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Seasonal Patterns of Protoplankton and Calanoid Copepods in the Firth of Forth: an Investigation of Copepod Grazing and the Effect of Diatoms upon Reproductive Processes

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Doctor of Philosophy

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Abstract

Seasonal variability in the zooplankton of the Firth of Forth, including mesozooplankton and microplankton, were investigated across a full annual cycle. Grazing rates and prey selectivity by the dominant calanoid copepods (*Acartia discaudata*, *Acartia clausi*, *Centropages hamatus* and *Temora longicornis*) were also examined. Investigations were conducted upon *Acartia discaudata* and field collected prey under simulated diatom bloom conditions, using the species *Thalassiosira rotula,* in order to test the hypothesis of diatom inhibition of calanoid copepod reproductive processes.

There were fundamental differences between the spring and autumn blooms, typical of temperate estuaries. The spring bloom was composed of highly abundant, small cells amounting to less than half the biomass of larger, less numerous microplankton present in autumn. Copepods dominated the mesozooplankton undergoing seasonal shifts in species' dominance primarily in response to physical factors.

Results of grazing experiments indicate a disproportionate preference for motile prey compared to ubiquitous concentrations of diatoms. Copepods switch to blooming diatom species when present in concentrations > 80 cells ml^{-1}. Ciliates generally contributed < 25% to copepod carbon ingestion. *Acartia discaudata*, *Acartia clausi*, and *Centropages hamatus* selectively consumed dinoflagellate and ciliate taxa whereas *Temora longicornis* remained an indiscriminate grazer during the entire study.

Despite increasing rates of egg production in *Acartia discaudata*, at high concentrations (> 1 x 10^3 cells ml^{-1}), *Thalassiosira rotula* inhibited hatching success such that recruitment to naupliar stage 2 was severely impaired compared with eggs hatched from females fed ~0.3 x 10^3 cells ml^{-1} concentrations of *T. rotula*. This is the first recorded evidence of embryogenic inhibition in *A. discaudata*.

Dedication

To E, for her love and faith.

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Table of Contents

Abstract ii

Acknowledgements iv

Table of Contents v

Chapter 1 - General Introduction 1

1.1 Biodiversity of calanoid copepods 1

1.2 Life cycle strategies 1

1.3 Growth and Reproduction 2

1.4 Feeding 3

1.5 Estuarine copepod populations 5

1.6 Trophic status of calanoid copepods 6

1.7 Diatom embryogenic inhibition 7

1.8 Study location and general description 8

1.9 Study aims 8

Chapter 2 – Seasonal variability of the microbial web and calanoid copepods in the Firth of Forth 11

2.1 Introduction 11

2.2 Methods 13

2.2.1 Salinity and temperature 13

2.2.2 Pigments 15

2.2.3 Protoplankton 16

2.2.4 Mesozooplankton 17

2.2.5 Statistical analyses 17

2.3 Results 19

2.3.1 Salinity and temperature 19

2.3.2 Phytoplankton 19

2.3.3 Microzooplankton 27

2.3.4 Calanoid copepods 30

2.4 Discussion 34

2.4.1 Phytoplankton succession and the microbial web 34

2.4.2 Calanoid copepod succession 38
Chapter 3 – Seasonal patterns in feeding and prey selectivity by four calanoid copepods in Scottish coastal waters. 46
3.1 Introduction 46
3.2 Methods 48
3.2.1 Incubation copepods 48
3.2.2 Grazing rate calculations 49
3.2.3 Statistical analyses 54
3.3 Results 56
3.3.1 Salinity and temperature 56
3.3.2 Microplankton seasonal dynamics 58
3.3.3 Prey ingestion 58
3.4 Discussion 67
3.4.1 Ingestion of ciliates 67
3.4.2 Ingestion of dinoflagellates 70
3.4.3 Ingestion of flagellates 71
3.4.4 Ingestion of diatoms 71
3.4.5 Concluding remarks 73

Chapter 4 – Effects of *Thalassiosira rotula* on egg production, hatching success and naupliar development of *Acartia discaudata* 74
4.1 Introduction 74
4.2 Methods 79
4.2.1 Copepod collection 79
4.2.2 Thalassiosira rotula cultures 80
4.2.3 Food media preparation 82
4.2.4 Egg production, viability and naupliar development 85
4.2.5 Prey Ingestion Rates 86
4.2.6 Statistical analyses 87
4.3 Results 90
4.3.1 Food media and grazing rates 90

4.3.2 Egg production rates 93
4.3.3 Stage duration 99
4.3.4 Hatching success 104
4.3.5 Female mortality 106
4.4 Discussion 109
4.4.1 Egg production and hatching success 109
4.4.2 Nauplii mortality and stage durations 113

Chapter 5 – General Discussion 117
5.1 Summary 117
5.2 Further research 121
References 123

Chapter 1 - General Introduction

1.1 Biodiversity of Calanoid copepods

Copepods are possibly the most numerous multicellular organisms on earth (Turner 2000, Kiørboe 1998, Mauchline 1998) and are mesoplanktonic metazoan crustaceans, ranging in size from 500µm to ~5mm. Divided into 10 orders the Copepoda are dominated in the marine pelagic environment by the order Calanoida (Humes 1994), which regularly constitute up to 80% of total mesozooplankton biomass (Jónasdóttir 1998). Accordingly calanoid copepod biomass can exceed that of all other copepod orders combined (Longhurst 1985, Coyle 1990) particularly following periods of high primary productivity in temperate latitudes. In the oligotrophic open oceans and Polar regions the diversity of calanoid copepods tends to be lower with the major proportion of the biomass dominated by larger bodied species (Vidal and Smith 1986, Kobari *et* a*l*. 2003) in direct contrast to the smaller bodied neritic copepods prevalent in the more productive coastal areas (Williams *et al*. 1994).

As a consequence of their biomass dominance, copepods are the major metazoan consumer of both primary and microbial production, and the main conduit through which such production reaches higher trophic levels such as fish larvae (Runge 1988, Purcell and Grover 1990). Understanding the demographics of copepod populations (e.g. growth dynamics and reproductive capacity) and feeding preferences and rates, are fundamental to our understanding of copepod mediated regulation of microbial foodwebs and critical to our ability to understand energy flow.

1.2 Life cycle strategies

The growth and reproduction of calanoid copepods in temperate estuarine and coastal regions varies radically from that of other calanoid copepods in the open oceans, polar and tropical regions. Temperate estuarine calanoid copepods generally exhibit multi generational life cycle strategies in which species are able to span the time between one growing season and another by producing resting (diapause) eggs at the onset of

winter. Diapause eggs are believed to be genetically predetermined maternally as a response to the onset of environmental cues (Katajisto 2003) and are able to persist in the sediment ready to hatch as temperatures increase and the food environment improves again the following spring. Copepods also produce normal (subitaneous) eggs which can enter quiescence, which is a retarded state resulting in prolonged embryonic development which allows eggs to survive short lived periods of environmental adversity (Grice and Marcus 1981, Sørensen *et al*. 2007).

Generation times of small bodied estuarine calanoid species are typically very short, on the order of weeks, with many generations produced during the course of a single season. In contrast, the larger species present in the open ocean and polar regions will be characterised by either annual or multi-year cycles in which late stage copepodites (usually CIV or V) undergo periods of over-wintering diapause in deeper waters ascending in spring to moult to CVI and begin reproduction (Conover 1998, Atkinson 1998, Peterson 1998). Finally, tropical copepod communities although again dominated by small bodied species as in temperate estuaries exhibit very little or no seasonal patterns in either biomass fluctuations or egg production rates (Satapoomin *et al*. 2004).

1.3 Growth and Reproduction

Despite the broad variations in life cycle strategy, described above, calanoid copepods follow the same generalised pattern of growth and development. Copepod reproduction is sexual, the male transferring sperm via spermatophores, which are attached externally to the genital segment (first urosome segment) of females. Egg fertilisation and oogenesis then occurs within the female oviduct. Fertilised eggs are subsequently exuded into the external environment for further development prior to egg hatching. Females release eggs in two ways; a) broadcast spawning species release individual eggs into the surrounding seawater; b) sac spawning species release eggs which remain adhered to the female's body surface in clumps or within a membrane, and the eggs hatch whilst still attached to the female. The implications for egg production as a result of copepod grazing is one of the most thoroughly studied processes in copepod ecology (Hopcroft 1998) and egg production has historically been used as the primary measure of growth and consequently carbon flux in adult females (Hirst and McKinnon

2001). Eggs hatch into the naupliiar stage NI, which along with the subsequent NII and on occasion NIII stage are generally non-feeding stages. Naupliar growth then proceeds through further stages of development to NVI and then into the first juvenile copepodite stage CI. Pelagic copepods pass through 5 juvenile copepodite stages prior to the adult stage. The development of adult sexual structures begins commonly following the moult from CIII to CIV at which time P5 leg male/female development begins along with genital segment development in females. The final developmental stage is CVI, the mature adult stage, which produces the next batch of eggs. It is generally accepted that food availability and temperature are the fundamental factors affecting egg production rates (Kleppel 1992) and also that these two factors intrinsically govern both female and male adult body size (Koski 1999).

The fundamental growth processes in calanoid copepods, although highly studied, remain unresolved because it is extremely difficult to study somatic growth in field populations. These difficulties arise due to the complexity of *in situ* population structures (Lee *et al*. 2003) in which, at the same point in time, there can be several generations of the same species present at the same growth stage. In water masses where multi-year and annual life strategies prevail, there are commonly distinct differences in the size of copepodids of the same stage from different generations which can facilitate cohort analyses.

1.4 Feeding

Calanoid copepod prey comprises a wide range of particle types. Prey include pico (0.2 – 2-µm) nano (2 - 20µm) and micro (20 - 200µm) phyto and zooplankton (Gifford and Dagg 1988), detritus (Ohtsuka *et al*. 1996), other metazoans (Ohtsuka and Onbé 1989) and the cannibalism of smaller stages and eggs (Kang and Poulet 2000, Pers.Obser.). As copepods develop somatically through the feeding nauplii stages and the 6 copepodid stages the particle size range and consequently the types of prey consumed changes as does the vulnerability of copepods to predation (Takahashi and Tiselius 2005).

Using complex detection systems (Kiørboe and Saiz 1995, Kiørboe and Visser 1999, Kiørboe *et al.* 1999) which are still poorly understood, copepods are able to

perceive and differentiate particles types prior to capture. Motile and non-motile items are known to be detected by copepods using mechanoreception by the distortion of flow fields created by the feeding appendages (Bundy and Paffenhöfer 1996). Additionally copepods are also able, via chemoreception, to differentiate between various non-motile particles which are either food or are unsuitable for consumption (Paffenhöfer and Van Sant 1985).

Once prey is detected most calanoid copepods exhibit a variety of fundamental feeding patterns or modes in response to the particle type detected (Jonsson and Tiselius 1990). Generally during feeding bouts copepods are passive filter feeders spending the greater proportion of time cruising or sinking whilst maintaining a constant water flow over the feeding appendages. Until relatively recently it was assumed that suspension feeding copepods use maxillae as sieves which filter particles large enough to be retained between the setae. Contemporary studies, however have demonstrated that because of the water viscosity at the low Reynolds numbers relevant to copepods maxillae are predominantly used as paddles rather than as filters (Paffenhöfer 1984).

In complement with the relatively passive suspension feeding behaviour of copepods it is now clearly established that omnivorous copepods can rapidly switch to more active raptorial feeding activity (Vanderploeg *et al.* 1990, Kiørboe *et al.* 1996). Such behaviour involves a direct reaction towards individual planktonic prey items as a response to the detection of either larger items or motile prey or as an alternate behaviour when food becomes scarce (Rosenberg 1980, Landry 1981). The proportion of time allocated to raptorial (also termed ambush) feeding varies between different copepod species and also adult and juvenile copepodites of the same species. In the case of *Acartia clausi* for example adults allocate a greater amount of time to ambush jumping than juveniles but such behaviour represents less than 5% of the time across a broad range of food concentrations (Takahashi and Tiselius 2005). Such feeding modes are common in the literature and have also been described for *Centropages hamatus* (Conley and Turner 1985, Tiselius and Jonsson 1990) which also exhibits the cruising, sinking and jumping behaviour typical of ambush feeders, but to an even lesser degree in terms of time allocation than *Acartia clausi* (Takahashi and Tiselius 2005). *Temora longicornis* displays little or no deliberate raptorial behaviour and instead captures both animal and

plant prey via almost continuous filter feeding regardless of prey concentration and composition (Tiselius and Jonsson 1990).

1.5 Estuarine copepod populations

Estuaries are at the boundary between oceanic and continental regions and as such are subject to complicated hydrodynamic regimes and erratic chemical composition. This environmental complexity is a reflection of the interaction of tidal mixing coupled with the short term and seasonal unpredictability of precipitation based fluvial flow. Estuaries and coastal waters are the most productive ecosystems in the marine environment (Lam-Hoai *et al*. 2006). Estuaries are also highly dynamic in nature and are constantly flushed which leads to the continuous movement and replacement of planktonic populations. Consequently the organization of highly complex and diverse plankton communities is limited (Livingston *et al*. 1997) resulting in low species diversity despite high productivity.

A number of recurring features are nonetheless applicable to the occurrence of calanoid copepods in estuarine areas and have been observed in several geographically separate studies. Calanoid copepods generally dominate the holoplankton in estuarine regions (Paranagua 1982, Rainville and Marcotte 1985) yet copepod species diversity can be low (Goswami 1985, Islam *et al.* 2006) compared with offshore communities (Alcaraz 1983). The most regularly recorded estuarine genera is that of the *Acartia* spp. (Paffenhöfer and Stearns 1988) of which several congeners can co-occur in the same water mass at the same time. In such instances of co-occurrence slight morphological differences in the feeding apparatus have been observed (Alcaraz 1983). Such subtle variations could result in equally delicate differences in the diets between species thereby indicating divergence in food particle sizes utilized by the various congener species. These differences in diet should effectively reduce inter- and intraspecific competition between species and life stages.

In addition to *Acartia* spp. several other generalized, omnivorous calanoid copepod species are consistently found in estuaries or the near shore regions associated with an estuary. Representatives of *Centropages* (Ojaveer *et al.* 1998), *Eurytemora* (Lawrence *et al.* 2004), *Paracalanus* (Ramfos *et al*. 2006), *Pseudocalanus* (Corkett and

McLaren 1978) and *Temora* (Mouny and Dauvin 2002) are omnivorous, euryhaline species that can occasionally dominate numerically on an individual basis above all other mesozooplankton. Such species are commonly reported inhabiting estuaries and are able to persist within limited regions of the same estuary, which may be particularly suitable in terms of salinity, temperature or oxygen content, for prolonged periods. The resilience of estuarine copepods is due to several adaptations which counteract the effects of tidal flushing. High fecundity and growth rates along with short generation times achieve spatial stability when biological conditions are favourable. In addition the production of diapause eggs facilitates inter-annual, or longer, (Marcus and Murray 2001) survival during adverse conditions.

1.6 Trophic status of calanoid copepods

Of the 7 orders of Copepoda, calanoid copepods are widely regarded as the primary link between phytoplankton and higher trophic levels (Tiselius 1990, Xu and Wang 2003) due to their numerical dominance in coastal and oceanic regions. Historical perspectives of copepods which promoted the view of copepods as opportunist grazers upon the most numerous prey particles (commonly diatoms) forming a straightforward link in a simple ‘classical’ food-chain prevailed until relatively recently. Not only does this classical hypothesis fail to hold true in coastal and estuarine systems where prey biomass is much greater than in the open oceans, but it is rarely the case in the regions of the ocean where prey is meagre. In such areas despite commonly being available in proportionally lower concentrations heterotrophs and protists such as ciliates can have disproportionate effects upon reproductive success as a consequence of their nutritional content and are regularly recorded to be a preferential food source for calanoid copepods (Gifford & Dagg 1988).

An examination of the reproductive and growth dynamics of copepods requires a fundamental understanding of the copepod’s interactions with both the prey and the environment. In terms of pelagic calanoid copepods a highly significant mechanism of control of the mesozooplankton community is the quantity and quality of prey available (Saiz and Calbet 2007). Seasonally the abundance of prey for calanoid copepods in temperate marine systems fluctuates dramatically through the seasons. As a result of

both physical and biological factors the environment for a calanoid copepod in coastal and estuarine waters changes rapidly and drastically in much shorter periods of time. Consequently, when food availability declines behavioural mechanisms rather than physiological are of greater significance in maximising food intake in order to sustain growth and reproduction (Cowles 1979).

1.7 Diatom embryogenic inhibition

Our view of a simple model of tightly coupled mesozooplankton population growth linked to bursts of high primary productivity is at present under a radical reappraisal. This reassessment is as a consequence of the integration of our improved understanding of allelopathic defence mechanisms of autotrophic prey counteracting the accepted notion that 'diatoms are good for copepod egg production and recruitment'. Our perception of the role of diatoms in the copepod diet is continuing to develop increasing our understanding of some fundamental aspects of copepod growth and behaviour (Frost 2005). Significantly such research may help to explain why mesozooplankton growth is sometimes poor where the food environment would indicate the contrary to be expected. Anecdotal observations of poor copepod growth during diatom blooms are recorded as long ago as the 1950s (Marshall and Orr 1955). Additionally greater insight into the physiological effects and palatability of diatoms for copepods may significantly enhance the importance researchers attach to behavioural mechanisms of prey selection. Mechanisms which until relatively recently have been traditionally viewed as a means to maximise food intake rather than as a key factor in ensuring an adequate intake of varied and beneficial nutrients along with the avoidance potentially harmful prey.

It is now clear that copepods need a complex diet in order to provide a more rounded suite of biochemical nutrients, which are essential for egg production. A recently emerging issue suggests that the presence of toxic compounds in diatoms inhibits embryogenesis of copepod eggs and prevents hatching and also affects naupliar development and survivorship in eggs which do hatch (see Paffenhöfer 2002 for review). Several studies (Miralto *et al.* 1999, Turner *et al.* 2001, Pierson *et al.* 2005) have indicated that not only is a complex diet essential for nutrient requirements but it also

reduces the proportion and the effect of embryogenic toxins ingested by egg producing females.

1.8 Study location and general description

The Firth of Forth, Scotland is a wide estuary with an expansive, shallow coastal embayment which remains fully marine in terms of both salinity and species composition all year round as far as the upper estuary. Samples were collected at Port Edgar which is on the south bank of the lower Forth estuary, upstream of Queensferry and where salinity is > 31 PSU for much of year (Greenwood and Hill 2003). Port Edgar is a shallow marina bounded to the east and west by manmade breakwaters. Queensferry forms a natural constriction separating the Firth of Forth from the upper regions of the estuary which is a narrow meandering channel up to the tidal limit at Stirling (Neill and Elliot 2004). Immediately downstream of Queensferry the shores widen abruptly into the Firth of Forth proper. The Firth of Forth is an area of sheltered embayments and extensive intertidal areas which encompass approximately 57% of the shoreline (Buck 1993). The Firth of Forth then flows directly east to the North Sea. In the lower estuarine region near Port Edgar water column turbidity is high and suspended particulate material (SPM) is in the range 50 to 100 mgl^{-1} (Forth River Purification Board 1986) which contrasts with the Firth proper which has a mean SPM of 20 mgl^{-1}. The tidal range is approximately 5m at springs and 2.5m at neaps (Craig 1971, Webb and Metcalf 1987).

1.9 Study aims

Over the past few decades there have been many significant advances in our understanding of the factors affecting the population dynamics of copepods in the world's estuarine, coastal and oceanic regions. It is apparent, however, that there is a general lack of information available relating to many aspects of production of copepods (Lee *et al.* 2003). Our understanding of questions such as factors affecting natural mortality and energy transfer from microbial compartments via copepods to higher trophic levels are still emergent and developing. So too are many aspects of copepod grazing such as prey selection, prey switching, particle capture and retention efficiency and also the intrinsic biochemical effects of prey items towards copepod reproductive output and offspring

viability. All of these questions are at present the subject of much interest and debate. In order to address some of these questions in a geographical region where both proto- and mesozooplankton have rarely been examined, this study was undertaken. The intention was to provide an in-depth seasonal analysis, at high temporal resolution, of both the fluctuations of copepod populations and biotic factors that might affect these dynamics. Studies of plankton in the Firth of Forth still remain limited to a handful of sporadic studies dating as far back as the early 19th century (Goodsir 1843, Scott 1906a, b, Elliot 1983, Krause and Martens 1990, Roddie *et al.* 1984, Taylor 1987, Taylor 1993).

Detailed studies of the microbial foodweb in the Firth of Forth have never been carried out and to date there are no descriptions of the annual cycle of the micro- or nanoplankton which comprise much of the food available to copepods from the microbial web. The aims of this study given the paucity of available data were primarily focussed upon describing the population dynamics of pelagic copepods. With as regular a sampling interval as possible our intention was to focus predominantly on calanoid copepods over a complete annual cycle for a more detailed analysis of growth, grazing and egg viability.

Along with following the developmental growth and succession of the various calanoid copepod species in the Forth of Forth we also aimed to gain insight into the ecological factors that fundamentally impact upon copepod growth; factors such as grazing, prey selection, competitive interactions for potentially limited nutritionally important prey, growth in somatic tissue and in terms of egg production. As an overriding goal in attempting to study these complex and interlinked processes we set out to provide as much temporal resolution by sampling as regularly (~10 day intervals) as the workload would allow. Adopting such a regime of regular sampling was fundamentally important to such a comprehensive baseline study as this due to the rate processes of the organism under investigation. Not only can the copepod populations change rapidly over time but even more rapidly the populations of their prey (nano- and microplankton) with divisional times as short as hours or days can develop, impact copepod rate processes and then become numerically insignificant again in very short periods of time.

Finally, in order to investigate in more detail the effect of diet upon the reproductive processes of *Acartia discaudata,* which is a poorly studied species generally, we conducted experiments designed to analyse the cumulative effect of diatoms in the female diet. Of the four dominant calanoid copepod species present in the Forth all, with the exception of *A. discaudata,* have previously been examined in terms of diatom reproductive inhibition. Fecundity and hatching success of both *Acartia clausi* and *Centropages hamatus* have been shown to be adversely affected by diatoms (Ban *et al.* 1997). On the contrary, the reproductive responses of *Temora longicornis* appear unaffected by the polyunsaturated aldehyde (PUA) compounds considered responsible for inhibition (Dutz *et al.* 2008). In addition to determining the potential for diatoms to cause adverse effects upon the reproductive biology *A. discaudata* we considered a quantitative investigation of diatom concentration in the diet an important contribution to the progress of this developing field of copepod research.

Chapter 2 – Seasonal variability of the microbial web and calanoid copepods in the Firth of Forth

2.1 Introduction

Copepods are the major secondary producers in the ocean and play a significant role in the transfer of energy and organic matter from primary producers to higher trophic levels such as carnivorous zooplankton, fish larvae and planktivorous fish (Parsons *et al.* 1984). The major food types in the diets of many copepods are composed of nano- and microplankton collectively form much of the available biomass of the microbial web (see Kleppel 1993 for review, Thibault *et al*. 1999, Hansen *et al.* 2003, Calbet and Saiz 2005, Koski *et al*. 2005). Most calanoid copepods, however, are inefficient at grazing particles below ~5µm (Berggreen *et al*. 1988, Froneman 2001, Pagano 2003). Microzooplankton such as ciliates and heterotrophic dinoflagellates on the other hand are highly capable of removing particles smaller than those copepods are able to capture. Protist microzooplankton are also able to reproduce asexually almost as rapidly as the phytoplankton upon which they prey. For that reason microzooplankton may commonly remove a greater proportion of primary production through the ingestion of smaller sized phytoplankton such as nanoflagellates than copepods are able to capture. Hence copepods feeding on microplankton indirectly contribute to the flow of biological material derived from nanoplankton (< ~5µm) and picoplankton (0.2 – 2µm) production to higher trophic levels such as larval fish and chaetognaths. Accordingly, were it not for both the numerical abundance and ability of copepods to capture microzooplankton in greater quantities than all other mesozooplankton groups (Gifford and Dagg 1988) much of the biologically produced material consumed by microzooplankton would be lost from the euphotic zone via sedimentation, subduction and remineralisation (David *et al*. 2006).

Due to the high abundance and low diversity of copepods in temperate estuaries it is assumed that a few predominant species play an important role in energy transfer (Kimmerer 1993). Estuarine systems are often highly productive regions which exhibit extremely complex physical and chemical regimes (Laybourn-Parry *et al.* 1992). Simultaneously there is commonly a high degree of temporal, spatial and tidally induced

variability in the species structure of the microbial foodweb. As a result of such complexity the role of the microbial food web in the nutrient flux of temperate estuarine systems is intricate and can change seasonally.

During the course of an annual cycle in temperate regions heterotrophic microplankton may act as both a sink resulting in the loss of biologically derived material from surface waters via both respiratory losses and fecal pellet sedimentation (Buck and Newton 1995) and also as a conduit in the flow of nano- and picoplankton production to mesozooplankton at other times (Calbet and Saiz 2005). Consequently in temperate estuarine regions prey availability and composition for mesozooplankton is highly dynamic spatially and also over very short time periods (Dagg 1977). The seasonal complexity and the wide variety of trophic interactions occurring within the microbial web make the measurement of flows of material between compartments and trophic levels problematical (Bode and Alvarez-Ossorio 2004). Nonetheless, appreciating the function of copepods in such flows requires an understanding of the factors affecting growth, reproduction and mortality of these animals (Hirst and Lampitt 1998).

The mesozooplankton biomass in nearshore and estuarine regions are dominated by small bodied neritic calanoid copepods typically ~1mm prosome length (Lawrence *et al*. 2004). These small calanoid species characteristically reproduce continuously when conditions are favourable. This strategy is in contrast to the larger offshore calanoid copepods which are able to persist through periods of insufficient prey availability via the synthesis of lipid compounds. The smaller estuarine calanoid copepods have little or none of this energetic storage ability. As a consequence of this physiology, smaller calanoid copepods are susceptible to the rapid changes in abundance and variability of the food available in estuarine waters (Landry 1981). Given their dependence upon the immediately available food sources a seasonal succession of calanoid copepods is regularly reported in response to temporal changes in the nutritional quality and composition of the prey (Bode *et al*. 2005, Mazzocchi and Ribera d'Alcala 1995).

Food, temperature and female body size are all acknowledged to have an impact upon rates of somatic growth and reproductive output in copepods, and of these factors the quality and quantity of food is considered a very significant controlling factor (Arnott *et al*. 1986, Støttrup and Jensen 1990, Jónasdóttir 1994, Pond *et al*. 1996). Consequently

in order to understand the development and decline of populations of different copepod species and the growth of individuals within a species a thorough understanding of food availability is essential.

Generally calanoid copepod diets are diverse but the seasonal succession of calanoid species commonly observed in estuaries indicates that the optimum conditions for a particular species changes over time. The seasonal fluctuations and successions of mesozooplankton populations in the Firth of Forth are at present poorly documented. Additionally the timing and extent of protist plankton primary and secondary production have never been formally studied in direct relation to their effect upon copepod population dynamics in this region. Consequently a comprehensive field sampling program was conducted to more extensively document the temporal changes in calanoid copepod abundance and the factors affecting these changes.

The specific aims of this chapter were:

(1) to describe the composition, abundance, diversity and seasonal variability of the numerous auto- and heterotrophic protoplankton species which collectively make up the microbial web and to similarly describe calanoid copepod seasonal dynamics;

(2) to associate the seasonal shifts in the relative importance of the various primary producers i.e. autotrophic nanoflagellates, diatoms, autotrophic dinoflagellates and mixotrophic protists both to the prevailing hydrographic regimes and trophic interactions with protist and metazoan grazers;

(3) to relate the protoplankton dynamics described above to the numerical dominance and competitive interactions between the ecologically significant calanoid copepods in the lower estuary of the Firth of Forth.

2.2 Methods

2.2.1 Salinity and temperature

At Port Edgar (55°59'44.77''N, 03°24'37.60"W) in the Firth of Forth, Scotland (Figure 2.1) physical parameters (temperature and salinity) at 1m intervals from the bottom to surface were routinely measured using a temperature/salinity probe (VALEPORT Model 8008/602) on 36 occasions during an entire annual cycle from 6

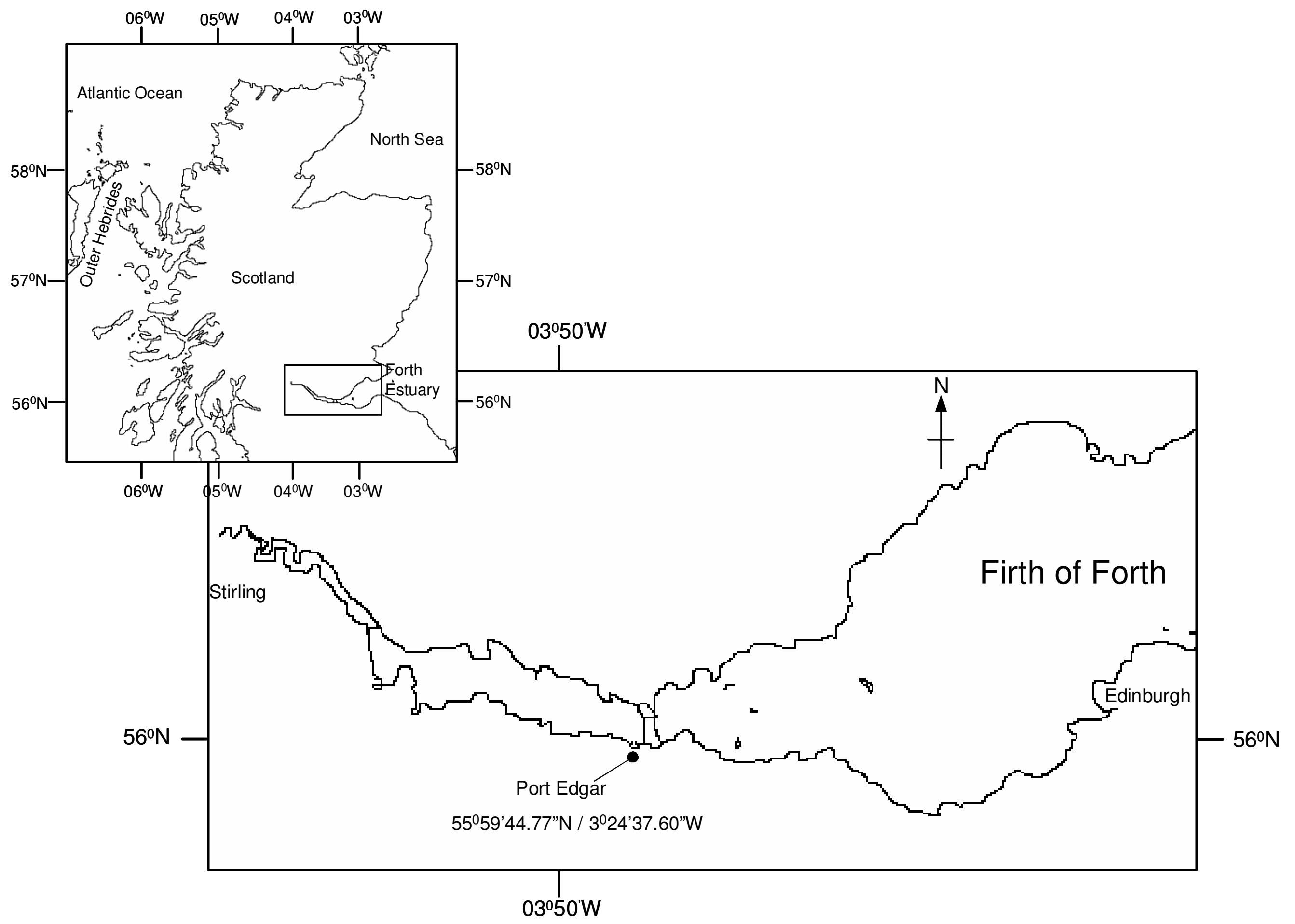


Figure 2. 1. Study site in the Firth of Forth from which all samples were collected. Inset map showing coastline of Scotland.

November 2001 to 13 November 2001. Field sampling was typically conducted at times which coincided with night time high tides in order to both ensure maximum water column depth and to ensure that mesozooplankton would be concentrated in surface waters where sampling would be less problematic than nearer to the bottom sediment.

2.2.2 Pigments

Using a clean 25 l carboy ambient surface seawater was collected for chlorophyll *a* analysis. Triplicate chlorophyll *a* samples were filtered within 4 hrs of field collection and transferred directly to foil envelopes for frozen storage at -20°C for ~1 month until analysis. Prior to fluorometric analysis pigments were extracted from filters for 24h duration at 4°C in 90% aqueous acetone.

Size fractioned (<10µm, 10 - 30µm and >30µm) chl *a* (µg^{-1}) was determined using a TURNER TD 700 flourometer. Chlorophyll a concentrations were calculated using the flourometric methods of Strickland (1972) after phaeopigment correction and following the equations of Parsons *et al.* (1984).

$$Chlorophyll\ a\ (\mu g\ l^{-1}) = F_d \frac{r}{r-1} \times (R_{sb} - R_{sa}) \times \frac{v_e}{V_f}$$

(2.1)

Where R_{sb} is the fluorescence of the sample before acidification

R_{sa} is the fluorescence of the sample after acidification

v_e is the volume of acetone (ml)

V_f is the volume of seawater filtered (ml)

F_d is a calibration term relating chl *a* concentration determined via spectrophotometry of primary standards to raw fluorescence and is calculated thus:

$$F_d = \frac{C_a}{R}$$

(2.2)

Where R is the raw fluorescence reading from the fluorometer and C_a is the concentration of chlorophyll *a* determined thus:

$$Chlorophyll\ a\ =\ 11.85\ E_{664} - 1.54\ E_{647} - 0.08\ E_{630} \quad (2.3)$$

Where E denotes the absorbance at the specified wavelength.

In order to make phaeopigment measurements for correction it is also necessary to calculate chlorophyll *a* ratios before and after acidification as follows.

$$r = \frac{R_b}{R_a} \quad (2.4)$$

Where R_b and R_a are the fluorescence readings of the pure chlorophyll *a* standards before and after acidification respectively.

In order to minimise destruction of soft-bodied aloricate ciliates, of which some phototrophic (e.g. *Mesodinium rubrum*) and mixotrophic (e.g. some *Strombidiid*) genera contain chlorophyll, filtration was carried out under the lowest practical vacuum pressure provided by our pump.

2.2.3 Protoplankton

Triplicate 250ml ambient seawater samples were also collected from ~1m depth on the same dates as copepod collection and preserved in acidified Lugol's iodine to a final concentration of 2% for later microscopic analysis. Enumeration of protoplankton cells and measurements of linear dimensions were carried out on an Olympus IX70 inverted microscope using image analysis software (Axiovision, Imaging Associates Limited) allowing resolution to within 0.1µm. Samples were settled for >24h following the Utermöhl method (Hasle 1978) and nano- and microplankton identified to species when possible but generally most cells were categorized to the level of genera. In all samples >200 cells were counted and identified and the entire chamber's surface examined. Small phototrophic nanoflagellates were counted by examining 20 random microscope fields (which always contained >200 cells) at x600 magnification and cell

concentrations ml^{-1} calculated from the total fields of view in the entire settling chamber. Cell volumes were calculated following the equations and shape formulae of Hillebrand *et al.* (1999) and cell shrinkage, where relevant, accounted for following the corrections of Montagnes *et al.* (1994). Cell carbon content (µg C µm^{-3}) was then determined from the regression analyses of Menden-Deuer & Lessard (2000) (their table 4).

