$	
	made up to 500ml with distilled water.	

1) 40 ml sodium hypochlorite and 10 ml of solution A were added to 20 litres of chemically sterilised seawater, in an algal culture flask. N.B. for diatoms, 10ml of the silicate solution was added.
2) Leave for 5 hours. Reconnect the airline, but do not switch on.
3) Sterilise pipettes with ethanol.
4) Add 20ml sodium thiosulphate to neutralise the bleach.
5) Add 2ml of solution C to the flask.
6) Approximately 1l of previously cultured algae was added to the flask as an inoculum. Artificial lighting was provided for the algae, the cultures were kept at 20°C and air was bubbled through at approximately 5l minute $^{-1}$ to provide 1-2% CO_2.

Appendix 2: Length and dry weight of individual *A. bifilosa, A. discaudata*, *A. clausi* and *A. tonsa* females

Squares of GF/F filter paper 1.5cm x 1.5cm were ashed at 550°C for 24 hours and weighed. 10 replicates of 5 freshly caught mature females of each *Acartia* species, (*bifilosa, discaudata, clausi* and *tonsa*) were rinsed in fresh water and placed on the squares and left to dry for 24 hours at 60°C and then weighed. The mean dry weight of an individual *Acartia* female of each species was then calculated.

Species	Length of adult female (mm) (Rose 1933, Bradford-Greive 1999)	Dry weight of a single adult female (mg)
A. bifilosa	1.00-1.10	0.595 ± 0.14
A. discaudata	1.00-1.20	0.523 ± 0.08
A. clausi	1.15-1.22	0.454 ± 0.23
A. tonsa	1.30-1.50	0.604 0.11

Appendix 3: Chemicals for fatty acid analysis

- KCl (0.88%) – 0.88g KCl in 10ml H_2O
- KOH (95% ethanol) - 500µl KOH, 9.5ml ethanol
- HCl (0.6M) – 1ml HCl, 9ml H_2O
- Acetonitrile – 10ml
- Diethyl ether – 10ml
- PFB (3.5% in acetonitrile) - 350µl PFB, 9.65 ml acetonitrile
- Triethylamine – 10ml
- Isooctane – 10ml
- $NaHCO_3$ (2%) – 0.2g $NaHCO_3$ in 10ml H_2O

Appendix 4: Annual temperature and photoperiod readings

(Temperature values from Castro-Longoria 1998, hours of daylight values from David Pond, personal communication).

Month	Hours of daylight	Temperature (°C)
Jan	8	7.8
Feb	10	5.0
Mar	12	5.4
Apr	14	8.8
May *	14	11.6
Jun *	14	16.0
Jul *	16	20.0
Aug *	14	20.0
Sep	12	18.0
Oct	10	16.0
Nov	8	11.8
Dec	8	10.6

* = months of *A. bifilosa* diapause (Castro-Longoria 1998)

Appendix 5: SIMPER results for Chapter 5

Similarity Percentages - species contributions

Factor groups
O=October
N=November
D=December
J=January
F=February
M=March
A=April

Group O

Average similarity: 92.89

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
18:0	48.12	45.81	20.06	49.32	49.32
16:0	17.52	17.06	41.89	18.37	67.69
18:1(n-7)	12.84	12.35	20.18	13.30	80.99
22:6(n-3)	5.08	4.25	3.80	4.58	85.57
14:0	4.30	3.83	6.01	4.12	89.69
20:5(n-3)	3.05	2.45	3.58	2.64	92.33

Group N

Average similarity: 94.48

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
18:0	62.68	61.28	50.87	64.86	64.86
18:1(n-7)	14.88	14.33	10.89	15.16	80.02
16:0	14.30	13.54	11.23	14.33	94.35

Group D

Average similarity: 93.46

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
18:0	56.61	55.77	81.93	59.67	59.67
18:1(n-7)	17.33	15.64	10.89	16.73	76.40
16:0	16.09	15.56	23.27	16.65	93.05

Group J

Average similarity: 90.24

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
18:0	51.82	49.56	17.29	54.92	54.92
16:0	21.94	20.79	15.26	23.04	77.97
18:1(n-7)	14.83	12.70	5.34	14.08	92.04

