POPULATION DYNAMICS AND SEASONAL LIPID CYCLES IN HYPERBENTHIC CRUSTACEANS FROM A COLD-OCEAN ENVIRONMENT (CONCEPTION BAY, NEWFOUNDLAND): MYSIS MIXTA (MYSIDACEA) AND ACANTHOSTEPHEIA MALMGRENI (AMPHIPODA)

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POPULATION DYNAMICS AND SEASONAL LIPID CYCLES IN HYPERBENTHIC CRUSTACEANS FROM A COLD-OCEAN ENVIRONMENT (CONCEPTION BAY, NEWFOUNDLAND): *MYSIS MIXTA* (MYSIDACEA) AND *ACANTHOSTEPHELA MALMGRENI* (AMPHIPODA)

by

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Abstract

Life cycles and seasonal changes in density, biomass, growth, secondary production and lipids in *Mysis mixta* and *Acanthostepheia malmgreni* from a 240-m deep site in Conception Bay, Newfoundland, were studied from October 1998 to November 2000. The primary aim was to provide life-history and ecological information on populations inhabiting the hyperbenthos of Conception Bay, and to relate the seasonal lipid and fatty acid dynamics to each species' life cycle and to the occurrence and quality of the annual phytoplankton bloom.

Life spans and reproductive cycles were remarkably similar in the two species, with the release of free-living juveniles from mature females occurring in April and May when bloom material was settling to the hyperbenthos. Females reproduced once and then died at age-2.5 years following a 5-month brooding period of larval stages. The biennial life cycles of both populations resulted in the presence of 2 cohorts of each species co-existing at most times, although a marked annual alternation in cohort dominance occurred in the amphipod population. Annual production biomass (P/B) ratios in the mysid population were similar in both years, indicating consistent recruitment from year to year. In contrast, the alternation in cohort strength in *A. malmgreni* had significant repercussions in the success of this population, reflected in a lower P/B ratio in 1999 than in 2000.

Divergent patterns in lipids, fatty acids, and specific fatty acid marker ratios reflected differing life styles, diets and critical periods of energy accumulation and utilisation in *M. mixta* and *A. malmgreni*. Influenced by the seasonally productive and perpetually cold environment created by the Labrador Current, *M. mixta* and *A. malmgreni* both accumulated high levels of lipid reserves similar to those attained by other cold-water zooplankton. Maximum lipid stores were observed in females, although the maximum lipid content in *M. mixta* was over twice that in *A. malmgreni*. Lipid levels increased with maturity, and developing mysids exhibited a particularly rapid accumulation of triacylglycerols and diatom-associated fatty acids at the start of the spring bloom. Unlike the mysids, juvenile amphipods did not begin to develop sexual characteristics or

accumulate significant amounts of triacylglycerol or phytoplanktonic fatty acids until after the spring bloom material had already reached the hyperbenthos in May. This study reveals a dependence of *M. mixta* and *A. malmgreni* on seasonal lipid accumulation for reproduction, and the existence of trophic links between their populations and the pelagic production cycle (i.e. benthic-pelagic coupling). Increased mobility and a broad range of prey types provided *M. mixta* with sufficient opportunities to accumulate essential fatty acids rapidly. However, *A. malmgreni* was restricted to a lower quality diet than was *M. mixta*, and the trophic connection between *A. malmgreni* and production in the euphotic zone was less pronounced.

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and, thanks for the music.

I dedicate this work to my grandmother, Amy Richoux... I wish you were here.

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List of Abbreviations and Units

AFDM	Ash-free dry mass (mg)
ALC	Alcohol(s)
AMPL	Acetone-mobile polar lipid(s)
B	Biomass (g DM per 100 m^3)
C	Carbon (mg)
C1	Cohort 1
C2	Cohort 2
C3	Cohort 3
C4	Cohort 4
Chl *a*	Chlorophyll *a* (µg l^{-1})
CHN	Carbon, hydrogen, nitrogen
CTD	Conductivity, temperature, depth
C:N ratio	Carbon:Nitrogen ratio
CO	Cohort
CV	Coefficient of variation [= (standard deviation × 100)/mean]
D	Density (ind. per 100 m^3)
DG	Diacylglycerol(s)
DM	Dry mass (mg)
Eq	Equation number
Fc	Fecundity (embryos $brood^{-1}$)
FFA	Free fatty acid(s)
FID	Flame-ionization detection
GC	Gas chromatography
HC	Hydrocarbon(s)
IF	Immature female(s)
IM	Immature male(s)
ind.	individual(s)
IU	immatures undifferentiated

JV	Juvenile(s)
KET	Ketone(s)
L_1, L_2, L_3	Length measurements (mm)
LI	Lipolysis index ((FFA + ALC)/(SEWE + ME + TAG + FFA + ALC + DG + AMPL + PL))×100
ME	Methyl ester(s)
MF	Mature female(s)
MM	Mature male(s)
MUFA	Monounsaturated fatty acid(s) (1 double bond)
NS	Non-sexed
P	Protein (mg)
P:B ratio	Production:Biomass ratio
P_C	Carbon production (mg per 100 m^3)
P_{DM}	Dry mass production (mg per 100 m^3)
PL	Phospholipid(s)
PUFA	Polyunsaturated fatty acid(s) (≥ 2 double bonds)
RFU	Relative fluorescence units
SE	Steryl ester(s)
SFA	Saturated fatty acid(s) (no double bonds)
SP	Spent female(s)
ST	Sterol(s)
TFA	Total identified fatty acids
TAG	Triacylglycerol(s)
TL	Total lipid (% DM, mg $individual^{-1}$)
TLC	Thin-layer chromatography
WE	Wax ester(s)

Chapter 1. Introduction and overview

1.1 The hyperbenthos

The hyperbenthos is the region of interaction between the sea floor and the water column. This oceanic region is characterized by a high concentration of suspended matter and supports a variety of invertebrates of both benthic and pelagic origin (Marcus & Boero 1998). Sampling problems resulting from the inaccessibility of the hyperbenthos have resulted in a general paucity of studies on organisms living in this region, but evidence suggests that hyperbenthic animals successfully exploit a diversity of food resources and are important links among numerous trophic levels (Mees et al. 1995, Mees & Jones 1997). Invertebrates living in this region, including mysids, amphipods, decapods, chaetognaths, copepods and a variety of other taxa, play key roles in the conversion of energy and nutrients from algae, detritus, bacteria and small zooplankton into animal biomass that can be consumed by fish and other predators (Alldredge & King 1985, Gardner et al. 1985, Stefanescu & Cartes 1992, Goedkoop & Johnson 1994). To date, most studies on the hyperbenthos have focussed on faunal descriptions, distributions and relative abundance, but few studies have examined the general biology, life cycles, energy storage cycles and ecological roles of species within this realm (Cartes et al 2001).

1.2 Benthic-pelagic coupling

1.2.1 Seasonal production in the euphotic zone

Benthic-pelagic coupling refers to the links between the pelagic and benthic regions of aquatic systems. Measurements of vertical flux connecting plankton and benthos in the ocean were not commonplace before the 1970's (Hargrave 1973), and many researchers continue to study the two regions as relatively separate entities. Only recently have benthic and pelagic processes been incorporated into a more cohesive unit in the marine ecosystem (e.g. Wassmann 1998). For example, Asper et al. (1992) made direct measurements of primary production, shallow flux and deep flux at a 3,200-m deep site off the coast of Bermuda in the Atlantic Ocean. They found a close correspondence

between primary production and organic particle flux to the benthos, thus supporting growing evidence that benthic and pelagic systems are closely connected even in very deep areas.

Although a variety of biological and physical processes link benthic and pelagic regions (Parsons et al. 1977, Alldredge & King 1985, Alldredge & Silver 1988, Graf 1992, Lehtonen 1995, Tyler 1995, Marcus & Boero 1998, Wassmann 1998), the concept of benthic–pelagic coupling is most often referred to as a vertical trophic link, whereby particulate organic matter originating in the euphotic zone is deposited to the benthos where its energy is consumed, stored and metabolized (e.g. Kemp et al. 1999). Uptake and transport of nutrients from benthic organisms and sediments back to the euphotic zone to support primary production completes the trophic loop. The cyclic transformation of nutrients between dissolved inorganic and particulate organic forms, and the vertical flux of solutes and particles, can facilitate the retention and recycling of nutrients within an ecosystem (Kemp et al. 1999 and references therein). Every oceanic region is characterized by some degree of benthic-pelagic coupling, although more accessible areas like the Baltic Sea have been more extensively studied than deeper, more remote regions (Vitto & Sarvala 1991, Rudstam et al. 1992, Lehtonen & Andersin 1998).