2.2.4 Mesozooplankton

Copepods were collected with quantitative vertical net hauls (bottom to surface) using a 55µm mesh, 50cm diameter mouth ring net. Multiple hauls were carried out to ensure a total tow length >30m dependant upon water column depth at the time of sampling. The net was fitted with a 0.5L non-filtering solid cod-end and the volume filtered simply derived from tow distance based upon water column depth. The contents of the cod end from each of the hauls were combined into a single sample. All mesozooplankton samples were preserved in 4% formaldehyde: seawater borax buffered solution. In order to estimate *in situ* abundance whole samples were subsampled and copepods were identified to species, sex and stage under a stereomicroscope until >200 individuals had been identified from each sampling date. Copepodite stages CI to CIII of the 2 calanoid species *Paracalanus parvus* and *Pseudocalanus elongatus* are extremely difficult to differentiate and are therefore collectively termed *Pseudo-paracalanus* spp. On occasions when mesozooplankton abundances were extremely low the whole sample was analysed. Prosome lengths were measured to within ±17µm using a Leica MZ8 dissection microscope at x80 magnification and the carbon content (µg C ind^{-1}) determined using relevant published species-specific regressions (Table 2.1). As no equations were available for *A. discaudata*, we used those for *A. bifilosa* determined by Koski (1999), these two species having very similar sizes and body proportions.

2.2.5 Statistical analyses

Variability and seasonality in the composition of phytoplankton species was summarised by way of principal component analysis (PCA) and non-metric

Table 2. 1. Summary of length/weight regression equations used in the calculation of the carbon content of numerically important calanoid copepod species where C is carbon (µg) and L is prosome length (µm) for all copepod species. In some cases length-weight regressions were calculated by converting from dry weight (DW) and in such instances DW was assumed to be 40% of total body weight

Species/Group	C content length/weight relationship	Source
Acartia clausi	$C = 10^{(3.055\log L-8.444)}$	Uye 1982
Acartia discaudata	$C = e^{-2.285+(0.003793L)}$	Koski 1999
Acartia bifilosa	$C = e^{-2.285+(0.003793L)}$	Koski 1999
Acartia tonsa	$C = e^{-7.955+(0.003793L)}$ 3.955L-7.955	Berggreen *et al*. 1988
Centropages hamatus	$C = 10^{(2.449\log L-6.0984)}*0.417$	Klein Breteler *et al*. 1982
Paracalanus parvus	$C = 10^{(3.128\log L-8.45)}*0.4$	Uye 1991
Pseudocalanus elongatus	$C = 10^{(2.7302\log L-6.9121)}*0.437$	Klein Breteler *et al*. 1982
Temora longicornis	$C = 10^{(1.79\log(L/100)-0.51)}*0.4$	Klein Breteler *et al*. 1982

multidimensional scaling (NMDS) ordination based on a Bray–Curtis similarity index (Field *et al.*1982) which is the most robust ordination algorithm when dealing with largeproportions of zero values in the data. These analyses were conducted on the $\log_{10}$ (x+1) transformed abundance data collected during the entire study period (Fernandez and Bode 1994). Prior to these analyses the most abundant taxa i.e. those with frequencies higher than 10% of total sample abundance, were calculated and species below these values removed from analyses. All of these tests were carried out using the statistical software package PRIMER (Plymouth Marine Laboratory, UK).

Spearman rank correlation coefficients between concentrations of numerically important microzooplankton and micrometazoans were correlated against physical and biological factors in order to determine specific interactions which may be important in determining species densities and community structure.

2.3 Results

2.3.1 Salinity and temperature

At Port Edgar (Figure 2.1) seawater temperature and salinity ranged between 3.5 to 15.5^{o}C and 21.8 to 33.5 PSU respectively (Figure 2.2) during the study. From mid-June to late August 2001 mild thermal stratification occurred resulting in a 2°C temperature decrease (16 to 14 ^{o}C) from the surface to the bottom compared with a variation of < 0.3^{o}C at all other times. Generally though due to the shallow nature and strong tidal mixing at Port Edgar the water column was relatively homogeneous. Salinity was always lowest and highly variable in the upper metre reflecting the numerous freshwater inputs.

2.3.2 Phytoplankton

An intense spring bloom of diatoms developed in late March reaching a peak in late April at 136 cells ml^{-1} followed by a less concentrated bloom of microflagellates (*Euglenophyceae* spp. and *Rhodomona*s spp.) peaking at 88 cells ml^{-1} at the end of May (Figure 2.3). The flagellate bloom resulted in the highest chl *a* concentration (2.6 μg l^{-1}) recorded on the 24th May 2001 (Figure 2.4). Dinoflagellate concentrations were depressed during March to June until the spring diatom bloom ended. A secondary

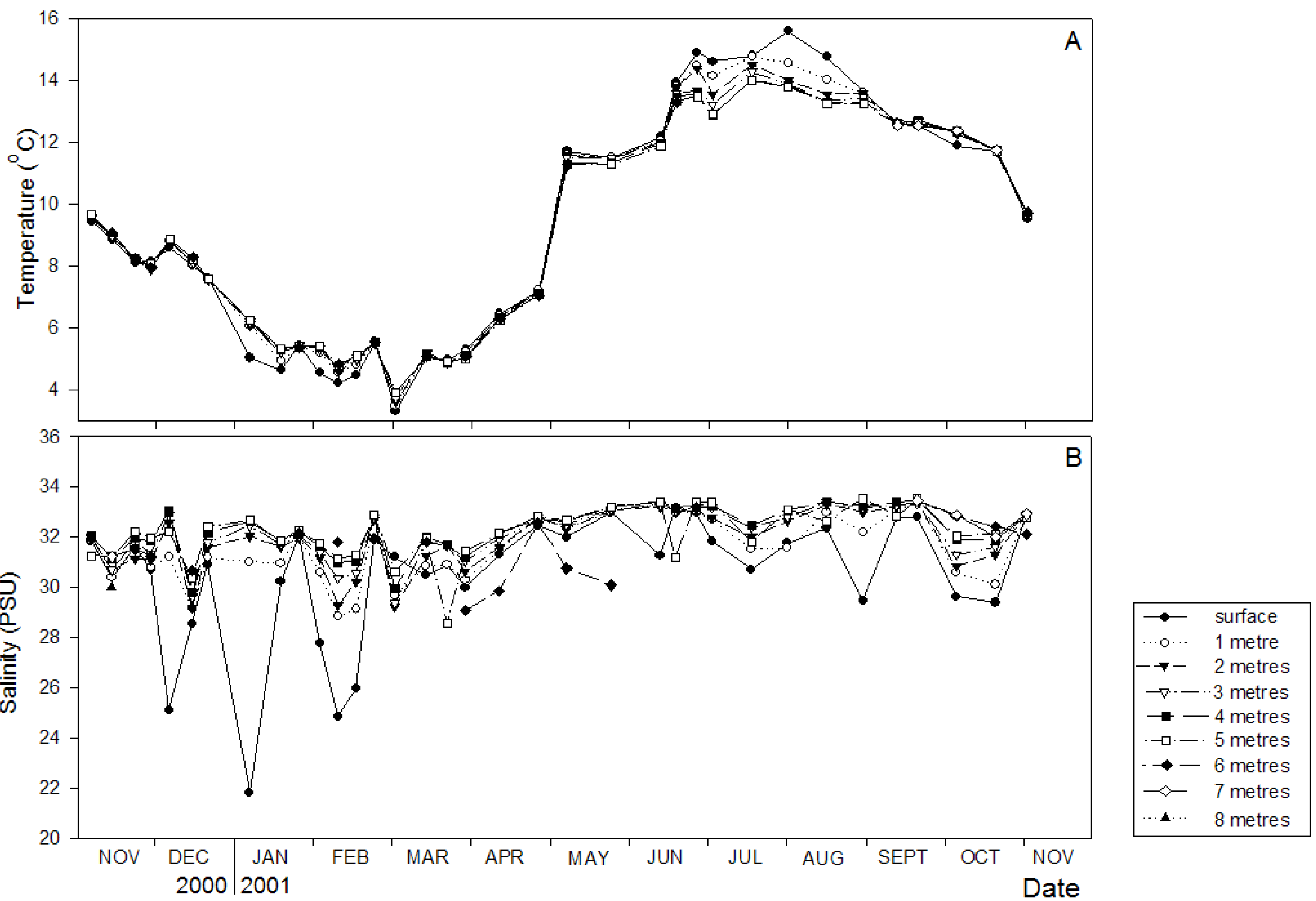


Figure 2. 2 Annual fluctuations of (A) seawater temperature (°C), and (B) salinity (PSU) at Port Edgar for a full annual cycle.

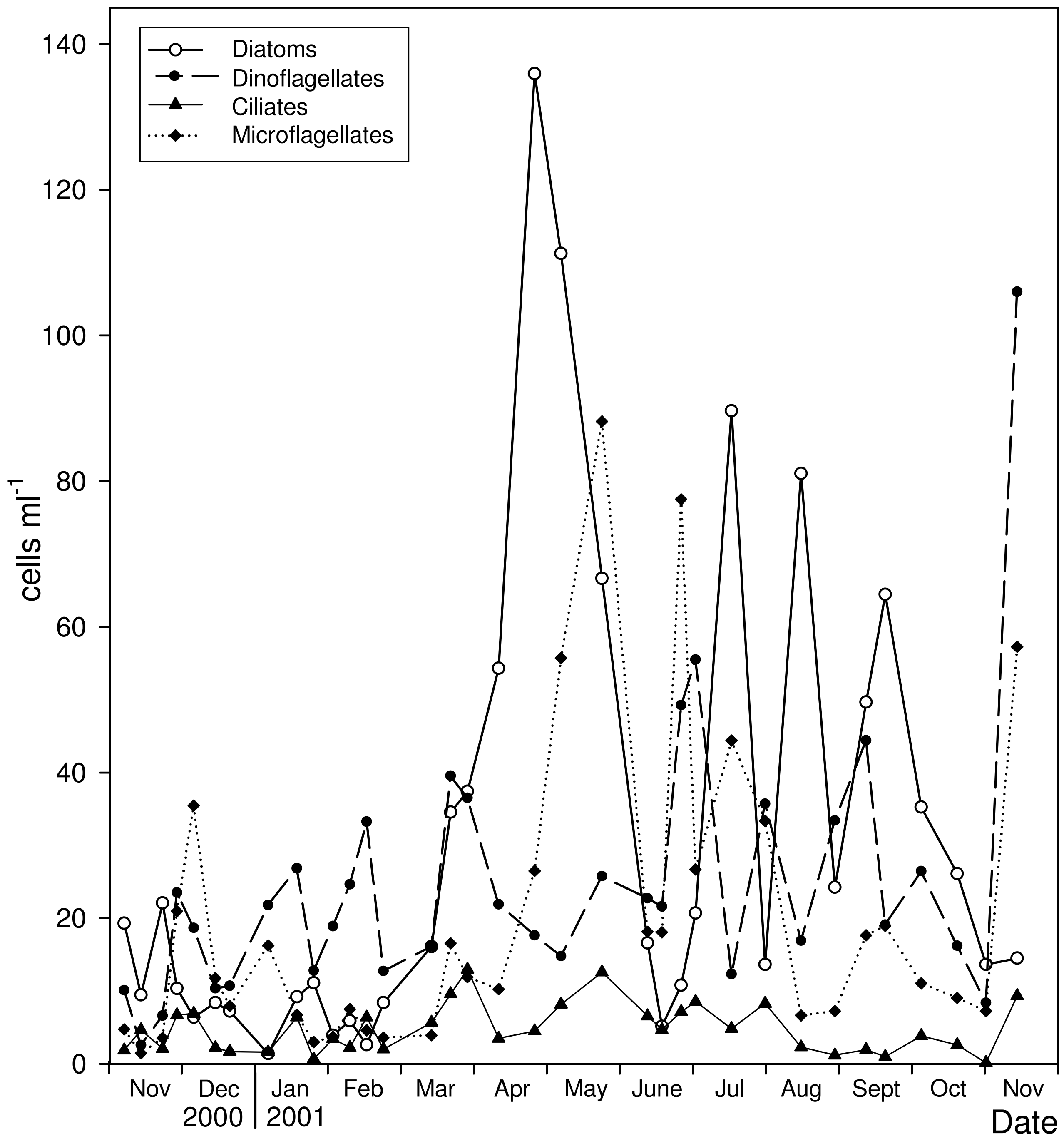


Figure 2. 3 Seasonal composition of major microplanktonic taxa during the course of the study.

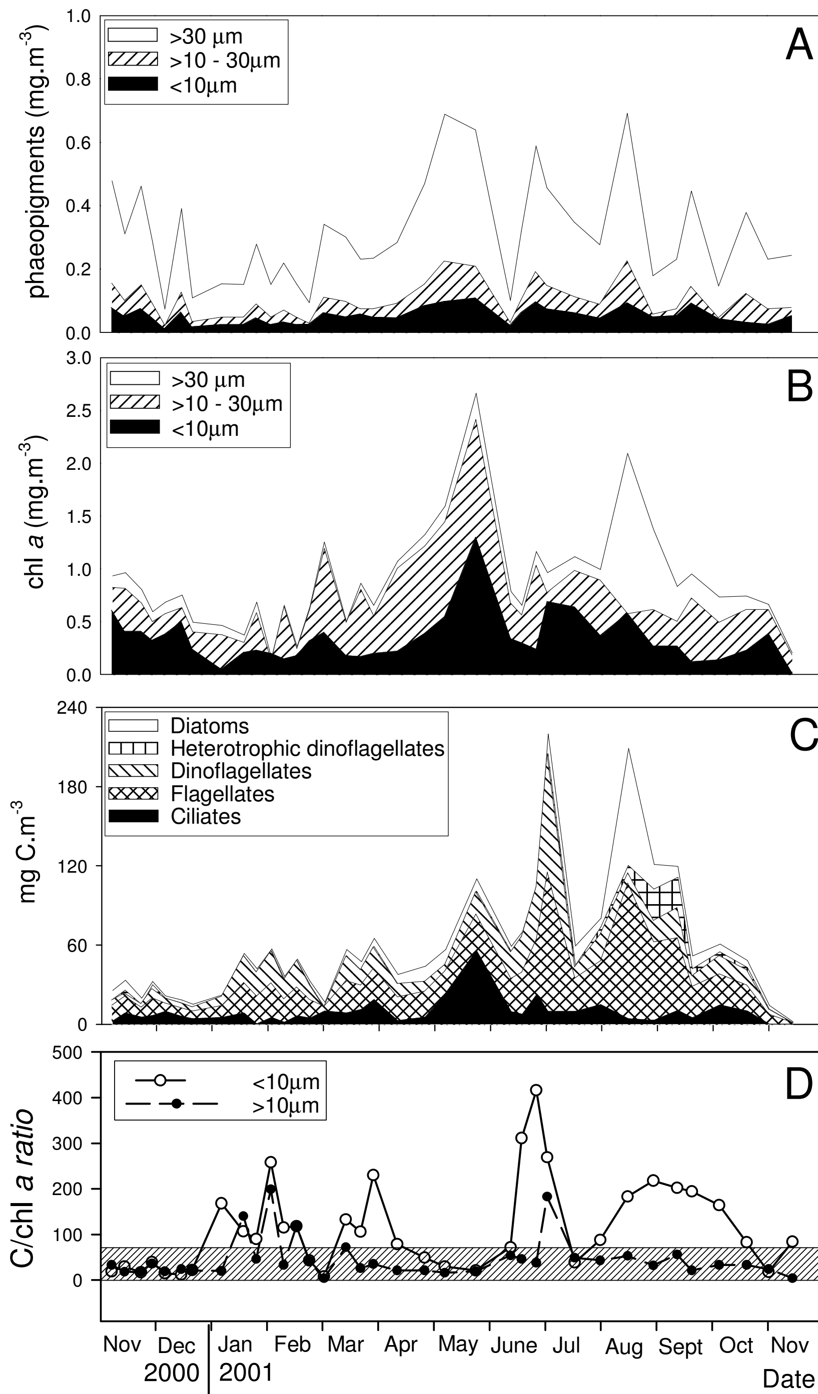


Figure 2. 4 Size fractioned phaeopigment (A) and chlorophyll *a* (B) seasonal concentrations (note scale change on Y-axes). Carbon concentration of major pigmented protoplanktonic taxa (C) and carbon : chl *a* ratio (D) of phytoplankton (i.e. ciliate and heterotrophic dinoflagellate carbon excluded) during study. The shaded region denotes carbon : chl *a* ratios indicative of nutrient replete phytoplankton (see text, Riemann *et al.* 1989).

'autumn' diatom bloom developed in mid July declining gradually through August and September eventually reaching pre-spring bloom levels by mid October (Figure 2.3).

Comparisons of chl *a* to phytoplankton community carbon which were independently calculated yielded C:chl *a* ratios ranging from 8 to 416 (0.2 to 18% chl *a* in organic carbon) for microphytoplankton and 4 to 199 (0.5 to 13%) for nanoflagellates (Figure 2.4d). These values signify that during much of the annual cycle C:chl *a* ratios were comparable to those of nutrient replete phytoplankton (Riemann *et al.,* 1989) indicating that phytoplankton in Port Edgar were physiologically healthy and that the estimates of phytoplankton carbon from biovolume estimates were not markedly inaccurate.

The PCA performed upon the annual abundance data of all nano- and microphytoplankton taxa (Table 2.2) separated species typical of both bloom events within 2 factors which collectively accounted for 53% of variance (Figure 2.5). The spring bloom, for example was dominated by several species of which *Navicula* sp.A, *Cylindrotheca closterium* and medium *Coscinodiscus/Thallassiosira* spp., were highly correlated with both PCA factors. Accordingly all 3 species which represented the late summer/autumn bloom (*Rhizosolenia* spp., *Ceratium lineatum* and *Leptocylindricus danicus*) were highly correlated to and separated from the spring bloom taxa by factor 1 of the PCA. Several other taxa which were present in moderate concentrations for the whole duration of the study are also correlated with one or other of the PCA factors but do not form distinct groupings. Consequently factor 1 of the PCA should be considered a measure of diatom and autotrophic dinoflagellate species' groupings which dominated the spring and autumn blooms.

Non-metric multidimensional scaling analysis (NMDS) was also used to clarify seasonal patterns in community structure. In the plot of samples expressed seasonally (Figure 2.6) bloom periods are again distinguished whereby the ordination plot separates the winter samples from the spring and autumn bloom periods whilst also separating much of the spring bloom period from summer. Additionally the NMDS ordination highlights the differences between periods of high primary productivity and rapid changes in the relative abundances of the various phytoplankton during summer and spring as evidenced by the wide dispersion of samples. The wide separation of individual

Table 2. 2. Mean (±SE) abundance (cells ml^{-1}) of protoplankton species representing >10% of total abundance in all of the samples collected during the study.

Group	Code	n	Mean	SE
Bacillarophyceae (diatoms)				
Asterionellopsis glacialis	ASTGLA	7	0.6	0.2
Baclillaria spp.	BACILL	4	0.4	0.2
Cylindrotheca closterium	CYLCLO	34	2.8	1.1
Guinardia delicatula	GUIDEL	8	0.6	0.3
Guinardia striata	GUISTR	8	0.2	0.1
Helicotheca tamesis	HELTAM	11	0.2	0.1
Large *Coscinodiscus/Thalassiosira*	LCOSTH	22	0.2	0.1
Leptocylindrus danicus	LEPDAN	18	1.4	0.7
Lithodesmium tricornutum	LITTRI	6	0.2	0.1
Medium *Chaetoceros* spp.	MCHAET	7	3.3	4.2
Medium *Coscinodiscus/Thalassiosira*	MCOSTH	35	2.2	0.5
Navicula spp.	NAVISP	22	4.0	2.3
Diploneis spp.	DIPLSP	19	0.1	0
Odontella aurita	ODOAUR	14	0.2	0.1
Odontella mobiliensis	ODOMOB	6	0.1	0
Phaeodactylum spp.	PHEASP	5	0.1	0
Pleurosigma directum	PLEDIR	17	0.2	0.1
Pleurosigma normanii	PLENOR	10	0.1	0
Pleurosigma spp.	PLEUSP	21	0.3	0
Pseudo-Nitzschia spp.	PNSSPP	22	0.6	0.3
Rhizosolenia setigera	RHISET	6	0.1	0
Rhizosolenia spp.	RHIZSP	13	2.7	3.1
Skeletonema costatum	SKECOS	7	0.5	0.4
Small *Chaetoceros* spp.	SCHAET	5	0.1	0.1
Small *Coscinodiscus/Thalassiosira*	SCOSTH	34	1.1	0.2
Thalassionema nitzschioides	THANIT	32	5.5	1.2
Large rhaphioidae diatoms	LRHAPH	26	0.2	0.1
Medium rhaphioidae diatoms	MRHAPH	33	1.9	0.6
Small rhaphioidae diatoms	SRHAPH	16	0.2	0.1
Navicula sp. A	NAVICA	20	0.6	0.2
Flagellates				
Monads <10µm	MONADS	35	2492.6	209.7
Euglenophyceae spp.	EUGSPP	35	0.2	0.1
Rhodomonas spp.	RHODSP	33	13.4	2.4
Dictyocha speculum	DICSPE	10	0.1	0.1
Dinophyceae (dinoflagellates)				
Alexandrium spp	ALEXSP	12	0.1	0
Ceratium furca	CERFUR	6	0.1	0.1
Ceratium lineatum	CERLIN	9	1.6	1.4
Dinophysis acuta	DINACU	6	0.2	0
Diplopsalis spp.	DIPSPP	7	0.1	0
Large *Gyrodinium* spp.	GYRLGE	17	0.1	0
Medium *Gyrodinium* spp.	GYRMED	31	1.2	0.3
Small *Gyrodinium* spp.	GYRSML	18	0.2	0.1
Large *Gymnodinium* spp.	LGYMNO	20	0.1	0
Medium *Gymnodinium* spp.	MGYMNO	35	10.9	1.0
Peridinales/Gonyaulax spp	PREGON	22	0.5	0.3
Prorocentrum micans	PROMIC	15	0.2	0.1
Protoperidinium spp.	PROTSP	23	0.4	0.2
Small *Gymnodinium* spp.	SGYMNO	35	7.0	0.9
Gyrodinium lachryma	GYRLAC	10	0.2	0.1

The species employed in the PCA on phytoplankton and the codes employed in Figure 2.5 are listed. n, number of sampling dates where each species was present.

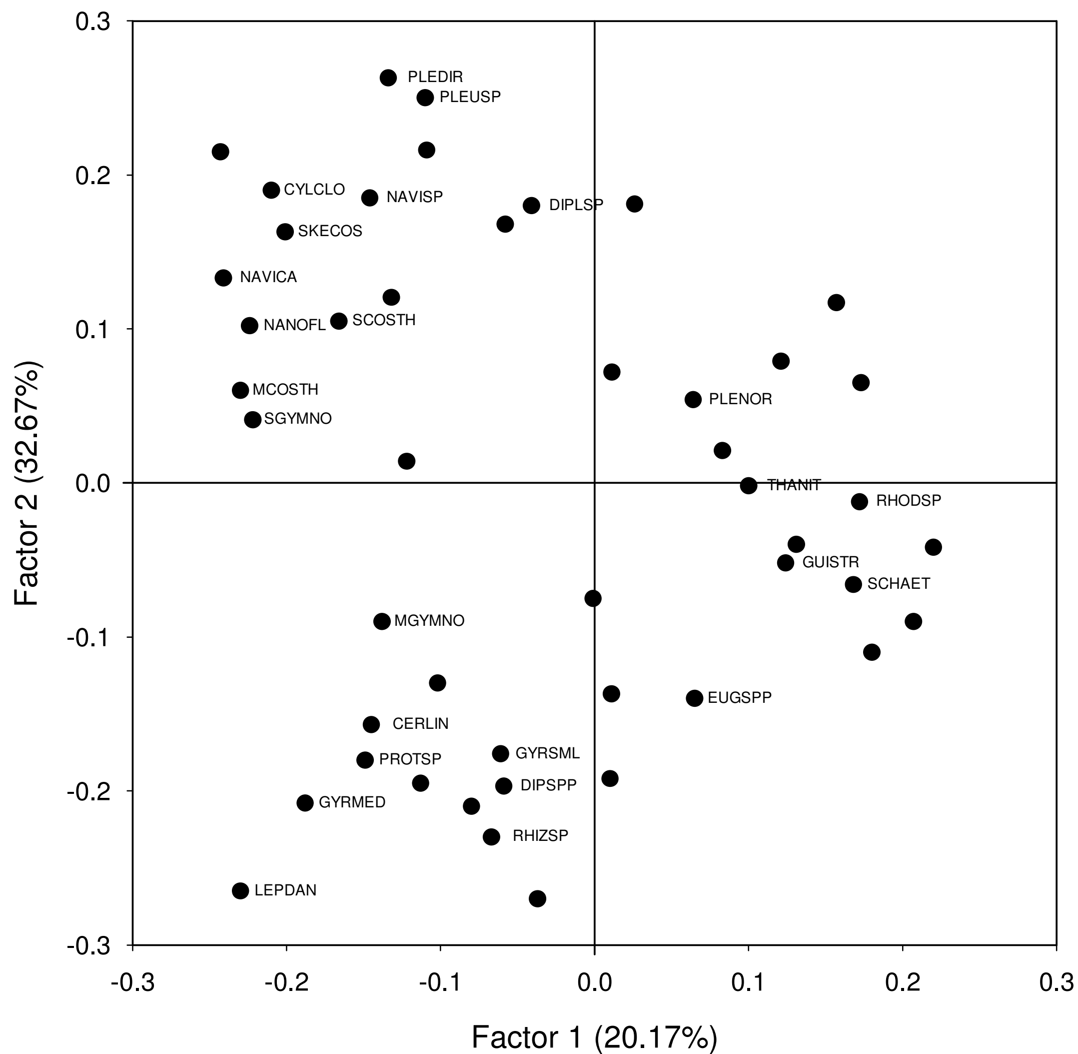


Figure 2. 5. Phytoplankton species distribution related to the first two factors of the PCA performed on phytoplankton species abundances. Phytoplankton species which showed the highest correlation values with both factors are indicated (see species codes in Table 3).

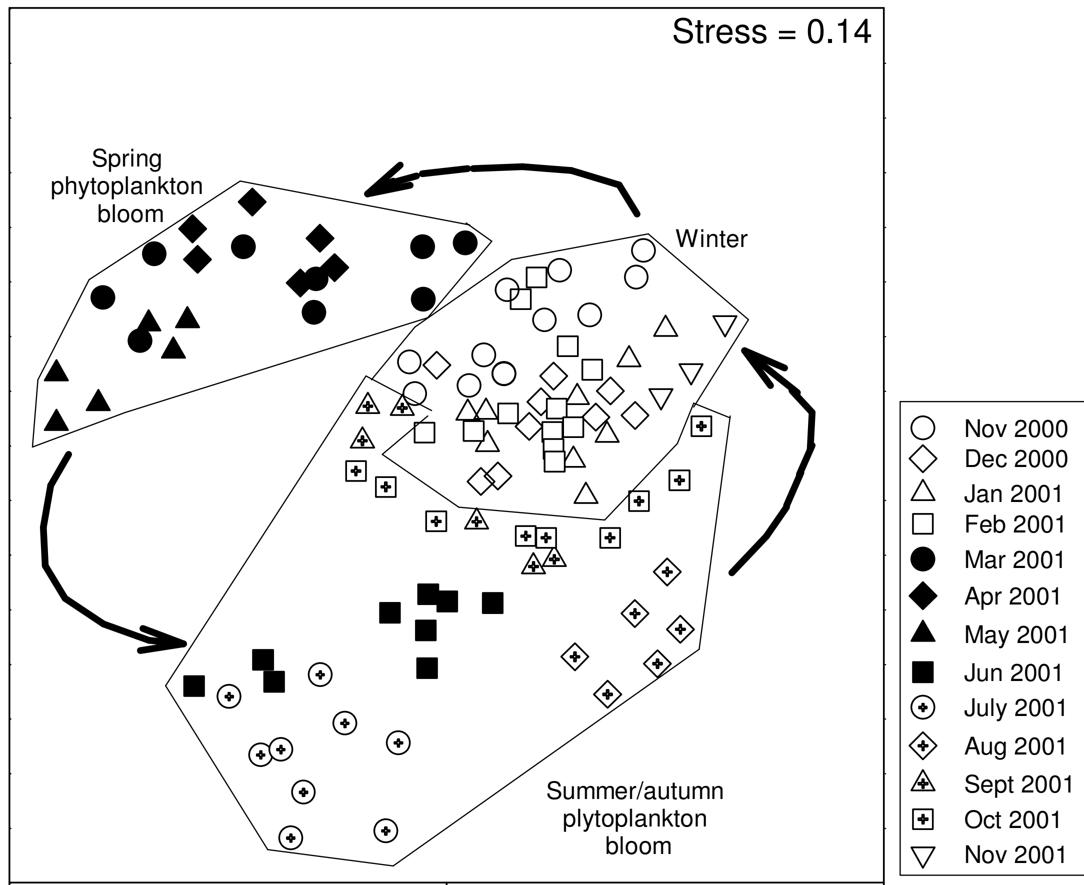


Figure 2. 6. NMDS plot of samples performed on phytoplankton species abundance values. The samples are coded according to sampling date. The seasonal cycle of phytoplankton community structure is indicated by arrows linking the main seasons. The stress value of 0.14 indicates the adequacy of the dimensional scaling in representing the seasonal ordination.

samples particularly during the summer/autumn period clearly contrasts with winter samples which form a tighter cluster group in the factorial space indicating much greater similarity, i.e. less change in the species composition and the relative concentrations of species between individual sampling dates.

2.3.3 Microzooplankton

Numerically dominant species of heterotrophic dinoflagellates (HDIN) varied seasonally (Table 2.3) as opposed to the species composition of aloricate ciliates which remained relatively diverse for much of the study period. The toxic dinoflagellate genus *Dinophysis* spp. was the numerically dominant heterotrophic dinoflagellate group on two distinct occasions in winter 2000 and again in late summer 2001 eventually being superseded in the water column by several *Ceratium* spp. in early autumn, most notably *Ceratium lineatum*. Apart from reaching concentrations exceeding 10,000 cells L^{-1} in August 2001 *Ceratium lineatum* was absent from the water column for most of 2001.

Densities of all the heterotrophic dinoflagellate taxa tested showed highly significant ($p < 0.001$) positive correlations with temperature (Table 2.4). Additionally both *Ceratium lineatum* ($p < 0.01$) and other grouped *Ceratium* spp. ($p < 0.001$) were positively correlated with the largest size fraction (>30µm) of chl *a*.

Most aloricate ciliate species were present all year round and underwent moderate rises in abundance in line with spring and autumn diatom blooms. Most ubiquitous was the small autotrophic ciliate *Myrionecta rubra* which occurred consistently all year round in densities exceeding 100 cells L^{-1}. Generally however smaller ciliates less than ~30µm in length, i.e. *Askenasia* spp., *Balanion* spp., *Halteria* spp., and smaller sizes of *strobilidiids* and *strombidiids* were numerically dominant during the study with larger ciliates such as *Tontonnia* spp. and *Laboea strobila* intermittently present at the study site. Additionally *strobilidiids* and *strombidiids* were positively correlated with both the <10µm and 10 – 30µm chl *a* size fractions in 10 out of 12 comparisons. Strongest positive correlations ($p < 0.001$) were recorded for the medium sized *strobilidiids* and *strombidiids* compared with the <10µm chl *a* fraction.

Tintinnids were present for much of the study but aloricate ciliates always outnumbered them despite tintinnids showing a moderate increase for the duration of the

Table 2. 3.Abundance of heterotrophic dinoflagellates, aloricate ciliates, tintinnids and metazoa at Port Edgar, Firth of Forth, Scotland.

	2000							2001																												
	Nov				Dec			Jan			Feb				Mar				Apr		May		June			July			Aug		Sept		Oct			Nov
	6	13	22	28	5	14	20	5	17	24	1	8	15	22	2	14	22	29	11	26	7	24	12	18	26	2	17	31	15	29	11	19	4	19	31	13
Dinoflagellates		2																																		
Ceratium lineatum				3																						4	6	6	8	8	6	6	5		1	4
Other *Ceratium* spp.			4	4																							4	4	2	5	5	2	2	4		
Dinophysis spp.	4	5		4	3	2	2															2	3	4		3	2	4	2						4	
Other HDIN <30µm																													3							
Other HDIN >30µm								2				3		2		4		5	2	3		4	6	6	5	5	2	5	4			2				1
Ciliates																																				
Askenasia spp.									2		4	4	6		3	4	2	6					1	4	4	4	2	4					5			1
Balanion spp.		2	2					2				3		2	3	3		5	2	3		4	6	6	5	5	2	5	5			2				1
Halteria spp.	4	2		3	2		2		4		2		4		4	4	4	5			2			3		5		2	2				2			
Laboea strobila	5	5	4	6	6	2	4										2				4	3			4			3								
Lohmanniella spp.									4	2					4	4	2		2					2	2											
Myrionecta rubra		4	4	4	5	4	4	4	5		4	5	5	5	5	5	6	6	6	6	6	4	6	6	6	6	6	6	4	4	3		4	4		1
Strobilidiids >30µm			5	3	2															4		6	2													
Strobilidiids >12 - ≤30µm	2	4	4	4	4	4	5	4	5		4	2	5			4	6	6	3	4	6	6	4	4	4	4	4	4			2		4	4		
Strobilidiids >5 - ≤30µm	4	6	4	6	4	4	4		4	2	3	2	3				4	3					2		3	4		3	3							
Strombidiids >30µm	3	4	4	4			5		4	2	4	2	5	2		4		6	4	4	6	5	6	3	4	4	4	4	3	2	2	3	6	4		2
Strombidiids >12 - ≤30µm		4						4	6	4	6	5	6	4	6	6	6	5	5	5	5	6	5	5	6	6	6	6	6	4	4	4	5	5	3	2
Strombidiids >5 - ≤30µm								2			2	3			3	4	4	2	3			2	2	4	4	4		4		3		3				
Tontonnia spp.	4	4	2		4												3	2		3				2		2	2									
Other ciliates <30µm											2			4		5	4				3	4	3	3		4				2						
Other ciliates >30µm			3		3											2	4				2	4		4	4		4									
Tintinnids																																				
Eutintinnus spp.																									3									3		
Helicostomella subulata																						5	5	5	5											
Stenosemella spp.	3	4	4	4	5	5	4																				4									
Tintinnopsis acuminata																						4														
Other tintinnids				1			1	5	4		4	3	4	5		4	4	6	4	4	5	6	4	4	6	4	4	5	2	4	6	5	4	6	2	2
Metaazoa			1	1		1	1																													
Appendicularia											1	1										2			1				2							
Balanus spp. nauplii			1	1	1	1	1	1		1	1	1	1	1	2	3	2					4			3				2							
Chaetognatha					1	1	1							1	1	1	2		1	1	2	1					1		2	1						
Copepod nauplii									1	1	1	1	1	1	1		1	1	2	1	2	2					1	1	1	1	1					
Meroplankton	1	1	1	1												1	2	2	3	2	4	3			1	1	2	2	4	4	1					
Ostracoda															1	1	1			2	2															
Trochophore larvae																	1	2	2		1	1														

Individuals (L^{-1}): 1 = 1-10; 2 = 11-50; 3 = 51-100; 4 = 101-500; 5 = 501-1000; 6 = 1001-5000; 7 = 5000 -10 000; 8 = 10 001-20 000.