Group F

Average similarity: 88.48

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
16:0	44.65	42.66	22.81	48.22	48.22
18:0	18.99	17.09	9.24	19.31	67.53
14:0	13.90	11.54	3.09	13.05	80.58
20:5(n-3)	4.73	3.80	2.88	4.30	84.87
18:1(n-7)	3.29	2.97	9.02	3.36	88.24
22:6(n-3)	3.91	2.96	3.56	3.34	91.58

Group M

Average similarity: 84.97

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
16:0	33.01	30.88	23.74	36.34	36.34
14:0	14.40	12.53	6.21	14.75	51.09
18:0	12.02	10.93	16.07	12.86	63.95
18:1(n-9)	11.90	10.68	8.32	12.56	76.51
16:1(n-7)	9.22	7.18	4.09	8.45	84.97
22:6(n-3)	4.79	3.31	1.63	3.90	88.86
18:1(n-7)	3.12	2.87	20.84	3.38	92.24

Group A

Average similarity: 89.71

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
16:0	31.17	30.72	25.90	34.25	34.25
14:0	24.69	23.63	17.12	26.34	60.59
16:1(n-7)	12.97	11.50	3.83	12.82	73.41
18:0	6.86	5.98	4.07	6.67	80.08
20:5(n-3)	5.97	5.55	10.89	6.18	86.27
18:1(n-7)	3.26	2.52	2.44	2.81	89.08
16:2(n-3)	2.52	2.36	8.17	2.63	91.71

Groups O & N

Average dissimilarity = 17.31

	Group O	Group N				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:0	48.12	62.68	7.28	3.59	42.05	42.05
22:6(n-3)	5.08	0.78	2.15	2.71	12.41	54.46
16:0	17.52	14.30	1.61	2.24	9.28	63.75
14:0	4.30	1.78	1.26	2.77	7.29	71.04
20:5(n-3)	3.05	0.64	1.21	2.33	6.97	78.00
18:1(n-7)	12.84	14.88	1.09	2.08	6.28	84.28
16:1(n-7)	2.69	0.95	0.87	1.35	5.02	89.30
18:1(n-9)	2.35	1.14	0.61	1.95	3.50	92.80

Groups O & D

Average dissimilarity = 13.57

	Group O	Group D				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:0	48.12	56.61	4.24	2.30	31.27	31.27
18:1(n-7)	12.84	17.33	2.24	1.71	16.53	47.79
22:6(n-3)	5.08	1.44	1.82	2.35	13.40	61.19
14:0	4.30	2.01	1.14	2.34	8.43	69.63
20:5(n-3)	3.05	1.19	0.93	1.64	6.86	76.49
16:1(n-7)	2.69	1.52	0.86	1.47	6.37	82.86
16:0	17.52	16.09	0.73	1.41	5.35	88.21
18:1(n-9)	2.35	1.35	0.58	1.26	4.31	92.52

Groups N & D

Average dissimilarity = 8.49

Species	Group N Av.Abund	Group D Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:0	62.68	56.61	3.04	2.41	35.74	35.74
18:1(n-7)	14.88	17.33	1.38	1.15	16.27	52.01
16:0	14.30	16.09	0.94	1.40	11.01	63.02
22:6(n-3)	0.78	1.44	0.62	1.41	7.24	70.26
16:1(n-7)	0.95	1.52	0.54	1.05	6.30	76.56
18:1(n-9)	1.14	1.35	0.46	2.36	5.44	82.00
20:5(n-3)	0.64	1.19	0.43	1.42	5.03	87.03
18:3(n-3)	1.56	1.17	0.40	1.28	4.66	91.69

Groups O & J

Average dissimilarity = 12.72

Species	Group O Av.Abund	Group J Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:0	48.12	51.82	2.49	1.48	19.62	19.62
16:0	17.52	21.94	2.21	2.41	17.39	37.00
22:6(n-3)	5.08	1.12	1.98	2.42	15.57	52.58
18:1(n-7)	12.84	14.83	1.62	1.67	12.72	65.29
20:5(n-3)	3.05	0.44	1.31	2.47	10.27	75.56
16:1(n-7)	2.69	1.13	0.79	1.22	6.19	81.75
14:0	4.30	3.62	0.66	1.06	5.22	86.97
18:1(n-9)	2.35	2.39	0.59	1.32	4.68	91.65