Primary production in the world's oceans occurs predominantly within the euphotic zone. Generally, particulate organic matter in the euphotic zone consists of fine particles, algal cells, detritus, freely-suspended bacteria, fecal pellets and aggregates. Aggregates and fecal pellets are the vehicles that provide the main passive flux of material (Emerson & Roff 1987, Alldredge & Silver 1988, Lampitt et al. 1993), although active deposition of pelagic material via animal movements also provides food for benthic and hyperbenthic organisms. In coastal ecosystems such as the northern Adriatic Sea, annual deposition of particulate organic matter has been calculated at 45 to 50% of primary production (Kemp et al. 1999). Even in the deep ocean, where only 1 to 3% of surface organic material reaches the seabed (Gage & Tyler 1991), the dominant source of organics for heterotrophic feeding is primary production within the euphotic zone (Tyler 1995).

The occurrence of seasonal food pulses originating in surface waters may be more important to benthic community structure and dynamics than the total annual input of

particulate organic matter to the seafloor. In the Kiel Bight, as much as one third of the annual vertical input to the benthos is delivered during seasonal phytoplankton blooms (Graf 1992). Similarly, in Conception Bay, Newfoundland, most sedimentation occurs following the spring phytoplankton bloom, which provides the benthos with large influxes of fresh particulate organic matter each year (Pomeroy et al. 1991, Redden 1994). A gradual reduction in primary production during non-bloom seasons results in periods of low flux to the benthos. Overall, the flux of organic material within an ecosystem depends on a number of changing physical and biological factors, therefore the nature and degree of coupling between pelagic and benthic systems varies both regionally and seasonally.

1.2.2 Responses in the hyperbenthos

Seasonal sedimentation events can elicit a variety of responses in benthic and hyperbenthic organisms that can be measured in individuals (e.g. changes in growth, digestion, diet, respiration, energy storage and reproduction) or populations (e.g. changes in density, biomass, recruitment and secondary production) (Hopkins et al. 1984, Christensen & Kanneworff 1985, Rudnick et al. 1985, Gage & Tyler 1991, Goedkoop & Johnson 1996, Lehtonen & Andersin 1998, Stead & Thompson 2003). The quantity and nature of material originating during a spring bloom that is consumed by hyperbenthic organisms remains largely unknown. Different species may exhibit a variety of behavioural and physiological adaptations that allow them to take advantage of this seasonal influx. These adaptations may be both multi-faceted and interrelated; therefore, the most informative studies on benthic-pelagic coupling encompass several potential responses of any benthic or hyperbenthic population.

1.2.3 Zooplankton migrations

In addition to the direct trophic links that return benthic biomass to the pelagic system (e.g. via predator movements), tidal or diel vertical migrations by hyperbenthic fauna constitute a significant link to the pelagic realm. Nocturnal vertical movements into the water column are common in organisms such as amphipods, isopods, cumaceans,

copepods, decapods, crab larvae, polychaetes and mysids living in coral reef, kelp bed and soft-bottom habitats (Alldredge & King 1985 and references therein). Such vertical migrations allow organisms to take advantage of increased food availability in the upper water column, decreased metabolism in cold water, horizontal dispersion, breeding migrations, and/or avoidance of visual predators (e.g. Rudstam et al. 1989). One important ecological impact of this phenomenon is a type of conveyor-belt system of feeding and egestion that affects the downward and upward flux of particulate material in oceanic regions inhabited by vertically migrating zooplankton.

1.3 Lipids and fatty acids

1.3.1 Importance of lipids

Lipids are carbon-rich components of marine food webs that serve three functions. First, lipids are an efficient source of metabolic energy. As storage products, they provide twice as much potential metabolic energy per unit mass as carbohydrate or protein (Quigley et al. 1989, Lehtonen 1994). Second, lipids have vital structural and functional roles in membranes and are therefore required for growth and reproduction (Arts 1999). Consumers derive all their lipid requirements either directly from the diet or indirectly by the transformation of protein and carbohydrate precursors into lipids. As a third function, lipids are used by some marine invertebrates, particularly pelagic larvae, to regulate buoyancy or adapt to changing physical factors such as oxygen levels or temperature (Sargent & Henderson 1986, Hall et al. 2000).

Lipid and protein account for most of the organic material in crustacean zooplankton, carbohydrate being only a minor constituent (Raymont & Conover 1961, Clutter & Theilacker 1971, Childress & Price 1983). Lipids are particularly important in polar zooplankton, which contain lipid levels ranging from 14 to 70% of total dry mass (Falk-Petersen et al. 1981). Very high lipid reserves in animals stem from high levels of neutral lipid, usually in the form of wax esters or triacylglycerols (Sargent & Falk-Petersen 1988). Phospholipid content remains relatively stable with season due to its role as a structural and functional component of membranes and organelles (Vanderploeg et al. 1992).

Species living in highly variable or extreme habitats have developed metabolic energy-saving mechanisms triggered by food limitation. Lee & Hirota (1973) postulated that lipids are accumulated in large quantities by marine zooplankton that experience short periods of food abundance followed by prolonged periods of food shortage. Numerous studies have since been undertaken to explore the validity and applicability of this early hypothesis, and the field of zooplankton energetics in general. Investigations of lipid content and composition provide essential information on the energetic importance of lipids in the survival and reproduction of zooplankton, as well as the energetic role of zooplankton species in marine food webs.

1.3.2 Lipids and benthic-pelagic coupling

Lipid content and composition of individuals in a zooplankton population, particularly one that relies heavily on annual spring bloom material to fuel reproduction and survival, usually vary in some seasonal and predictable manner (e.g. Gardner et al. 1985, Sargent & Falk-Petersen 1988). In contrast, populations not dependent on annual bloom production generally do not exhibit substantial fluctuations in lipid content (e.g. Moore 1976, Napolitano & Ackman 1989, Choe et al. 2003). Lipid analysis is therefore an effective and versatile tool with which to study benthic-pelagic coupling. Unfortunately, lipid content and composition in hyperbenthic zooplankton are affected by a wide variety of factors, some of which may not be related to seasonal nutrient flux from the euphotic zone (e.g. diet preference, body size, physiology, developmental stage, reproductive strategy and temperature; Clarke et al. 1985, Tande & Henderson 1988, Kattner et al. 1994, Hagen & Shnack-Scheil 1996). As a result, it is necessary to incorporate information on the biology and ecology of a population when determining changes in lipids relative to seasonal food availability.

Because lipids are major biochemical constituents of most marine organisms and lipid composition can be species-specific, lipid classes and fatty acids have been used to study trophic interactions between marine consumers and their food supply (Falk-Petersen et al. 1987, Sargent et al. 1987, Graeve et al. 1994, Kattner et al. 1994, Virtue et al. 2000, Scott et al. 2001). Fatty acid biomarkers are particularly useful as dietary indicators, and they

provide an alternative or complementary method of diet determination that may be particularly helpful in environments like the hyperbenthos, where organisms cannot easily be observed. Essential ω3 fatty acids originating in algae are considered appropriate trophic markers since most zooplankton are not able to synthesize them, but require them for normal development and reproduction (Sargent & Falk-Petersen 1988). The lipid composition of consumers can therefore vary quantitatively and qualitatively according to the lipid composition of the phytoplankton (Sargent & Falk-Petersen 1988, Gardner et al. 1989). Since dietary fatty acids are incorporated into body tissues relatively unmodified (Lee et al. 1971), the fatty acid composition of zooplankton can provide information on both diet and foraging strategy over an ecologically meaningful time scale (weeks to months) (Arts 1999). Little direct information is available on the feeding ecology and trophic niches of hyperbenthic species in any region, and such information must often be deduced from a general biological knowledge of morphological features or related species. The potential of fatty acids and specific fatty acid marker ratios to reveal trophic relationships is a useful tool for any ecological investigation.

1.4 Thesis overview

One objective of this research was to describe the basic ecological characteristics of *Mysis mixta* and *Acanthostepheia malmgreni* inhabiting the hyperbenthos of Conception Bay, Newfoundland. Preliminary information on year-round abundance and lipid storage abilities indicates that these species are important components of the Conception Bay ecosystem (Deibel et al. unpublished, Parrish et al. unpublished). Life cycles and annual secondary production, and seasonal changes in growth, density and biomass in *M. mixta* and *A. malmgreni* over a 2-year period, are described in Chapters 2 and 3. These chapters provide the basic ecological data necessary in any study on trophic interactions between production in the euphotic zone and zooplankton populations living in the hyperbenthos. Physical characteristics of the spring phytoplankton blooms occurring in 1998, 1999 and 2000 are described in Chapter 2.

In Chapters 4 and 5, seasonal changes in lipid classes and total lipids in *M. mixta* and *A. malmgreni*, respectively, are presented. Variations were related to each species' life

cycle and to the occurrence and quality of the annual phytoplankton bloom. Areal concentrations of lipid energy reserves were calculated using density data (Chapters 2, 3) to compare the relative ability of each population to sequester organic material from the environment. Effects of starvation on the lipid content in *A. malmgreni*, and comparative observations on the lipid dynamics of the sympatric species, are included in Chapter 5.