Table 2. 4. Spearman correlation coefficients between densities of major microzooplankton species and temperature (Temp.), Salinity, size fractioned chlorophyll *a*, size fractioned phaeopigments and copepod abundance.

Taxa	Temp	Salinity	Chl *a* <10µm	10-30µm	>30µm	Phaeopigments <10µm	10-30µm	>30µm	Calanoid copepods
Ceratium lineatum	0.651***	0.323	-0.126	0.014	0.510**	0.259	0.045	0.045	0.312
Other *Ceratium*	0.634***	0.204	0.053	0.116	0.595***	0.181	0.066	0.052	-0.376*
Dinophysis spp.	0.506**	0.431**	0.489**	-0.129	0.170	0.103	0.184	0.092	0.029
Total heterotrophic dinoflagellates	0.751***	0.471**	0.178	0.055	0.170	0.284	0.199	0.173	0.222
Askenasia spp.	-0.112	-0.222	-0.401*	-0.401	-0.338*	-0.128	-0.260	-0.176	-0.029
Balanion spp.	0.277	0.285	0.298	0.044	0.121	0.123	0.235	0.301	-0.120
Halteria spp.	-0.118	-0.114	-0.171	-0.227	-0.147	0.007	-0.054	-0.009	0.030
Laboea strobila	0.175	0.163	0.273	0.509**	0.462**	0.346*	0.390*	0.364*	0.263
Lohmanniella spp.	-0.260	-0.090	-0.081	-0.091	0.244	-0.178	-0.036	-0.021	-0.379*
Myrionecta rubra	-0.106	-0.056	0.245	0.244	0.244	0.020	0.076	0.091	-0.069
Strobilidiids >30µm	0.044	0.344*	0.278	0.333*	0.137	0.204	0.137	0.214	0.153
Strobilidiids >12 - ≤30µm	-0.218	-0.156	0.687***	0.388*	-0.254	-0.540**	0.329	0.262	0.032
Strobilidiids ≤12µm	-0.032	-0.131	-0.064	-0.362*	-0.117	-0.193	-0.081	-0.059	-0.235
Strombidiids >30µm	0.113	-0.033	0.429**	0.379*	0.336*	0.365*	0.286	0.311	0.159
Strombidiids >12 - ≤30µm	-0.109	-0.083	0.604***	0.502**	0.302	0.380*	0.021	0.035	0.172
Strombidiids ≤12µm	0.021	-0.019	0.479**	0.362*	-0.297	0.231	0.292	.0249	-0.355*
Tontonnia spp.	0.099	0.053	0.445**	0.447**	0.392*	0.248	0.206	0.226	0.140
Total aloricate ciliates	-0.021	-0.017	0.345*	0.432**	0.209	0.035	0.081	0.104	0.052
Eutintinnus spp.	0.170	0.008	0.069	0.068	0.196	-0.042	0.426**	0.136	-0.647***
Helicostomella subulata	0.258	0.451**	0.176	0.246	0.167	0.245	0.160	0.179	0.064
Stenosemella spp.	0.203	0.008	0.252	0.346*	0.114	0.365*	0.106	0.231	0.359*
Tintinnopsis acuminate	0.089	0.236	0.285	0.285	0.237	0.285	0.252	0.264	0.291
Total tintinnids	0.334*	0.179	0.055	0.315	0.372*	0.128	0.491**	0.304	0.073
Total microzooplankton	0.358*	0.302	0.473**	0.422*	0.468**	0.248	0.072	0.106	0.135
Calanoid copepods	0.254	0.169	0.217	0.379*	0.212	0.267	0.477**	0.407*	-

Significant correlations indicated by: *P < 0.05, **P < 0.01, ***P < 0.001.

annual bloom period from spring through to autumn. Prolonged peaks in tintinnid abundances occurred during May and again in September. Only two tintinnid taxa displayed pronounced seasonality, *Stenosemella* spp. which was only present for any length of time during the winter of 2000 and *Helicostomella subulata* which was only present during May and June 2001.

Micrometazoan species number was low for much of the study period but increases in both diversity and density occurred during March 2001. These changes in the micrometaozan community began approximately 1 month after abundances of both phyto- and heterotrophic protoplankton began to approach the first major peak which formed the spring plankton bloom (Figure 2.2) at the beginning of March 2001. Barnacle nauplii larvae were the first metazoa to appear in increased numbers in the water column in mid March followed closely in late March 2001 by a complex of meroplankton larvae made up of several species i.e. zoea larvae, trochophore larvae, cyprid larvae, scyphonautes of *Membranipora membranacea*, large numbers of foraminifera and the larvae of several species of gastropods and bivalves.

2.3.4 Calanoid copepods

Calanoid copepod abundance varied from 0.026 x 10^3 to 2.02 x 10^3 ind. m^{-3} (mean: 0.33 x 10^3) over the study period (Figure 2.7). Calanoid copepod abundance during the first part of the study (Nov 2000 – February 2001) was composed of *Pseudocalanus elongatus*, *Acartia bifilosa* and *Paracalanus parvus* (Figure 2.7b) but dominated by the latter species. The pattern of annual abundance of total calanoid copepods was manifest in 3 distinct peaks of abundance the first of which, an exceptionally large peak of very short duration almost exclusively dominated by *Centropages hamatus* and *Temora longicornis*, occurred in May 2001. In August and October 2 further peaks of lesser magnitude occurred and were composed of a complex of calanoid copepods which were dominated numerically by *Acartia* spp. in both instances. The October peak exceeded that in August due to a co-occurrence of increased densities of younger (CI – CIII) copepodites of *Pseudocalanus/Paracalanus* spp.

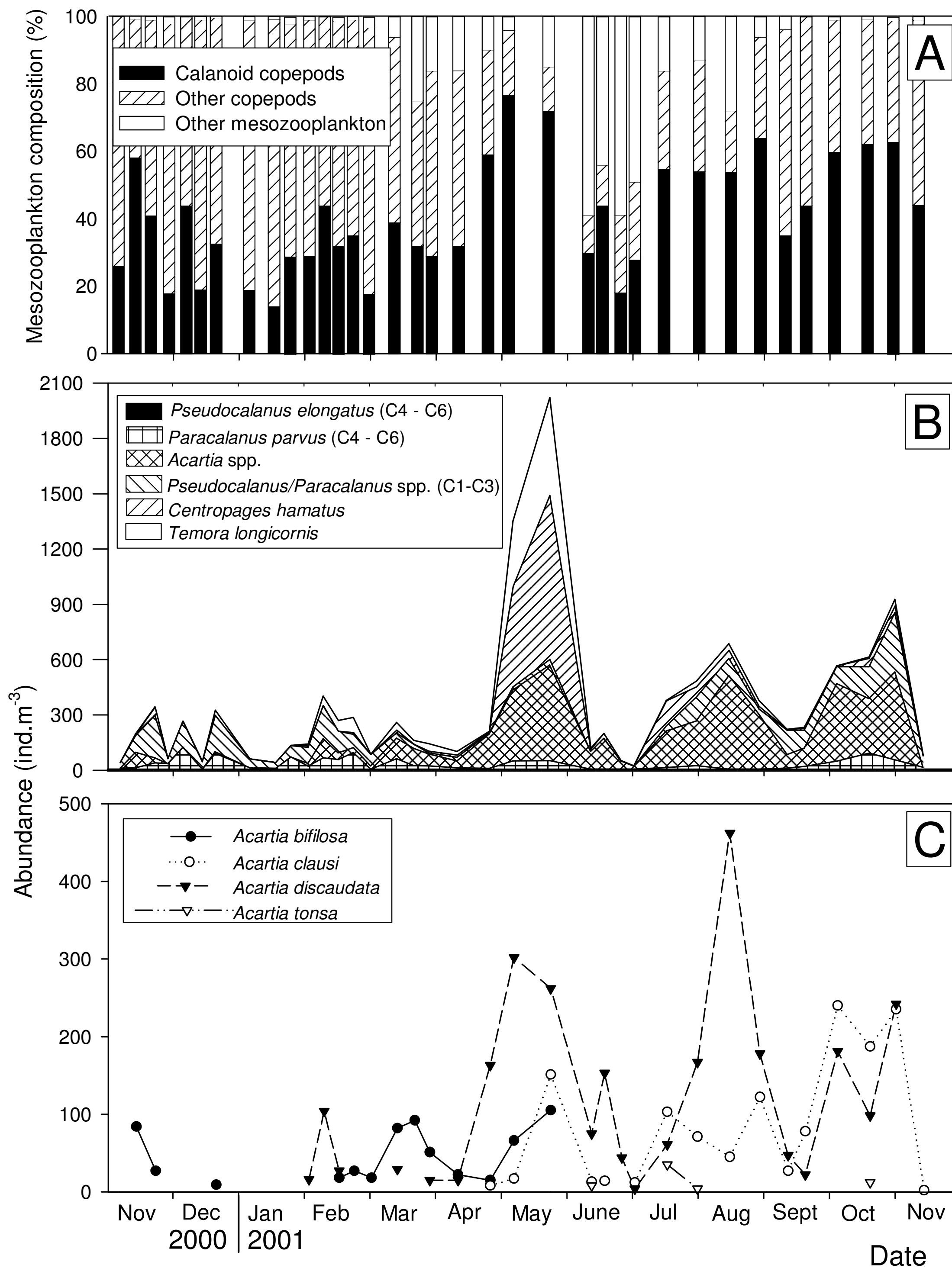


Figure 2. 7. Mesozooplankton abundance, (A) higher taxonomic composition (abundance expressed as a percentage of the total), (B) calanoid copepod species cumulative composition, and (C) the *Acartia* species composition.

Pelagic copepods (including Cyclopoida and Poecilostomatoida) utterly dominated the mesozooplankton at Port Edgar but calanoid copepods did briefly dominate numerically over all other mesozooplankton groups during May 2001 following the spring diatom bloom in April. Subsequently pelagic copepods were extremely rare for the entire duration of June during an intense bloom of ctenophores composed almost exclusively of *Pleurobrachia pileus* (Pers. Obs.) at which time concentrations of pelagic copepods in the water column was less than 150 ind. L^{-1}. Whilst ctenophores were present in high numbers, the concentrations of all pelagic copepods were lower than at any other time during the study. During this period the mesozooplankton was then dominated by epibenthic copepods of the families Harpacticidae and Ectinosomatidae and this was the only period during the study when pelagic copepods did not dominate mesozooplankton.

No individual copepod species dominated overwhelmingly and all of the major calanoid genera underwent clear seasonal trends. Of the 3 important *Acartia* congeners *A. bifilosa* was present only in winter and early spring and had disappeared by the time of the ctenophore bloom and was not recorded again during the rest of the study (Figure 2.8b). With the onset of the spring bloom during March 2001 all life stages of *A. bifilosa* rapidly increased in numbers culminating in the highest concentrations for the year during May. Once ctenophore numbers declined A. *clausi* then replaced *A. bifilosa* and continued to co-occur along with *A. discaudata* for the rest of the study (Figure 2.8c,d). Both *Acartia* species then followed similar peaks and troughs in abundance, although the magnitude of the latter species was greater. In addition *A. discaudata* had also been present earlier in the year from February onwards but when co-occurring with *A. bifilosa* had been present in much lower numbers. The final Acartiid, *A. tonsa* was recorded on only 4 occasions and in such low densities each time that it was considered those individuals present were displaced from lower salinity regions further up the river by advective processes.

There was an extraordinarily high peak in both biomass (2800 ugC m^{-3}) and abundance (2024 ind. m^{-3}) on 24 May 2001 which was dominated by *Centropages hamatus* and *Temora longicornis* but was mainly composed of the younger stages of both species, predominantly stages CI-III but in the case of *T. longicornis* CIV also. By 2 July

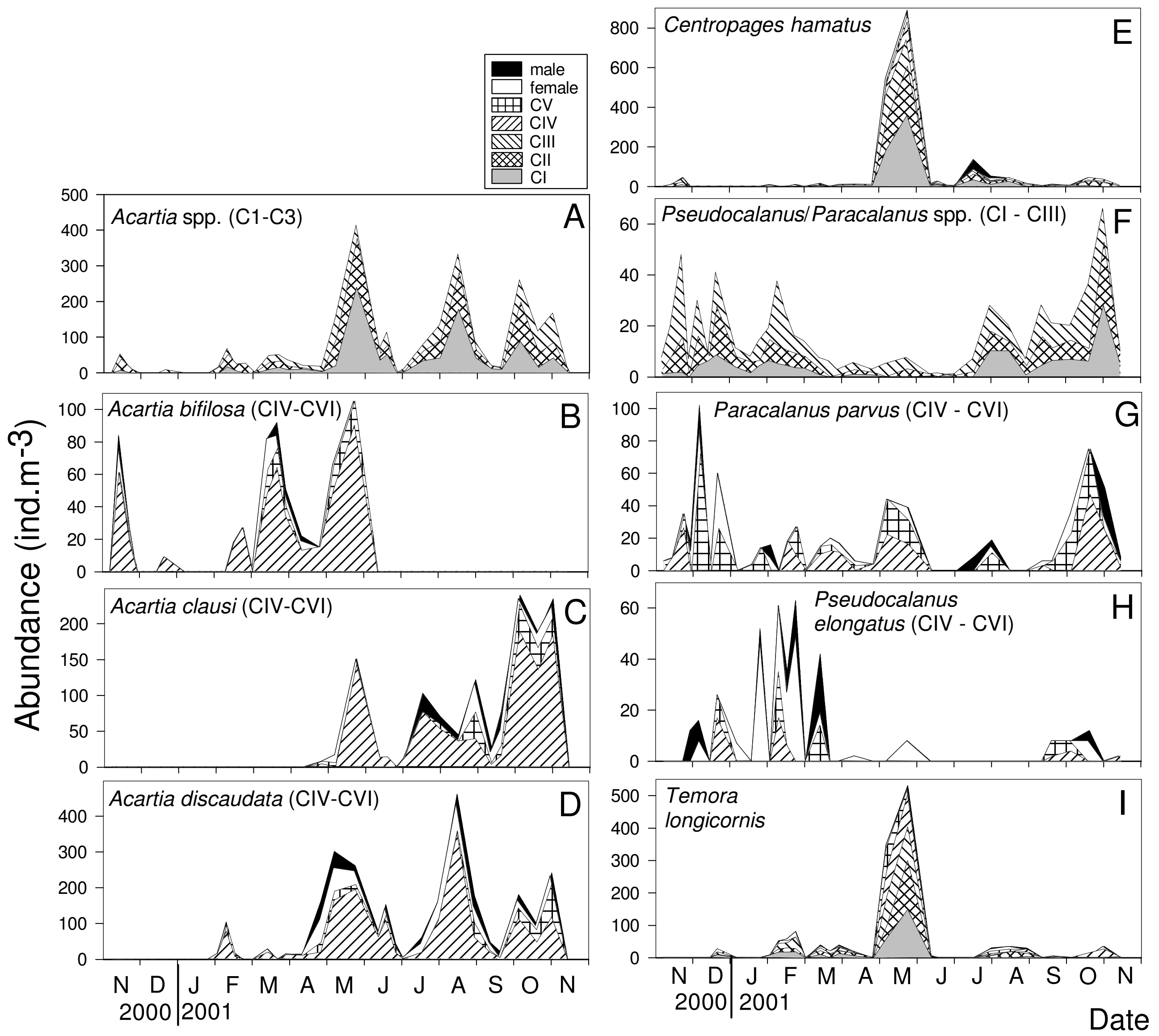


Figure 2. 8. Annual abundances and stage composition of the major calanoid copepod species at Port Edgar.

2001 these large numbers of copepods had been entirely eliminated from the study location and neither *Centropages hamatus* nor *Temora longicornis* were again numerically dominant. Indeed for the remainder of the study neither species exceeded 150 ind. m^{-3}.

The relative dominance of all copepodid stages of both *Paracalanus parvus* and *Pseudocalanus elongatus* was low in comparison to all the other calanoid species. Both of these species however showed sharply contrasting patterns of seasonal abundance compared with all the other species with the exception of the winter/early spring species *A. bifilosa*. At the end of Feb 2001 numbers of both species had declined to < 20 ind. m^{-3} and, aside from a brief period in May, remained at such low levels until October 2001. At this time the CI-CIII stages of both species exceeded the previous winter's concentrations and later copepodid stages of *P. parvus* had also attained similar densities as in winter 2000.

The prosome length of the smallest CI copepodites was 280 ± 24 µm (0.31 ± 0.02 µg C) for *Pseudocalanus/Paracalanus* spp. ranging up to 349 ± 28 µm (0.44 ± 0.04 µg C) for C1 copepodites of *Acartia* spp. The size range of CVI adults was 729 ± 37 µm (2.41 ± 0.12 µg C) for *Paracalanus parvus* and 950 ± 76 µm (8.69 ± 0.70 µg C) for *Centropages hamatus* (Figure 2.9). In the copepod species and life stages which were present for prolonged periods during the study the time-series data is relatively complete and copepod prosome lengths display little if any pronounced seasonal variability in response to fluctuations in temperature. Only in *Acartia discaudata* was there a clear reduction in body size as temperatures peaked in July and August 2001. Patterns of relative abundance and relative biomass composition of the numerically dominant calanoid copepods present are very similar and separate graphs of biomass are not presented.

2.4 Discussion

2.4.1 Phytoplankton succession and the microbial web

The results reported here, with the exception of potentially short-term (hours to days) and finescale (100s metres) variability in phytoplankton blooms, are probably

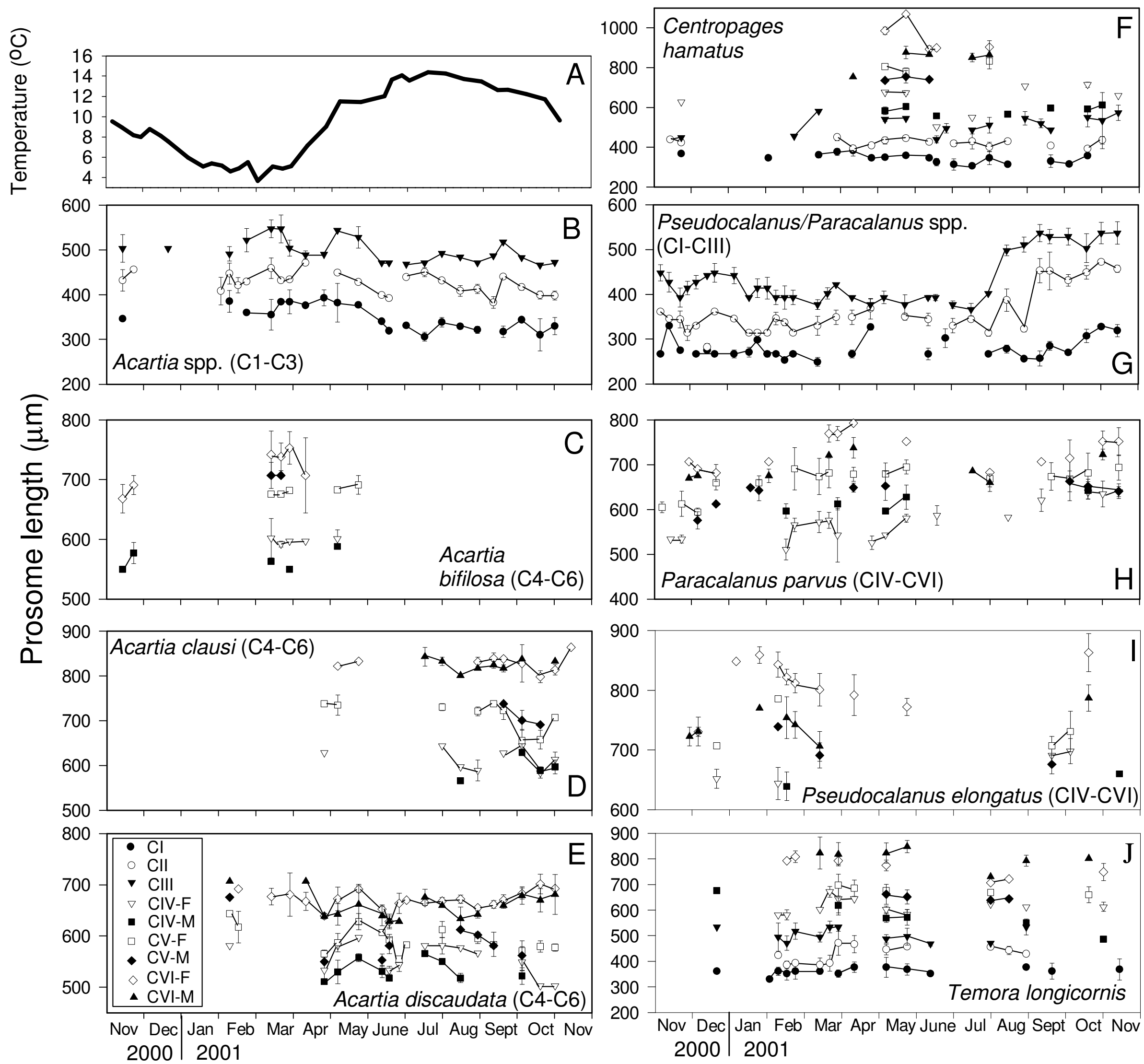


Figure 2. 9. Average seawater temperature (A) and stage specific prosome lengths (B – J) of the major calanoid copepod species at Port Edgar. Note scale changes on y axes. All y axes except plot A define copepod prosome length. Error bars ± 1SE.

representative of a much larger geographical region of the lower estuary of the Firth of Forth. Port Edgar is a relatively shallow embayment and tidal flushing will lead to an extensive turnover rate of plankton populations from the main water mass particularly during periods of spring tides. The study shows that this region of the Forth estuary is subject to the annual bicyclical occurrence of plankton blooms typical of many temperate European estuaries (Kromkamp *et al.* 1995, Burdloff *et al.* 2002). Underlying these generalised seasonal plankton dynamics however there is a great deal of complexity in the shifts in abundance of both proto- and metazoan plankton.

It is noteworthy that the clear differences in the species composition between spring and autumn phytoplankton blooms produced an inverse relationship between biomass and abundance. The spring bloom was dominated by microflagellates (*Euglenophcyeae* spp., *Rhodomonas* spp.) and diatoms (*Navicula* spp. *Cylindrotheca closterium* and *Coscinodiscus/Thallassiosira* spp.) of species which occurred as small individual cells. These small cells are fast growing, typical *r* strategists, and competitive in well mixed waters (Bode *et al.* 2005). In contrast later blooms were characterised by much larger slower growing phytoplankton species composed of both diatoms and dinoflagellates (*Rhizosolenia* spp. and *Ceratium lineatum*). As a consequence of both their small size and lower relative carbon content (Menden-Deuer and Lessard 2000) the phytoplankton comprising the spring bloom, despite being of greater abundance, resulted in less than half the biomass of a later bloom in July (109 mg C m^{-3} in May 2001 compared to 221 mg C m^{-3} in July).

Seasonal patterns of phytoplankton biomass in estuaries are linked to a variety of factors including light, nutrient availability (Campbell *et al.* 1991, Mallin *et al.* 1991, 1993, Mallin and Paerl 1992), and biological effects i.e. zooplankton grazing (Grange *et al.* 2000, Froneman, 2002, 2004). Blooms form when conditions favourable to exponential growth occur and once such conditions cease the continued maintenance of the bloom in the water column is regulated almost exclusively by mortality (Olsen *et al.* 2006). On the basis of the imbalance of C:chl *a* ratios following bloom events nutrient limitation probably governed the magnitude of successive phytoplankton blooms. The ensuing diminution of phytoplankton standing stocks however is initially in response to clearance by heterotrophic protists given the limited delay between the peak of the spring

bloom and the immediate response in the growth of microzooplankton. There were tightly coupled increases in both the ciliate biomass during the spring bloom and dinoflagellate biomass (almost completely composed of the heterotrophic dinoflagellate *Ceratium lineatum*) possibly in response to the autumn bloom of *Rhizosolenia* spp. To be more specific, the seasonal increase in numbers of small phytoplankton cells at the start of the spring bloom was closely followed by enhanced abundance and diversity of oligotrich ciliates. The relationship between this group of ciliates and small phytoplankton cells is apparent in light of the number of highly significant positive correlations when compared with *strobilidiids* and *strombidiids*. The immediate response by ciliates to the spring bloom is supportive of the rapid growth potential of these essentially indiscriminate feeding organisms (Dolan 1991).

Neither tintinnids nor heterotrophic dinoflagellates showed a similar response in terms of such a rapid population growth. Tintinnids have been shown to feed selectively on occasion (Strom and Loukos 1998, Stoecker *et al.* 1981) and this is one mechanism by which consumers can become decoupled from particular food components (Jansen *et al.* 2006, Schultes *et al.* 2006). Consequently the seasonal disparity between phytoplankton and tintinnids may be in part due to selective grazing. Alternatively the extremely low concentrations of tintinnids in relation to faster growing free swimming ciliates may have led to an inability of these microzooplankton to reproduce in sufficient numbers to impact bloom formation. Selective grazing by dinoflagellates on the other hand has rarely been observed and heterotrophic dinoflagellates appeared in significant numbers only later in the study, where they contributed significantly to the autumn peak in plankton abundance. During spring, dinoflagellates may have been out-competed for nutrients by diatoms in the well mixed conditions which prevailed before the onset of thermal stratification which began to occur moderately during June in Port Edgar. It is highly likely that a greater degree of stratification would occur in the deeper waters of the estuary proper. Accordingly, given the extensive tidal replacement of the plankton populations found in Port Edgar, bottom up nutrient limitation of dinoflagellates in the main body of the river resulted in the patterns observed in Port Edgar.

Seasonal patterns of microzooplankton numbers indicate the potential to exert top down control and regulate to a large degree the seasonal patterns of microphytoplankton

(>10μm). Nanoflagellates on the other hand appear to be bottom up regulated via nutrient availability for much of the study after the spring bloom period when C:chl *a* ratios were in excess of 70. During the spring bloom the carbon contribution from nanoflagellates remained relatively stable and while surface waters were well mixed a classical diatom dominated food chain prevailed. Once the bloom had been removed and ciliate densities had declined markedly to lower than pre-spring bloom levels nanoflagellate carbon then increased dramatically and the microbial loop became of greater significance. After the spring bloom, particulate organic carbon was dominated by flagellate mass but notably C:chl *a* ratios of the phytoplankton fraction <10μm indicated nutrient depletion during the whole of this period. It is well recognized that nutrient depletion favours nanoflagellates (Tiselius 1989) via the suppression of the growth of larger phytoplankton competitors. Indeed the dominance of nanoflagellate biomass ended and their abundance declined when an extremely dense concentration of the very large diatom *Rhizosolenia* spp. arrived in late August.

In general terms then seasonal evolution of the food environment for copepods in the lower estuary of the Firth of Forth is characterised by a gradual increase in the number of potential prey species after an initial spring outburst of diatoms. As summer develops and microbial complexity increases a heterotrophic dinoflagellate dominated microzooplankton community is sustained due to high primary productivity by nanoflagellates.

2.4.2 Calanoid copepod succession

The mesozooplankton community in this region of the Forth was dominated for much of the year by copepods, most notably during the winter period when *Oncaea* spp. and *Oithona similis* were the major mesozooplankton component. The contribution from other zooplankton such as the meroplanktonic larvae of benthic animals and gelatinous forms was generally insignificant. The impact however of ctenophores which appeared in unprecedented numbers in June was responsible for a brief but complete restructuring of the calanoid copepod community from those species present in May to the calanoid copepods present afterwards.

The prominent and sudden appearance of high densities of *Centropages hamatus*, *Temora longicornis* and increased numbers of *Acartia* spp. (mostly *A. discaudata*) at the end of May 2001 can be closely related to the seasonal protoplankton dynamics described above. But the equally sudden and extensive elimination of > 90% of the calanoid copepod standing stock may have been as a result of extremely high predation pressure by a dense community (> 500 ind m^{-3} during the whole of June 2001) of gelatinous animals dominated by *Pleurobrachia pileus* which are able to clear > 16 copepod $cten^{-1}$ d^{-1} (González *et al*. 2004) Following the disappearance of the ctenophores by July 2001 *Acartia bifilosa, Centropages hamatus* and *Temora longicornis* never again played a significant role in the calanoid copepod community at the study location and were instead supplanted by co-occuring *Acartia clausi* and *A. discaudata*.

Essentially the seasonal patterns of calanoid copepod community composition and abundance can be structured into 3 distinct time periods. Firstly the winter period from November 2000 to April 2001 then secondly the brief peak in biomass and abundance of calanoid copepods during May and finally the remainder of the study from July until November in which the onset of the calanoid community which dominated during autumn began to develop in July.

The winter copepod species community was mainly dominated by *A. bifilosa*, *Paracalanus parvus* and *Pseudocalanus elongatus* and despite minor fluctuations in abundance this community was relatively stable and persisted until the increase in primary production beginning in March 2001. Despite the food concentration being relatively low prior to the spring bloom the moderate fluctuations in both the size structure and available biomass of prey did not result in corresponding changes in the copepod population structures. Of the three species *Paracalanus parvus* and *Pseudocalanus elongatus* are extremely well adapted to conditions in which food availability is limited (Schnack 1982). Moreover, *Pseudocalanus elongatus* has a low susceptibility to food limitation (Frost 1985).

Along with *Paracalanus parvus* the abundance of P. *elongatus* declined as the spring bloom approached but, unlike any other calanoid copepod species examined, this species was totally absent from samples all through the summer and the early autumn period until September. The suggestion that *P. elongatus* is a cold-water species with an

upper temperature limit of occurrence of about 13 ^{o}C (Unal *et al.* 2006) is in close accord with this study. Furthermore the suggestion that *Pseudocalanus elongatus* has the ability to suspend development during winter and enter resting stages during summer (Möllmann and Köster 2002, Casini *et al.* 2004) may explain the absence of the C4 to C6 stages of copepodites for much of the highly productive spring and summer period from May to September 2001. Alternatively the absence of *P. elongatus* may be as a consequence of high mortality rates similar to those which have resulted in very low abundances during summer months in coastal waters in several previous studies conducted in northern European waters (Eriksson 1973, Fransz and Van Arkel 1980, Soetaert and Herman 1994).

The copepod *Paracalanus parvus* regularly co-occurs along with *P. elongatus* (Schnack 1982, Aksnes and Magnesen 1988, Hay 1995, Fernandez de Puelles *et al.* 1996) which is unsurprising given the similar physio-ecological requirements of each species. There is a great deal of evidence that *P. parvus* is highly competitive during periods of lower food concentrations and is morphologically and behaviourally adapted to sustain constant levels of egg production even when food is limited (Paffenhöfer and Stearns 1988, Gómez-Gutiérrez and Peterson 1999). The facility to maintain fecundity coupled with an ability to selectively consume more nutritionally beneficial phytoplankton (Checkley 1980) and capture very small cells more efficiently than *Acartia* spp. may explain why *P. parvus* was able to dominate the calanoid copepod assemblage over winter.

The low relative abundance during winter of *Acartia bifilosa* in comparison with *Pseudocalanus elongatus* and *Paracalanus parvus* indicates that a strategy of overwintering in the free-swimming copepodid stages by *Acartia bifilosa* is less successful than the latter 2 species. In addition to this difference in prey partitioning due to morphological differences it has been conclusively demonstrated that *A. bifilosa* is highly selective for autotrophic nanoplankton (Gasparini and Castel 1997). In particular *A. bifilosa* shows a strong preference for cryptophyte genera (Cotonnec *et al*., 2001, Meyer-Harms and von Bodungen, 1997) which the researchers suggest was due to high concentrations of the precursor fatty acids which are highly beneficial in terms of egg production. The autotrophic cryptophyte genera *Rhodomonas* spp. was a ubiquitous and

abundant prey all year round in the Forth (Table 3.5) and well within the size range of prey (14µm) easily captured by *A. bifilosa* (Berggreen *et al.* 1988, Sopanen *et al.* 2006).

The response of *A. bifilosa* to the spring bloom was more immediate than any other calanoid copepod species which was another indication that *A. bifilosa* may be less well adapted than either *Pararacalanus parvus* or *Pseudocalanus elongatus* to effectively exploit the much lower densities of food prey during the winter months. Additionally, a large proportion of the spring population of *Acartia bifilosa* may come from resting eggs and be temperature or food initiated. The youngest copepodid stages of both *Pseudo* and *Paracalanus* spp. declined until ultimately CI copepodites disappeared from the plankton by May 2001. In contrast at the same point in time abundances of CI *A. bifilosa* had reached an annual peak of abundance. Seasonal patterns of A. *bifilosa* in other North European estuaries have been linked to temperature on several occasions (Baretta and Malschaert 1988, Soetaert and Van Rijswijk 1993, Tackx *et al*. 2004). The egg production of *A. bifilosa* is lower than other *Acartia* congeners at higher temperatures (Chinnery and Williams 2003) and *A. bifilosa* in that study even continued to produce eggs at temperatures as low as those recorded in Port Edgar. In our study pulses of *A. bifilosa* CI copepodites had already begun to appear as early as January which was far in advance of the spring bloom but coincided with seasonal low temperatures.

The seasonal patterns of *A. bifilosa* abundance and of its summer diapause we observed in the Firth of Firth are similar to those in Southampton Water (Chinnery and Williams 2003) where it is suggested such patterns are induced by changes in photoperiod. The only marked difference between their study and this was the early disappearance of *A. bifilosa* from May 2001 onwards, as opposed to June in Southampton. The premature disappearance of *A. bifilosa* at our study location was, as previously stated, the result of a ctenophore bloom rather than photoperiodicity which in any case will be modified due to the higher latitude.

Both *Centropages hamatus* and *Temora longicornis* show a similar yet slightly delayed response, compared with *A. bifilosa,* to the increased abundance of food in the spring bloom. In addition to both species having to a limited degree similar feeding strategies (Martynova 2005) the seasonal patterns of hatching in *T. longicornis* and *C. hamatus* are similar (Engel and Hirche 2004). Additionally both species also have highly

analogous generation times (18 - 21 and 19 – 21 days for *Centropages hamatus* and *Temora longicornis* respectively) and growth rates (0.250 – 0.292 and 0.255 – 0.291 d^{-1} respectively) at the ambient temperature (15°C) when they were co-occurring in our study (Huntley and Lopez 1992 and references therein). Accordingly it is unsurprising that the population dynamics of both species responded to the spring phytoplankton bloom in very similar ways.

It is clear from the close correlation in the numbers of younger copepodite stages of both *Centropages hamatus* and *Temora longicornis* with chl *a* and phaeopigments in spring that food limitation is the primary factor regulating the growth of these species. Previous studies have linked short term bursts in the reproductive rates of *T. longicornis* (Peterson 1985, Peterson and Kimmerer 1994) and *C. hamatus* (Durbin and Casas 2006) to phytoplankton blooms. Therefore it is proposed that the lower levels of chl *a* and phaeopigments (<0.5 mg. m^{-3} and <1.5 mg. m^{-3}) before March 2001 are limiting for both egg production and growth resulting in the negligible population growth for the period from January to March inclusive.

It is suggested that the extremely rapid response to favourable conditions by *Centropages hamatus* and *Temora longicornis* is quicker than can be expected by merely ascribing extremely high egg production rates to the very few females present (2 and 3 females. m^{-3} respectively with corresponding maximum egg production rates of 70 and 32 eggs $female^{-1}$ d^{-1}). It is more probable that, given the reproductive strategies of both species includes the production of diapause eggs, (Marcus 1996, Marcus and Lutz 1998), the cohort present in May was from the synchronous hatching of resting eggs produced and deposited in the benthos the previous year.