Groups N & J

Average dissimilarity = 14.98

Species	Group N Av.Abund	Group J Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:0	62.68	51.82	5.43	2.74	36.23	36.23
16:0	14.30	21.94	3.82	3.66	25.49	61.72
18:1(n-7)	14.88	14.83	1.43	1.75	9.53	71.25
14:0	1.78	3.62	1.10	1.97	7.36	78.60
18:1(n-9)	1.14	2.39	0.86	2.17	5.71	84.31
22:6(n-3)	0.78	1.12	0.57	1.18	3.81	88.12
18:3(n-3)	1.56	0.60	0.50	1.12	3.32	91.45

Groups D & J

Average dissimilarity = 11.63

Species	Group D Av.Abund	Group J Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
16:0	16.09	21.94	2.93	3.16	25.18	25.18
18:0	56.61	51.82	2.43	1.38	20.87	46.05
18:1(n-7)	17.33	14.83	1.93	1.43	16.60	62.65
14:0	2.01	3.62	1.03	1.86	8.82	71.48
18:1(n-9)	1.35	2.39	0.80	1.36	6.86	78.33
22:6(n-3)	1.44	1.12	0.58	1.32	4.98	83.31
16:1(n-7)	1.52	1.13	0.53	1.07	4.52	87.83
20:5(n-3)	1.19	0.44	0.48	1.36	4.11	91.94

Groups O & F

Average dissimilarity = 42.34

Species	Group O Av.Abund	Group F Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:0	48.12	18.99	14.56	6.58	34.39	34.39
16:0	17.52	44.65	13.56	8.46	32.03	66.42
14:0	4.30	13.90	4.80	2.57	11.33	77.75
18:1(n-7)	12.84	3.29	4.78	10.99	11.28	89.03
20:5(n-3)	3.05	4.73	1.00	1.62	2.37	91.40

Groups N & F

Average dissimilarity = 56.11

Species	Group N Av.Abund	Group F Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.
18:0	62.68	18.99	21.84	12.47	38.93	38.93
16:0	14.30	44.65	15.17	9.04	27.04	65.97
14:0	1.78	13.90	6.06	3.26	10.80	76.77
18:1(n-7)	14.88	3.29	5.80	9.91	10.34	87.10
20:5(n-3)	0.64	4.73	2.05	2.69	3.65	91.80

Groups D & F

Average dissimilarity = 52.62

Species	Group D Av.Abund	Group F Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:0	56.61	18.99	18.81	12.20	35.74	35.74
16:0	16.09	44.65	14.28	8.89	27.14	62.88
18:1(n-7)	17.33	3.29	7.02	5.48	13.34	76.22
14:0	2.01	13.90	5.94	3.18	11.29	87.52
20:5(n-3)	1.19	4.73	1.77	2.23	3.37	90.88

Groups J & F

Average dissimilarity = 45.33

Species	Group J Av.Abund	Group F Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:0	51.82	18.99	16.42	7.55	36.21	36.21
16:0	21.94	44.65	11.35	6.41	25.05	61.26
18:1(n-7)	14.83	3.29	5.77	3.61	12.73	73.99
14:0	3.62	13.90	5.14	2.58	11.34	85.33
20:5(n-3)	0.44	4.73	2.15	2.79	4.74	90.06

Groups O & M

Average dissimilarity = 48.17

Species	Group O Av.Abund	Group M Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:0	48.12	12.02	18.05	9.39	37.47	37.47
16:0	17.52	33.01	7.75	4.50	16.08	53.56
14:0	4.30	14.40	5.05	3.55	10.48	64.04
18:1(n-7)	12.84	3.12	4.86	11.66	10.09	74.13
18:1(n-9)	2.35	11.90	4.77	4.79	9.91	84.04
16:1(n-7)	2.69	9.22	3.27	2.00	6.78	90.82

Groups N & M

Average dissimilarity = 62.97

Species	Group N Av.Abund	Group M Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:0	62.68	12.02	25.33	18.55	40.23	40.23
16:0	14.30	33.01	9.35	5.22	14.85	55.08
14:0	1.78	14.40	6.31	4.48	10.02	65.10
18:1(n-7)	14.88	3.12	5.88	10.29	9.34	74.44
18:1(n-9)	1.14	11.90	5.38	5.58	8.54	82.99
16:1(n-7)	0.95	9.22	4.14	2.69	6.57	89.56
22:6(n-3)	0.78	4.79	2.05	1.81	3.25	92.81