In Chapter 6, seasonal and ontogenetic changes in fatty acid composition and specific fatty acid marker ratios in juveniles through adult stages of *M. mixta* and *A. malmgreni* are profiled. Patterns in fatty acid composition were expected to differ in the two species, as they exhibited distinctive feeding strategies, population dynamics and lipid accumulation characteristics. Fatty acid markers were used to provide further evidence for the presence and degree of the benthic-pelagic connections established in Chapters 2 - 5.

Chapter 7 integrates the information provided in Chapters 2 to 6 and includes suggestions for future studies on hyperbenthic zooplankton and benthic-pelagic coupling.

1.5 Publication and submission status

Chapter 2 (Richoux, Deibel, Thompson) is published in *Marine Biology* as "Population biology of hyperbenthic crustaceans in a cold water environment (Conception Bay, Newfoundland). I. *Mysis mixta* (Mysidacea)" 144:881-894 (reproduced with permission)

Chapter 3 (Richoux, Thompson, Deibel) is published in *Marine Biology* as "Population biology of hyperbenthic crustaceans in a cold water environment (Conception Bay, Newfoundland). II. *Acanthostepheia malmgreni* (Amphipoda)" 144:895-904 (reproduced with permission)

Chapter 4 (Richoux, Deibel, Thompson, Parrish) has been accepted in *Canadian Journal of Fisheries and Aquatic Sciences* as "Seasonal changes in the lipids of *Mysis mixta* (Mysidacea) from the hyperbenthos of a cold-ocean environment (Conception Bay, Newfoundland)"

Chapter 5 (Richoux, Thompson, Deibel, Parrish) has been accepted in *Journal of the Marine Biological Association of UK* as "Seasonal and developmental changes in the

lipids of *Acanthostepheia malmgreni* (Amphipoda) from the hyperbenthos of a cold-ocean environment (Conception Bay, Newfoundland)"

Chapter 6 (Richoux, Deibel, Thompson, Parrish) has been submitted to *Journal of Plankton Research* as "Seasonal and ontogenetic variation in the fatty acid composition of two crustaceans from the hyperbenthos of a cold-ocean environment (Conception Bay, Newfoundland)"

1.6 Co-authorship statements

As the first author of each 'stand alone' chapter prepared for publication, I developed the overall concepts, designed the sampling protocols, directed the field team in all sample collections, performed or directed all laboratory analyses, statistically analysed all data and wrote the manuscripts/chapters. My co-authors, Drs. Thompson, Deibel and Parrish contributed ideas, advice and editorial suggestions.

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Chapter 2. Population biology of *Mysis mixta* (Mysidacea)

2.1 Introduction

Studies on the population structure of deep-living hyperbenthic crustaceans are scarce in the literature. In this study, the hyperbenthic mysid *Mysis mixta* was sampled in Conception Bay, Newfoundland, for two consecutive years to determine its life-cycle and seasonal population dynamics, and to investigate the ecological position of mysids in cold-water, hyperbenthic communities. Owing to its high mean lipid content relative to 17 other macrofauna examined in Conception Bay (Parrish et al. unpublished), high abundance, diel vertical migrations and year-round presence (Deibel et al. unpublished), *M. mixta* may play an important energetic role in coastal cold-water ecosystems. Little is known about the hyperbenthic component of any ecosystem, and even basic population information on predominant species is required. Population dynamics and seasonal vertical distribution of the most commonly occurring macroinvertebrate in the hyperbenthos of Conception Bay (the chaetognath *Parasagitta elegans*) are known, although annual secondary production of this population has not been estimated (Choe & Deibel 2000). *M. mixta* and the amphipod *Acanthostepheia malmgreni* are among the most commonly occurring hyperbenthic species in Conception Bay, in addition to *P. elegans* and several other species (i.e. three species of mysids, two decapods, two cumaceans and one euphausiid; Deibel et al. unpublished), and relevant population data are entirely lacking. Thus, the present study lays the foundation for further work to establish the role of invertebrates in the hyperbenthos of Conception Bay and similar cold-ocean regions.

M. mixta is a boreal relict that originated in the North Atlantic Ocean and later adapted to brackish waters in some regions of the world (Salemaa et al. 1986). This species' distribution extends from east Atlantic regions of the White Sea, Spitsbergen, Scandinavia, the Baltic Sea and Iceland (Apstein 1969, Wigley & Burns 1971, Gorokhova & Hansson 2000), to west Atlantic areas off the coasts of Greenland, eastern Canada (Tattersall 1939, Black 1957, Brunel 1978) and the eastern United States (Wigley

& Burns 1971, Grabe & Hatch 1982). *M. mixta* has been described as an annual, semelparous species, with few individuals surviving for 2 years (Grabe & Hatch 1982, Salemaa et al. 1986, Rudstam & Hansson 1990). Owing to differing environmental conditions, particularly temperature and food availability, *M. mixta* populations inhabiting the coastal waters of insular Newfoundland are likely to have different life-history characteristics and life cycles than populations in other regions of the world.

M. mixta is a brooding species, with the embryos developing inside the brood pouch of the female. This mysid is an opportunistic omnivore that alters its diet at different life-cycle stages, with small juveniles feeding on phytoplankton and other small particles and larger individuals feeding on rotifers, cladocerans, tintinnids, copepods and detritus (Rudstam 1989, Hansson et al. 1990a, 1990b). Mysids are important globally as prey for various fish including cod, hake, smelt, perch, herring, turbot and sculpins (Black 1957, Mauchline 1980, Rudstam & Hansson 1990, Viherluoto 2001), as well as for invertebrates, birds and seals (Mauchline 1980), thereby linking primary and secondary production to higher trophic levels. Furthermore, vertical migrations of *M. mixta* toward surface waters at dusk contribute to benthic-pelagic coupling (Hansson et al. 1990a). While residing in the hyperbenthos, the mysids swim continuously and do not appear to utilise the benthos for food or substrate (Rudstam et al. 1989). This motility, in addition to the inaccessibility of the hyperbenthos, has caused considerable difficulty and logistical problems in the study of mysid populations.

The flux of energy in a marine ecosystem depends on seasonal patterns in physical factors such as light, nutrients, temperature and salinity, as well as biological factors including the abundance, distribution and life cycles of the species present. Seasonal phytodetritus deposition and biological processes such as digestion, energy storage, gametogenesis and recruitment of deep- or cold-water species are often correlated (e.g. Hopkins et al. 1984, Gage & Tyler 1991, Stead & Thompson 2003). This link is especially strong in some deep-sea species that are subject to a highly seasonal food supply and to stable low temperatures that allow for low basal metabolic rates and efficient nutrient utilisation (Clarke 1983). In the present study I examine the density, biomass, growth, production and overall life cycle of *M. mixta* in Conception Bay. These

population parameters probably vary in relation to seasonal phytodetritus flux. In particular, major reproductive events and increases in growth are likely following sedimentation of the primary phytoplankton bloom.

2.2 Materials and methods

2.2.1 Study site

Conception Bay is a large fjord-like bay on the east coast of Newfoundland, Canada. The bay is ~100 km long by ~30 km wide at the mouth, with a maximum depth of 300 m in the central basin. A 150 m deep sill at the mouth restricts access of deep water to the bay, and bottom slopes within the bay are steep near the shores (deYoung et al. 1993). The Labrador Current supplies Conception Bay with <0°C water throughout the year. The study site (Fig. 2.1) lies within the deep depositional region of the bay at ~240-m depth (tow start coordinates 47°30.5´N; 53°07.5´W; tow end coordinates 47°32.5´N; 53°07.0´W), where mean currents are weak (1 - 2 cm s^{-1}, deYoung & Sanderson 1995).

2.2.2 Sample collection

Samples of *Mysis mixta* Lilljeborg and other hyperbenthic crustaceans were collected monthly from December 1998 to November 2000 (weather permitting). Sampling was done from a 13 m boat with an epibenthic sled (original design by Rothlisberg & Pearcy 1977, modified by Brattegard & Fosså 1991), which collected organisms living directly on the sea floor and those living within 60 cm of the bottom (Buhl-Jensen 1986, Brattegard & Fosså 1991). Organisms entering the sled (mouth area 0.3 m^2) were retained by a 500-μm mesh net, tapering in a cod end, which was adequate to sample all life-history stages of *M. mixta,* from newly released larvae to full-sized adults. The mouth of the sled was guarded by a door that was opened when a lever on the sled bottom was triggered by contact with the sea floor. This ensured that samples were not collected while the sled was being deployed or retrieved. Depth of the sled and position of the door were monitored with a hydrophone and an acoustic transmitter (Vemco, Nova Scotia).