The mechanisms by which the initiation of diapause egg hatching occurs is unknown but is regularly linked to an increase in ambient temperatures in both species (Marshall 1949, Bakker and van Rijswijk 1987, Peterson and Kimmerer 1994, Chen and Marcus 1997, Marcus and Lutz 1998). Water column temperature at Port Edgar on 26 April 2001 was 7°C and rose to ~12°C by 24 May, a period of 28 days. Assuming the equivalent generation times stated above were relevant to this study it is possible that the adult copepods (certainly the later stage copepodites) had sufficient time to develop from eggs which had hatched when temperatures began to rise in the intervening period. The

range of temperatures observed during this period is within the range at which almost 100% egg hatching success was observed for *T. longicornis* (Castellani 2003) and 60% hatch success for *C. hamatus* (Marcus 1989) when investigators specifically attempted to incubate diapause eggs.

During the period in late summer and autumn when *Acartia clausi* and *A. discaudata* became the dominant calanoid copepods it was difficult to explain why there was no resurgence of the populations of *C. hamatus* and *T. longicornis*. Given the larger body size of the latter species in comparison to both *Acartia* congeners they were even better suited to capture the wider range of prey types and sizes available during the autumn bloom (Tiselius 1990, Tiselius and Jonsson 1990). It was initially assumed that the elimination of all mesozooplankton due to the ctenophore bloom and the lack of a similar sharp increase in temperatures such as that in April indicated unsuitable conditions for either *C. hamatus* or *T. longicornis* to again develop solely from diapause eggs. It seems however that for *T. longicornis* there may be an evolutionary adaptation which predisposes females to begin producing great proportions of resting eggs even when the population is peaking and conditions are favourable. Similar blooms of *Pleurobrachia pileus* have been observed in the Menai Strait and resulted in the lowest hatching success of *T. longicornis* eggs at a time when ctenophore densities had begun increasing (Castellani 2003). Such an adaptive response therefore may be initiated as a reaction to overcrowding. As populations peak the production of high proportions of resting eggs would thereby minimise the effects of starvation caused by the decline of the spring bloom in addition to diminishing predation mortality. The premature disappearance of *Acartia bifilosa* from the Firth of Forth may also have occurred for similar adaptive reasons as *T. longicornis*. Again during the peak of its population numbers *A. bifilosa* was recorded producing large numbers of resting eggs in proportion to all eggs spawned during spring blooms in Southampton Water (Castro-Longoria and Williams 1999).

The appearance of *Acartia tonsa* in the July, August and November samples is characteristic of the typical seasonal occurrence of this species in northern European estuaries (Bakker and de Pauw 1975, Durbin and Durbin 1981, Baretta and Malschaert 1988). However the maximum density recorded was only 35 ind. m^{-3} on one of the 4

occasions when its presence was recorded. *A. tonsa* is regularly recorded as occurring higher up in estuaries at lower salinities and geographically separated from *A. clausi* (Cervetto *et al*. 1999, Gaudy *et al*. 2000, Vieira *et al.* 2003). Although *A. tonsa* has been found in regions where salinity ranged from 5‰ to 35‰ (Brylinski 1981, Gaedke 1990, Miller and Marcus 1994, Cervetto *et al*. 1999) it has been demonstrated that at higher salinities within this range *A. tonsa* has higher respiration and excretion rates (Gaudy *et al.* 2000) and that at salinities greater than 25‰ survival of early naupliar stages declines (Tester and Turner 1991). The sporadic occurrence of *A tonsa* was therefore attributed to the displacement of small numbers of individuals from much larger populations farther up the estuary. Consequently it is suggested that *A. tonsa* is not representative of the region of the Firth of Forth under investigation in this study.

Following the re-development of calanoid copepod populations after the ctenophore bloom the establishment of a complex of congeneric *Acartia* spp. is not an unusual occurrence (Guerrero and Rodriguez 1997, Martens 1980). Along with the quality and quantity of available food the most fundamental factors which govern copepod growth and fecundity include temperature and salinity. Both *Acartia clausi* and *A. discaudata* were subject to the same physical regimen leading to the conclusion that there is sufficient biological complexity in the food supply from July 2001 onwards to sustain the populations of both species. Indeed the highly complex, constantly changing biological complexity in estuarine systems ensures that equilibrium between omnivorous calanoid copepod species and prey which could lead to a monopoly of the food source is never achieved (Hutchinson 1961). Consequently the coexistence of species with very similar resource requirements is possible (Alcaraz 1983) in the dynamic environment of the Forth.

Although many studies exist which estimate secondary production of *Acartia clausi* and *A. discaudata* (Guerrero and Rodriguez 1997) the influence of various environmental (abiotic and biotic) factors on the spatio-temporal co-occurence of both species has never been analysed. It has been argued that when food is abundant, as it was from June to the beginning of October, intraspecific and interspecific competition for food is probably unimportant in the biology of copepods (McLaren 1978). There is however disagreement with this suggestion (Landry 1978) and growth, development and

egg production of *Acartia clausi* was affected by food availability and positively correlated with chl *a*. Both *Acartia* congeners were able to persist all through June until the end of October and for much of this period were present in excess of 100 ind. m^{-3} $species^{-1}$. Despite the positive correlations with chl *a* and phaeopigments for grouped calanoid copepods, when *A. clausi* and *A. discaudata* were examined in isolation no such correlations were observed for either chl *a* (0.323, $p = 0.055$ and 0.335, $p = 0.093$ respectively) or phaeopigments (0.191, $p = 0.348$ and 0.270, $p = 0.180$). As the regulation of *Acartia* spp. populations is not resource limited it may be asserted that either 'top down' predation or shifts in physical conditions are controlling. Predator control of *Acartia clausi* has been previously documented in lagoonal systems (Landry 1978) coastal bays (Uye 1982) and estuarine inlets (Sekiguchi *et al.* 1980). The mechanism of control in all of these previous studies was stage-specific mortality focused upon the adult stages of *A. clausi*. An examination of the age structure of *A. discaudata* before, during and after its population peak in August shows a distinct decline in the abundance of CVI males and females. The resulting collapse in the *A. discaudata* population may then have permitted the subsequent ascendancy of *A. clausi* to dominate the calanoid copepod community until the end of the study through the elimination of congeneric competition for food resources. Finally, once again at the peak of its abundance *A. clausi* undergoes the same modification in age structure during October as *A. discaudata* in August.

This study shows that, on the one hand, whilst there is a progressive increase in biomass and diversity of microbial organisms from spring through summer and autumn the diversity of mesozooplankton remains limited. In addition the seasonal succession and temporal separation of copepod species, apart from the outstanding impact of ctenophores in June 2001, appears to be regulated through physiological responses to the physical environment rather than external biological effects such as food availability, food composition diversity and competitive interactions between calanoid species.

Chapter 3 – Seasonal patterns in feeding and prey selectivity by four calanoid copepods in Scottish coastal waters.

3.1 Introduction

Mixotrophic and heterotrophic microzooplankton are undeniably significant in retaining and recycling particulate organic matter (POM) in the upper water column by the consumption of bacteria, hetero- and autotrophic nanoflagellates and picophytoplankton (Azam *et al.* 1983, Sherr and Sherr 1988). This recognition has led to increased focus in developing our understanding of the microzooplankton-copepod link in pelagic systems. This trophic link is of even more significance given that there is a great deal of evidence indicating that copepods are ineffective at consuming the major proportion of phytoplankton (Gifford and Dagg 1988, Calbet *et al.* 2002, Gowan *et al.* 1999) and that microzooplankton can remove extremely high proportions of daily primary production which would otherwise be lost, through sinking, to pelagic microbial foodwebs.

The role of feeding preference (prey selection) by copepods has the potential to regulate the transfer of organic matter from the microbial web to higher trophic levels such as fish larvae (Mousseau *et al.* 1998). Directly the flux of POM upwards may be adversely affected by prey selection due to reduced impact on ubiquitous food sources such as phytoplankton. Indirectly however the flow of organic matter from lower trophic levels may be modified by the removal of the top predators (Calbet and Saiz 2005) in the microbial loop (ciliates and microphagous dinoflagellates) which may cause unregulated growth of cells too small to be consumed by copepods (bacteria and nanoflagellates).

Microplankton abundances can vary dramatically in relatively short periods of time in terms of food concentration, size structure and species composition (Hassett and Landry 1990), yet feeding selection studies have commonly been undertaken with little or very limited consideration of seasonality. We sampled repeatedly over a 9 month period in order to better appreciate temporal changes in feeding and selectivity of the dominant pelagic copepods in this area: *Acartia discaudata*, *Acartia clausi*, *Centropages hamatus* and *Temora longicornis*. Additionally the general acknowledgment that

microzooplankton are a fundamental link between the microbial web and 'classical' food chains is mostly supported from the increasing number of studies conducted in oceanic and coastal regions. Studies which investigate the behaviour of prey preference and dietary diversity in estuarine regions are not only less numerous but at present conclusions are conflicting. Estuarine studies which detail highly preferential grazing upon microzooplankton (Gifford and Dagg 1988) contradict research reporting copepod ingestion of high levels of autotrophic prey in comparison to heterotrophic microplankton.

Many rate processes in marine pelagic copepods are intrinsically linked to food availability and its variability (Kiørboe and Sabatini 1994, Hirst and Lampitt 1998, Peterson 2001), and there is strong evidence that food limitation of growth for example is commonplace (Hirst and Bunker 2003). With the exception of a few highly specialised carnivorous species e.g. *Paraeuchaeta norvegica*, *Chiridius armatus* (Olsen *et al.* 2000) and *Tortanus dextrilobatus* (Lougee *et al.* 2002), it is now apparent that the application of the older descriptions of 'herbivores' and 'carnivores' is incorrect and that the vast majority of calanoid species exhibit omnivorous feeding.

Copepods can be considered as both 'carnivorous predators' upon the protist portion of the microplankton and also 'herbivorous grazers' upon the fraction of microplankton responsible for primary production (Saiz and Kiørboe 1995). The balance of both feeding modes fundamentally depends upon the behavioural mechanisms of the copepods to detect and capture prey, and the ability of the prey to avoid capture. Indiscriminate as opposed to selective raptorial (Lonsdale *et al.* 1979) behaviour can be considered as the effective extremes along a spectrum of opportunistic omnivory (Kleppel 1993, Dam and Lopes 2003). Nevertheless, feeding behaviour has been shown to be modified by the prey composition, with normally indiscriminate grazers which use artificially generated feeding currents able to switch to 'silent' ambush mode in order to catch more nutritious motile prey (Tiselius 1989).

It is well established that when phytoplankton concentrations are low heterotrophic microplankton and protozoans can form an important and highly nutritious food for copepods (Fessenden and Cowles 1994). In the intervening periods between phases of high phytoplankton abundance when small protist prey are relatively more

abundant active predatory behaviour may represent an entirely distinct and separate feeding method for copepods (Landry 1981). Adaptive feeding responses to changing food conditions whilst difficult to isolate in field incubations may be detectable firstly through changes in the relative sizes of prey items consumed under contrasting phytoplankton concentrations throughout a temperate seasonal cycle and secondly by the disproportionate consumption of smaller motile prey items before, between and after temperate blooms.

As part of a wider study into the seasonal dynamics of calanoid copepods and the composition of protoplankton (nano- and micro-) composition in the Firth of Forth, Scotland, we conducted a series of experiments on grazing by the dominant calanoid copepods at Port Edgar, a mid-estuary site in the Firth of Forth. Salinity at the study site is almost fully marine for much of the year (Greenwood and Hill 2003) and the tidal range is approximately 5m at springs and 2.5m at neaps (Webb and Metcalf 1987).

3.2 Methods

Samples for grazing experiments were collected at Port Edgar (Figure 2.1) from March 22 to October 31 2001. The timing of sampling and measurement of physical parameters are described in section 2.2.1.

3.2.1 Incubation copepods

Copepods were collected from ~1m depth with slow, non-quantitative hand tows using a 200µm mesh, 50cm diameter mouth ring net, fitted with a 2L non-filtering solid cod-end. The contents of the cod-end were transferred into a 50L plastic lined polystyrene container and diluted with ambient seawater. The polystyrene container was immersed in a bath of ambient seawater for immediate transport back to the laboratory, which was always less than 1h after collection.

In the laboratory actively swimming, undamaged adult female copepods were transferred to triplicate 1L Duran bottles which were filled with seawater collected at the Port Edgar site at the time of collection and which had been screened through 55µm mesh to remove any other large metazoan grazers. On each sampling event a qualitative judgement was made as to which were the numerically dominant calanoid copepods. In

all experiments females of the 2 most abundant species were then incubated (Table 3.1). Incubation always commenced within 4 h from copepod collection in the field. Grazing incubations were run for 24 h on a rotating plankton wheel set at ~ 0.5 rpm rotating end over end in a controlled temperature room set to within ± 0.5^{o}C ambient sea temperature and a light regime of 12:12 L:D. Controls without the addition of copepods were also run in triplicate.

At the end of incubations the condition of the females (dead or alive) was noted, dead copepods were identified and excluded from grazing calculations, mortality was <1% over all incubations. Female prosome lengths were measured to within ±17μm using a Leica MZ8 dissection microscope at x80 magnification and the carbon content (μg C female^{-1}) determined using the length-weight regressions of Klein Breteler *et al.* (1982) for *Centropages hamatus* and *Temora longicornis*, and Uye (1982) for *Acartia clausi*. As no equations were available for *A. discaudata*, we used those for *A. bifilosa* determined by Koski (1999), these two species having very similar sizes and body proportions. Values were converted to carbon where necessary assuming this to be 40% of dry weight (Parsons *et al.* 1984, Båmstedt 1986).

On April 11, April 26 and July 2 2001 (experiments 3, 4 and 7 respectively) only *Centropages hamatus* was incubated as all other copepod species were rare and not present in sufficient numbers to allow incubations. Additionally, for the whole of June 2001 adult copepods were very rare at the study site, while a ctenophore bloom composed almost exclusively of *Pleurobrachia pileus* was present. We continued to monitor the study site weekly throughout June 2001, but populations did not recover sufficiently to provide enough females for incubation until July.

3.2.2 Grazing rate calculations

To establish cell concentrations of prey at time zero (T_0), triplicate 250ml water samples were taken from the screened incubation water prior to addition of copepods. These samples were preserved in acid Lugol's iodine to a final concentration of 2%. At the end of incubations 250ml subsamples of the incubation water were taken

Table 3. 1. Summary of experimental conditions during the sixteen incubation experiments during 2001.

Experiment	Date	Microplankton prey concentration (μg C l^{-1})	Copepod Species Incubated	No. of live females in each of 3 replicate bottles after experiment	Seawater temp. (^{o}C)	Mean female size (μm)	Mean female C content (μg C)
1	22 March	52.66	*Acartia discaudata*	5, 3, 4	4.9	794	2.1
			Temora longicornis	5, 5, 4		931	14.2
2	29 March	66.92	*Acartia discaudata*	3, 3, 5	5.1	798	2.1
			Temora longicornis	2, 3, 5		951	15.1
3	11 April	52.06	*Centropages hamatus*	5, 5, 4	6.3	1002	7.4
4	26 April	62.23	*Centropages hamatus*	10, 10, 10	7.1	1085	9.1
5	7 May	80.55	*Acartia discaudata*	10, 10, 10	9	896	3.2
			Temora longicornis	9, 10, 8		1022	19.2
6	24 May	120.5	*Acartia discaudata*	10, 10, 10	11.5	876	2.8
			Temora longicornis	10, 8, 10		1051	20.8
7	2 July	138.25	*Centropages hamatus*	10, 10 10,	14.1	993	7.3
8	17 July	53.34	*Acartia clausi*	10, 9, 12	13.6	1047	6.7
			Centropages hamatus	10, 9, 10		1084	9.1
9	31 July	77.11	*Acartia clausi*	8, 9, 7	14.4	1007	5.9
			Centropages hamatus	10, 10, 10		1011	7.7
10	15 Aug	116.69	*Acartia clausi*	11, 8, 11	14.3	959	5.0
			Centropages hamatus	9, 9, 8		1005	7.5
11	29 Aug	82.92	*Acartia clausi*	8, 10, 5	13.7	929	4.5
			Centropages hamatus	9, 10, 10		1017	7.7
12	11 Sept	88.89	*Acartia clausi*	7, 7, 8	13.5	928	4.5
			Centropages hamatus	5, 5, 5		986	7.2
13	19 Sept	56.63	*Acartia clausi*	8, 9, 6	12.6	950	4.8
			Centropages hamatus	5, 5, 5		991	7.3
14	4 Oct	57	*Acartia clausi*	7, 8, 6	12.7	948	4.8
			Acartia discaudata	8, 8, 8		773	1.9
15	19 Oct	49.63	*Acartia clausi*	8, 6, 8	12.2	920	4.4
			Acartia discaudata	8, 8, 8		758	1.8
16	31 Oct	15.58	*Acartia clausi*	7, 8, 7	11.7	917	4.3

from each of the six (3 bottles per species incubated) bottles which contained copepods, and the three control bottles and preserved with Lugol's iodine once again. The remaining incubation water was filtered through 30µm mesh for the collection of copepods.

From each Lugol's preserved sample 10ml aliquots were settled (for a minimum period of 12h) following the Utermöhl method (Hasle 1978). Counting and measuring were conducted using an Olympus IX70 inverted microscope using bright field and phase contrast microscopy. Identification was carried out to the lowest possible taxonomic category using various descriptions and keys of Dodge (1982), Montagnes *et al.* (1988), Tomas (1997) and Foissner *et al.* (1999). For some diatoms and dinoflagellates, light microscopy is insufficient to identify separate species and several taxa were placed into morphological groups and counting was conducted upon size categories within these groups (Table 3.2).

When enumerating microplankton the entire settling chamber was counted and typically >200 to ~1000 cells were examined (Sandgren and Robinson 1984). However, when a single species was present in large numbers (~500 ind. ml^{-1} or greater) counts of random fields were made, >200 cells were counted and then the number of cells ml^{-1} calculated after determination of the total number of fields covering the entire surface of the chamber. Nanoplankton (primarily small monads and *Rhodomonas* spp.) ~5µm or smaller were always enumerated by counting >20 random fields at x400 until at least 200 individual cells were counted.

Clearance rates (F, L. cop^{-1}. d^{-1}) and ingestion (I, cells. cop^{-1}. d^{-1}) on each microplankton taxa were calculated using the equations of Frost (1972) thus.

$$F = V\,(k_c - k_t)/Z \qquad (3.1)$$

Where V is the volume of the incubation bottle (litres), Z is the number of live copepods l^{-1} in the incubation bottle after 24h, and k_c and k_t are the net growth rates of microplankton in the controls and treatments respectively. k values (d^{-1}) are calculated as:

$$k = \text{Log}_e\,(C_o/C_e) \qquad (3.2)$$

Table 3. 2. Size categories used to define morphologically similar microplanktonic taxa.

Category Name	**Size groupings**
Small *Chaetoceros* spp.	< 12 µm width
Medium *Chaetoceros* spp.	≥ 12µm < 30 µm width
Large *Chaetoceros* spp.	≥ 30 µm width
*Small *Coscinodiscus/Thalassiosira* spp. (incl chain forming spp.)	< 12 µm diameter
*Medium *Coscinodiscus/Thalassiosira* spp. (incl chain forming spp.)	≥ 12µm < 30 µm diameter
*Large *Coscinodiscus/Thalassiosira* spp. (incl chain forming spp.)	≥ 30 µm diameter
Small rhaphioid and pennate diatoms	< 12 µm
Medium rhaphioid and pennate diatoms	≥ 12 µm < 30 µm length
Large rhaphioid and pennate diatoms	≥ 30 µm length
Small dinoflagellates	< 12 µm length
Medium dinoflagellates	≥ 12 µm < 30 µm length
Large dinoflagellates	≥ 30 µm length
Small ciliates	< 12 µm length
Medium ciliates	≥ 12 µm < 30 µm length
Large ciliates	≥ 30 µm length

* In the case of *Coscinodiscus/Thalassiosira* chains, measurements were made on individual cells rather than entire chain length and the number of cells in the chain recorded.

Where for a 24 h incubation C_o is the initial concentration of prey at time zero (T_0) and C_e is the concentration of prey in the control and treatment bottles at the end of the incubation. Ingestion rate (*I*, cells copepod^{-1} d^{-1}) is then calculated as:

$$I = C \times F \quad (3.3)$$

Where C is the mean concentration of prey throughout the 24h incubation period, which is calculated by

$$C = C_o\,(e^{kt}-1)/k_t \quad (3.4)$$

Volumes of prey cells were estimated from linear dimensions by comparing the cells to appropriate geometric shapes (Hillebrand *et al.* 1999) and carbon content of the cells was then calculated following the regression analyses in table 4 of Menden-Deuer and Lessard (2000). In order to examine feeding selection as a function of prey size it was necessary to determine a representative size of cell for each taxon upon which grazing by copepods was observed. Thirty individual cells of each prey taxon were measured microscopically from 3 distinct periods in the annual cycle, pre-spring bloom from December 2000 to January 2001, bloom from late March 2001 to the end of June 2001, and post bloom from September to November 2001. We ensured that measurements of microplankton were from 3 distinct and temporally separate periods in order that any seasonal changes in the relative size of a prey type would become apparent. Of all the microplankton taxa measured and analysed many did not display significant seasonal differences in mean cell size (ANOVA, $\alpha = 0.05$) and a single volume applied for the entire duration of the study when calculating carbon content. Where there were significant seasonal differences in cell size different bloom and post bloom values were applied.

Grazing rates for chain forming species are based on the calculation of grazing upon individual cells, as breakage of chains is likely to occur during sample preparation and handling. Attempting to infer grazing as a function of chain length reduction is consequently erroneous, whereas reduction in total cell numbers of chain forming species

will still permit grazing rate and carbon ingestion measurements over the course of incubations.

3.2.3 Statistical analyses

Ingestion (*I*, cells copepod^{-1} d^{-1}) rates upon individual microplankton were plotted as a function of the initial concentration of prey (C_0, cells ml^{-1}) at the start of incubations (T_0) and the correlation coefficients for each of the prey taxa i.e. diatoms, ciliates, dinoflagellates and flagellates (hereafter referred to as 'prey groups') calculated. The slope and intercepts of each regression were then compared using ANCOVA and the regressions for each prey group were combined where appropriate. Linear regressions were also conducted upon ingestion as a function of prey cell length (*L*, µm) and ANCOVA was again used to combine regressions where appropriate.

When cells which are present at T_0 are completely removed by the end of an incubation (T_1) natural logarithm transformations used in equations which calculate clearance rates make it impossible to determine the volume of such prey types swept clear by copepods. So additionally Ivlev electivity (E) indices, which are capable of being calculated when cells are completely consumed, were also calculated for each prey group (Chesson 1983). Ivlev's E is scaled from +1 to -1 with 0 indicating neutral preference for a particular prey taxa and values tending towards +1 or -1 indicating increasing preference or avoidance of a prey type respectively. Preference for various prey types was tested statistically using Kruskal-Wallis (Tukey test) one-way ANOVA on ranked clearance data and electivity indices (Rollwagen Bollens and Penry 2007).

The identification of groups of microplankton taxa which followed similar patterns of seasonal abundance was examined using multivariate tests which were conducted in Plymouth Routines in Multivariate Ecological Research (PRIMER 5) software. Species which were present in < 10% of samples were considered rare and not included in the analyses conducted upon the raw dataset of cells ml^{-1}. Given the high number of zero values in the data a Bray-Curtis similarity matrix was constructed upon $\log_{10}(x + 1)$ transformed data (Field *et al.* 1982). The resulting similarity matrix was then used to produce a cluster dendrogram (Figure 3.1) with group average linkage

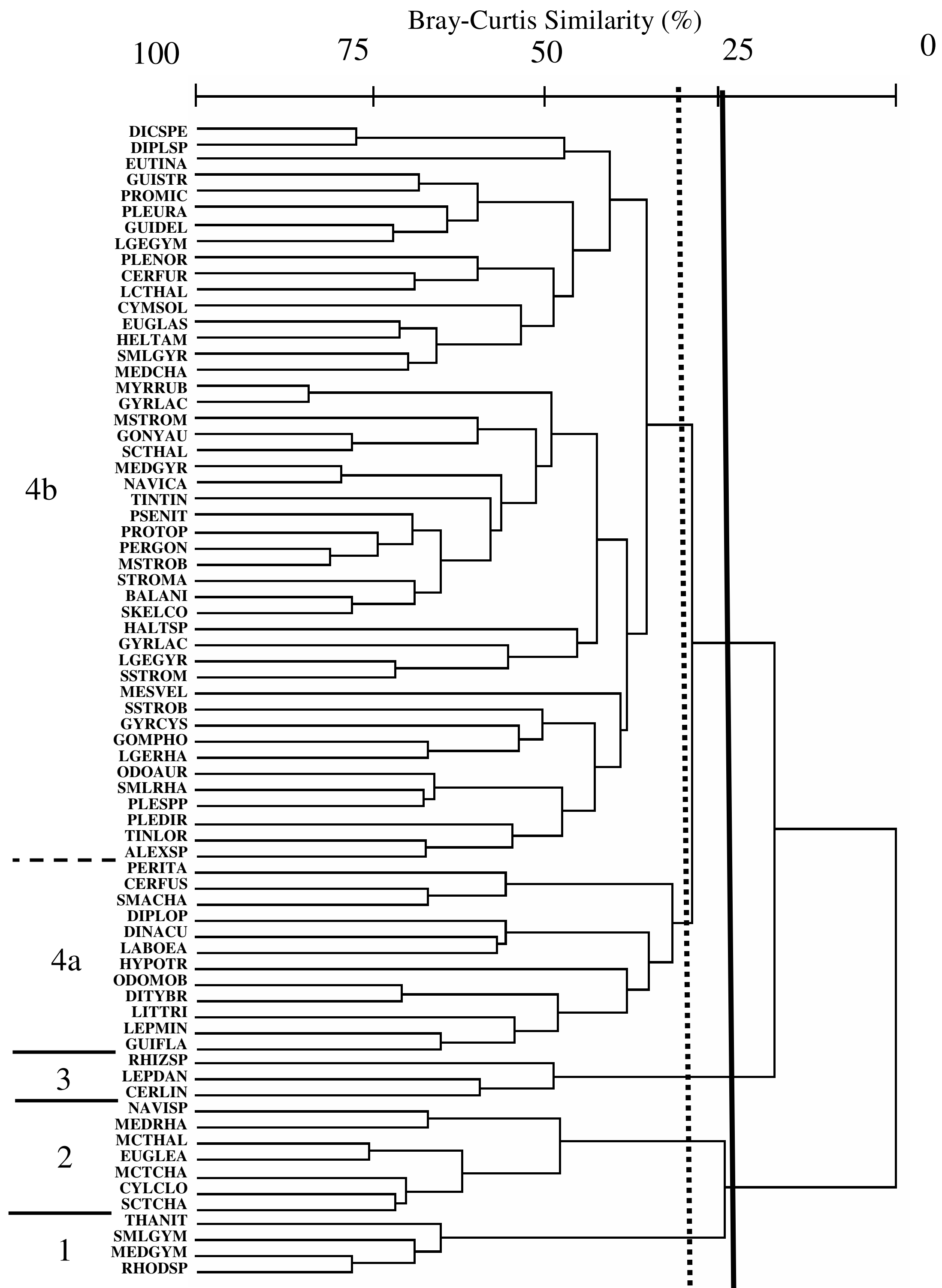


Figure 3. 1. PCA cluster dendrogram of microplankton taxa occurring in > 10% all samples collected during the study based on a Bray-Curtis similarity matrix and Log x+1 transformed data. Primary cluster groups (1 to 4) formed at a similarity level of 25% and clusters 4a and 4b at a similarity level of 29%. Species codes in table 3.5.

(Clifford and Stephensen 1975). Primary (25% similarity - solid line) and secondary (29% similarity - dashed line) cluster groupings were verified by superimposition onto a Non-Metric Multidimensional Scaling (NMDS) ordination plot (Figure 3.2). In order to examine copepod grazing in relation to initial prey concentrations and the behavioural and morphological characteristics of the microplankton prey a further set of NMDS analyses were conducted upon subsets of the microplankton species analysed in the initial cluster analysis. Again, given the large number of zero-zero comparisons in each of the copepod prey data sets NMDS ordination plots were constructed based upon Bray-Curtis $\log_{10}(x + 1)$ similarity matrices. As an aid to better visually represent the grazing data and allow comparisons between copepod species all NMDS plots were rotated in order to maximise R^2 values of cell volumes from left to right along the x-axes.

3.3 Results

During the course of sampling 4 species dominated the calanoid copepods numerically, *Acartia discaudata, Acartia clausi, Centropages hamatus* and *Temora longicornis*. These species underwent abundance and dominance shifts over the course of the sampling period. At the start of the study in mid March 2001 *A. discaudata* and *T. longicornis* were the main species incubated until June 2001. This switched to *A. clausi* and *C. hamatus* on 2 July 2001, these 2 species dominated until mid-October (except on the two sampling occasions in April when *C. hamatus* outnumbered all other species). In October *C. hamatus* was replaced by *A. discaudata*, which together with its congener *A. clausi*, dominated until the end of the study in October. On the final incubation date (Oct 31 2001) only *A. clausi* was present in sufficient numbers to allow our incubations (Table 3.1).

3.3.1 Salinity and temperature

See section 2.3.1

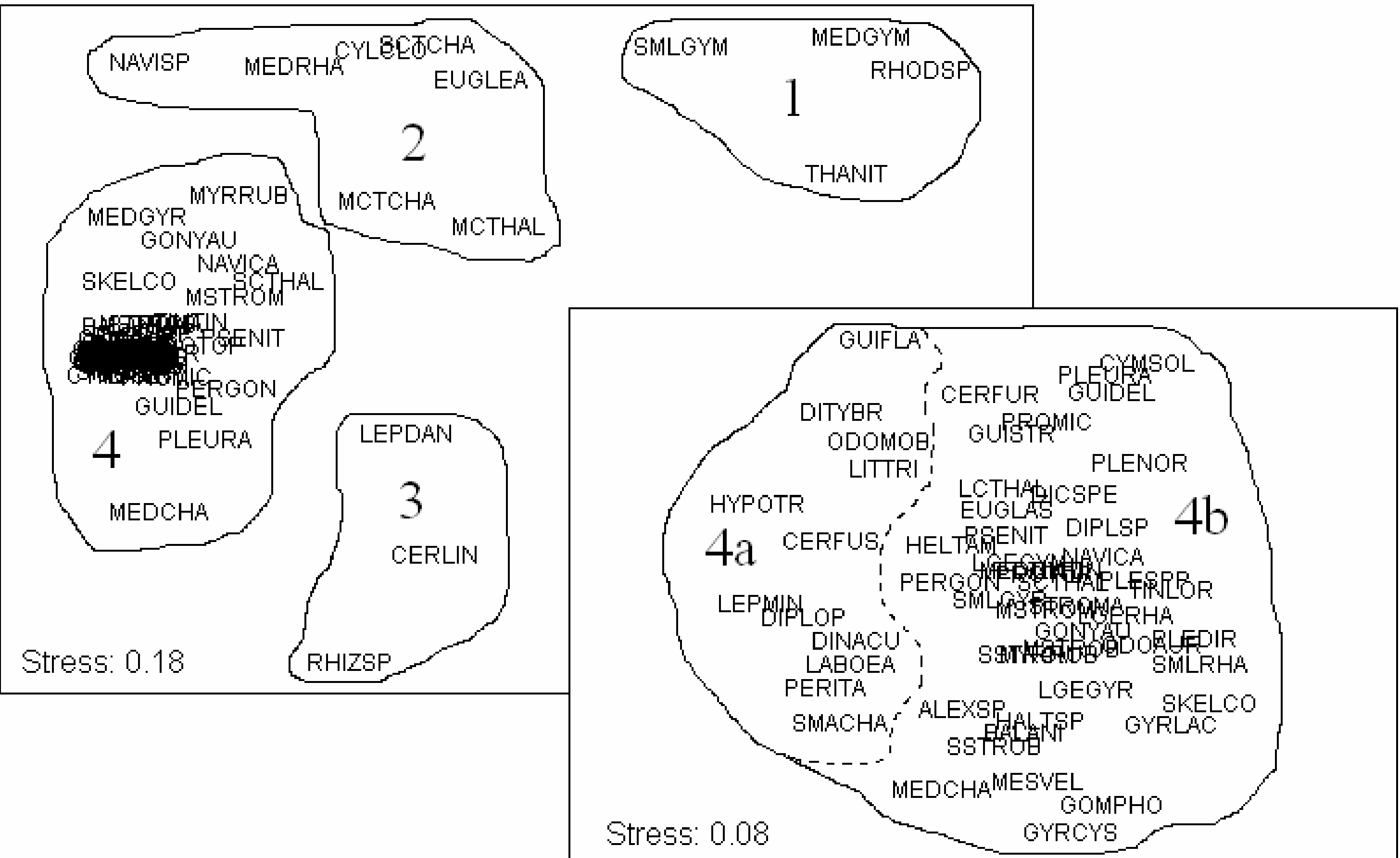


Figure 3. 2. NMDS ordination plot of the comparison of prey taxa in the cluster dendrogram (Figure 3.1) using the same Bray–Curtis (Log x+1) similarity index. Cluster groups are superimposed onto the NMDS plot and cluster boundaries are drawn subjectively by eye. Inset, results of second ordination conducted upon only primary cluster group 4 species using the same similarity matrix parameters. See species codes in table 3.5.

3.3.2 Microplankton seasonal dynamics

Cluster and NMDS analyses conducted upon both hetero- and autotrophic microplankton identified 4 groupings (Figures 3.1 and 3.2). Cluster group 1 was the "seasonally consistent" group made up of species present during the grazing study which increased in concentration with both the spring and autumn blooms. Group 2 was the "spring bloom" group comprised of species which peaked and were responsible for the spring bloom in April 2001. Cluster group 3 the "autumn bloom" group were absent before May 2001 and peaked in concentration during the autumn bloom. The autumn bloom was initially composed of *Rhizosolenia* spp. until the bloom peak on 15 August. As the autumn bloom declined the heterotrophic dinoflagellate *Ceratium lineatum* became numerically dominant in September. Cluster group 4 was made up of species which appeared "sporadically" and contained 2 secondary clusters. Group 4a was distinguished from 4b in that 4a species were present less frequently and concentrations never exceeded 1 cell ml^{-1}. The stress value of the initial NMDS plot (0.18) indicated that the compactness of group 4 adversely affected the goodness of fit of the ordination plot to the similarity matrix (Figure 3.2). Consequently the microplankton species in group 4 were examined in isolation with a second NMDS using the same similarity matrix parameters as previously (Fig 3.2 Inset).

3.3.3 Prey ingestion

Particle sizes grazed during incubations ranged from small *Gyrodinium* spp., ~9µm in length to *Ceratium fusus* at ~330µm long. Linear regressions were used to examine ingestion ($Log_{10}I$, cells d^{-1}) versus prey concentration ($Log_{10}C_0$, cells ml^{-1}) (Figure 3.3) and also ingestion versus cell length ($Log_{10}\ L$, µm). Separate regressions upon each microplankton prey group were not statistically different from each other (ANCOVA, $p > 0.05$) and a single regression was used to describe ingestion rates (Table 3.3) versus both C_0 and L for all 4 copepod species. The fit of all linear regressions of ingestion versus prey concentration were highly significant ($p < 0.001$).