Groups D & M

Average dissimilarity = 59.18

Species	Group D Av.Abund	Group M Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:0	56.61	12.02	22.30	20.60	37.67	37.67
16:0	16.09	33.01	8.46	4.90	14.30	51.97
18:1(n-7)	17.33	3.12	7.10	5.57	12.01	63.98
14:0	2.01	14.40	6.19	4.36	10.46	74.44
18:1(n-9)	1.35	11.90	5.27	4.93	8.91	83.36
16:1(n-7)	1.52	9.22	3.85	2.33	6.51	89.86
22:6(n-3)	1.44	4.79	1.76	1.67	2.98	92.84

Groups J & M

Average dissimilarity = 51.93

Species	Group J Av.Abund	Group M Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:0	51.82	12.02	19.90	10.60	38.33	38.33
18:1(n-7)	14.83	3.12	5.86	3.67	11.28	49.61
16:0	21.94	33.01	5.54	2.94	10.66	60.27
14:0	3.62	14.40	5.39	3.40	10.38	70.65
18:1(n-9)	2.39	11.90	4.75	4.01	9.15	79.80
16:1(n-7)	1.13	9.22	4.05	2.62	7.79	87.59
20:5(n-3)	0.44	4.50	2.03	1.23	3.91	91.51

Groups F & M

Average dissimilarity = 24.60

Species	Group F Av.Abund	Group M Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
16:0	44.65	33.01	5.82	2.54	23.65	23.65
18:1(n-9)	2.50	11.90	4.70	4.82	19.11	42.76
18:0	18.99	12.02	3.49	2.14	14.18	56.94
16:1(n-7)	2.30	9.22	3.46	2.17	14.08	71.02
14:0	13.90	14.40	1.82	1.34	7.42	78.44
20:5(n-3)	4.73	4.50	1.44	1.43	5.85	84.29
22:6(n-3)	3.91	4.79	1.16	1.63	4.72	89.02
16:3(n-3)	2.27	1.75	0.79	1.14	3.21	92.23

Groups O & A

Average dissimilarity = 55.06

Species	Group O Av.Abund	Group A Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:0	48.12	6.86	21.04	10.40	38.21	38.21
14:0	4.30	24.69	10.40	6.11	18.89	57.10
16:0	17.52	31.17	6.96	5.03	12.64	69.75
16:1(n-7)	2.69	12.97	5.24	3.24	9.52	79.26
18:1(n-7)	12.84	3.26	4.89	6.47	8.88	88.14
22:6(n-3)	5.08	1.46	1.85	2.84	3.35	91.50

Groups N & A

Average dissimilarity = 68.28

Species	Group N Av.Abund	Group A Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:0	62.68	6.86	28.46	18.71	41.68	41.68
14:0	1.78	24.69	11.69	6.89	17.12	58.80
16:0	14.30	31.17	8.60	5.83	12.60	71.39
16:1(n-7)	0.95	12.97	6.12	4.04	8.97	80.36
18:1(n-7)	14.88	3.26	5.93	6.91	8.69	89.05
20:5(n-3)	0.64	5.97	2.72	4.95	3.98	93.03

Groups D & A

Average dissimilarity = 64.63

Species	Group D Av.Abund	Group A Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:0	56.61	6.86	25.37	20.43	39.25	39.25
14:0	2.01	24.69	11.57	6.78	17.90	57.15
16:0	16.09	31.17	7.69	5.54	11.90	69.05
18:1(n-7)	17.33	3.26	7.18	4.95	11.10	80.15
16:1(n-7)	1.52	12.97	5.83	3.57	9.03	89.18
20:5(n-3)	1.19	5.97	2.44	4.07	3.77	92.95

Groups J & A

Average dissimilarity = 57.28

Species	Group J Av.Abund	Group A Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:0	51.82	6.86	22.93	11.54	40.03	40.03
14:0	3.62	24.69	118:0	5.82	18.77	58.79
16:1(n-7)	1.13	12.97	6.03	3.96	10.53	69.32
18:1(n-7)	14.83	3.26	5.90	3.39	10.31	79.63
16:0	21.94	31.17	4.71	2.98	8.22	87.85
20:5(n-3)	0.44	5.97	2.82	5.02	4.92	92.77