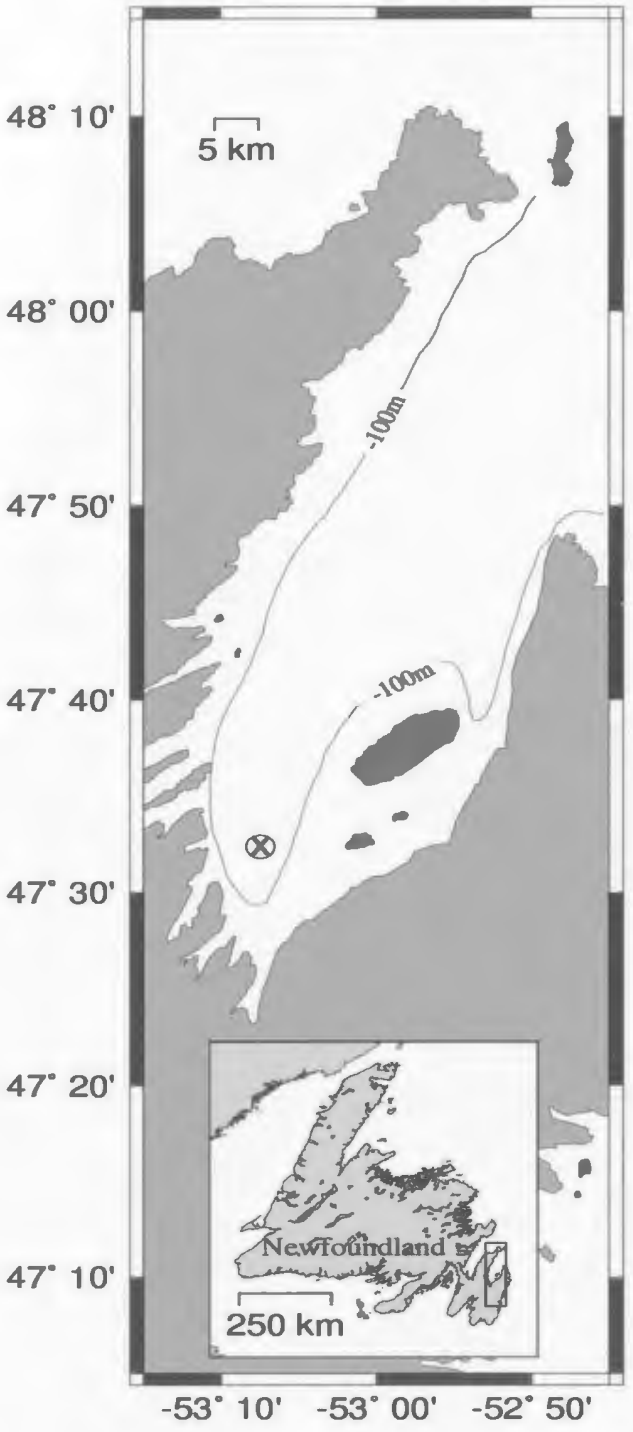

Fig. 2.1 Study site ⊗ within Conception Bay, Newfoundland

All tows lasted 20 - 30 minutes at a speed of 1.0 – 1.5 knots (1 knot = 0.51 m s^{-1}); therefore the distance covered during one tow was 620 - 930 m. A TSK flowmeter at the mouth of the sled recorded the volume filtered (87 - 196 m^3 tow^{-1}). For between-study comparisons, volumetric (m^3) units are considered as approximately equivalent to areal units (m^2) by integrating the data over 1 m (an estimate of the water column depth sampled by the sled). Tows were completed between 1100 and 1400 hours, a time when mysids aggregate in the hyperbenthos (Rudstam 1989).

One tow was completed per sampling day, with the exception of February, May, August and November 2000, when three successive tows were done to determine the variance among replicate tows. On retrieval, live samples from each tow were maintained in insulated containers filled with seawater recently obtained from below the thermocline. Excess mysids in extremely dense samples were fixed in 4% buffered formaldehyde to prevent anoxia in the containers. Upon return to the laboratory, the containers were kept at ~3°C to allow live mysids to acclimate and depurate overnight.

A Seabird SBE25 CTD with a SeaTech fluorometer was deployed on each sampling day to measure water column temperature (°C), salinity (psu), depth and relative fluorescence. Relative fluorescence units (RFU) were converted to micrograms chl *a* per litre using an algorithm developed previously from Conception Bay water samples (chl $a = 0.3973 \times RFU + 0.3015$, $r^2 = 0.65$, $n = 253$; Ru Cheng Tian, personal communication).

2.2.3 Sample analyses

During the 2 - 3 days following sampling, live mysids were categorized into life-history stages (Table 2.1) and counted. The density and biomass of mysids in each tow were standardised to the number of individuals per 100 m^3 and milligrams dry mass (DM) per 100 m^3, respectively. Embryos protected by brooding females were counted under a Zeiss microscope, and bitmap images of several embryos per brood were captured using a Sony CCD camera and Image Pro or Matrox software for determination of larval length by image analysis. Larval length was measured from terminal to frontal tip of the body, including eyes when present (Fig. 2.2). For each free-living stage, the lengths of 1 - 12

straightened mysids (depending on availability) were measured to the nearest 0.5 mm under the stereomicroscope. On occasion, insufficient mysids were available from some life-history stages, and sample size was necessarily reduced. Because several length measurements are commonly used in the mysid literature, three body length measurements were taken: L_1 is the tip of the scales to the uropod tips (Fig. 2.3), L_2 is the rostrum tip to the telson tip (Fig. 2.3), and L_3 is the carapace length (Fig. 2.4). The most common measurement, L_2, is used for presentation of data here, but equations to convert L_2 values to L_1 and L_3 are provided.

Table 2.1 *Mysis mixta*. Demographic categories (modified from Mauchline 1980) and approximate size ranges (free-living juveniles <7 mm were not sampled due to a higher depth distribution). Body length = L_2, except for larval stages. Size gap between larval stages I and II results from the uncurling of the embryo once hatched

Life-history stage	Body length (mm)	Dry mass (mg)	Characteristics
Larval stage I	0.88 – 0.95	0.13 – 0.19	Early egg-like embryo contained within an egg membrane
Larval stage II	2.16 – 3.03	0.10 – 0.26	Embryo is hatched and has rudimentary eyes, antennae and thoracic appendages
Larval stage III	3.15 – 4.08	0.14 – 0.30	Embryo has stalked eyes and well developed thoracic appendages
Juvenile	7.0 – 15	0.9 – 18	No visible secondary sexual characteristics
Immature	14 – 30	6 – 69	*Female*: has developing oostegites
	14 – 30	4 – 41	*Male*: has developing penes
Mature female	21 – 32	19 – 79	Fully developed marsupium containing embryos
Spent female	25 – 32	36 – 57	Fully developed empty marsupium
Mature male	24 – 27	23 – 41	Well developed penes, and 4th pleopods extend beyond the uropods

Mysids reserved for determination of DM were rinsed with filtered seawater, dried for 5 - 6 days at 55°C in preweighed aluminum foil envelopes or capsules, weighed (±0.1 mg for developing and mature mysids; ±0.1 μg for small juveniles and embryos), combusted

for 9 - 12 hours at 450°C, cooled in a desiccator and re-weighed to determine ash-free dry mass (AFDM). Embryos within each brood were counted and each brood was treated as a separate sample. Between June and November 2000, 17 mysids were prepared for CHN analysis and 24 mysids for determination of protein content. Mysids intended for protein or CHN assays were frozen (-80°C), lyophilized, homogenized and sub-sampled. The protein content of an individual mysid was estimated spectrophotometrically from two replicate samples (modified Lowry method, Hartree 1972), with bovine serum albumin as standard. The carbon and nitrogen content of replicate samples was determined with an elemental analyzer (Perkin-Elmer model 2400, acetanilide standard). Carbon, nitrogen, protein and AFDM were determined primarily to facilitate comparisons between studies.