Ingestion rates increased with prey concentration but there was a moderate decrease in ingestion rates with increasing prey cell length (Tables 3.3, 3.4 and Figure 3.3)

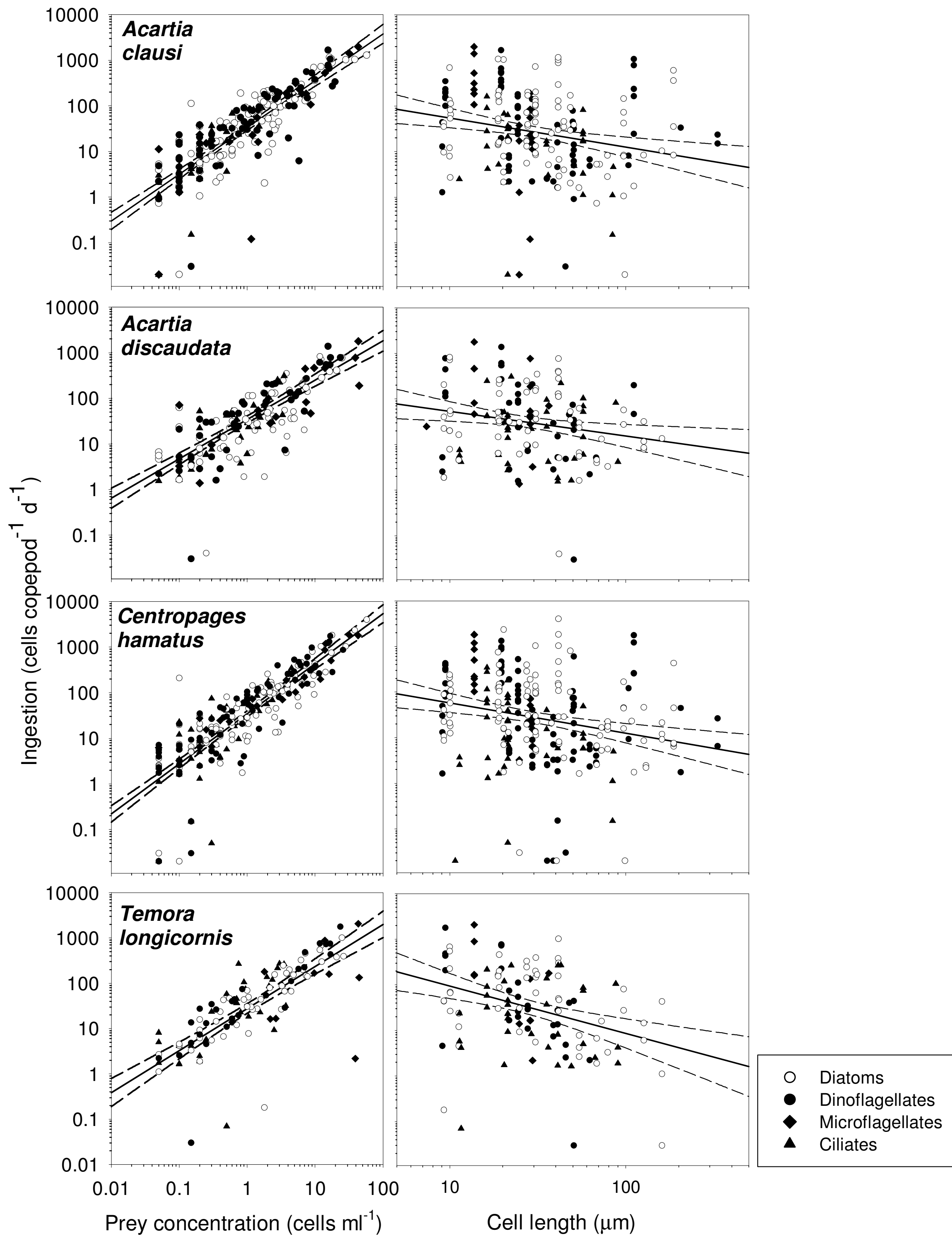


Figure 3. 3. Ingestion (I, cells copepod^{-1} d^{-1}) versus initial cell concentration (C_0, cells ml^{-1}) at (T_o) and prey cell length (µm) of food taxa for the 4 copepod species incubated during the course of the study. Equations for the regression lines and correlation coefficients are detailed in tables 3.3 and 3.4. Dashed lines indicate ± 95% confidence intervals.

Table 3. 3. Summary of regression analyses to describe the effect of initial prey concentrations ($Log_{10}C_0$, cells ml^{-1}) on ingestion ($Log_{10}I$, cells $copepod^{-1}$ d^{-1}) by the 4 copepod species studied. Data set based upon all copepod incubation experiments during the study period.

Copepod species	Prey group	Regression Equation	N	R^2	p
Acartia clausi	All Prey	$Log_{10}I = 35.955\ Log_{10}C_0 + 19.811$	220	0.77	<0.001
Acartia discaudata	All Prey	$Log_{10}I = 31.615\ Log_{10}C_0 + 27.518$	128	0.63	<0.001
	Round diatoms	$Log_{10}I = 48.191\ Log_{10}C_0 - 41.062$	38	0.68	<0.001
Centropages hamatus	All prey	$Log_{10}I = 55.814\ Log_{10}C_0 - 14.036$	247	0.84	<0.001
Temora longicornis	All prey	$Log_{10}I = 25.452\ Log_{10}C_0 + 38.382$	121	0.45	<0.001

Table 3. 4. Summary of regression analyses to describe the effect of prey length ($Log_{10}L$, µm) on ingestion ($Log_{10}I$, cells $copepod^{-1}$ d^{-1}) by the 4 copepod species studied. Data set based upon all copepod incubation experiments during the study period.

Copepod species	Regression Equation	N	R^2	p
Acartia clausi	$Log_{10}I = 0.363\ Log_{10}L + 145.21$	220	0.05	0.386
Acartia discaudata	$Log_{10}I = -1.767\ Log_{10}L + 180.84$	166	0.04	0.009
Centropages hamatus	$Log_{10}I = -0.661\ Log_{10}L + 197.55$	247	0.05	0.239
Temora longicornis	$Log_{10}I = -2.268\ Log_{10}L + 233.86$	121	0.12	0.011

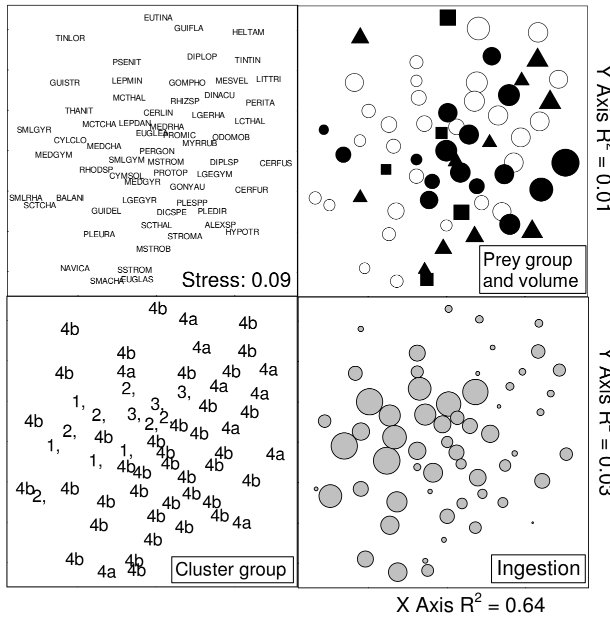
This relationship was further also evident using multivariate analyses. This interaction is emphasised by the decreasing bubble size (Figure 3.4) of prey ingestion which is inversely related to the increasing symbol size of cell volume along the x axes of the NMDS plots for each copepod species.

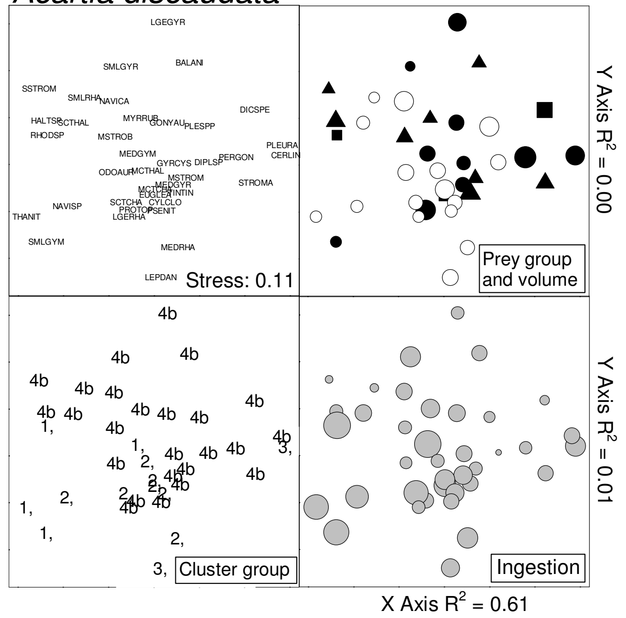
The structure of cluster groupings from the dendrogram of initial prey concentrations (Figure 3.1) remained in evidence in the NMDS plots of ingestion by *Acartia clausi*, *Centropages hamatus* and *Temora longicornis* with prey taxa from the same cluster remaining tightly grouped. *Acartia discaudata* differed from the other copepod species in that when the cluster grouping was overlaid onto the NMDS, prey taxa from the same clusters were more widely dispersed suggesting more discriminate feeding and a reduced influence of initial prey concentration.

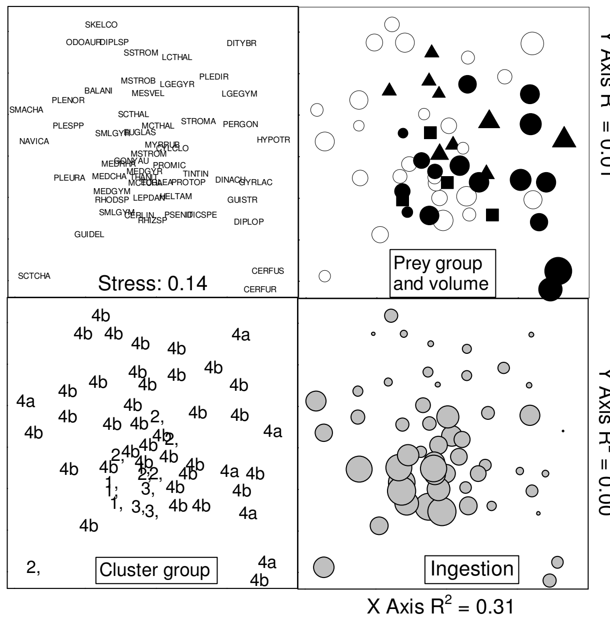
Clearance rates (ml copepod^{-1} h^{-1}), prey group electivity (E*) and ingestion rates (ngC copepod^{-1} d^{-1}) are presented in Figure 3.5. Dinoflagellates were consistently cleared in greater volumes than other prey groups by all copepods except *Temora longicornis*, for which clearance rates were similar to proportions available. The relative proportion of dinoflagellate carbon in the diet of the copepods generally exceeded that available (Figure 3.6).

Clearance and carbon ingestion of dinoflagellates by both *Acartia* spp. is similar on 4 and 19 Oct the only dates upon which direct comparisons of grazing by these 2 congeneric species were possible. The heterotrophic dinoflagellate groups *Gymnodinium* spp. and *Gyrodinium* spp. were ubiquitous and the most numerous dinoflagellate taxa during our study (Table 3.5). Multivariate analyses of ingestion (cells copepod^{-1} d^{-1}) show that the balance of grazing upon the smaller size fractions of both dinoflagellate taxa was more effective by *A. discaudata* and that *A. clausi* ingested a greater proportion of medium *Gymnodinium* and *Gyrodinium* spp. than small sized cells (Figure 3.4).

Ciliate carbon contribution to copepod diets was generally low during the whole study. Both *Acartia* congeners showed significant ($p < 0.01$) and positive electivity for ciliates but *A. discaudata* was able to clear greater volumes of ciliates than *A. clausi* (Figure 3.5) and cleared the highest volume measured (10.6 ml copepod^{-1} h^{-1}) on 24 May. Ciliate carbon ingested never surpassed 1 ngC copepod^{-1} h^{-1} or contributed meaningfully







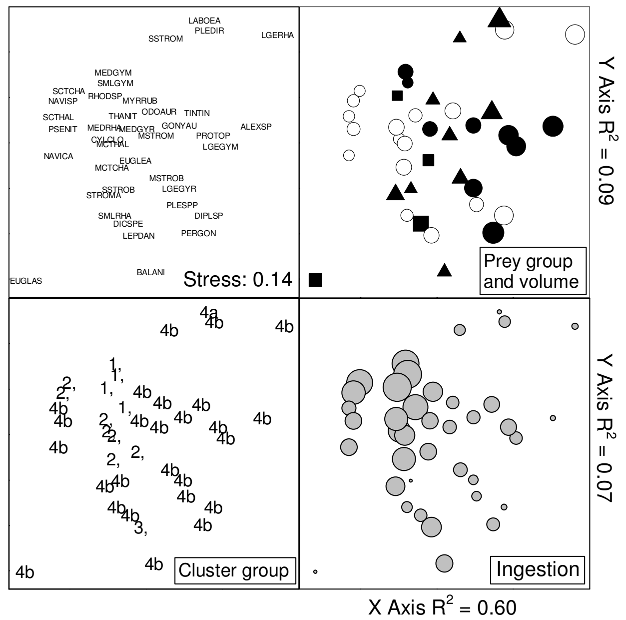


Figure 3. 4. NMDS ordination plots of copepod grazing data (cells ingested cop.$^{-1}$ d^{-1}). The NMDS ordination for each copepod is presented using four symbolic approaches. Upper left panel: The NMDS ordination of prey species (see species codes in table 3.5). Lower left panel: The same ordination coded using the PCA prey cluster groups (see figure 3.1). Upper right panel: The ordination related to prey group and volume. Symbol size is related to prey volume, open circles – diatoms, black circles – dinoflagellates, triangles – ciliates, squares – flagellates. Lower right panel: The ordination showing ingestion rate, symbol sizes are related to mean ingestion (cells ml^{-1}). Correlation coefficients of symbol sizes related to each axes are also given for both right hand panels.

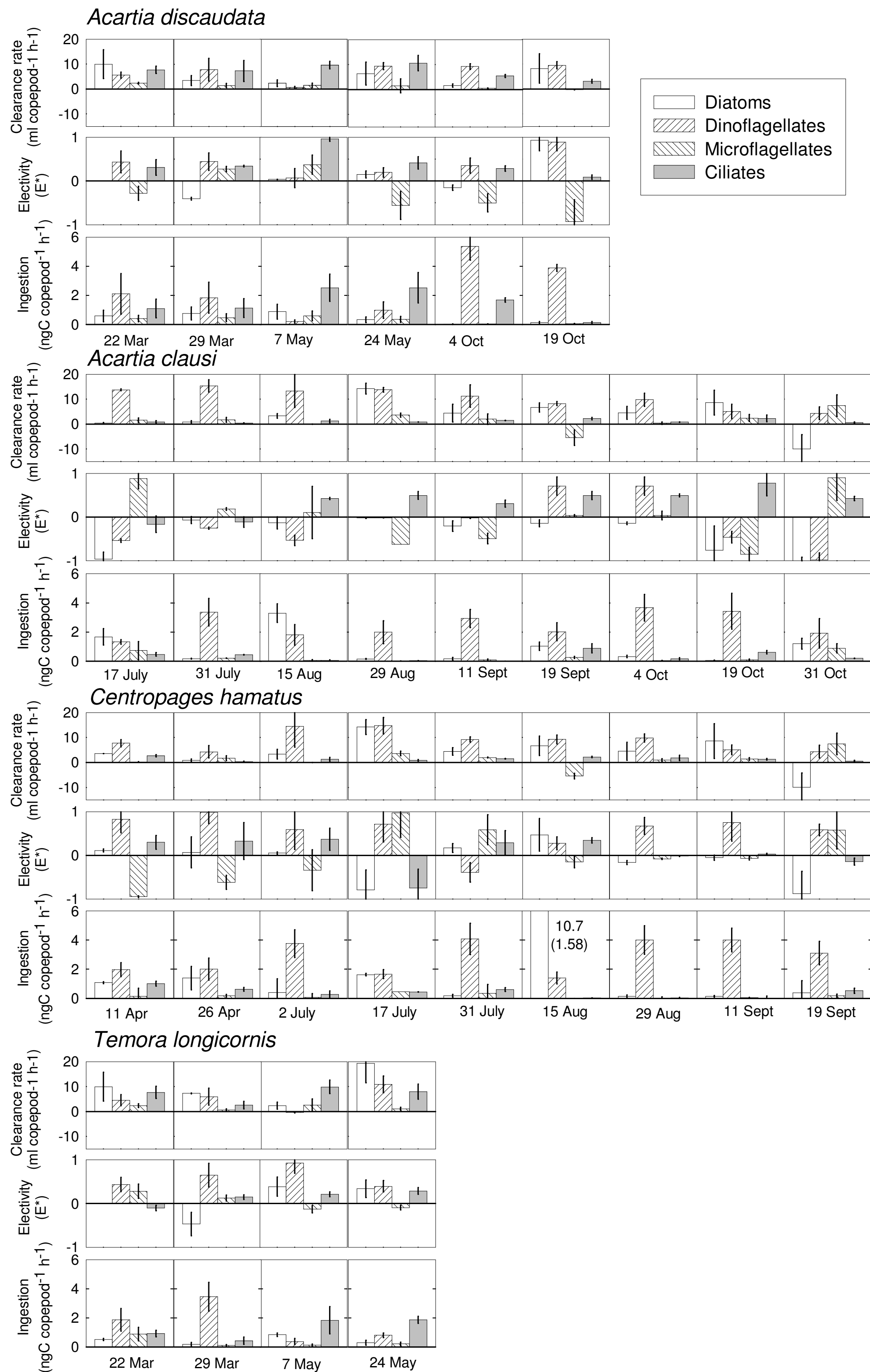


Figure 3. 5. Electivity, clearance rates and ingestion by the copepods during incubations between March and October 2001. Error bars = 1 SE.

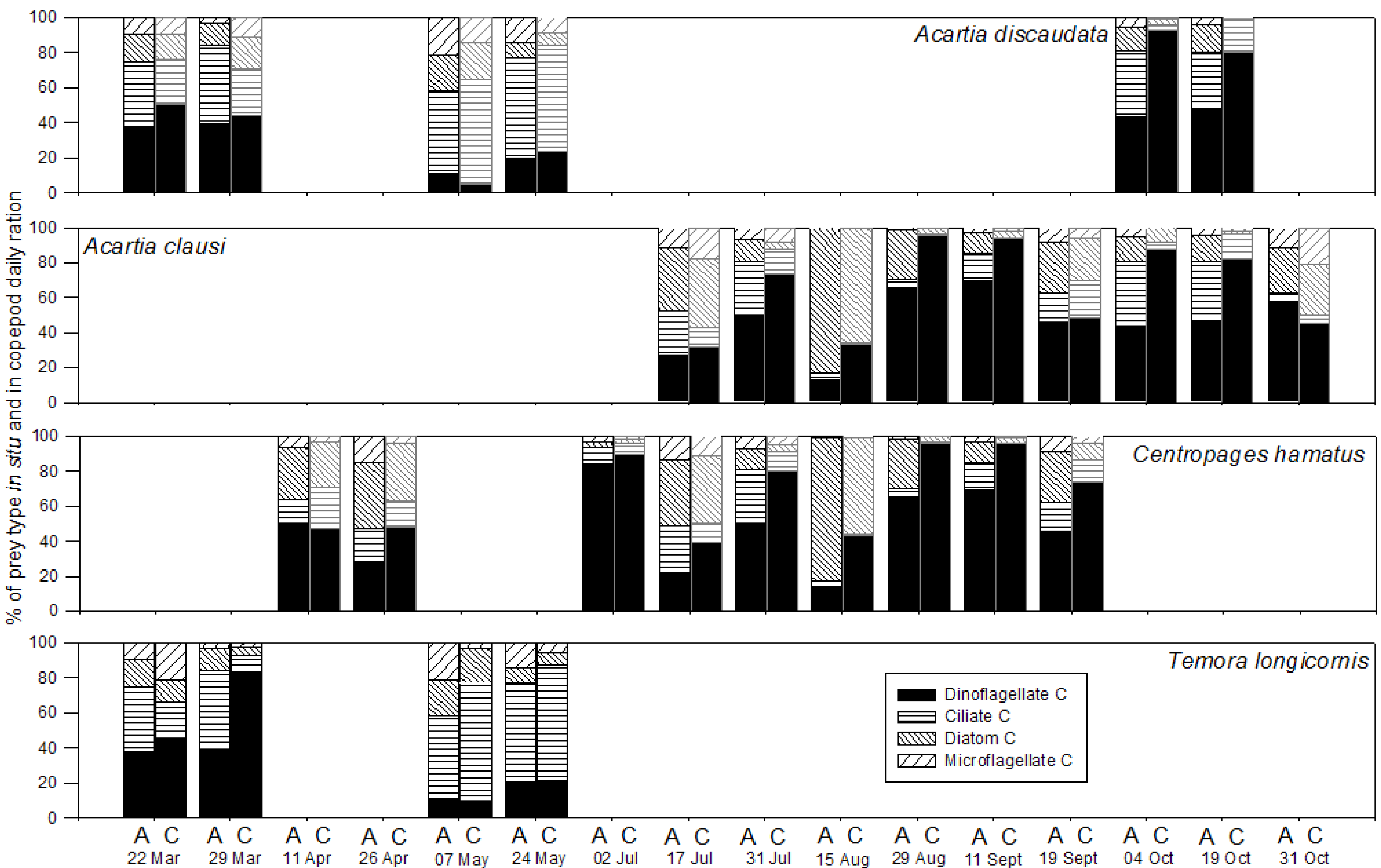


Figure 3. 6. Relative proportions of carbon biomass of prey available (A on x-axis) (% of total prey biomass available in screened seawater) vs. prey biomass consumed (C on x-axis) by copepods (% of total prey biomass consumed during 24h incubation) from Port Edgar during study period.

Table 3. 5.Details of phyto- and microzooplankton species used in cluster and NMDS analyses. Mean (±SE) abundance (cells ml^{-1}). Species codes correspond to those used in Figs. 3.3,3.4 and 3.8, *n*, number of dates when each species was recorded.

Taxa	Code	*n*	Mean (±1SE)	Length (µm)
Dinophycaea (Dinoflagellates)				
Alexandrium spp.	ALEXSP	4	0.1 (0.0)	45.5
Ceratium furca	CERFUR	6	0.2 (0.1)	205.4
Ceratium fusus	CERFUS	4	0.2 (0.0)	332.2
Ceratium lineatum	CERLIN	8	5.7 (2.3)	111.0
Dinophysis acuta	DINACU	4	0.1 (0.0)	62.4
Diplopsalis spp.	DIPLOP	3	0.1 (0.0)	35.7
Gonyaulax sp. A (*grindleyi*?)	GONYAU	16	0.9 (0.3)	29.4
Gyrodinium cysts	GYRCYS	3	0.4 (0.2)	15.8
Gyrodinium lachryma	GYRLAC	5	0.3 (0.1)	67.8
Large *Gymnodinium* spp.	LGEGYM	12	0.2 (0.0)	40.9
Large Gyrodinium spp.	LGEGYR	9	0.2 (0.1)	38.8
Med *Gymnodinium* spp.	MEDGYM	16	11.7 (1.4)	19.6
Med *Gyrodinium* spp.	MEDGYR	16	1.9 (0.4)	24.4
Peridinales/Gonyaulax spp.	PERGON	13	1.0 (0.6)	50.6
Prorocentrum micans	PROMIC	8	0.6 (0.1)	50.0
Protoperidinium spp.	PROTOP	16	0.6 (0.3)	21.7
Small *Gymnodinium* spp.	SMLGYM	16	7.5 (1.4)	9.4
Small Gyrodinium spp.	SMLGYR	11	0.4 (0.1)	9.1
Ciliates				
Balanion spp.	BALANI	6	0.6 (0.4)	20.4
Halteria spp.	HALTSP	7	0.2 (0.1)	29.1
Hypotrichs	HYPOTR	2	0.1 (0.1)	84.0
Laboea strobila	LABOEA	4	0.1 (00)	90.1
Med *Strobilidium* spp.	MSTROB	12	0.5 (0.1)	21.3
Med *Strombidium* spp.	MSTROM	16	1.0 (0.2)	21.3
Mesodinium velox	MESVEL	4	0.2 (0.4)	16.4
Myrionecta rubra	MYRRUB	14	1.9 (0.1)	18.9
Peritrichia sp.A	PERITA	3	0.2 (0.1)	85.0
Small *Strobilidium* spp.	SSTROB	5	0.2 (0.1)	11.3
Small *Strombidium* spp.	SSTROM	9	0.2 (0.0)	11.6
Strombidium sp. A	STROMA	12	0.5 (0.2)	35.5
Tintinnids	TINTIN	16	0.6 (0.2)	57.2
Tintinnids (without loricae)	TINLOR	7	0.1 (0.0)	36.2
Flagellates				
Dictyocha speculum	DICSPE	9	0.3 (0.1)	24.8
Euglenophyceae sp.A	EUGLEA	16	8.2 (3.4)	28.6
Euglenophyceae spp.	EUGLAS	9	0.2 (0.0)	29.5
Rhodomonas spp.	RHODSP	16	16.0 (3.1)	13.8

Taxa	Code	n	Mean (±1SE)	Length (µm)
Bacillarophycea (Diatoms)				
Cylindrotheca closterium	CYLCLO	16	5.6 (2.0)	30.8
Cymatopleura solea	CYMSOL	4	0.3 (0.2)	36.4
Diploneis spp.	DIPLSP	12	0.2 (0.0)	68.4
Ditylum brightwellii	DITYBR	3	0.1 (0.0)	130.4
Gomphonema spp.	GOMPHO	3	0.1 (0.1)	111.3
Guinardia delicatula	GUIDEL	5	1.2 (0.4)	27.8
Guinardia flaccida	GUIFLA	2	0.2 (0.2)	71.2
Guinardia striata	GUISTR	7	0.3 (0.1)	44.5
Helicotheca tamesis	HELTAM	8	0.4 (0.1)	78.8
Large *Coscinodiscus/Thalassiosira*	LCTHAL	9	0.3 (0.1)	41.7
Large rhaphioidae diatoms	LGERHA	10	0.2 (0.1)	54.3
Leptocylindrus danicus	LEPDAN	13	2.9 (0.9)	37.6
Leptocylindrus minimus	LEPMIN	2	0.5 (0.1)	21.5
Lithodesmium tricornutum	LITTRI	5	0.5 (0.2)	37.8
Med *Chaetoceros* spp.	MEDCHA	6	6.7 (6.3)	20.2
Med *Coscinodiscus/Thalassiosira*	MCTHAL	16	3.7 (1.0)	18.9
Med *Coscinodiscus/Thalassiosira* (chain)	MCTCHA	15	3.0 (0.5)	19.1
Med rhaphioidae diatoms	MEDRHA	16	3.7 (1.2)	27.4
Navicula sp. A	NAVICA	14	1.4 (0.3)	10.1
Navicula spp.	NAVISP	8	10.2 (6.0)	41.3
Odontella aurita	ODOAUR	8	0.3 (0.1)	24.9
Odontella mobiliensis	ODOMOB	4	0.1 (0.0)	98.9
Pleurosigma directum	PLEDIR	7	0.5 (0.1)	160.5
Pleurosigma normanii	PLENOR	6	0.2 (0.0)	72.7
Pleurosigma sp. A	PLEURA	4	3.1 (2.0)	48.4
Pleurosigma spp.	PLESPP	10	0.4 (0.1)	126.5
Pseudo-Nitzschia spp.	PSENIT	11	1.5 (0.5)	97.2
Rhizosolenia spp.	RHIZSP	7	10.1 (8.0)	187.1
Skeletonema costatum	SKELCO	5	1.8 (0.7)	31.3
Small *Chaetoceros* spp.	SMACHA	3	0.6 (0.5)	10.1
Small *Coscinodiscus/Thalassiosira*	SCTHAL	16	1.5 (0.4)	9.2
Small *Coscinodiscus/Thalassiosira* (chain)	SCTCHA	14	6.4 (1.4)	10.0
Small rhaphioidae diatoms	SMLRHA	6	0.4 (0.1)	11.2
Thalassionema nitzschioides	THANIT	16	9.6 (2.1)	41.7

to the diet of *A. clausi* whereas ciliate carbon was secondary only to dinoflagellate carbon in the diet of *A. discaudata* and was > 1 ngC copepod^{-1} h^{-1} on 3 out of 6 occasions.

Ciliate concentrations peaked on the 29 March at which time there was no statistical difference for clearance, electivity or ingestion between dinoflagellates and ciliates for either *A. discaudata* or *T. longicornis* because dinoflagellates were present in much higher numbers. During May, however, when dinoflagellate concentrations were only marginally higher than ciliates the same grazing parameters were higher and significantly different ($p < 0.05$) for *A. discaudata* and *T. longicornis* grazing on ciliates. *Centropages hamatus* displayed the least consistent patterns of grazing upon ciliates. On only 3 occasions out of 9 incubations was ciliate electivity by *C. hamatus* significantly positive ($p < 0.05$) on Apr 11, July 2 and Aug 15. In April and July when *C. hamatus* was first incubated consumption of various prey types followed similar patterns with the highest electivity indices for dinoflagellates ($p < 0.05$) followed by ciliates and then diatoms and finally strongly negative E* indices for microflagellates. These electivity relationships were mirrored in both clearance rates and ingestion.

The proportion of dinoflagellate carbon in the diet of *C. hamatus* increased radically on July 2 when the dinoflagellate group *Peridinales/Gonyaulax* spp. which is a relatively large (7.55×10^4 μm^3) taxon was present at higher concentrations (~8 cells ml^{-1}) than at any other time in the year and therefore consumed in large numbers (~250 cells *C. hamatus*$^{-1}$ d^{-1}). When *A. clausi* and *C. hamatus* were both incubated on the same dates from July 17 to Sept 19 (Table 3.1) *A. clausi* cleared higher volumes of dinoflagellates but consumed less dinoflagellate carbon than *C. hamatus*. This result reflects consumption of fewer dinoflagellates which were of greater volume by *C. hamatus* than those ingested by *A. clausi*.

This interaction also occurred between the same 2 copepods in response to large diatoms on Aug 15 during an almost monospecific bloom of the diatom *Rhizosolenia* spp. *C. hamatus* ingested over 3 times more *Rhizosolenia* spp. Cells (58 diatoms copepod^{-1} h^{-1}) than *A. clausi* and electivity of diatoms was highly significant ($p < 0.01$) and strongly positive as opposed to electivity by *A. clausi* which was weakly negative and not statistically significant ($p = 0.309$). *C. hamatus* also ingested very high numbers of medium *Chaetoceros* spp. (36 diatoms copepod^{-1} h^{-1}) on July 17 whilst *A. clausi* was

unable to clear these chain forming spinous diatoms to any measurable degree. On both of the above dates diatoms were present in very high concentrations (>80 cells ml^{-1}). By contrast when diatoms were at similarly high concentrations but composed of very much smaller cells (*Navicula* sp. A, *Cylindrotheca closterium* and small *Coscinodiscus-Thalassiosira* spp.) there was no evidence of elevated ingestion by *C. hamatus*.

3.4 Discussion

Wide varieties of microplankton prey were present at Port Edgar allowing comparative investigation of the feeding ecology of the copepods studied. Within the annual cycle of phytoplankton during the study period the 4 copepod species we investigated responded to the changes in the types of prey available in a variety of different ways. Despite subtle differences in grazing between the copepods all four copepod species were omnivorous to varying degrees and were ineffective at grazing nano-sized particles.

3.4.1 Ingestion of ciliates

Even the smallest of ciliates in this study were large enough to be captured by *Acartia clausi* which has been recorded consuming cells between 3 and 9µm (Pagano *et al.* 2003). *Acartia clausi* is also able to expand the size range of prey eaten to include larger sized items following a period of exposure to a smaller spectrum of prey up to the point whereby the size of the prey items lead to handling and/or capture difficulties (Nival and Nival 1976). *Acartia discaudata*, however, selected for and cleared ciliates more effectively than *A. clausi* and a consideration of the morphological differences between both congeners may provide some insight. Size based flexibility of selectivity is naturally not limited to *Acartia clausi* (Bartrum 1980, Gaudy *et al.* 1996) or even the genus *Acartia* (Vincent and Hartmann 2001) and particle retention in calanoid copepods is highly influenced by the mesh size formed by intersetular distances of the maxillae (Nival and Nival 1976, Paffenhöfer 1984). The important point to note is that generally the upper size limit of prey consumed is commonly considered to be a product of the overall length of the copepod (Berggreen *et al.* 1988). In the Ria of Vigo, Spain (Alcaraz

1983) the relative size differences of co-occurring adult females *A. discaudata* and *A. clausi* (755 and 900µm respectively) are very similar to the prosome lengths of both species in our study (Table 3.1). Additionally, descriptions of the morphology of the feeding appendages of an *A. discaudata* and an *A. clausi* of the same prosome length from the same region indicate the ability by the former to retain smaller particles due to closer spacing of maxilla setules (M. Alcaraz Personal Communication). Although *A. discaudata* retained smaller ciliates than *A. clausi* on the whole such small protists were only present sporadically in our study. Consequently as *A. clausi* was able to consume larger sized cells and given its greater prosome length the choice of prey sizes was therefore wider overall for *A. clausi*. The narrower choice of food for *A. discaudata* may result in biased impressions of selective feeding when directly comparing both congeners from the same food environment because of physical limitations rather than behavioural response to prey in the surrounding media. Selection of ciliates by *Acartia* spp. is regularly reported (Tiselius 1989, Wiadnyana and Rassoulzadegan 1989, Saiz and Kiorboe 1995, Rollwagen Bollens and Penry 2003) but as far as we are aware this study is the first to report selective ciliate grazing by *A. discaudata*.

Evidence to support the electivity of ciliates by either *Centropages hamatus* or *Temora longicornis* in this study was inconsistent and in accord with several previous studies for which limited preference for ciliates by *Centropages hamatus* (Turner and Graneli 1992, Sommer *et al.* 2005) and *Temora longicornis* (Gasparini *et al.* 2000) has also been observed. Previous investigations of grazing by *C. hamatus* indicate that under food saturating conditions they spend a great deal of time passively suspension feeding compared to ambushing prey (Hwang *et al.* 1993). *Temora longicornis* however captures prey by almost continuous filter feeding (Tiselius and Jonsson 1990) regardless of the amount or composition of food. *Centropages hamatus* has nonetheless occasionally been recorded exhibiting ambush jumping feeding modes (Conley and Turner 1985, Tiselius and Jonsson 1990) but to a lesser degree in terms of time allocation than *Acartia* spp. (Takahashi and Tiselius 2005). Evidence is however contradictory in the literature and *Centropages hamatus* also displayed permanent suspension feeding behaviour under a variety of food concentrations and turbulent regimes. The same study calculated that for

Centropages hamatus the continuous generation of feeding currents is energetically efficient when preying upon phytoplankton (Marrase *et al.* 1990).