Groups F & A

Average dissimilarity = 29.64

Species	Group F Av.Abund	Group A Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
16:0	44.65	31.17	6.87	3.33	23.17	23.17
18:0	18.99	6.86	6.19	3.70	20.89	44.06
14:0	13.90	24.69	5.51	2.21	18.59	62.65
16:1(n-7)	2.30	12.97	5.44	3.44	18.35	81.00
22:6(n-3)	3.91	1.46	1.25	1.70	4.22	85.23
18:4(n-3)	0.60	2.50	0.97	3.39	3.27	88.49
20:5(n-3)	4.73	5.97	0.84	1.28	2.84	91.33

Groups M & A

Average dissimilarity = 23.86

Species	Group M Av.Abund	Group A Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
14:0	14.40	24.69	5.25	2.43	22.02	22.02
18:1(n-9)	11.90	1.95	5.08	4.63	21.28	43.30
18:0	12.02	6.86	2.63	2.15	11.04	54.34
16:1(n-7)	9.22	12.97	2.39	1.52	10.04	64.38
16:0	33.01	31.17	1.81	1.22	7.58	71.96
22:6(n-3)	4.79	1.46	1.72	1.57	7.22	79.18
20:5(n-3)	4.50	5.97	1.67	2.02	6.99	86.17
18:3(n-3)	2.10	0.41	0.86	1.45	3.62	89.79
18:4(n-3)	1.54	2.50	0.73	1.76	3.07	92.85

Appendix 6: Copepod collection and identification

Collection of samples

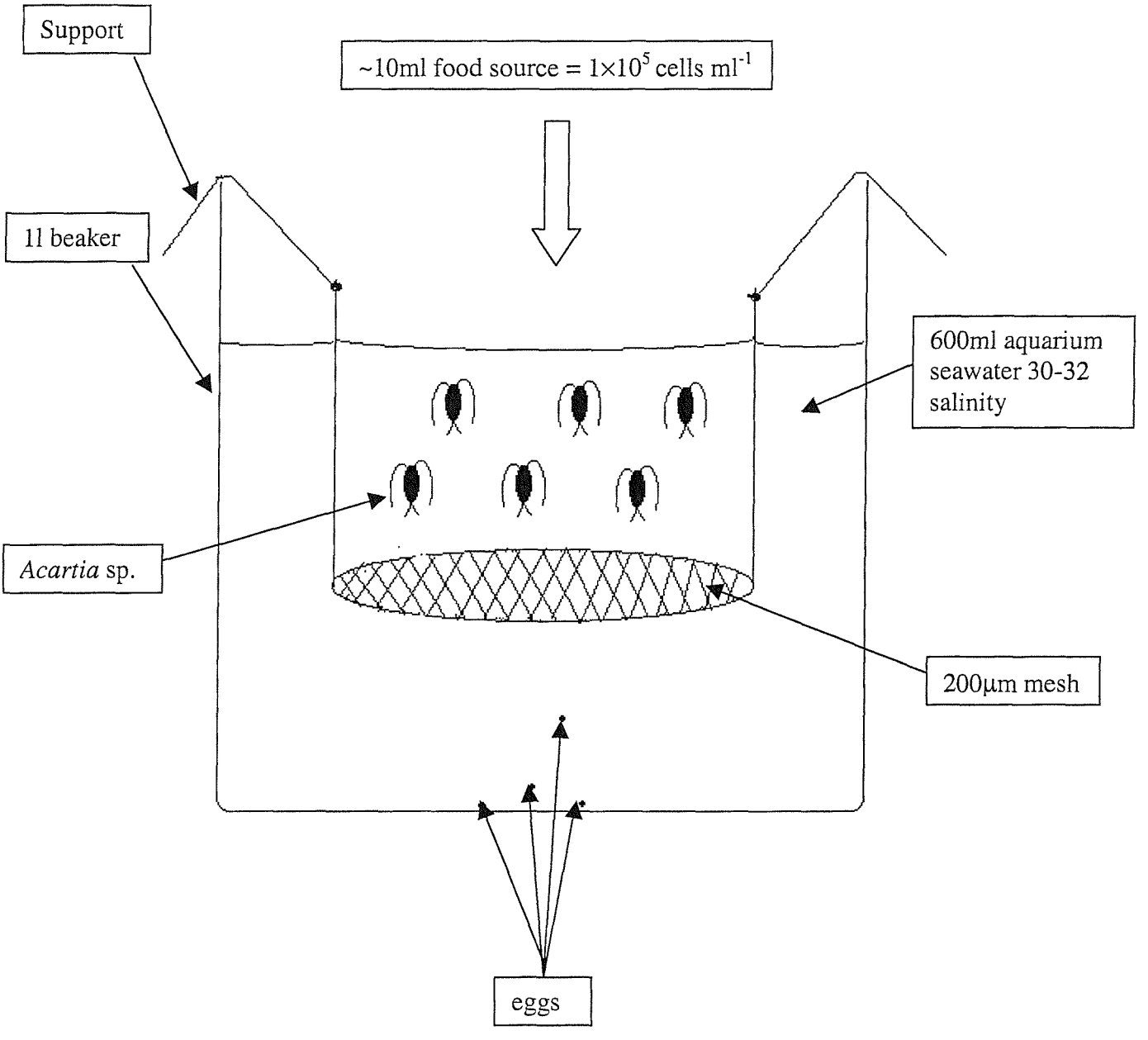
Owing to the seasonal succession of the *Acartia* species in the estuary, it was necessary to carry out qualitative sampling at regular intervals over the course of the year. Experimental individuals were collected using a net with a 120μm mesh during horizontal tows of 5 minutes duration at approximately 1 knot and 2-4m depth, on or as near to high water as possible.