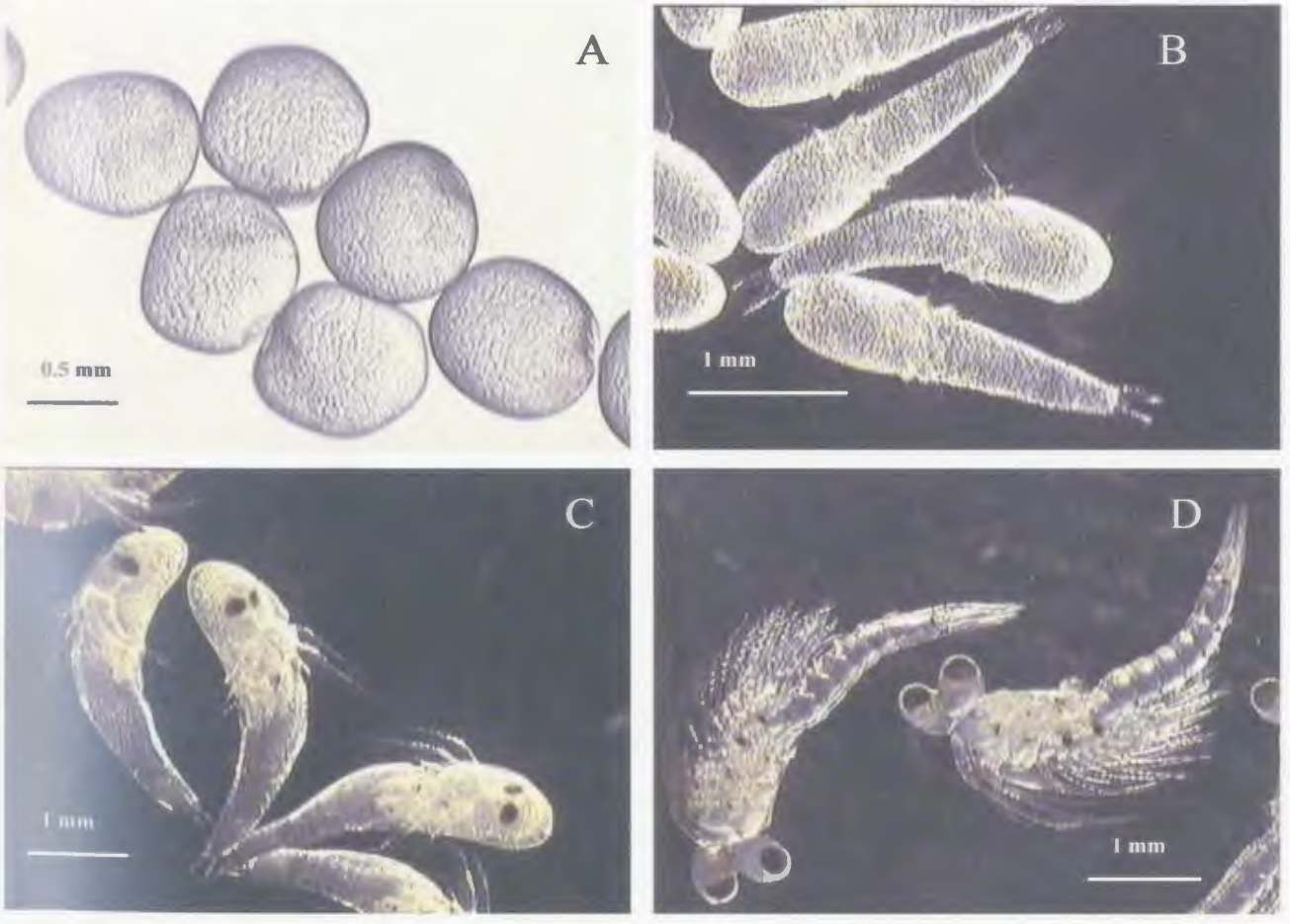

Fig. 2.2 A,B,C,D *Mysis mixta*. Larval stages **A** LI, **B** early LII, **C** late LII and **D** LIII within the brood pouch of a female

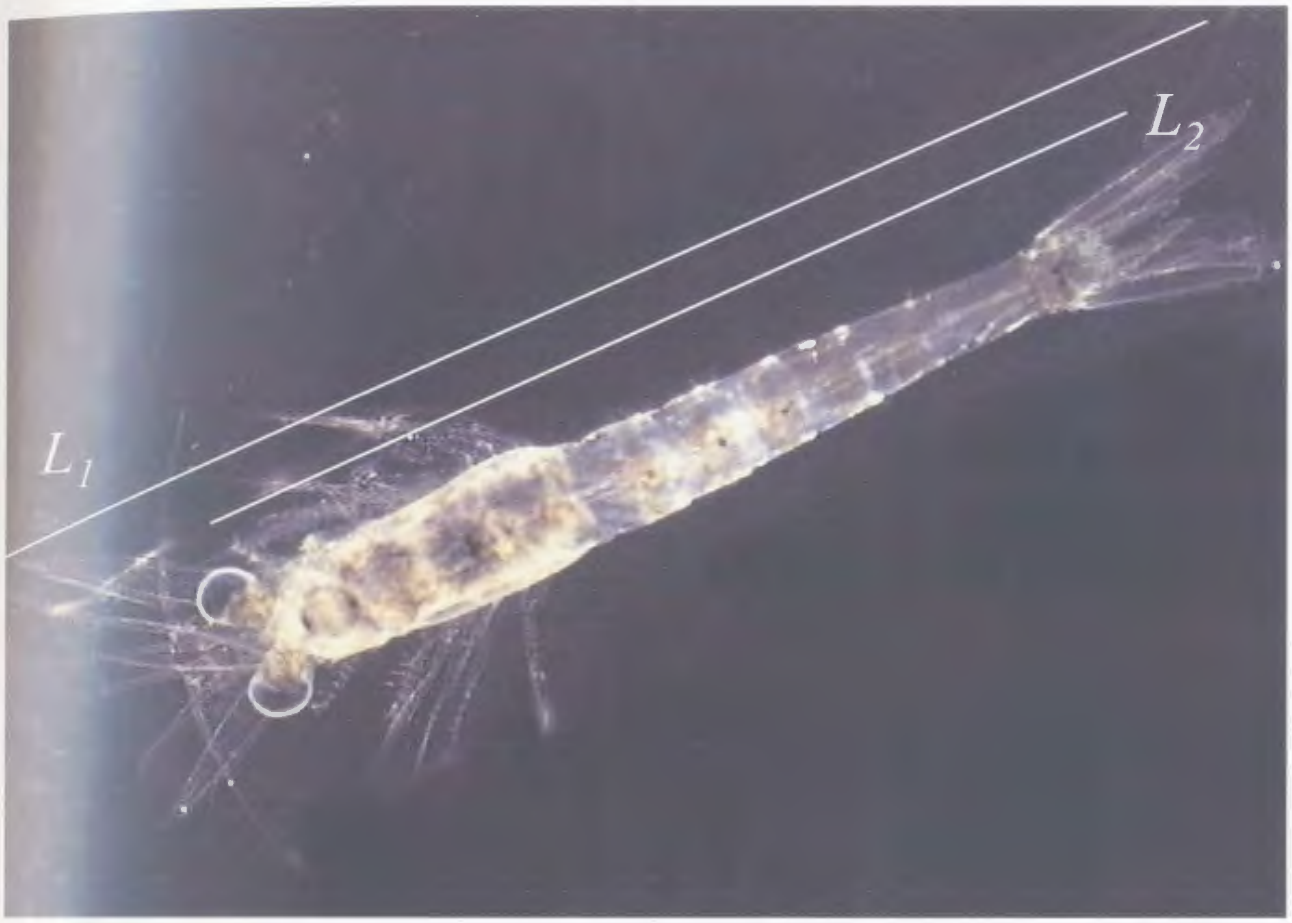

Fig. 2.3 *Mysis mixta.* Full body view of a free-living juvenile showing length measurements (L_1 and L_2)

Size-frequency histograms (L_2, 1 mm intervals) were constructed to determine the population structure and life cycle of *Mysis mixta*. Growth rates of distinct cohorts (analytical cohort-identification techniques were not necessary) were determined from slopes of linear sections of untransformed L_2 and DM data (regression analyses; linear sections were selected by eye from each plot). Mass-specific growth rate ($month^{-1}$) was calculated by dividing growth rate by mean DM. Biomass at each sampling time was calculated as the product of density and mean DM for each life-history stage. Secondary production was calculated for each year by a growth increment summation method (Rigler & Downing 1984):

$$P = \sum [\overline{N}_{(k-1),k} (\overline{dm}_k - \overline{dm}_{(k-1)})] \qquad \text{Eq. 2.1}$$

Fig. 2.4 *Mysis mixta*. Head and partial body regions of a juvenile showing the carapace measurement (L_3)

where P is production in milligrams DM per cubic metre per year, $\overline{N}_{(k-1),k}$ is the average population density over the interval between sample number *k-1* and sample number *k* (arithmetic mean), and $\overline{dm}$ is the average DM of an individual in sample numbers *k* and *k-1*. Since newly released juveniles <7 mm were not retained by the epibenthic sled, production was calculated for the size range 4 mm (embryos within marsupia) to 7 mm (size at first capture) as one interval (Johannsson 1995). Annual production was calculated by summing the cohort production of all stages. Raw density data were used to estimate minimum production, and back-calculated density data were used to derive maximum production estimates. It was necessary to back-calculate to derive maximum estimates because density in later samples was frequently higher than in earlier samples

within a cohort. It follows that mysids present later in a cohort occurred at the same or higher densities earlier within the same cohort, although mortality was not taken into consideration and maximum values of production are therefore conservative estimates. Secondary production estimates include both reproductive and somatic production, because brooded embryos were included in all calculations involving mature females.

2.3 Results

2.3.1 Environmental data

The seasonal temperature profile for Conception Bay indicated little variation from -0.5°C at depths >200 m, although surface water temperatures varied markedly and exceeded 14°C in September each year (Fig. 2.5A). Mysids had to migrate to depths <120 m from the surface to experience temperatures >0°C. Water column data collected prior to November 1998 were included to allow between-year comparisons and to show ecosystem conditions in 1998 (Fig. 2.5). At 240 m, salinity was 32.0 - 34.0 psu in 1999 and 2000 (Fig. 2.5B). The spring phytoplankton bloom began in March (as indicated by increased chl *a* concentrations) and reached a maximum in late April to mid-May (Fig. 2.5C). A smaller, secondary chl *a* pulse began in July of 1998 and 1999, although no secondary bloom was apparent in 2000. Chl *a* minima occurred in surface waters shortly after bloom periods (minima in 1998, 1999 and 2000 were 0.37, 0.36 and 0.43 $\mu g\ l^{-1}$, respectively), and maxima appeared in April and May each year at 10 - 60 m depth (maxima in 1998, 1999 and 2000 were 5.27, 2.22 and 3.64 $\mu g\ l^{-1}$, respectively). Sinking of this fresh material to the hyperbenthos was evident in May each year.