Of greater relevance to the interactions of copepods which allocate a great proportion of their time to indiscriminate suspension feeding may be the effect of prey avoidance upon encounter rates (Caparroy and Carlotti 1996). Suspension feeding is turbulently 'noisy' and both *C. hamatus* and *T. longicornis* generate feeding currents more consistently than *Acartia* spp. Consequently ciliates may be more effective at avoiding capture in bottles containing *C. hamatus* and *T. longicornis* (Sommer *et al.* 2005) given the greatly increased pre-attack hydro-mechanical warning given to microplanktonic prey by a suspension feeding copepod.

During March 2001 dinoflagellates greatly exceeded ciliates in abundance yet measures of grazing, i.e. clearance, electivity and ingestion by A. *discaudata* and *T. longicornis* were statistically similar for both prey groups. In May however, ciliates and dinoflagellates were available at similar concentrations but both copepod species had significantly higher measures of grazing upon ciliates than dinoflagellates. It is clear that the greater nutritional value per unit volume of ciliates (Menden-Deuer and Lessard 2000) is offset by the numerical superiority of dinoflagellates in March. But in May 2001 the high ciliate carbon concentration outweighs dinoflagellate carbon when concentrations of both prey are present in similar numbers. The outstanding feature of the ciliate assemblage in May 2001 which will also have significantly increased the contribution of ciliate carbon to copepod diets was the presence of higher concentrations of tinitnnids and *Laboea strobila* (Table 2.4) which are much larger (Table 3.3) than the ciliates present throughout the rest of the year.

Ciliate carbon is often negligible in the diets of small coastal calanoid copepods (Tiselius 1989, Halvorsen et al. 2001, Calbet & Saiz 2005) despite the nutritional importance of ciliates in sustaining growth (Heinle *et al.* 1977, Stoecker and Egloff 1987). Several reasons have been proposed to explain this seemingly paradoxical situation. It has been suggested that ciliate populations regularly comprise particle sizes below that which copepods are able to efficiently graze. Moreover copepods are recorded as experiencing handling difficulty following the capture of larger ciliates (Hodgkin and Rippingale 1971, Nival and Nival 1976). It has also been suggested that a

'threshold' level is required in order to elicit a copepod feeding reaction (Frost 1975, Cowles and Strickler 1983, Jonsson and Tiselius 1990) and also the high swimming velocity and random jumping of ciliates has been put forward as a mechanism which results in low copepod ingestion levels (Broglio *et al.* 2001). In our study ciliates were certainly a negligible component of the copepods' diets for much of the year. This was particularly evident when primary productivity was high but during times of food scarcity copepods were capable of fully exploiting even very low numbers of ciliates out of proportion to other available prey types.

3.4.2 Ingestion of dinoflagellates

Of all the prey groups, dinoflagellates are clearly the most significant prey in carbon ingestion terms to copepods in this study. In only 4 incubations was the proportion of dinoflagellate carbon ingested lower than the proportion available in the standing stock at the start of the experiment and three of these incubations directly correspond to instances of increased ciliate carbon contribution which occurred when dinoflagellates were at their lowest concentrations during the study. Thus, the most notable interaction observed here is that on the majority (> 75%) of occasions carbon ingested from diatoms, flagellates and ciliates is less than at the start of the experiment because dinoflagellate carbon ingested by the copepods has increased from that available at the start. There is clearly evidence of elevated levels of ingestion of dinoflagellate carbon in comparison to diatoms but this result is concomitant with the higher comparative carbon content in dinoflagellates in comparison to diatoms.

The indiscriminate grazing of dinoflagellates and a lack of clear patterns of repeated selection based upon either size or motility by T. *longicornis* reinforces the perspective of this species being a weakly selective grazer both in this study and several previous studies (DeMott, 1988a,b, Tiselius and Jonsson 1990, van Duren and Videler 1995, Koski *et al.* 2005).

3.4.3 Ingestion of flagellates

We observed fluctuations in the concentrations of nanoflagellates during the course of the incubations with wide variation in both the numbers ingested, and clearance rates recorded (e.g. *C. hamatus* ~ -52000 to 73000 cells d^{-1}). Negative clearance is commonly an artefact of the removal of grazing pressure upon nanoflagellates when larger cells such as ciliates and heterotrophic dinoflagellates are removed by copepod grazing which leads to faster growth in grazing bottles compared with controls. We also observed greatly reduced numbers of nanoflagellates in some experiments without corresponding increases in the concentrations of protists. Such unpredictability in nanoflagellate densities clearly reflects shunting of the importance of the various pathways in the microbial web and feeding modes of microplankton at trophic levels below copepods. Both ciliates and heterotrophic dinoflagellates are capable of feeding over more than one trophic level (Fenchel 1980a,b, Drebes and Schnepf 1998) potentially resulting in modified top-down predation effects and also bottom up effects as a consequence of both ciliates and dinoflagellates grazing upon bacteria (Calbet *et al.* 2001). Bacterivory leads to the removal of prey for heterotrophic nanoflagellates and also the reduced availability of dissolved organic nutrients from bacterial cells impacting upon the growth rates of autotrophic nanoflagellates. Essentially however very small cells apart from nanoflagellates were not significantly cleared by any of the 4 copepods species and our analyses were not able to distinguish the complexity of trophic interactions of nano-sized cells and below.

3.4.4 Ingestion of diatoms

On the whole, the copepods incubated here exhibited higher grazing rates upon motile prey compared to diatoms. There were occasions when diatoms of both short, round shape, (medium sized *Chaetoceros* spp.) and elongated forms (*Thalassionema nitzschioides* and *Rhizosolenia* spp.), were grazed at much higher rates than any motile group. These occasions are strictly limited to periods when diatoms were present at much higher concentrations than all the motile prey groups (e.g. *Rhizosolenia* spp. on Aug 15).

Selective grazing upon diatoms is regularly observed when there is a lack of other more nutritious prey (Paffenhöfer and Knowles 1980, Conley and Turner 1985, Teegarden *et al.* 2001, Miralto *et al.* 2003). Diatom selectivity is also commonly proposed as a side-effect of the deliberate avoidance of other prey groups by copepods, an interaction most notable in response to toxic phytoplankton cells (Teegarden *et al.* 1999, Frangópulos *et al.* 2000). We recorded the presence of both toxic diatoms and dinoflagellates throughout the course of the year of the genera *Pseudo-nitzschia* spp., *Alexandrium* spp and *Dinohysis* spp. but their presence was sporadic and in very low numbers hindering our ability to measure avoidance. We did however observed repeated avoidance of *Gyrodinium* spp., amongst which toxic forms are known to occur (da Costa and Fernández 2002, Delgado and Alcarez 1999).

Cell elongation by diatoms appears no defence against grazing, certainly for *Rhizosolenia* spp. for which extremely high ingestion rates were observed. Quite the reverse was the case in relation to *Cylindrotheca closterium* which is also an elongated form but was not heavily ingested. The basis of this outcome may result in the thickness of the cells rather than the maximum length given that Visser and Jonsson (2000) suggest that diatom cells are reoriented along their long axis during entrainment in the flow field of copepods, and such orientation potentially facilitates the escape of cells even when the long axis exceeds the spaces between feeding apparatus. The *Rhizosolenia* spp. is much thicker across the short axis than *C. closterium* indicating that the width of *Rhizosolenia* spp. was larger than the spaces between feeding setae leading to greater capture rates of the latter diatom even when reoriented cells were encountered lengthwise.

Consequently whilst it is clear that cell elongation was no defence against grazing it may be useful to note that rather than trying to understand prey selection as a function of shape in diatoms it may better to focus upon the dimensions of the shortest axis when dealing with elongated cells and not place too much emphasis upon ratios of longest to shortest dimensions.

3.4.5 Concluding remarks

Taken in isolation our observations of the feeding interactions of each copepod species with microbial prey are consistent with much of the literature. The *Acartia* spp. appear to be selective for motile prey with ciliated protists eliciting greater selective responses than dinoflagellates but both congeners are unable to consume prey in similar quantities to the larger species *C. hamatus* and *T. longicornis*. Additionally the *Acartia* spp. appear to be poorly adapted to low food concentrations which resulted in less discriminate feeding prior to and after the seasonal phytoplankton blooms. Although temporally separated for much of the bloom period, when they did co-occur niche separation between the two *Acartia* spp. appears to be prey size based, with *A. discaudata* more efficient at grazing the smaller sized prey. It is clear that for the entire duration of our study the copepods consistently failed to exploit the largest phototrophic resource available given the disparity in diatoms available to that consumed.

Chapter 4 – Effects of *Thalassiosira rotula* on egg production, hatching success and naupliar development of *Acartia discaudata*

4.1 Introduction

As a ‘first line’ of defence planktonic diatoms have evolved thick silicaceous frustules which enclose and protect the cell protoplasm (Hamm *et al.* 2003). Nevertheless, most calanoid copepods are easily able to rupture the frustule in order to facilitate the ingestion of diatoms and allow gut absorption of the intracellular nutrients (Sullivan 1975). It has recently been discovered that in many species of diatom once the frustule is ruptured secondary defence mechanisms are activated which result in the production of allelopathic (chemical inhibitory) compounds. Within seconds of rupture, via metabolic pathways as yet incompletely understood (d'Ippolito *et al.* 2002), cell membrane polyunsatured fatty acids are transformed into oxylipins and unsaturated aldehyde compounds (Pohnert *et al.* 2002, d'Ippolito *et al.* 2004). These compounds have now been shown to have considerable negative effects upon the reproductive success of copepods feeding predominantly upon diatoms (Ban *et al.* 1997, Ianora *et al.* 2003).

Instances of allelopathic defence mechanisms by plants upon grazing herbivores are common in both terrestrial (Robbins *et al.* 1968, Rosahl 1996) and aquatic ecosystems (Fistarol *et al.* 2003, Reynolds 1990). Notably however, allelopathic systems are typically based upon either repelling or poisoning the grazer organism (Shaw *et al.* 1995). Accordingly, such interactions have been recorded on several occasions related to calanoid copepod grazing on marine phytoplankton (Ives 1985, Carlsson *et al*. 1995, Shaw *et al*. 1995, Shaw 1997, Delgado and Alcaraz 1999). Since copepods are the main consumers of diatoms in the sea (d’Ippolito *et al*. 2002, Paffenhöfer 2002), inevitably the copepod diet is often composed of this phytoplankton group. Several researchers have suggested that allelopathic defence based upon embryogenic inhibition is widespread in diatoms (Jüttner and Durst 1997, Pohnert *et al.* 2002). If such deleterious interactions are indeed ubiquitous this would cast serious doubt upon the long held paradigm that

enhanced egg production is stimulated and sustained by diatom blooms resulting in increased populations of copepods (Frost 2005).

The classical view of copepod-diatom interactions has long been one of a tightly coupled response in growth and fecundity linked to the seasonal variations of phytoplankton (Riley 1947, Mann 1993, Ceballos and Ianora 2003). In particular the spring diatom bloom is considered responsible for initiating pulses of mesozooplankton recruitment and production, and sustaining the subsequent reproductive output during summer and autumn in temperate marine foodwebs. It is now becoming apparent that despite this seemingly straightforward relationship, increased fecundity during periods of high primary production does not necessarily translate into enhanced recruitment (Mazzocchi *et al*. 2006). The evidence to support insidious, less direct allelopathic effects which adversely influence calanoid copepods via reproductive failure has now begun to accumulate (see review of Turner *et al.* 2001). Such a convoluted interaction between a grazing animal and its plant food is at present a dynamic plant response unique to marine planktonic ecosystems (Paffenhöfer 2002).

It is now apparent that egg quality and viability is closely related to the recent feeding history of adult females, and that the detrimental effect of diatoms can potentially result in greater than 90% egg mortality (Laabir *et al.* 1995, Ianora 1998, Ceballos and Ianora 2003). Research regarding the influence of diatoms on the embryogenesis of copepod eggs to date has revealed that there are 4 distinct effects depending upon the particular diatom and copepod combination: (1) reduced fecundity and hatching; (2) reduced fecundity with hatching unaffected; (3) reduced hatching with fecundity unaffected; (4) no effect on either (Ban *et al*. 1997).

Furthermore there is evidence to suggest that diatoms may also be in part responsible for sub-lethal effects in copepod nauplii (Williams and Wallace 1975, Poulet *et al.* 1995) with the viability of nauplii being compromised in a variety of ways. Anomalous embryonic development can result in abnormal hatching and consequently poor swimming ability due to congenital morphological defects (Carotenuto 2002) or simply reduced fitness resulting in higher non-predator based mortality (Poulet *et al.* 1995). Our classical understanding of naupliar stages N1 and N2 based upon their non-feeding status is that these early stages are reliant upon maternal yolk reserves (Marshall

and Orr 1955). Thus the survival of early naupliar stages is expected to be strongly affected by the recent feeding history of the spawning female. Accordingly in several laboratory (Frangópulos *et al.* 2000, Lacoste *et al.* 2001, Carotenuto *et al.* 2002) and field (Guisande and Harris 1995, Laabir *et al.* 1995, Pond *et al*. 1996, Ban *et al.* 2000, Ianora *et al.* 2004) studies naupliar survivability has been coupled to the maternal diet.

Historically anecdotal observations of low egg hatching success were generally ascribed to eggs being either dead, unfertilised or diapausal (Marshall and Orr 1952, 1955, Parrish and Wilson 1978, Watras and Haney 1980, Laabir *et al.* 1995) and not examined further. Unsurprisingly, the initial investigations which specifically focussed upon examining low egg hatching success and diatom inhibition in the field were unclear as to the mechanisms underlying poor recruitment. Much effort was expended examining the general effects of how female nutrition (Ianora *et al.* 1992, Jónasdóttir 1994, Guisande and Harris 1995, Jónasdóttir and Kiørboe 1996) and anoxia (Ambler 1985) affected hatchability. The major portion of the current research is now focussed upon the biochemical inhibition of copepod reproductive processes.

In the seminal study of Ianora and Poulet (1993), laboratory cultures of the diatom *Thalassiosira rotula* were found to reduce egg viability in *Temora stylifera* from ~77% for females consuming *in situ* prey to as low as 25% when females were fed pure diatom cultures. This relationship has since been reproduced several times for a variety of different copepod:diatom combinations over the past decade (see Paffenhöfer 2002 for review). At present much of the evidence to support the inhibitory effects of diatoms on reproductive failure is based upon laboratory experiments with convincingly high proportions of eggs, up to 100%, failing to hatch (Poulet *et al.* 1994, 1995, Ianora *et al.* 1995, 1996, 1999, Uye 1996, Starr *et al.* 1999, Lacoste *et al.* 2001). Upon further laboratory investigation however it has been demonstrated that the inhibitory effects of diatoms is alleviated with increased dietary complexity (Kang and Poulet 2000, Lacoste *et al*. 2001, Turner *et al.* 2001, Lee *et al.* 2006). Additionally, through factors which are yet to be investigated, it has been demonstrated that the same species of diatom (e.g. *Thalassiosira rotula*) whilst having a major impact upon egg embryogenesis in one region (Turner *et al*. 2001) are ineffective elsewhere (Pohnert *et al.* 2002). Given that the inhibition of egg hatching and the production of reactive aldehydes is not only highly

variable between species of diatoms but also within geographical sub-populations predictions of copepod growth parameters based upon the presence and concentration of known inhibitory diatoms cannot be given without a detailed analysis.

In addition to such inherent inter- and intraspecific variability of reproductive inhibition by diatoms the application of laboratory results to field populations is further complicated when considering the ability of many calanoid copepods to feed selectively. Copepods are able to discriminate between various types of prey (Kleppel 1993 and references therein) and are also able to avoid prey types which contain toxins (Turriff *et al*. 1995, Engstrom *et al.* 2000). Consequently by feeding selectively and maintaining a nutritionally diverse diet during periods of high diatom concentrations (i.e. spring and autumn blooms) copepods may mitigate negative reproductive effects (Jones and Flynn 2005). As a result of discriminatory behaviour, at low or intermediate diatom concentrations the insidious embryogenic effects upon reproduction may not become apparent as copepods have the potential to selectivity increase ingestion on other prey items (Poulet *et al.* 1995).

With the exception of Starr *et al*. (1999), to date no study has been made to quantitatively determine the concentration, or range thereof, at which diatoms consumed become detrimental to reproductive success. Further, some studies have utilised high concentrations of diatom extracts, which are unrealistically high compared to what may be expected in the field (Laabir *et al.* 1995). Also, many laboratory studies (Poulet *et al.* 1994, 1995, Ianora *et al.* 1995, Miralto *et al.* 1995) have used diatom concentrations several orders of magnitude higher than would normally be expected *in situ*, even under bloom conditions (Grall 1972, Martin-Jézéquel 1983). In reality decreased egg hatching in natural populations rarely reaches 100%, which is in accord with the suggestions of Poulet *et al.* (1994) that inhibition is caused by accumulation of harmful diatom exudates in the oocytes of females. This hypothesis is based upon the assumption that increasing concentrations of diatoms have a proportional effect upon inhibitor accumulation (Lacoste *et al.* 2001). It is, logically, much more difficult in the field to discern if diatom effects are impacting fecundity or hatching rates due to the inability of the investigator to both realistically measure egg production rates *in situ* and, where shipboard incubations are carried out, accurately represent prey consumed over a full diel cycle. As a

consequence such impacts upon copepod reproductive processes have rarely been observed (Nejstgaard *et al.* 1997, Miralto *et al.* 1999, Starr *et al.* 1999, Irigoien *et al.* 2000).

The aim of this work was to examine the various effects of diatoms on female fecundity, the hatching viability of eggs spawned and the nauplii survivorship beyond N2 of a numerically dominant calanoid copepod in the Firth of Forth, *Acartia discaudata*. This species was chosen because it was present in excess of 100 ind. m^{-3} for a protracted period from June until October 2001 (Figure 2.8). During much of this period *A. discaudata* was the single most numerous copepod species present. Additionally, adult females of *A. discaudata* are distinctive, having characteristic paddle shaped uropods allowing rapid identification and isolation. Finally, *A. discaudata* females retain spermatophores for a prolonged period after copulation, indicating likely sexual maturity and breeding status.

In order to examine the extent to, or indeed if, *A. discaudata* females were able to maintain dietary diversity despite an increasingly poor prey choice we exposed females to increasing concentrations of the small diatom *Thalassiosira rotula*. This diatom has been shown to produce inhibitory compounds (Caldwell *et al.* 2002) and negatively affect several calanoid copepod species including representatives of the genus *Acartia* (Ianora *et al.* 1996, Ban *et al.* 1997, Ianora 1998).

We hypothesise that across a broad range of realistic field diatom concentrations it should be possible to detect the number of diatom cells ingested which results in the onset of embryogenic inhibition. We further suggest that the onset of embryogenic effects by *Thalassiosira rotula* may be retarded at lower concentrations when females are presented with alternative food sources, and hence higher concentrations of *Thalassiosira rotula* will be required to produce similar effects in monospecific dietary conditions.

4.2 Methods

4.2.1 Copepod collection

Adult stages of *Acartia discaudata* were collected from Port Edgar, Firth of Forth, Scotland, with surface night-time tows using a hand-towed plankton net (diameter 50 cm, mesh size 200 µm) fitted with a 2 l non-filtering solid cod-end. Animals were transported back to the laboratory within 1 h of capture in a 50 l insulated box. Upon return to the laboratory live copepods were sorted immediately within a controlled temperature room set to within ± 0.5°C of the ambient seawater temperature (13°C). Active undamaged females were transferred to 100 l glass aquaria (x3) filled with seawater from the sampling location, which had been filtered through a 55µm plankton mesh in order to remove any other mesozooplankton. In addition actively swimming CVI males were also sorted and placed in the aquaria in order to allow continued mating to take place.

Collection of females was carried out for 4 consecutive days in order to collect and process sufficient numbers (> 240) for experimentation. In order to prevent food depletion during the collection and acclimation period, males and females were gently collected on a 100µm mesh and transferred to another 100 l aquarium filled with freshly collected 55µm screened seawater. Greater than 300 individuals of each sex were isolated prior to the start of experiments. More females were collected than required in order to ensure sufficient numbers of spermatophore bearing females were present. Females with spermatophores attached were selected and used in all subsequent experiments.

Aquaria were aerated to maintain oxygen levels and keep plankton suspended and all copepods were allowed to acclimate to the 12:12 L:D lighting cycle in the controlled temperature room for at least 3 days. Following acclimation animals were individually measured [prosome length (±15µm)], checked to be in an apparent undamaged state, spermatophore bearing females were then transferred individually into 300ml sterile plastic beakers containing 200ml of experimental food media (see section 4.2.3). Females were transferred to fresh food media daily using a small bore pipette and for each food type 30 replicate females were used. The daily process of transfer never exceeded 1 hour for all females which permitted a closer estimate of egg spawning and

hatching times. Such large numbers of females were used to ensure significant numbers of eggs for examination as *Acartia discaudata* produces very few eggs (typically ~7 – 10 eggs female^{-1} d^{-1}) in the field (unpublished data). At the termination of the experiment (or when females were found dead) prosome lengths were again measured.

4.2.2 Thalassiosira rotula cultures

An isolate (strain no CCAP 1085/1) of the diatom *Thalassiosira rotula* (henceforth referred to as TR) (equivalent spherical diameter [ESD] ca. 6 to 14 µm) from Dunstaffnage Marine Laboratory, Oban, Scotland was grown in batch cultures in *f*2 medium (Guillard 1975) silicon enriched with 1:100 v:v of acidified sodium metasilicate solution. Cultures were prepared using seawater collected from the same site as experimental copepods and were then GF/C filtered and sterilised in an autoclave. Cultures were incubated at 15^{0}C under a constant 24h light regime.

In order to facilitate rapid estimation of diatom cell densities after the egg viability experiment had begun, TR 'test' cultures had previously been prepared and examined several weeks in advance of experimentation. Replicate (x5) test cultures were grown using a 25ml seed culture of exponentially growing TR inoculated into 5L of sterile GF/C filtered nutrient enriched seawater. Test cultures were then sampled daily (3 x 5ml sub samples) without nutrient replenishment for over a month for the purposes of determining the time required to reach the various growth stages i.e. exponential - end of exponential – stationary – decline. Test culture samples were analysed using spectrophotometry, fluorometry (section 2.2.1) and light microscopy and a growth curve calculated which would allow the rapid estimation of TR cell densities based upon spectrophotometry readings. Actual TR cell densities of batch culture media used during experiments were established at a later date by counting >200 cells (Hasle 1978) in random fields of a Sedgewick-Rafter chamber under bright field microscopy at x400 magnification (Table 4.1). During experimental runs time was not sufficient to make all necessary cell counts, hence the algal culture method design described here.

After analysis of test cultures 50 l volumes of seawater prepared under the same conditions as test cultures were inoculated with a 1:200 vol:vol seed of TR 17 days prior

to day 1 of the experiment. After ~17 days of growth TR test cultures were at the end of exponential growth. Additionally, both cell volume and variability of cell densities was

Table 4. 1. Concentrations of *Thalassiosira rotula* cells (x10^3 cells ml^{-1}) in media fed to female copepods. See section 4.2.3 for details of food media.

Food Media	Real Concentration
1	0.015* ± 0.001
2	0.27 ± 0.16
3	0.32 ± 0.09
4	1.86 ± 0.4
5	1.90 ± 0.4
6	10.65 ± 2.05
7	9.82 ± 1.89

* This value is > 0 due to the presence of diatom cells in natural seawater which were morphologically indistinguishable from cultured *Thalassiosira rotula* cells under light microscopy.

relatively stable from day 15 to 18 (Figure 4.1). Identical 50 l experimental culture volumes were seeded during the following days until the start of experiments began. The various 50 l culture volumes were then used to provide synchronised TR cells at the same growth stage on each of the 10 days of planned copepod experimentation because all cultures were harvested 17 days after their initial seed inoculation

4.2.3 Food media preparation

In addition to a control treatment, which consisted of GF/C filtered, autoclaved seawater (FSW) there were 7 different experimental food treatments. Food media codes used throughout this work are given in parentheses.

1) 55µm filtered ambient seawater from the same location as copepods (ASW).

2) FSW + ~100 *Thalassiosira rotula* ml^{-1} (TR100)

3) ASW + ~100 *Thalassiosira rotula* ml^{-1} (ASW100)

4) FSW + ~1000 *Thalassiosira rotula* ml^{-1} (TR1000)

5) ASW + ~1000 *Thalassiosira rotula* ml^{-1} (ASW1000)

6) FSW + ~10 000 *Thalassiosira rotula* ml^{-1} (TR10 000)

7) ASW + ~10 000 *Thalassiosira rotula* ml^{-1} (ASW10 000)

The control treatment was used in order to determine the time taken for females to cease the production of eggs when feeding had stopped. Consequently, because control treatment females continued to produce eggs for 2 days (Figure 4.2a) eggs produced by females on days 1 and 2 in food media treatments 2 to 7 were excluded from further analyses of egg viability and naupliar development. Additionally as a result of the time lag in food grazed leading to eggs spawned day 1 in statistical analyses of EPR corresponds to the beginning of the 3rd day of female feeding upon the various food media types.

To achieve the target concentrations required for treatments 2 to 7 the volume of culture medium was first highly concentrated to 50 000 TR cells ml^{-1}. Cultures were concentrated using reverse filtration (Dodson and Thomas 1978) following the estimation of TR cell concentrations by spectrophotometry. In order to reduce excessive modification of the natural prey concentrations in food treatments which contained both natural prey and TR, 20% of the seawater volume was removed by means

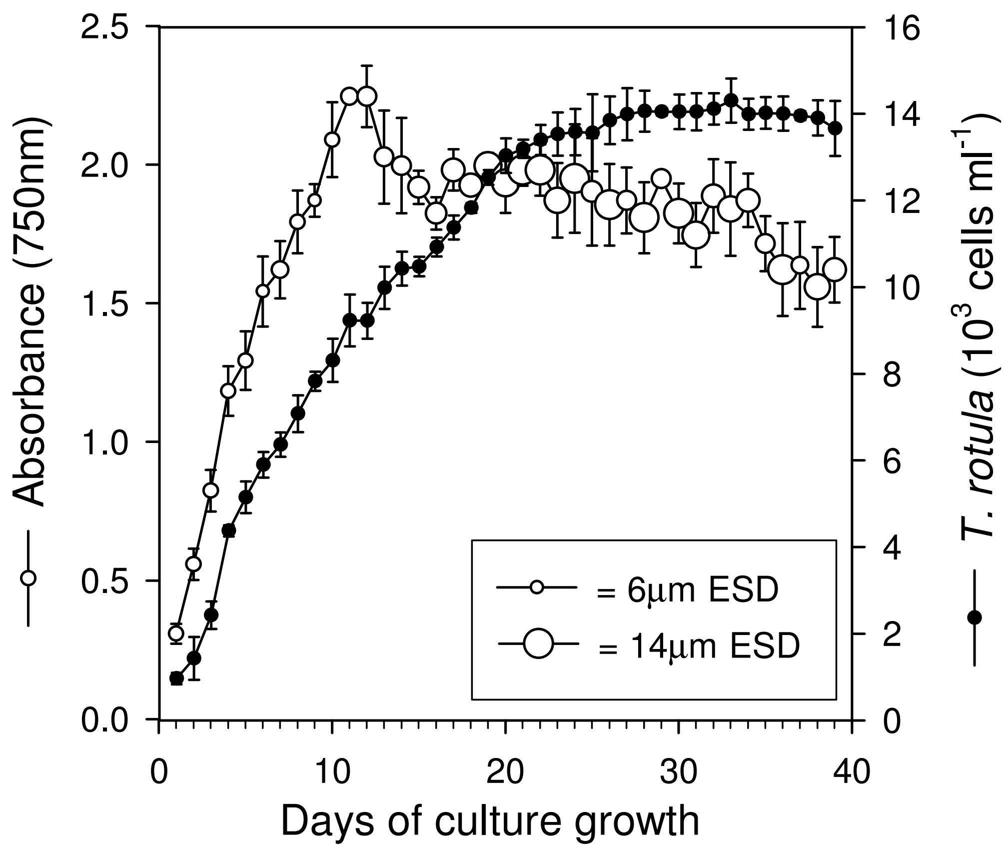


Figure 4. 1. Growth curve of *Thalassiosira rotula* cultures used in experiments. Open symbols (absorbance at 750nm) vary in size related to diatom cell size as per inset legend. Filled circles indicate cell concentrations ml^{-1}.

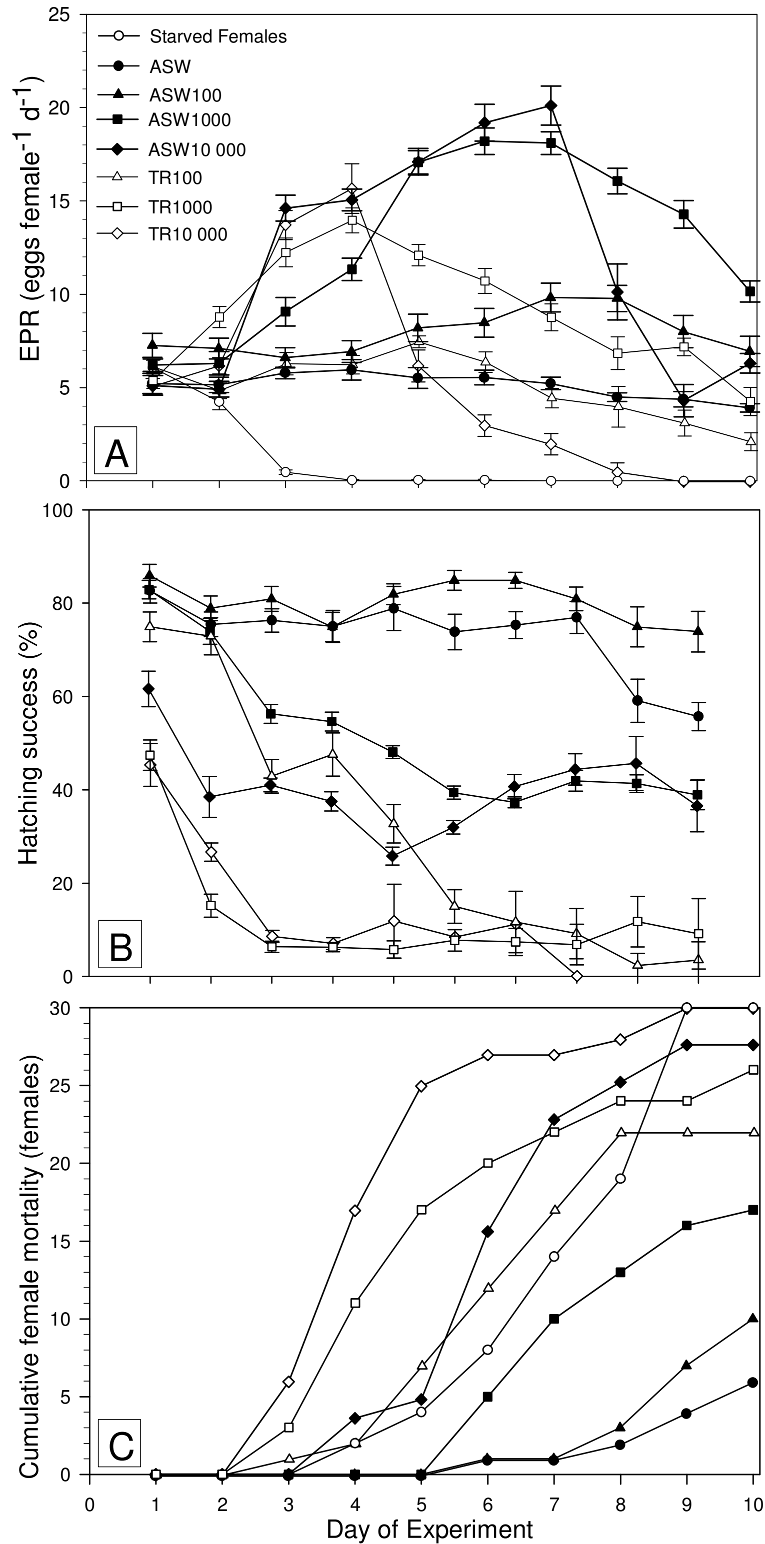


Figure 4. 2. (A) mean EPR, (B) mean egg hatching success (starved females excluded) and (C) mean female mortality of each media type during the progression of the experiment. All error bars indicate ± 1SE.

of reverse filtration from a 21 litre carboy of ASW. This 21 litre volume of concentrated ASW water was then split into 3 aliquots and the 20% volume of water removed was then replaced by a similar volume of (a) 50 000 TR cells ml^{-1} (b) 5 000 TR cells ml^{-1} (c) 500 TR cells ml^{-1} in order to produce food media 3, 5 and 7. A similar process was carried out to produce treatments 2, 4 and 6 by adding the 3 TR concentrations above into a 1:4 dilution of FSW. It should be noted that during the time it took to concentrate the ASW sample grazing by protists in the reduced volume may be modified as a result of higher prey densities. In order to minimise this effect the 3 TR concentrations were prepared ready for immediate addition to the ASW after removal of 20% volume. Additionally 3 large 15cm GF/C filters were used and the whole process of concentration and TR addition took less than 1h.

In order to account for small centric diatoms present in the field collected seawater, and which we cannot be sure are not *Thalassiosira rotula,* the variation in concentrations of all small centric diatoms including TR cells were compared between food media types 1,2 and 3 i.e. ASW, TR100 and ASW100 using t-tests. Tests were conducted upon 30 replicates of the initial incubation media before the addition of copepods.

4.2.4 Egg production, viability and naupliar development

Females were gently pipetted into new containers with fresh media daily. The incubation media from which animals were taken was passed through 5cm discs of 55µm plankton mesh which retained *A. discaudata* eggs whilst allowing food items to pass. The mesh discs were then immersed in sterile Petri dishes containing (55µm screened) natural seawater, egg diameters (±1.49µm) were then measured using a calibrated microscope graticule and eggs/nauplii were then monitored for hatching success and moulting. Egg production was recorded daily over the course of the experiment by counting the eggs spawned by the females. Cannibalism of eggs was accounted for by adding crumpled egg membranes to daily egg production counts. Hatching success was determined as the percentage of nauplii hatched in relation to egg production minus cannibalised eggs. Spawned eggs were initially monitored for hatching at 8 hourly

intervals during the first 4 days after spawning. Eggs were also examined at 8 hourly intervals during the period when females were in the food media in order to account for those eggs which hatched in the first 24h after spawning. Hence, because we assumed a constant rate of egg spawning by females and the initial observation of eggs was 8 hours after females had been placed into incubation vessels, the mean age of eggs was assumed to be 4h upon first examination. After 4 days eggs were monitored daily up to a maximum of 10 days, after this time all unhatched eggs were assumed to be non-viable. At the same time intervals as eggs, nauplii were also staged so that we could determine moulting and stage duration. Counting and staging of nauplii was carried out for a total of 15 days post-spawning. Nauplii were also examined daily for morphological deformity, viability (actively swimming) and mortality. Potential recruitment was calculated as the product of egg production rate (including crumpled egg membrane counts) and the number of NII nauplii without morphological malformations. We continued to monitor for egg hatching until all the nauplii which had hatched from eggs spawned by females on day 10 of the experiment had moulted to NIII or died.

Any adults which had died during the previous day were excluded from measurements of grazing or egg production but we continued to monitor eggs spawned prior to the day of death. Individual egg production for each female in relation to ingestion of TR was plotted for all surviving females each day. Starved females continued to produce eggs for 2 days and on day 3 of starvation the average EPR were < 1 egg female^{-1} d^{-1}. Consequently individual female EPR rates were plotted against the TR cells consumed 3 days prior to egg production. Females were not transferred to newly prepared food media after day 10, eggs which were spawned on days 11 and 12 were also monitored for hatching success and naupliar viability.