No set site for sampling was adhered to, but it is known that *A. tonsa* prefers fresher water (Castro-Longoria 1998) as so was sampled for at Cracknore. Both *A. bifilosa* and *A. discaudata* are considered "intermediate" species in terms of salinity (Castro-Longoria 1998) and were collected off Netley. In contrast, *A. clausi* as the more marine of the congeners was collected from Calshot (Figure 1.1). After each tow, the net was rinsed with seawater to contain all of the zooplankton in the cod end. The resultant sample was then stored in a 2l brefin bottle filled with seawater until the return to the laboratory, this was no more than 2 hours from the time of collection. Samples were then filtered through a 1.5mm mesh to remove the larger organisms, specifically seasonal predators like *Sagitta, Aurelia* and *Pleurobranchia*. The copepods and smaller zooplankton were placed in a glass container with 2l of 26μm mesh filtered seawater. 10ml of one of the three phytoplankton species, (*Isochrysis, Phaeodactylum* or *Skeletonema*) was added at the start as a food source. This gave a cell concentration of 1×10^4 to 1×10^6 cells ml^{-1}. An airline was introduced which induced water and food circulation in addition to keeping the system aerated.

Acartia identification

After 24 hours, a sub-sample of approximately 100ml was taken from the stock population. *Acartia* copepods were removed using a wide-bore 2.5ml Pasteur pipette and placed in droplets of seawater on a petri dish. Each droplet contained one or two copepods which facilitated the identification of the individuals, and also meant that they could be kept alive during the process. The identification of the *Acartia* species was based on Rose (1933), Farran (1948) and Bradford-Grieve (1999). Adult females were then used in the experiments.

Appendix 7: *Acartia* incubation/egg production chamber



Appendix 8: Ammonia calibration curve

Chemicals

0.01M ammonium chloride: 0.535g NH_4Cl l^{-1}
Phenol solution: 10g phenol in 100ml 95% ethanol
0.5% sodium nitroprusside: 1g sodium nitroprusside in 200ml distilled water
1.5N sodium hypochlorite
Alkaline solution: 100g tri-sodium citrate and 5g NaOH in 500ml distilled water
Oxidising solution: 100ml alkaline solution and 25ml sodium hypochlorite

Method

1ml of 0.01M NH_4Cl was added to 1l of distilled water to give 0.535mg NH_4Cl l^{-1}. Sub-samples of this were then taken and added to vials containing100ml of distilled water.
0.2ml = 0.107µg NH_4Cl in 100ml = 1.07µg NH_4Cl l^{-1}
0.5ml = 0.2675µg NH_4Cl in 100ml = 2.675µg NH_4Cl l^{-1}
0.7ml = 0.3745µg NH_4Cl in 100ml = 3.745µg NH_4Cl l^{-1}
1.0ml = 0.535µg NH_4Cl in 100ml = 5.35µg NH_4Cl l^{-1}

The procedure consisted of successive adding of 400µl phenol solution, 400µl sodium nitroprusside and 1000µl oxidising solution to the sub-samples. The colour was allowed to develop for 1 hour at room temperature and the absorbency recorded at 640nm. These values were then converted into ammonia concentrations in µg l^{-1}.