2.3.2 Biometrics

Morphometric relationships for *Mysis mixta* are listed in Table 2.2. Eq. 2.2, derived from the combined data from juveniles, immature males, immature females, mature males and mature females, was used solely to predict the DM of juveniles. Predictions for males, females and spent females were made from Eqs. 2.3, 2.4 and 2.5, respectively (Table 2.2). Separate regressions were necessary because the length-DM relationships were

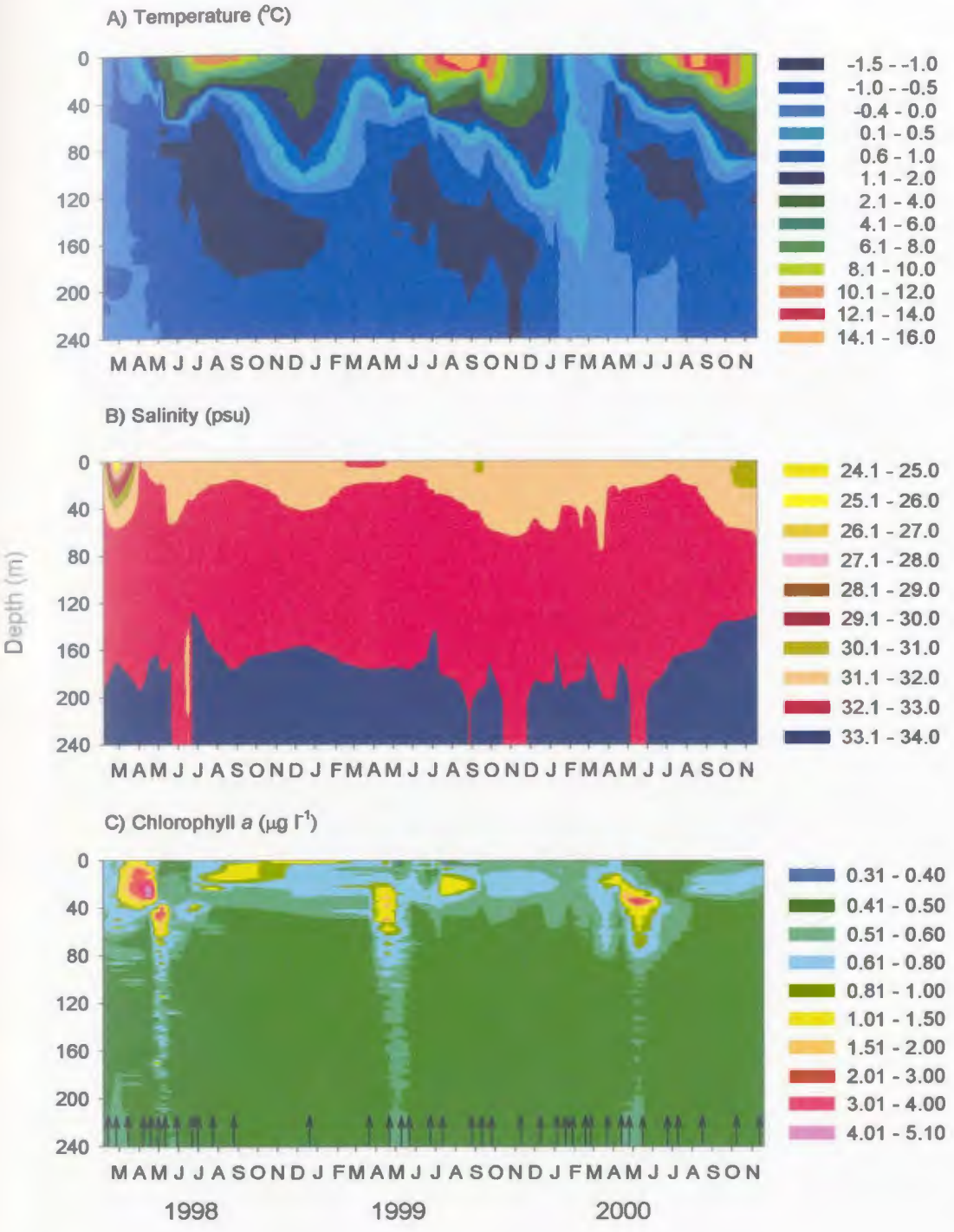

Fig. 2.5 A,B,C Seasonal temperature (**A**, °C), salinity (**B**, psu) and chl *a* concentration (**C**, µg l^{-1}) in the water column at the study site in Conception Bay. *Arrows* in panel C denote sampling dates. Note that interval scales of isopleths are not equidistant in the temperature or chl *a* plots

significantly different among life-history stages (ANCOVA, model: log DM = $\beta_0 + \beta_1 \cdot$sex + $\beta_2 \cdot \log L_2 + \beta_3 \cdot$sex$\cdot \log L_2$ + Error; F(interaction term)= $15.727_{1,222}$, $p < 0.001$). To facilitate comparisons among studies based on alternative body-length measurements, equations to calculate L_1 and L_3 from L_2 are provided (Eqs. 2.9, 2.10, Table 2.2).

Brood size ranged from 22 to 111, with a mean (±SD) of 79±20 embryos brood^{-1}. All broods appeared intact (incomplete broods were obvious as the larvae were arranged together in a loose formation, whereas larvae in intact broods were packed tightly together), and embryo loss was not observed in advanced embryo stages (ANOVA was used to determine whether brood size differed among broods of the 3 larval stages; $F = 0.294_{2, 47}$, $p = 0.746$). Size ranges of larval stages are provided in Table 2.1. Brood size increased with female body length (all larval stages included, Eq. 2.8, Table 2.2), and the DM of broods changed with brood size as given by: $Br_{DM} = 0.190 \times Fc - 0.137$, where Br_{DM} is brood dry mass (mg) and Fc is embryos per brood ($n = 21$, $r^2 = 0.50$, $p < 0.001$).

AFDM ranged from 74% DM in spent females to 85% DM in immature females, with a grand mean (±SD) of 83±6.2% DM for all life-history stages. Carbon and protein as functions of body length are provided in Table 2.2 (Eqs. 2.6, 2.7). Carbon and protein were 45±3.0% DM and 35±6.4% DM, respectively. Mean C:N ratio (by mass; all stages included) was 4.3±0.54.

2.3.3 Density, biomass and growth

Mysis mixta in the Conception Bay hyperbenthos exhibited a ~2.5 year life span and a semelparous life cycle with brood incubation periods of ~5 months. Four cohorts were identified during the 2-year sampling period (labelled C1 - C4; Figs. 2.6, 2.7, 2.8, 2.9, 2.10), with two cohorts coexisting on most sampling dates. Within-cohort density and biomass often fluctuated considerably from one sampling date to another in C2, C3 and C4 (Fig. 2.8). These fluctuations are to be expected, considering the high degree of vertical and horizontal migration by mysids. Food patchiness and aggregation of mysids could explain high variation between successive tow dates, particularly the high density of individuals in the September 2000 tow compared with the lower density in the October

2000 tow, and the high biomass in June 2000 compared with the low biomass in July 2000. Changes in bottom-water turbidity and salinity did not correlate with the large fluctuations in cohort density (Spearman's correlation analyses; coefficents < 0.16). In addition, there were no obvious associations between the density of *M. mixta* and substantial population increases observed in other hyperbenthic species including copepods, cnidarians, chaetognaths, ctenophores and large shrimps (qualitative data on these additional populations not shown). Reasonable coefficients of variation (CV; range 44.7 - 77.3%) for cohort density were recorded from the triplicate tows in February, May, and August, and a lower CV of 19.5% was observed in November (Fig. 2.8A). These CV values are generally lower than those obtained by Hesthagen & Gjermundsen (1977/78), who concluded that an epibenthic sled of similar design (Beyer's 50cm net sled) representatively samples the hyperbenthos.