4.2.5 Prey Ingestion Rates

Due to the large number of samples generated by incubating 240 females for 10 days, 5 randomly chosen females from each media type were chosen for measurements of ingestion each day during the 10 day feeding period. The start and end concentrations of prey cells in the incubation media of selected females were then calculated as described

in section 2.2.3. Triplicate control samples (without copepods) of 200ml were taken from excess food media left over from the preparation process detailed in Section 4.2.2 and decanted into similar 300ml sterile plastic beakers used for copepods. The start and end concentrations of cells in these control samples were used to calculate growth of protoplankton taxa in the absence of copepods.

The average cell volume of *Thalassiosira rotula* cells during each day of the 10 day feeding experiment was determined by measuring the cell dimensions of 50 individual cells which was then converted to ESD allowing the subsequent estimation of cell C content ($\mu g\ C\ \mu m^{-3}$) as per section 2.2.2. Along with the total *Thalassiosira rotula* carbon consumed by females in each media type over each day of the 10 day feeding period, total carbon ingestion was also calculated for the following taxonomic groupings when incubations included natural seawater - other diatoms, autotrophic dinoflagellates, nanoflagellates and microzooplankton (including ciliates, tintinnids and heterotrophic dinoflagellates).

4.2.6 Statistical analyses

Simple linear regression analysis was used to relate individual female egg production rate (EPR) to the daily ingestion of *Thalassiosira rotula* cells for each of the food media. Regression line slopes and elevations were compared using covariance analysis (ANCOVA). We performed multiple pairwise comparison tests on log transformed EPR vs. TR ingestion data using a Bonferroni correction to identify which pairs of regressions were significantly different.

In order to examine the response of female EPR to cell concentrations we employed non-linear analysis and compared the 2 main categories of food media i.e. pooled food media types 3, 5 and 7 (ASW + TR cells) versus pooled media types 2,4 and 6 (TR cells only in FSW). In describing the potential asymptotic response Ivlev functions using cell ingestion as the independent variable were fitted to each of the 2 groups of pooled food types. The Ivlev function is defined as:

$$EPR = EPR_{max}\ (1-e^{-k \times TR}) \qquad (4.1)$$

The Ivlev curve follows a curvilinear response where EPR_{max} is the asymptotic maximal EPR rate, k is the maximum rate at which EPR approaches this asymptote and TR is the daily ingestion rate of TR cells.

The effect of the various food media upon the stage duration (SD) and median development times (MDT) of eggs and the first 2 naupliar stages was investigated by fitting gamma distribution functions to stage proportion data. The MDT of each stage was defined as the time when 50% of the eggs or nauplii from each food media had passed that stage (Landry 1975, 1983), based upon estimates from stage–frequency data which was converted to stage proportion data i.e. the cumulative decrease in numbers from one stage to another.

Development times of calanoid copepods are commonly assumed to conform closely to a gamma distribution (Klein-Breteler *et al.* 1994, Leandro *et al.* 2006) with a probability density function of the form:

$$f(a-t_0) = \frac{(a-t_0)^{k-1} e^{-b(a-t_0)}}{\Gamma(k) b^k} \tag{4.2}$$

where $\Gamma(k)$ is the gamma function, b is the scale parameter which is > 0, k is the shape parameter which was set at 3 (Read and Ashford 1968) and $(a - t_0)$ is the development time of a particular stage. It is assumed that no animals will enter a certain stage at $a \leq t_0$ and the probability density function is therefore defined to be zero at $a \leq 0$. The mean and variance of each development time are expressed by a/b and a/b^2 respectively (Lee *et al.* 2003).

In order to facilitate the estimation of the two unknown parameters b and the starting time t_0 the gamma distribution equation was reparameterized following Klein-Breteler *et al.* (1994) and the MDT was used in place of the scale parameter b as:

$$b = 2.764/\,(\mathrm{MDT}-t_0) \tag{4.3}$$

For each development stage (j), the negative log-likelihood function (L_j) can be calculated from:

$$L_j = \Sigma\,[(n_t - x_{jt})\ln(1 - \pi_{jt}) + x_{jt}\ln(\pi_{jt})] \tag{4.4}$$

Where n_t is the total number of animals observed at time t and x_{jt} equals the number of animals remaining at stage j at the same time. Using the statistical software package MINITAB, t_0 and MDT were estimated by simplifying L_j using the maximum likelihood method (modified Newton-Raphson algorithm). Initially the stage duration of each stage up to N3 was then calculated as the difference in MDT between 2 successive stages. Using the stage-specific derived parameters t_0 and MDT gamma distribution curves were then fitted to stage proportion data using SIGMAPLOT. Finally, *post hoc* Anderson-Darling goodness-of-fit tests were conducted in order to evaluate how closely the estimated parameters, t_0 and MDT, and the gamma distributions specific to each copepod life stage and food media type followed the experimentally observed cumulative proportion data.

Variations in stage development among the food media were tested with one-way analysis of variance (ANOVA) and multiple comparison tests conducted when ANOVA indicated significant difference among the treatments. Separate multiple comparison tests were carried out upon each of the 3 life stages studied (egg, N1 and N2). Following arcsine transformation the cumulative stage proportion data for each of the 6 modified food media types (types 2 to 7) vs. the ASW food media were compared statistically using Dunnett's tests. For the purposes of the test patterns of stage development in eggs and nauplii produced from females fed only *in situ* prey items (ASW) was considered the 'natural condition' from which to examine any modification of development times.

Statistical regression models were used to describe hatching success (HS, %) as a function of TR cell ingestion. Regression models were initially approximated across the entire range of TR cells consumed for each of the 2 main types of food media i.e. ASW + TR cells (media types 3, 5 and 7) versus TR cells in FSW (media types 2,4 and 6) from all media types using a LOWESS (locally weighted regression) smoothing function (Cleveland 1979). The LOWESS derived relationship was then used as a basis to fit a regression curve iteratively using the Marquardt-Levenberg least squares algorithm (SIGMAPLOT 7).

The numerical response of hatching success in relation to TR cell ingestion was defined by fitting the following logistic equation to hatching data;

$$HS = \frac{HS_{max}}{1 + \left(\frac{TR}{TR_0}\right)^b} \qquad (4.5)$$

Where HS = hatching success (%); HS_{max} = maximum hatching success (%); TR = the number of *Thalassiosira rotula* cells ingested (cells female^{-1} d^{-1}); TR_0 = the number of *Thalassiosira rotula* cells ingested at which HS equals half HS_{max} and b = the number of TR cells ingested at which HS declines at its maximum rate. Initially several predictive equations of negative exponential and sigmoidal form were fitted to the data and the most appropriate selected based on its biological appropriateness and best fit to the data; i.e. where regressions were significant, $p < 0.05$, and had the highest adjusted R^2 value. Differences in hatching rates of eggs between ASW+TR vs. TR only media were compared statistically with a Kruskal–Wallis One Way Analysis of Variance on Ranks.

4.3 Results

4.3.1 Food media and grazing rates

The pattern of TR growth in cultures was characterised by the rapid increase in the concentration of individual cells of ~6µm equivalent spherical diameter (ESD) in size for the first 10 days of growth (Figure 4.1). On the 11th day after inoculation the maximum cells densities of 14.4 x 10^3 cells ml^{-1} were achieved. After the initial increase in TR cells there was then a small decrease in cell densities from days 10 to day 15 whilst the concentration of chl *a* continued to increase. The increase in chl *a* was due to increased size of individual TR cells which grew in size until day 17 at which time, and for several following days, TR cell volumes stabilised between 13 to 14 µm ESD. On day 20 of the culture development, during the period of maximum cell size, the rate of increase of chl *a* declined, indicating that the culture was at the end of exponential growth. On day 22 both the individual cell size and cell densities began to decrease moderately. Additionally chl *a* concentration became asymptotic and gradually began to decline on the 30th day after inoculation.

Student's t-tests on TR cell concentrations indicated highly significant differences ($p < 0.001$, $df = 29$) between food medium 1 and both of the food media to which 100 TR cells ml^{-1} had been added (media types 2 and 3). No statistical difference was found between food media 2 and 3 ($p > 0.5$, $df = 29$). These results indicate that the concentrations of small centric diatoms morphologically similar to TR already present in ambient seawater did not significantly alter our estimate of TR cell densities.

The composition of naturally occurring prey items was similar in terms of diversity and composition to September 2001, which was the same month in 2003 when the present study was carried out, (Section 2.3.2 and Table 2.3) with the notable exception that high densities of the dinoflagellate *Ceratium lineatum* and the chain forming diatom *Rhizosolenia* spp. were absent in this study. Within each food media type there was no interaction effect between the day of the experiment and carbon ingestion (Figure 4.3) for natural prey items (2-way ANOVA, $F = 0.412$, $p = 0.372$). Since carbon ingestion did not appear to be a function of experimental duration, then 'experimental day' was excluded from further analyses, thus allowing estimates of carbon ingestion from all days to be pooled. In contrast, interaction between TR carbon ingestion and experimental day was significant (2-way ANOVA, $F = 10.01$, $p < 0.001$) for media types ASW1000, ASW10 000 and TR1000. Further analysis indicated that from day 7 onwards grazing upon TR cells was significantly lower than from days 1 to 6 (Tukey pairwise test, days 1 to 6 $p > 0.5$, days 7 to 10 $p < 0.01$).

Two-way ANOVA highlighted the obvious dominance of autotrophic dinoflagellate carbon followed by carbon from other diatoms ($F = 684.145$, $p < 0.001$) in female diets in each of the ASW food media types. Additionally the 2-way ANOVA also indicated a statistically significant interaction between each of these media types and protoplankton prey groups ($F = 7.571$, $p < 0.001$). Regardless of the availability of natural prey items, carbon ingestion of TR biomass increased in line with TR cell availability i.e. ASW10 000, TR10 000 > ASW1000, TR1000 > ASW100, TR100. Increased *Thalassiosira rotula* carbon in female diets corresponded to a reduction in the carbon ingested from other prey types. The statistical interaction between prey types and ASW media types (ASW100, ASW1000 and ASW10 000) suggests that reductions in other

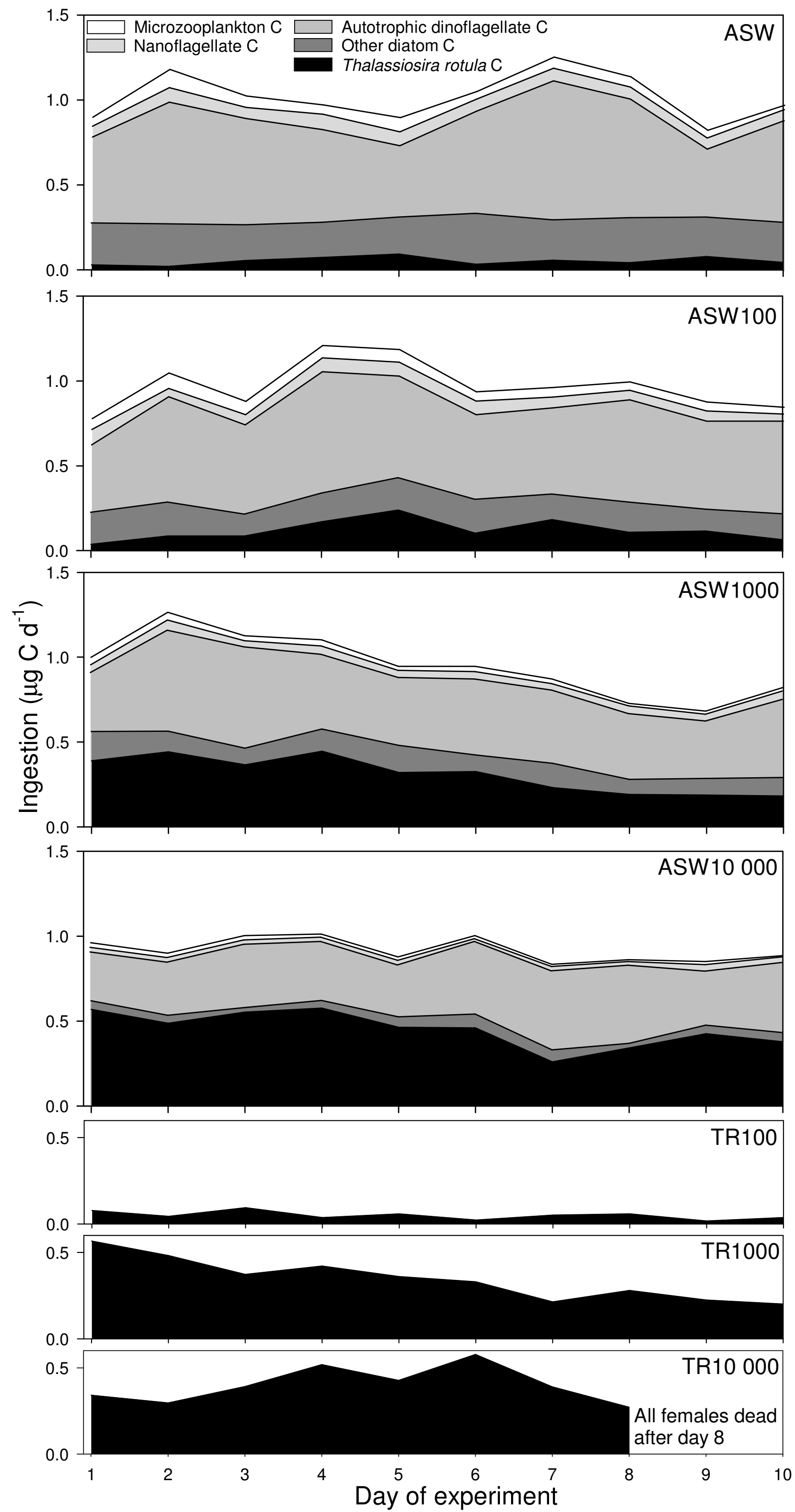


Figure 4. 3. Cumulative area plots of daily carbon ingestion from the major protoplankton taxa available in each food media. All charts of TR carbon ingestion are presented with the same y-axis scaling regardless of chart size.

prey carbon declines in proportion to the amount in the diet i.e. dinoflagellate carbon decreases to a greater degree than carbon from other diatoms and also that microzooplankton and nanoflagellate carbon declines much less than that of other diatom carbon. The media vs. prey type interaction was significant between all pairwise combinations of the 4 ASW media types with the exception of ASW vs. ASW100 (Tukey test $p = 0.05$) indicating that the small
increase in TR carbon ingestion by females in ASW100 media did not impact upon the ingestion of carbon from any other prey types.

4.3.2 Egg production rates

Linear regression analysis of female EPR vs. TR ingestion (Figure 4.4) for each food media type in isolation demonstrated highly significant relationships by females fed upon diets which contained low (100 cells ml^{-1}) and medium (1000 cells ml^{-1}) TR concentrations (Table 4.2). Pairwise comparisons (Bonferroni tests, $p < 0.05$) between ASW100 and TR100 (Table 4.3) showed that regression lines were parallel and intercepts were not significantly different between diets (ANCOVA, $F = 0.37$, $p = 0.917$). Additionally, the linear regression describing the effect of TR cell ingestion in food media ASW was not significantly different from either ASW100 or TR100 (ANCOVA, $p = 0.869$, $p = 0.841$ respectively). In the direct comparison of the mean daily EPR of females from ASW and ASW100 females from the latter food media produced significantly (t-test $p < 0.001$) more eggs on a daily basis.

In the cases of the food media types ASW10 000 and TR10 000 a linear fit was not significant for EPR vs. TR ingestion. Also correlation coefficients were extremely low for both of these food media types (Table 4.2), and pairwise comparisons resulted in no significant difference between both media for either slopes or intercepts (Table 4.3).

The cell ingestion response of *Acartia discaudata* to increasing concentrations of food types which contained only TR cells compared with food types which included natural prey types showed different patterns (Figure 4.5a) when investigated with non-linear functions. A rectangular hyperbolic (Holling type 2) response (Holling 1965) was observed for both of these food categories across the whole range of TR cell concentrations employed when fitted with the Ivlev (1955) equation (Table 4.4).

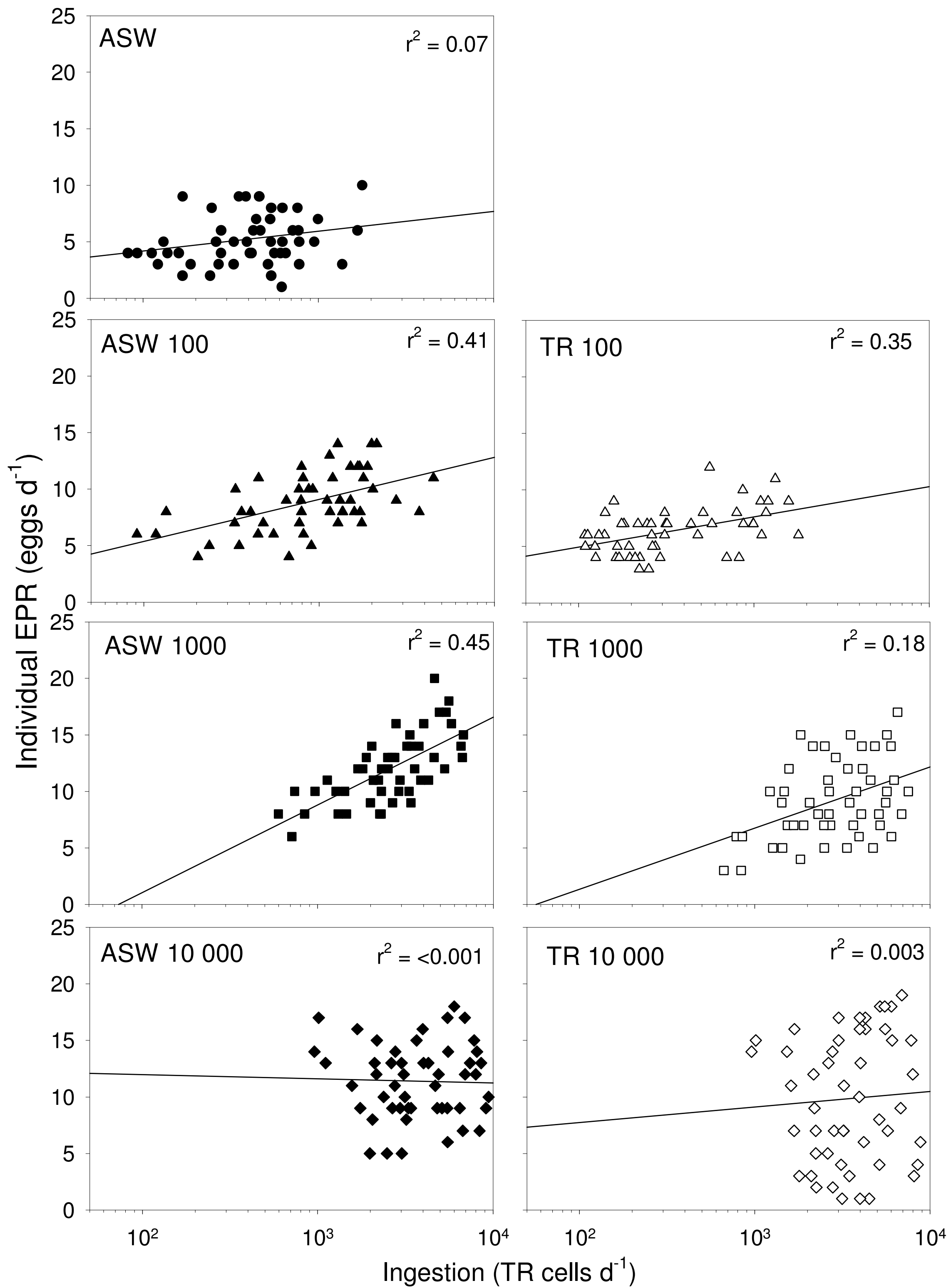


Figure 4. 4. Individual daily female EPR vs TR cells consumed. Solid lines indicate linear regressions. Linear regression parameters from each media type are detailed in table 4.2.

Table 4. 2. Parameters of the linear regression equation EPR = *a* + *b* ($\log_{10}$TR ingestion) used to describe the relationships between individual female egg production (eggs d^{-1}) and TR cell ingestion (TR cells d^{-1}) in each food medium (Figure 4.4). Values in bold type indicate a significant relationship ($P < 0.05$), n = 50 for all media types.

Food media	Intercept (*a*)	Slope (*b*)	Correlation coefficient	*P*-value
ASW	0.683	1.751	0.268	0.060
ASW100	-1.970	3.690	0.529	**<0.001**
TR100	-0.350	2.657	0.467	**<0.001**
ASW1000	-14.108	7.635	0.671	**<0.001**
TR1000	-9.586	5.435	0.418	**0.002**
ASW10 000	12.518	0.322	0.024	0.864
TR10 000	4.662	1.465	0.061	0.674

Table 4. 3. Results of multiple ANCOVA and Bonferroni simultaneous pairwise tests of TR ingestion ($\log_{10}$ cells ingested d^{-1}) versus individual female egg production (eggs d^{-1}) between all food media types. All values are *p*-values from comparisons of slopes (S) and intercepts (I). Values in bold type indicate a significant difference (P<0.05). For key to food media codes see Section 4.2.3.

	ASW100		TR100		ASW1000		TR1000		ASW10 000		TR10 000	
	S	I	S	I	S	I	S	I	S	I	S	I
ASW	0.515	0.869	0.506	0.841	0.245	**0.025**	0.143	0.211	0.248	**0.047**	0.358	**0.048**
ASW100	-		0.917	0.542	0.078	**<0.001**	0.057	**<0.001**	**0.015**	**0.012**	0.407	0.076
TR100	-		-		**0.016**	**<0.001**	0.194	**<0.001**	**0.023**	**0.007**	0.449	0.238
ASW1000	-		-		-		0.761	**<0.001**	**0.002**	**0.003**	0.363	**<0.001**
TR1000	-		-		-		-		**0.007**	**0.004**	0.212	0.242
ASW10 000	-		-		-		-		-		0.305	0.163

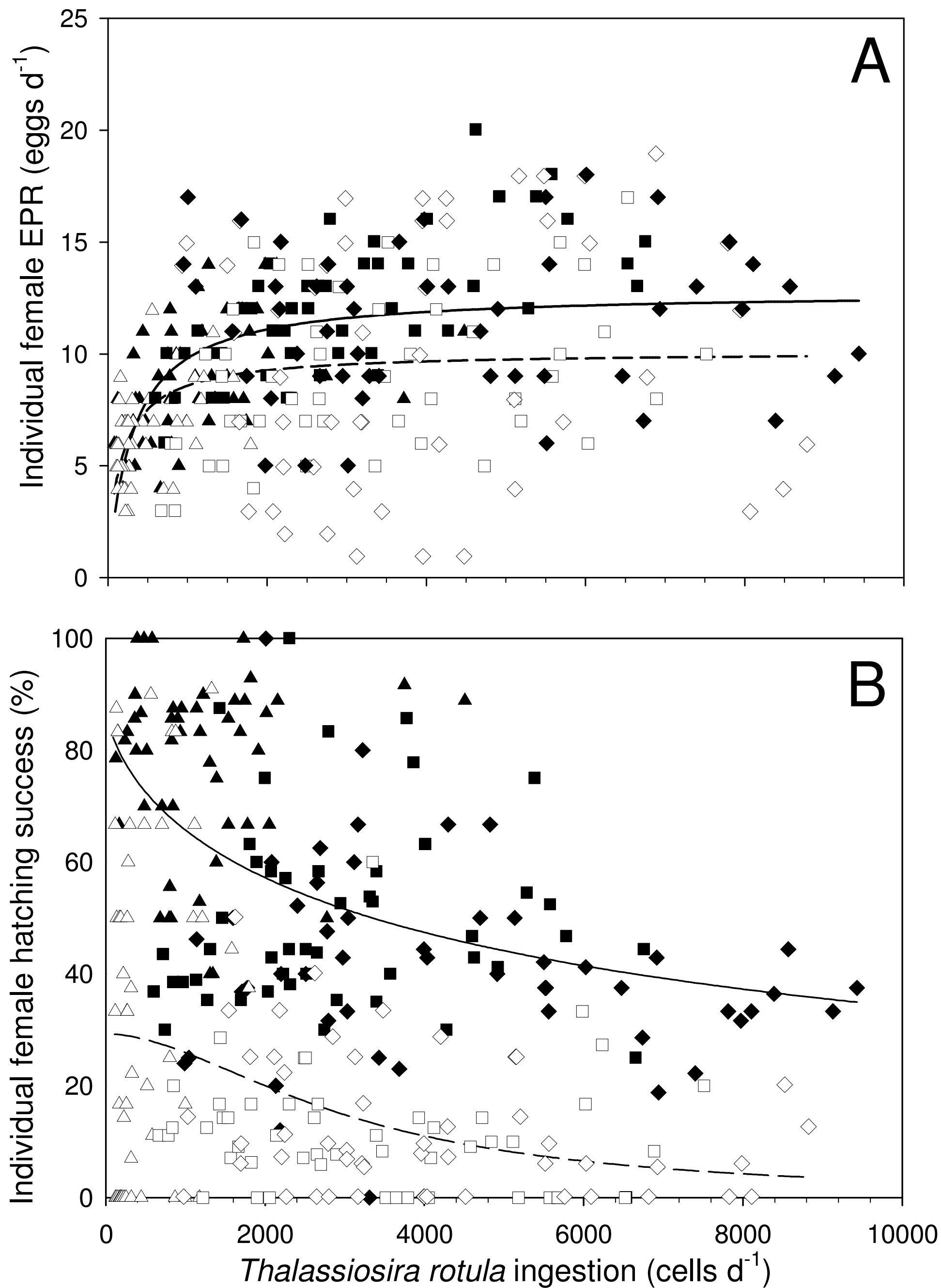


Figure 4. 5. Non-linear regressions between (A) individual female egg production (eggs d^{-1}) and *Thalassiosira rotula* cell ingestion (cells d^{-1}); data were fitted to Ivlev curves of the form detailed in equation 4.1: and (B) individual female egg hatching success and *Thalassiosira rotula* cell ingestion; antisigmoidal curves were fitted to the data iteratively using equation 4.5. Solid lines represent regression curves of females fed upon the 3 types of food media composed of ASW which had been enhanced with increasing concentrations of TR cells. The dashed lines represent the regression curves of females fed upon TR cells alone. Symbols used are as per figure 4.2.

Table 4. 4. Parameters of the Ivlev equation (equation 4.1) used to describe the relationships between individual female egg production and TR cell ingestion of food media containing ASW and food media containing only TR cells (see Figure 4.5a).

Food Source	EPR_{max} eggs d^{-1}	k	Adj R^2	Saturation conc. TR cells	p
ASW +TR treatments	12.78	10.64	0.24	304.19	<0.001
TR only treatments	10.10	8.97	0.15	182.07	<0.001

Although noisy, the relationships were statistically significant. The mixed diets which included microplankton prey from ambient seawater displayed the highest ingestion rate (I_{max}), had the highest rate at which saturation of EPR was reached (*k*-value) and the highest food saturation density. The data suggest egg production rate at which EPR increases began to decrease at TR concentrations above ~400 TR cells d^{-1} regardless of the availability of *in situ* prey items (Figure 4.5a). EPR ranged from 4 to 20 for media types composed of ASW enhanced with TR cells and 1 to 19 for media types which contained only TR with a mean value of 11 and 9 eggs $female^{-1}$ d^{-1} for ambient+TR and TR cultures respectively.

Egg diameters from all media types combined ranged from 76.05 to 91.26 μm. There was no statistically significant difference in diameters (Table 4.5) between all the media types (1-way ANOVA, $p > 0.05$), there was however a marginal increase in mean egg diameters across the spectrum of media types ASW > ASW100 > ASW1000 > ASW10 000. No such increase in size occurred across the range of TR only media. Cannibalism of spawned eggs, with the exception of females incubated in filtered seawater on day 2 of the experiment (56%), remained low regardless of media type, and mean cannibalism was always less than 7% of total eggs produced.

4.3.3 Stage duration

The cumulative proportion data for eggs of *A. discaudata* females fed upon ASW, ASW100, ASW10 000 and TR100 against time was well fitted to the gamma function (Figure 4.6). Cumulative development of stage 1 nauplii from all 4 of the ASW media types also followed gamma distributions. Additionally all nauplii stage 2 cumulative data conformed closely to a gamma function regardless of food media. Of the eggs which hatched, the majority (>90%) did so within 48h after spawning (Table 4.6). The estimated development times were shorter for eggs and nauplii from females fed on the TR1000 and TR10 00 food media types (Table 4.7) than food media 1 (ASW) but these results should be viewed with caution given the poor fit to a gamma distribution. The high mortality as a result of eggs failing to hatch in TR1000 and TR10 000 resulted in a large proportion of viable eggs hatching within a short space of time and very few

Table 4. 5. Summary of experimental data on mean female prosome length (µm), female body weight (µg C female^{-1}) and egg diameter (µm) in each food media type. Data presented for the initial prosome length of all females (n = 210) and the final prosome length after either 10 days incubation or immediately after the female was found dead (n = 30 per media type). Numbers in parentheses are ±1SE.

	Initial	ASW	ASW100	ASW1000	ASW10 000	TR100	TR1000	TR10 000
Prosome length	671 (2.6)	666 (2.5)	673 (3.1)	675 (2.6)	669 (3.1)	668 (2.7)	663 (2.8)	669 (2.5)
Weight	1.29 (0.01)	1.27 (0.01)	1.30 (0.01)	1.31 (0.01)	1.29 (0.01)	1.28 (0.01)	1.26 (0.01)	1.28 (0.01)
Egg diameter	83.6 (0.43)	84.4 (0.61)	84.6 (0.60)	85.3 (0.65)	85.01 (0.54)	84.16 (0.55)	83.76 (0.56)	83.71 (0.59)

For key to food media codes see Section 4.2.3.

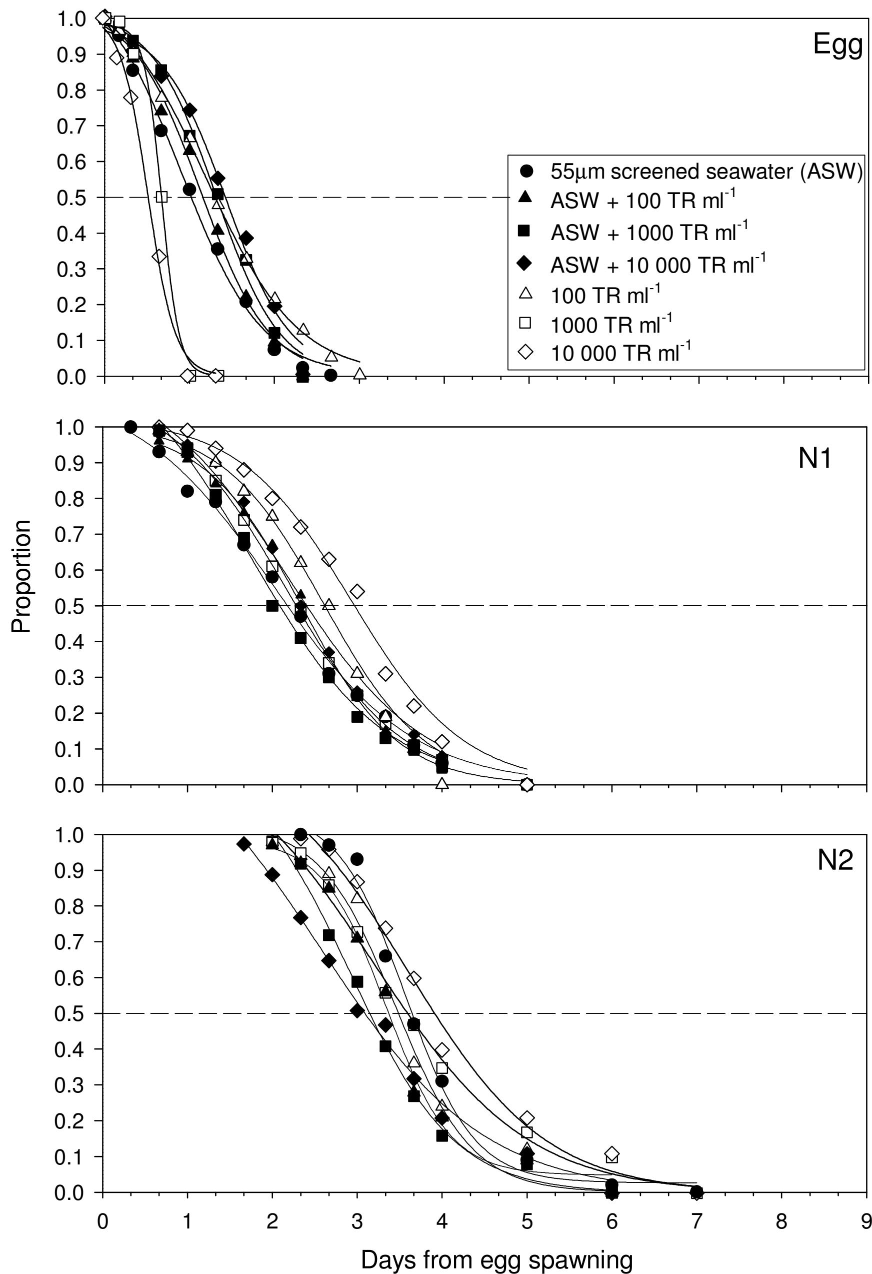


Figure 4. 6. Egg and post-embryonic development time in *Acartia discaudata* reared at 15°C and fed upon different food media. Cumulative proportions of all animals that have not yet passed a given stage were plotted against time. Gamma distributions were fitted to all experimental data. The final minimum proportion remaining within a given stage was recorded when, after 3 successive observations, the proportion remained unchanged. The gamma distributions are indicated by solid lines and each food media type is represented by a different symbol. The intersection between the dashed line (50%) and the fitted Gamma function defines the mean development time (MDT) for the particular stage.

Table 4. 6. The mean stage duration (days), Anderson-Darling goodness-of-fit statistic *p*-value and median development times (MDT, hours from spawning) of eggs and the first two naupliar stages of *Acartia discaudata*. For key to food media codes see Section 4.2.3. * Indicates that the experimentally measured cumulative proportion data used to derive stage duration and MDT are adequately described by the log–likelihood estimated gamma distribution parameters. † Stage durations of N1 nauplii were calculated using egg MDT estimated from the maximum likelihood method and where egg hatching success was <50% of viable eggs spawned. ‡ Indicates a modified gamma distribution with unrepresentative low MDTs as a result of extremely high mortality of eggs. Mortality (*m*) = the proportion of eggs or nauplii which either failed to hatch or moult to the next stage.