Table 2.2 *Mysis mixta*. Relationships describing dry mass (*DM*, mg), carbon (*C*, mg), protein (*P*, mg) fecundity (*Fc*, embryos brood^{-1}), length measurement L_1 (mm) and length measurement L_3 (mm) as functions of body length (L_2, mm) [*a*, *b* regression parameters; r^2 coefficient of determination; *n* sample size; *Eq.* equation number (all models significant at $p < 0.0001$); *JV* juveniles; *IM* immature males; *IF* immature females; *MM* mature males; *MF* mature females; *SF* spent females]

Model is to predict:	Stages Included:	Equation	*a*	*b*	r^2	*n*	*Eq.*
Juveniles	*JV;IM;IF;MM;MF*	$DM = a \times L_2^{\,b}$	0.00162	3.05	0.97	187	2.2
Males	*JV;IM;MM*	$DM = a \times L_2^{\,b}$	0.00259	2.89	0.98	102	2.3
Females	*JV;IF;MF*	$DM = a \times L_2^{\,b}$	0.00140	3.11	0.98	124	2.4
Spent females	*JV;SF*	$DM = a \times L_2^{\,b}$	0.00282	2.86	0.99	53	2.5
All life stages	*JV;IM;IF;MM;MF*	$C = a \times L_2^{\,b}$	0.00073	3.05	0.99	16	2.6
All life stages	*JV;IM;IF;MM;MF*	$P = a \times L_2^{\,b}$	0.00086	2.90	0.94	21	2.7
Fecundity	*MF*	$Fc = b \times L_2 + a$	-85.7	5.73	0.50	48	2.8
All life stages	*All*	$L_1 = b \times L_2 + a$	-0.15	1.32	0.98	155	2.9
All life stages	*all*	$L_3 = b \times L_2 + a$	0.16	0.27	0.97	147	2.10

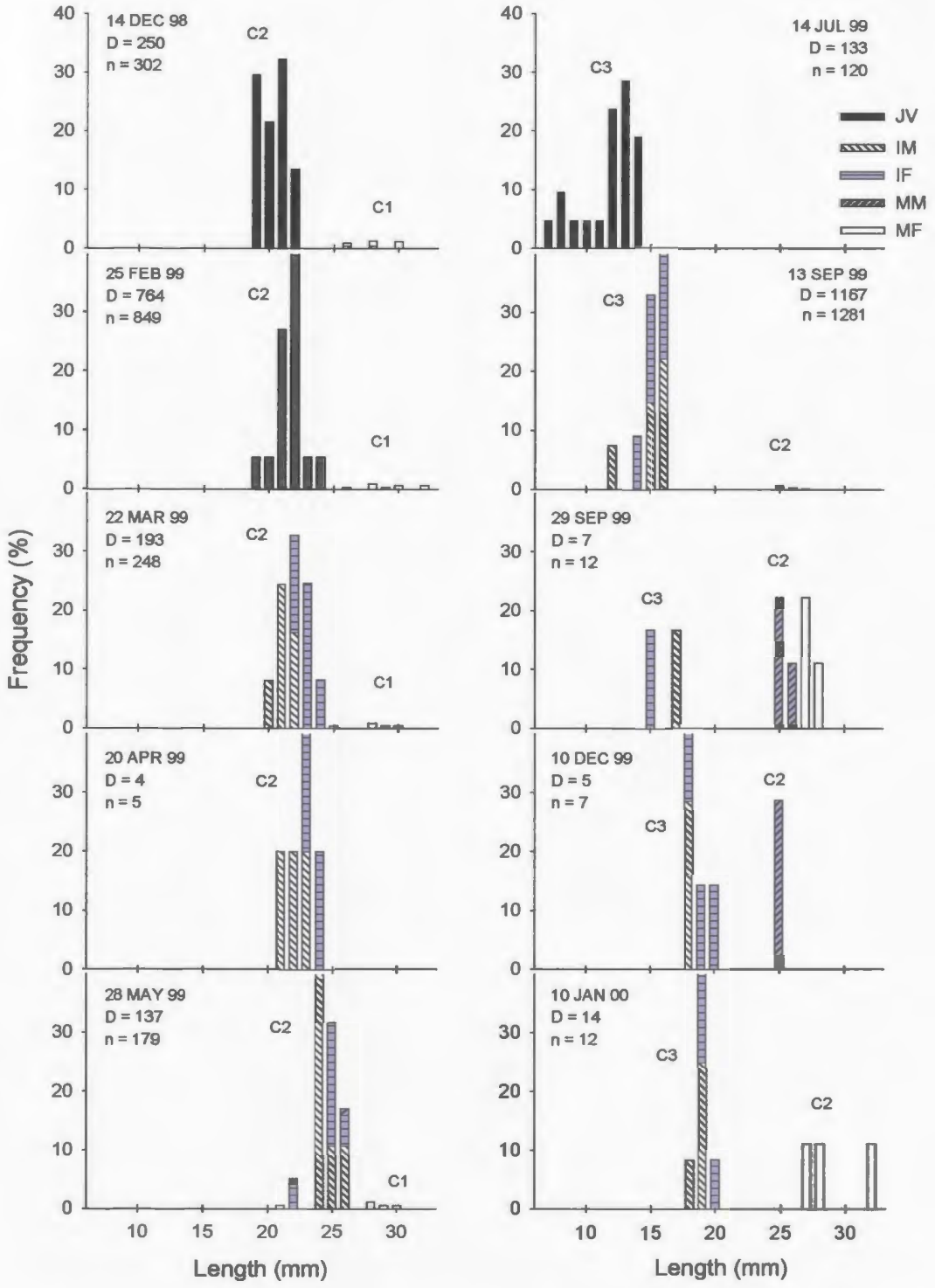

Fig. 2.6 *Mysis mixta*. Length-frequency histograms from 14 Dec 1998 through 10 Jan 2000 (histograms for Oct and Nov 1999 were not included due to low occurrence of mysids). Labels *C1, C2, C3* and *C4* indicate cohorts 1-4 [*JV* juveniles; *IM* immature males; *MM* mature males; *IF* immature females; *MF* mature females (includes both brooding and spent females); *D* density (ind. per 100 m^3); *n* number collected]

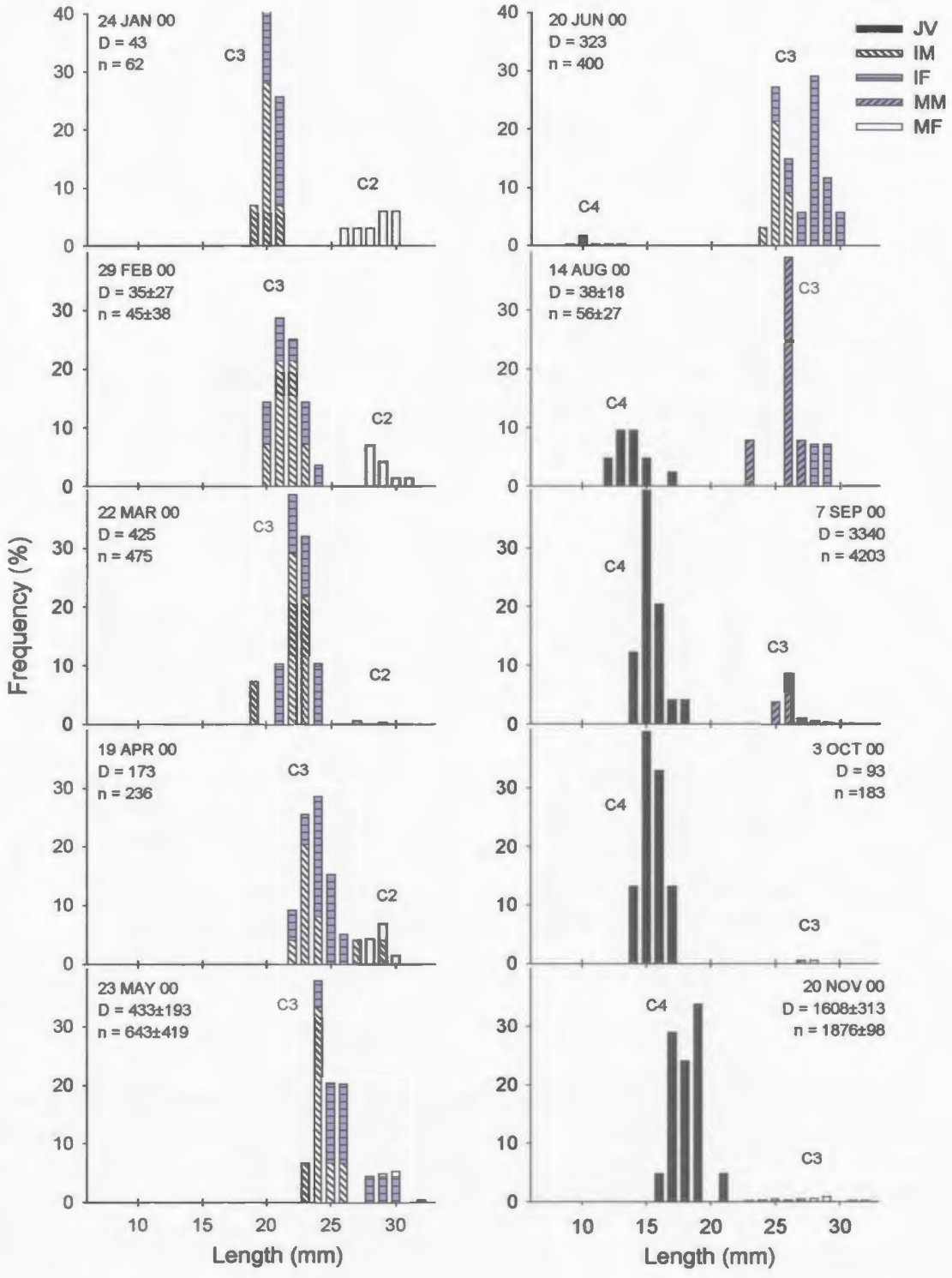

Fig. 2.7 *Mysis mixta*. Length-frequency histograms from 24 Jan through 20 Nov 2000 (July histogram not included due to low densities). Means (±SD) are provided for replicate tows in Feb, May, Aug and Nov 2000 (*abbreviations as in Fig. 2.6)*