Food Media	Stage	Stage duration	*p*	MDT	*m* (%)
ASW	Egg	1.16	0.406*	27.8	20
	N1	1.14	>0.25*	55.3	5
	N2	1.47	0.352*	90.5	0
ASW100	Egg	1.20	>0.25*	28.8	19
	N1	1.11	>0.25*	55.5	3
	N2	1.15	>0.25*	83.1	2
ASW1000	Egg	1.31	>0.223	31.4	51
	N1	0.77†	>0.25*	49.9	10
	N2	1.08	0.277*	75.9	5
ASW10 000	Egg	1.42	>0.25*	34.1	57
	N1	0.99†	>0.25*	57.9	17
	N2	0.68	>0.25*	74.3	4
TR100	Egg	1.31	>0.25*	31.5	72
	N1	0.97†	0.241	54.8	11
	N2	1.20	>0.25*	83.6	3
TR1000	Egg	0.66‡	0.045	16.0‡	89
	N1	1.92†	>0.213	62.1	7
	N2	0.98	>0.25*	85.7	7
TR10 000	Egg	0.51‡	0.177	12.3‡	90
	N1	2.46†	0.158	71.4	5
	N2	0.95	>0.25*	94.2	6

Table 4. 7. Results from 1-way ANOVAs comparing stage development times and of Dunnett's multiple pairwise comparison tests conducted upon arcsine transformed cumulative stage proportion data for each copepod life stage. Media types 2 to 7 (see section 4.2.3) were compared to media type 1 (ASW) which contained only natural prey items. Numerical *p* values in bold type indicate where significant differences between food media exist. *Development time should be viewed with caution due to extremely low hatching success (<10%)

Life Stage	Stage development time vs. ASW	df	*F*	*p*
Egg	TR10 000* ,< TR1000* < ASW = ASW100 < ASW1000 < TR100 < ASW10 000	6, 29	9.1	**<0.001**
N1	ASW = (ASW100,ASW1000,TR1000) < ASW10 000 < TR100 < TR10 000	6, 67	7.3	**<0.001**
N2	ASW1000 < ASW100 < ASW = (TR1000, ASW10 000, TR100, TR10 000)	6, 50	5.8	**<0.001**
	shorter ← **DT** → **longer**			

hatching later which caused highly positively skewed data. In contrast, the development times of eggs gradually increased with increasing concentrations of diatoms in the 4 ASW media types (ASW, ASW100, ASW1000 and ASW10 000). Development times of N1 from food media types ASW1000 and ASW10 000 were faster than any other media type, but the extremely long development times of N1 nauplii from TR1000 and TR10000 media should again be viewed with caution as a consequence of the skewed egg gamma distributions. The rate of development of N2 nauplii increased in line with increasing diatom concentrations in all of the artificially modified media types, the slowest N2 development time was that of nauplii from females kept in natural seawater (ASW). Additionally there was no statistical difference (1-way ANOVA) in the development times of N2 from media types TR100, TR1000, TR10 000 and ASW10 000 which were all similar in duration regardless of media type and took longer to develop than those N2 from ASW (Table 4.7). Similarly comparisons of development rate and stage duration of non-feeding N1 nauplii tended to be shorter than both eggs and N2 nauplii which were being maintained in natural seawater (Table 4.6).

4.3.4 Hatching success

Mean hatching success ranged from 0% to 92% and averaged 43% overall.
From day 4 to day 10 there was no interaction effect with media type between experimental duration (i.e. day of experiment) and hatching success (2 way ANOVA $p < 0.001$, Tukey multiple comparison tests $p > 0.05$). Days 1 and 2 were also similar in patterns of hatching success ($p = 0.104$) but day 3 appeared to be the transition day during the experiment when hatching success began to be affected by food media type (Figure 4.2b) it being statistically similar to both days 2 ($p = 0.572$) and 4 ($p = 0.974$) yet both of these days were different from one another ($p = 0.040$).

Logistic (3 parameter) sigmoidal curves (Figure 4.5b) produced the closest statistically significant fit to hatching success data. The diets which included ASW in combination with TR cells produced significantly higher (Kruskal-Wallis 1-way ANOVA, $H = 81.72$, $p = <0.001$) rates of hatching success than TR only diets (Table 4.8). The non-linear regression models indicated that the maximum hatching rate of eggs

Table 4. 8. Parameters of the logistic regression equation (equation 4.5) used to describe the relationships between individual female egg hatching success and TR cell ingestion of food media containing ASW and food media containing only TR cells in figure 4.5b.

Food Source	HS_{max} (%)	TR_0 (Cells)	b	Adj R^2	*p*
ASW +TR treatments	87.97	5071	0.67	0.23	<0.001
TR only treatments	30.50	1823	501	0.14	0.0158

(H_{max}) from females fed ASW + TR diets was more than double that of eggs spawned by females fed only TR cells.

Investigation of food types confirmed the non-linear regression analysis. Hatching success decreased progressively with increasing TR cell ingestion for ASW+TR media types (Figure 4.7). Hatching success of eggs from TR only media was also detrimental in a dose dependant fashion but even the highest median and average hatching rates (TR100) were reduced in comparison to ASW10 000. The hatching data of the TR100 food media was not statistically different from ASW, ASW100 and ASW1000. These comparisons should be viewed skeptically as an artifact of the highly variable hatching data of TR100 eggs (Table 4.9). The distribution of hatching data for the 2 media types ASW and ASW100 are very similar (Bonferroni test, $p > 0.05$). In this instance however the increased median and mean hatching success of the ASW100 data suggests a positive effect imparted by the moderate increase in TR cell concentration of the ASW100 media in comparison to the ASW media.

Females produced fewer eggs over 10 days when ingesting 0.1 x 10^3 to 1 x 10^3 TR cells d^{-1} and a maximum number of eggs when ingesting 2 x 10^3 to 10 x 10^3 cells d^{-1} (Figure 4.4). However, Figure 4.7 shows that the percentage of nonviable eggs was not constant, but rather increased from 22 % of the total egg production at the low concentration (ASW100) to a maximum of 59 % and 88 % at the highest concentrations (ASW10 000 AND TR10 000 respectively). A consequence of this is that the production of morphologically normal, actively swimming nauplii increased very little at concentrations greater than 1 x 10^3 cells ml^{-1}.

4.3.5 Female mortality

In food media types which contained only TR cells, the rates of cumulative female mortality increased as diatom cell numbers increased, TR10 000 > TR1000 > TR100 (Figure 4.2c). Additionally, mortality rates in the 3 feeding media where TR alone was included exceeded the mortality rates of the starved females. Also, female mortality increased as diatom cell numbers increased in the ASW + TR food media types. When

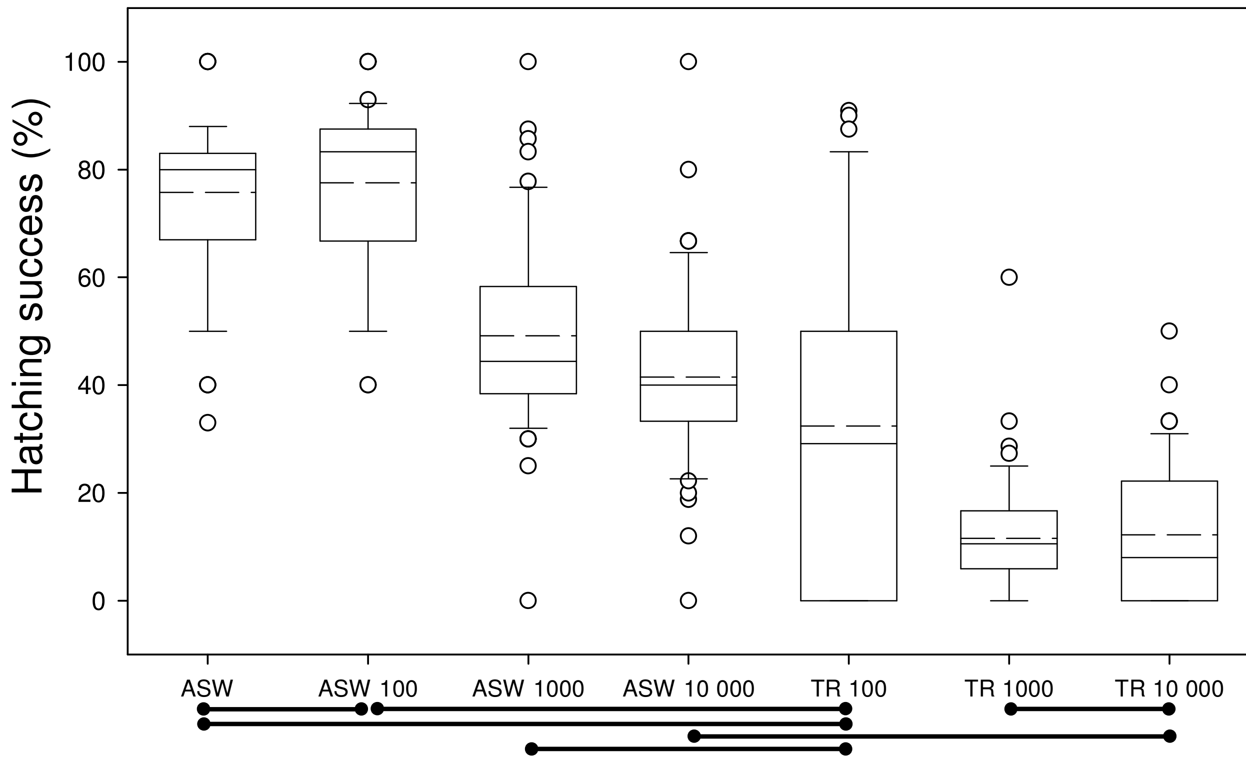


Figure 4. 7. Overall values of hatching success of eggs spawned by individual females fed on each food media type summarised as box plots. Bottom boundary of the box: 25th percentile; solid line within the box: median; dashed line within the box: mean; upper boundary: 75th percentile; whiskers above and below: 90th and 10th percentiles. In addition, outlier (O) values are displayed; Food media codes are as detailed in section 4.2.3. Black bars below the plot indicate pairs of food media types which were not significantly different (Bonferroni tests, $p > 0.05$).

Table 4. 9. Results of multiple ANCOVA and Bonferroni simultaneous pairwise tests of TR ingestion ($\log_{10}$ cells ingested d^{-1}) versus individual egg hatching success (%) between all food media types. All values are *p*-values from comparisons of slopes (S) and intercepts (I). Values in bold type indicate a significant difference (P<0.05). For key to food media codes see Section 4.2.3.

	ASW100		TR100		ASW1000		TR1000		ASW10 000		TR10 000	
	S	I	S	I	S	I	S	I	S	I	S	I
ASW	0.515	0.869	0.506	0.841	0.245	**0.025**	0.143	0.211	0.248	**0.047**	0.358	**0.048**
ASW100	-		0.917	0.542	0.078	**<0.001**	0.057	**<0.001**	**0.015**	**0.012**	0.407	0.076
TR100	-		-		**0.016**	**<0.001**	0.194	**<0.001**	**0.023**	**0.007**	0.449	0.238
ASW1000	-		-		-		0.761	**<0.001**	**0.002**	**0.003**	0.363	**<0.001**
TR1000	-		-		-		-		**0.007**	**0.004**	0.212	0.242
ASW10 000	-		-		-		-		-		0.305	0.163

compared with the corresponding TR only media group however, i.e. TR10 000 vs. ASW10 000, TR1000 vs. ASW1000 and TR100 vs. ASW100 rates of female mortality (i.e. females still alive on a day-to-day basis) were lower in the ASW media type for each of these pairings. In addition to the effect of food media type upon female mortality the effects of captivity duration also became apparent after day 5 of the feeding experiment. Prior to this time female mortality was zero in ASW, ASW100 and ASW1000. During the period between days 5 and 6 the first females were found dead in these food types and, in addition, female mortality increased sharply in the ASW10 000 food media. Finally, no statistical differences in female prosome length (Table 4.5) were apparent either between the initial body lengths measured before females were exposed to the various media types or between media types (1-way ANOVA, $p > 0.05$).

4.4 Discussion

4.4.1 Egg production and hatching success

Our results indicate that egg production by adult female *Acartia discaudata* in the Firth of Forth during our study was food limited and that rates of recruitment were enhanced by moderate increases (ASW100) in the diatom content of the diet of sexually mature fertilised females. Egg production of females fed extremely high diatom concentrations exceeded that of females ingesting moderate concentrations of diatoms but recruitment was compromised because very few eggs hatched when females ingested several ($2 \times 10^3 - 9 \times 10^3$ TR cells d^{-1}) 1000 diatoms d^{-1}. An understanding of the mechanisms which cause hatching failure may shed some light on the seemingly beneficial effect of moderate concentrations of *Thalassiosira rotula* on hatching success.

When female *Calanus helgolandicus* were fed between 1×10^3 to 5×10^3 *Thalassiosira rotula* ml^{-1} Poulet *et al.* (1994) found that the mechanisms of hatching failure was a result of prevention of cell division prior to fusion of the male and female pronuclei or during mitosis. Hatching success in that study fell from ~80% to levels between 15-30% after 4 days, this response is very similar to females fed *Thalassiosira rotula* in the present study in which hatching success was initially ~75% and declined to > 20% after 6 days. Much emphasis has been placed upon the accumulation of anti-

mitotic agents in the gonad tissue whereby the rate of accumulation is dependent upon the density of the diatom (Chaudron *et al.* 1996, Caldwell *et al.* 2004, Kang and Poulet 2000). Increased hatching failure during the course of our experiment could indicate that the rate of toxin accumulation in female oocyte tissue may become asymptotic. We suggest that oocyte tissues may have become saturated with anti-mitotic agents because patterns of decline in hatching success for both the TR1000 and TR10 000 food media were identical and reached minimum values in 3 days, whilst hatching success took 6 days to reach minimum values for the TR100 media. Hence, regardless of the initial diatom cell concentration, when copepods were only fed *Thalassiosira rotula* eventually the outcome was similarly low rates of hatching success. The greater degree of overall variability in hatching success we observe for the TR100 (Figure 4.7) compared to all other media types is as a consequence of the more gradual decline in hatchability. Consequently, it may be hypothesized that in the TR100 media the tissues of female oviducts either took longer i.e. 6 days, to saturate with anti-mitotic agents or alternatively did not accumulate toxins to such a degree as to inhibit hatching but females were instead able to sustain the production of greater proportions of healthy eggs due to the mobilization of female body tissues.

Several researchers have suggested that at sub-saturating food concentrations the egg production response of copepods is proportional to the range of experimental diatom concentrations used (Starr *et al.* 1999, Kang and Poulet 2000, Turner *et al.* 2001). We investigated the possibility of such a response in this study at low diatom concentrations using a simple linear analysis to examine egg production in the TR100 and ASW100 media purely as a function of the number of *Thalassiosira rotula* cells consumed on a daily basis by individual females. These analyses found no difference between individual female egg production rates and *Thalassiosira rotula* cell ingestion when comparing the ASW100 and TR100 (Figure 4.4, Table 4.3). This result was obtained when investigating individual copepods for the entire duration of the experiment. When we examined average daily egg production and hatching success rates the ASW100 and TR100 media diverge widely (Figure 4.2). Hatching success and egg production of females consuming ASW100 media improved in comparison to the ASW media but hatching success of TR100 females never improved upon ASW copepods, additionally,

egg production whilst initially higher than for ASW rapidly declined after day 5. Each of the two analyses (average vs. individual egg production rates) are based upon slightly different but fundamentally important parameters. Egg production rate analyses of individual females purely as a function of TR cells consumed are crucial in order to directly compare the media types which included both ambient prey types and cultured TR cells. Essentially however the variability of individual egg production data at lower TR cell concentrations fails to highlight the beneficial effect of the ASW100 media compared to ASW and equally the reduced mean daily egg production of females fed upon TR100 in comparison to ASW females (Figure 4.2).

In order to understand the differences in hatching success between the various TR only media and media which contained both natural prey and *Thalassiosira rotula,* it is necessary to view the presence of diatom derived compounds which may inhibit reproduction in female tissue as a dynamic rather than cumulative process. Kang and Poulet (2000) suggest that at diatom concentrations in the range 10^2 – 10^3 cells ml^{-1} embryogenic inhibition is masked by ambient food diversity. Additionally only at diatom cell concentrations of 1 x 10^4 ml^{-1} and when no alternative prey were present did Kang and Poulet (2000) observe clear detrimental effects upon both fecundity and hatching.

Unfortunately in their study Kang and Poulet (2000) do not present data related to prey ingestion of females which were producing eggs from solely *in situ* food. They do suggest that there may be a threshold concentration below their experimental food levels at which nutrient deficiency of females rather than biochemical inhibition is the main cause of egg mortality. From our results we suggest that such threshold concentrations may be much lower than many studies anticipate. When females were presented with modest increases in TR in the diet the accumulation of inhibitory compounds may be counteracted where there is a balance between a sustained increase in fecundity versus diatom ingestion, as was the case for females feeding upon the ASW100 media.

The optimal diet theory (Lehman 1976) proposes that when food becomes less limiting copepods may be increasingly able to acquire a more balanced, nutritionally beneficial diet through selective feeding. This theory and the corresponding toxin-dilution hypothesis advocate that under conditions which limit ingestion rates non-selective feeding behaviour prevails due to increased starvation (Uye and Takamatsu

1990) and when food limitation is alleviated or removed satiation permits discriminatory behaviour. The improvements in both egg production and hatching success of females consuming the ASW100 media compared with ASW females in our study (Figure 4.2 a,b) indicates that when food limitation was lessened females had the potential to upgrade the nutritional status of eggs produced

Previous studies have established that *Thalassiosira rotula* is a polyunsaturated aldehyde (PUA) producing species (Wichard *et al.* 2005, Poulet *et al*. 2006) and there is clear evidence which supports the presence of anti-mitotic agents in the *Thalassiosira rotula* strain we used given the extremely low hatching success of eggs from TR only media. Also when copepods are offered a limited amount of TR in the presence of diverse natural prey, then as long as TR concentrations do not overwhelm a female's ability to consume other prey the accumulation of harmful concentrations of PUAs may not occur. It may also be that females transferred anti-mitotic agents into reproductive growth in amounts too low to prevent embryogenesis or are able to partially metabolise such molecules (Chaudron *et al.* 1996).

An alternative to the toxin dilution hypothesis may be one of protist mediated dietary enrichment. It has been suggested that microzooplankton which have been feeding upon phytoplankton, provide a more beneficial food source for copepods because of the production of highly unsaturated fatty acids (HUFA) or sterols (Klein-Breteler *et al.*1999, Dam and Lopes 2003). Such fatty acids and sterols are essential for crustacean growth (Stoecker and Capuzzo 1990). Consequently, although diatoms were already available in the natural seawater for microzooplankton to prey upon, elevated amounts of fatty acids and sterols may have been produced as a result of our addition of *Thalassiosira rotula* in the ASW100 media and transferred to copepods and their eggs.

In reality it is difficult to measure maternal diet *in situ* and even where fecal pellet production fluctuates in accord with a natural diatom bloom, care must be taken when implying that diatoms may be a major cause of natural mortality. Consequently, observations of coupled increases in copepod excretion rates and egg production during a diatom bloom do not necessarily indicate a diet detrimental to rates of population recruitment when resultant hatchability is low. The most plausible explanation in circumstances where the diatom-copepod paradox (Ban *et al.* 1997) is not a causal factor

is that large numbers of unripe females are present which have not yet mated with males and are therefore producing unfertilised eggs. On the other hand there are several field based observations in which higher egg production during blooms has positively linked diatoms to poor recruitment (Ianora *et al.* 2004, Leising *et al.* 2005, Pierson *et al.* 2005).

Regardless of the food media type, females produced eggs which failed to hatch, at least during the period in which the eggs were observed. It may be the case that a significant proportion of these seemingly non-viable eggs were diapause or senescent to some degree. We made no attempt to quantify *A. discaudata* females *in situ* in order to follow field populations during the time period we carried out this study. Nevertheless data from 2001 during the same period in the annual cycle (late August – early September) indicated that populations of *A. discaudata* were declining (Figure 2.9) at a time when annual temperatures had peaked and were decreasing but when food was still abundant (Figure 2.4c). Consequently it is plausible that physiological responses by experimental females to ambient physical conditions may already have initiated to some degree the production of diapause eggs. If in fact this was the case in this study then it may be appropriate to consider the average proportion of eggs failing to hatch from females fed purely natural seawater (ASW) as a correction factor applicable to the other food media types. Unfortunately due to time and storage limitations we were unable to monitor eggs from females for a protracted period of time i.e. several months, whilst attempting to simulate *in situ* seawater temperatures over winter to confirm this hypothesis.

4.4.2 Nauplii mortality and stage durations

Accurate field estimates of 'natural mortality' of the youngest life stages of *Acartia* spp. and indeed calanoid copepods generally are rare. Despite the importance of early mortality in recruitment of pelagic copepods (e.g. Kiørboe and Nielsen 1994, Peterson and Kimmerer 1994, Hirst et al. 2007) identification of the causes of this mortality can be very difficult (Carotenuto *et al.* 2006). Where attempts have been made to examine the impact of maternal diet on mortality, laboratory (Turner *et al.* 2001) and field studies (Miralto *et al.* 2003) have highlighted the decoupling of important

parameters normally applied to estimates of natural mortality. In the latter of these studies, which examined *Acartia clausi*, neither fecal pellet production, egg production rates or hatching viability were linked.

Patterns of growth and stage development in the genus *Acartia* have been extensively described by several previous studies and are considered to be generally isochronal (Landry 1975, Miller *et al.* 1977, Sekiguchi *et al.* 1980, Uye 1980, Klein-Breteler et al. 1982, Berggreen et al. 1988, Klein-Breteler and Schogt 1994). In these previous studies the N1 stage was found to be much shorter than the N2. That pattern is also evident in this work when females were fed ASW, ASW100 and ASW1000 but as we either increased the concentrations of diatoms beyond 10^3 cells ml^{-1} or removed natural prey altogether this relationship broke down and N1 stage durations became longer than the N2. The first feeding stage in the genus *Acartia* is typically the N2 stage (Landry1983) and in this present study all N2 were maintained in the same 55µm pre-screened ambient seawater. Regardless of the nauplii feeding environment, development times of the stage 2 nauplii decreased progressively when hatched from eggs to which females had been exposed increasing concentrations of *Thalassiosira rotula* cells i.e. ASW > ASW100 > ASW1000 > ASW10 000. Whilst going some way towards explaining why N1 stage durations were longer than N2 for the ASW10 000 media type, the trend in N2 duration highlights a distinctly inverse relationship to egg stage durations, in that egg development times increased whilst N2 duration decreased. It is noteworthy that when examining the ASW10 000 stage duration data we observe both the longest egg development time and correspondingly the shortest N2 stage duration.

Whilst it is clearly recognised that temperature has the greatest impact upon both egg size and egg development times (McLaren 1965, Huntley and López 1992, Hirst and Sheader 1997) several researchers have investigated the effect of food quantity and quality upon egg development. The length of egg stage durations in the present study (Table 4.8) is similar to the variations observed in egg diameters. As the average diameter of eggs from females fed ASW, ASW100, ASW1000 and ASW10 000 food media increased slightly, the egg stage durations also gradually increased (Table 4.5). For the copepod species *Calanus helgolandicus*, Guisande and Harris (1995) observed that increased egg size had a positive effect upon hatching success as a consequence of

the increased organic content of eggs. On the other hand Jónasdóttir (1994) found no indication that egg size had any impact upon hatching success when studying *Acartia tonsa* and *A. hudsonica*, but did observe that the quality of food fed to females adversely affected egg development times. Our study is at variance with both of these observations because egg hatching success of the ASW1000 and ASW10 000 food types was markedly lower than the other 2 media types (ASW and ASW100). On the other hand, the enhanced naupliar development observed by Guisande and Harris (1995) concurs with our observations.

The relationships between egg size, egg development times and N2 stage durations are instructive. Intuitively, the concept of the first feeding stage in copepods is one in which it is considered the nauplii become autonomous of maternal effects and are then directly influenced by the prevailing physical, chemical and biological environment (Landry 1983, Ianora 1998). As a consequence of this separation of maternal effects in the ongoing development of N2 nauplii it is counter-intuitive to apportion more rapid development times of stage 2 nauplii to females which were fed more food and produced larger eggs. It should be noted that the comparisons of stage development of the N2 under discussion here are of course based upon only those animals which had hatched and survived the N1 stage successfully and then go on to moult to N3. Therefore it may be that for these animals which survived and developed apparently healthily, increased egg size may in fact reflect increased nutrient content. But more importantly the nutrient ratio was not imbalanced due to the excessive transfer of diatom material from females to eggs.

The effects of inhibitory diatom metabolites upon the reproductive biology of females within the context of the ongoing development of first feeding nauplii and beyond are poorly investigated. In only a single study conducted upon freshwater cladocera is there evidence that offspring from females fed better quality food had faster growth rates (Brett 1993). Undoubtedly, an important mechanism which can lead to improved survival is the reduction of developmental duration and, therefore, the reduction of cumulative mortality by predation in the plankton. Nowhere in the current literature focussed upon the various detrimental effects of diatoms on the population dynamics, reproductive biology and physiology of calanoid could we locate research

which looked beyond the fate of non-feeding stages of nauplii. Descriptions of hatching success and naupliar deformation are numerous but why despite experimental manipulation and exposure of females to extremely high concentrations of diatoms do some nauplii still survive beyond first-feeding stages at all? We suggest that further investigations are necessary in order to clarify the impact of the maternal diet on the ongoing development of early copepod larval stages, particularly the first feeding stage.

Chapter 5 – General Discussion

5.1 Summary

The annual cycle of calanoid copepods and the main food sources upon which they prey were sampled on an almost weekly basis from November 2000 to November 2001. Seasonal patterns of the abundance of microbial species i.e. diatoms, microflagellates, nanoflagellates, dinoflagellates, aloricate ciliates and tintinnids generally followed the broad pattern of seasonality typical of temperate estuaries. Nonetheless, some interesting results related to more detailed species-specific interactions were observed. In addition, the temporal succession and periodicity of the dominant calanoid copepod species showed roughly similar dynamics in terms of abundance and stage composition to studies of more southerly British estuaries (Castro-Longoria and Williams 1999, Chinnery and Williams 2003).

It is clear from this study that during the course of a year the lower estuary of the Firth of Forth sustains an abundant and at times diverse microbial food web. Despite general trends in protoplankton composition which are comparable to many European estuaries, there are differences, both subtle and obvious, in the microbial dynamics observed in our study. As a consequence of the well mixed nature of the Forth (Webb and Metcalfe 1987) and the tidally induced dispersion of phytoplankton the bicyclical blooms we observed were composed of a broad variety of species Even during the dense autumn bloom of *Rhizosolenia* spp. a complex of phototrophic species was present. Additionally the changes in the sizes and types of phytoplankton between the blooms which resulted in the inverse relationships between cell abundance and biomass (see section 2.4.1) appears to indicate a progressive development of conditions suitable to the growth of large phytoplankton cells later in the year.

Investigations of grazing rates by copepods highlighted distinct differences in responses by calanoid species to prey types, indicating potential resource partitioning. Principally however despite their importance in nutritional terms all copepod species were poor at utilising ciliates as a food source. Consequently incubations indicated that

top-down predation of ciliates by copepods was not sufficient to explain the failure of ciliate communities to respond to nanoplankton blooms. Only during May 2001 were ciliates selectively grazed upon by copepods (section 3.4.1) yet for much of the duration of the study ciliate concentrations remained limited and never exceeded 20 cells ml^{-1} (Figure 2.3). Despite limited predation upon ciliates during the entire period from July until the end of September (Figure 3.6) microbial biomass was dominated by nanoflagellates < 10µm in size (Figure 2.4c) which are considered ideal prey for protists. Ciliate populations however remained low during this entire period resulting in unregulated growth of nanoplankton leading to the nutrient depletion observed (Figure 2.4d). The reasons behind the failure of ciliates to regulate nanoplankton growth are unclear, the focus of the incubations we conducted being copepod grazing. In the field factors such as turbidity and turbulence (which can result in the masking or elimination of the hydromechanical signals produced by prey) may be posited as anti-predation mechanisms. In the bottle incubations however whilst turbidity may still potentially inhibit predation mechanisms turbulence will be greatly reduced and the reasons for unregulated nanoflagellate growth are unclear.

Various estimates of carbon flow through ciliate communities have indicated the importance of ciliates as the major consumers of algal and bacterial production through high grazing impact in several regions (Burkill 1982, Capriulo and Carpenter 1983, Verity 1987, Calbet and Saiz 2005). Regardless of the clearly diverse populations of heterotrophic pelagic ciliates in the Firth of Forth (Table 2.3) no single ciliate species or association thereof were able to utilize nanoplankton efficiently enough to allow a coupled growth response which would result in top down regulation of nanoplankton. Our data demonstrate a decoupling of nanoflagellate growth and microzooplankton herbivory suggesting that phytogenic carbon from the smallest primary producers may have been lost to higher trophic levels.

As a consequence of limited ciliate biomass and abundance, the behavioural response by copepods to ciliates tends to be unpredictable. Estimates of carbon consumption of ciliate biomass by copepods, electivity indices and ingestion rate measurements show that ciliate biomass was never the major dietary component of any of the calanoid species which we studied even when ciliate carbon was comparable to other

prey groups. Analysis of grazing rates and carbon ingestion, despite the high C content in ciliates, indicates that the more ubiquitous microzooplankton groups, heterotrophic dinoflagellates, formed the major portion of food in copepod diets in the Firth of Forth. Significantly therefore, given the poor retention and capture efficiency of calanoid copepods in relation to particles < 5µm, it is probable that a substantial proportion of the primary production from nanoautotrophs which ciliates could potentially consume fails to reach higher trophic levels.

In contrast to nanoplankton the various groups of microphytoplankton rarely underwent nutrient depletion (Figure 2.4d). Although the seasonal cycle typical of north temperate estuaries occur in the Firth of Forth the few predominant calanoid copepods present at any point in time were effective in removing diatoms and autotrophic dinoflagellates within days of a bloom. During the immediate approach to the spring bloom nutrient depletion occurred and analysis shows that the size spectra of primary producers at this time were at the lower end of the size spectrum efficiently retained by copepods i.e. cells < 10µm (Figure 2.4). Again for a very brief period in July 2001 carbon:chl *a* ratios indicated some nutrient depletion in the larger phytoplankton (Figure 2.4d). The peak in autotrophic dinoflagellate abundance which occurred during this period was able to grow unregulated as a consequence of the elimination of >99% of all mesozooplankton from the water column in June as a result of predation by ctenophores. Significantly however this event in June not only led to a complete restructuring of the metazoan community for the remainder of the study but profound modifications in the microphytoplankton occurred. In August there was a significant decrease in the concentrations of all other phytoplankton species and a virtually monospecific *Rhizosolenia* spp. bloom occurred. The decline in much of the previous phytoplankton biomass apart from *Rhizosolenia* spp. may possibly have been as a result of a continuation of grazing responses by the dominant mesozooplankton species following a bloom of *Ceratium lineatum* in July (see Section 3.3.3). This dinoflagellate bloom peaked suddenly in mid July and was rapidly grazed down and formed a significant component in the diets of the *Acartia clausi* and *Centropages hamatus* which we studied at this time (Figure 3.5). Immediately after the *Ceratium lineatum* bloom in mid August 2001 we recorded the highest grazing rate upon diatoms by *A. clausi* at any time and the

highest grazing rate upon diatoms of any copepod species by *Centropages hamatus* during the entire study. Concurrently, size fractioned chl *a* analysis indicated that diatoms 10 – 30μm in size were being cleared rather than the much larger *Rhizosolenia* spp. (Figure 2.4b).

The extremely high abundances and numerical dominance of phytoplankton species, particularly the diatom bloom in August 2001, prompted us to investigate the potential causes behind the precipitous decline in *Acartia discaudata* populations despite the availability of large quantities of food. The decline in numbers of this copepod may have been as a result of evolutionary physiological responses to the approach of winter in the region such as the production of diapause eggs, absence of remating (Parrish and Wilson 1978) or inefficiency of fertilisation (Ianora *et al*. 1989). Additionally, of course, coupled with the start of a period of diapause egg production by females there will be the diminution of existing animals. Several factors may be in effect in this respect, i.e. advection (both tidal and fluvial based), predation mortality and natural mortality. Equally, there may have been cause to question whether the available food type was inhibiting reproductive processes to some degree. We chose to examine the reproductive responses of *Acartia discaudata* when exposed to extremely high concentrations of diatoms, as described in the copepod-diatom paradox (Ban *et al.*1997). In concentrations much lower than those to which *A. discaudata* was exposed *in situ* in August 2001 (>80 diatoms cells ml^{-1}), egg hatching was inhibited by the diatom *Thalassiosira rotula* in the laboratory. These results indicate that *A. discaudata* is susceptible to embryogenic inhibition and that this is one mechanism by which recruitment could be suppressed resulting in the rapid decline of field populations. In addition to poor egg hatching the increased rates of mortality of females when fed very high concentrations of diatoms bears further examination. Although very little is known concerning the effects of copepod diets upon longevity several previous studies have also observed profound reductions in adult female stage duration when fed experimental diets. Reduction in female longevity caused by *Thalassiosira rotula* has also been demonstrated in the copepod *Temora stylifera* when egg producing females were fed unialgal cultures (Ianora and Poulet 1993). To confound our understanding of this aspect of female physiology the longevity of *Acartia clausi* females was unaffected when the same diatom was used yet

again (Ianora *et al.* 1996). As a consequence of the ambiguity and lack of consensus as to the inhibitory effects of even a single species of diatom and the as yet unclear biochemical and physiological effects upon both eggs and females, hypotheses as to the causes of reduced female longevity must at present remain conjectural.

5.2 Further research

In relation to calanoid copepods, investigations of longer term interannual variability in population structures would have been desirable. Although long-term databases of plankton populations in the Firth of Forth are maintained by SEPA (The Scottish Environmental Protection Agency) the data collected is generally of a baseline descriptive content with little ecological investigation of growth processes or population dynamics. In addition to more prolonged investigation, the absence in our study of data pertaining to the development and stage structure of nauplii is a clear shortcoming. In particular the high mortality rates of the earliest naupliiar stages i.e. N1 and N2 can often be linked directly to the population structures of the much later copepodite stages (Liang *et al.* 1996, Sell *et al.* 2001, Di Capua and Mazzochi 2004, Eiane and Ohman 2004). Therefore the differentiation of stage and species specific nauplii populations, certainly of the several dominant calanoid copepods studied here, would contribute significantly to our understanding of mesozooplankton production in this important European estuary.

Generally both cyclopoid and (epi-)benthic copepods were ubiquitous in samples for much of the annual study period (Figure 2.8a). Benthic copepod genera of the taxa Ectinosomatidae spp. were occasionally present in numbers comparable to calanoid copepod taxa in the water column. The presence of the benthic copepods in such numbers may impact indirectly upon pelagic genera by sustaining larger populations of holoplanktonic predators than calanoid and cyclopoid genera alone would sustain. Consequently, investigations of predators from higher trophic levels such as chateognaths and fish larvae along with detailed seasonal analysis of meroplanktonic larvae would help to clarify the fate of much of the mesozooplankton biomass.

The investigations of selective grazing conducted here, whilst certainly a step forward in our understanding of planktonic trophic interactions in the Firth of Forth, were

limited to a few dominant calanoid genera. The abilities of the less numerous species, or species which appeared only briefly, to compete, survive and reproduce would undoubtedly assist in the greater understanding of energy transfer and microbial web exploitation by the wider variety of mesozooplankton. In addition of course it would have also been desirable to investigate grazing responses to prey availability by the whole range of copepodite stages rather than simply the adult females analysed in this study.

Finally, whilst still a rapidly developing field of endeavour into our understanding of copepod population dynamics and growth processes, many aspects of the effect of diet upon reproduction by calanoid copepods in the Firth of Forth are worthy of further study. In particular two important aspects which remain relatively unexplored and consequently unexplained in the global literature which were observed in this study are those of the influence diatoms have upon egg size and female longevity. The significance of both factors in our understanding of the demographics and survival of field populations of calanoid copepods is of clear import. Fortunately, specific molecular and biochemical interactions upon the physiology of these important mesozooplankton are clarified with increasing frequency at the current time and may assist in a reappraisal of our data in relation to egg size and female longevity in the near future.

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