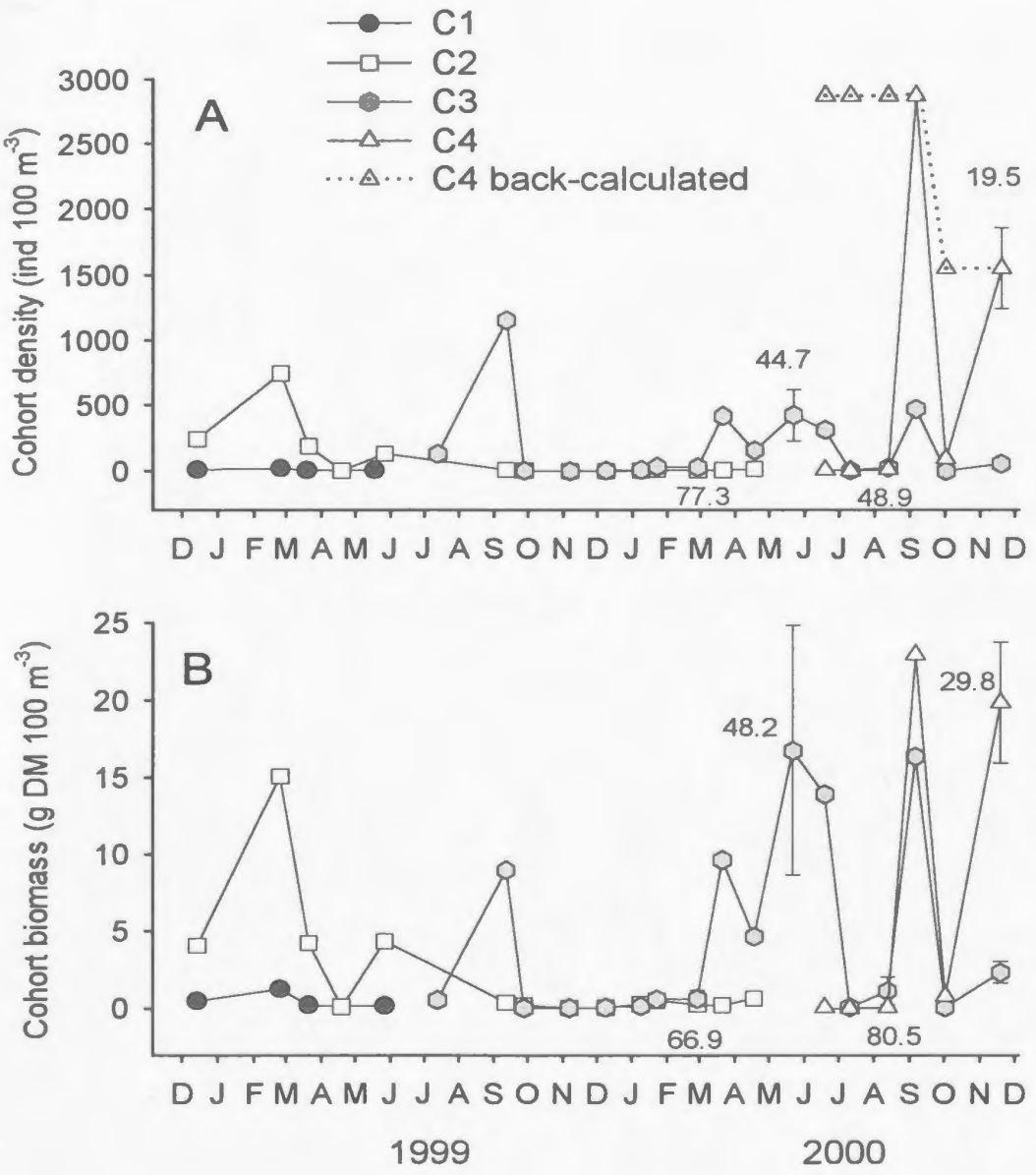

Fig. 2.8 A,B *Mysis mixta.* **A** Cohort density and **B** cohort biomass. Coefficients of variation and error bars representing SD around mean density/biomass are included for dates having replicate tow data (Feb, May, Aug, Nov 2000) (*C1, C2, C3, C4* cohorts 1 – 4). Back-calculated data for *C4* are included in *panel A* to demonstrate the method used to estimate maximum production (Table 2.3)

Despite high within-cohort variation, population abundance was similar between years (mean density 242±379 ind. per 100 m^3 in 1998/99 and 544±987 ind. per 100 m^3 in 2000; Table 2.3). Highest densities and biomass of mysids in 1998/99 occurred in February and September, and for the remainder of the year values were very low (Fig. 2.8). Highest densities in 2000 occurred in September and October, whereas highest biomass occurred in May, September and October (Fig. 2.8). Mean annual biomass in 1999 (4.0±5.3 g DM per 100 m^3) was similar to that in 2000 (9.3±12 g DM per 100 m^3; Fig 2.8B; Table 2.3). *P/B* ratio and secondary production estimates were similar between years (Table 2.3).

Table 2.3 *Mysis mixta*. Density, biomass and secondary production in Conception Bay [*C1, C2, C3, C4* cohorts 1 - 4; *D* mean density (ind. per 100 m^3, ±SD); *B* mean biomass (lower estimate, ±SD; g DM per 100 m^3); P_{DM} production (minimum estimate – maximum estimate; mg DM m^{-3}); P_C production (min. estimate – max. estimate; mg C m^{-3}); *P/B* ratio of annual production to mean annual biomass (using min. estimates of production and biomass)]

	C1	*C2*	*C3*	*C4*	1998/99	2000
D	10.2 ± 8.04	98.3 ± 202	191 ± 300	755 ± 1201	242 ± 379	544 ± 987
B	0.52 ± 0.50	2.2 ± 4.1	4.5 ± 6.2	7.3 ± 11	4.0 ± 5.3	9.3 ± 12
P_{DM}	-1.20 to -1.70	30.4 – 38.0	82.3 - 282	72.2 - 301	65.3 - 162	119 - 458
P_C	-0.540 to -0.764	13.6 – 17.0	36.9 - 127	32.4 - 135	29.1 – 72.5	53.3 - 205
P/B					1.6	1.2

The first cohort of the sampling series (C1) consisted of a small number of mature males and mature females in December, February, March and May 1998/1999 (Fig. 2.6). The females brooded their young from late autumn 1998 to spring 1999, with the release of juveniles (inferred from the presence of spent females) coinciding with the spring phytoplankton bloom in April/May (Fig. 2.5C). Density and biomass remained low throughout the sampling period (cohort mean density = 10±8 ind. per 100 m^3; cohort mean biomass = 0.52±0.50 g DM per 100 m^3; Fig 2.8), and the disappearance of C1 after May indicated the deaths of spent females. C1 mysids exhibited no increase in length or DM (Figs. 2.9, 2.10; Tables 2.4, 2.5).

C2 was first sampled in December 1998 as a group of established juveniles that were probably spawned in autumn 1997 and released in spring 1998 (Fig. 2.6). Mysids in C2 grew at 0.65 mm month^{-1} throughout 1999 and showed no growth during the first 4 months of 2000 (Table 2.4), with a few early maturing females (22 mm body length) spawning in May 1999 (Figs. 2.6, 2.9). The principal group of brooding females (smallest size at maturation 27.5 mm) spawned in October 1999, and juveniles were released in April 2000 (indicated by the presence of spent females). Following the release of juveniles, spent females from C2 disappeared from the hyperbenthos. A few small mature males were collected during the early spawning event in May 1999 (26 mm body length), and males disappeared entirely from the cohort after December 1999. From December to June 1999, density and biomass of C2 remained relatively high as juveniles slowly developed sexual characteristics. Once sexual maturation was complete in October and November 1999, density and biomass of C2 decreased and remained low until the cohort died (cohort mean density = 98±202 ind. per 100 m^3; cohort mean biomass = 2.2±4.1 g DM per 100 m^3; Fig 2.8). Unlike the increase in body length, the increase in DM differed between the sexes. Males grew at 2.1 mg month^{-1} and females at 2.8 mg month^{-1} until both reached maturity (and death, in the case of males) at age ~2 years, after which female growth ceased until death occurred at age ~2.5 years (Fig. 2.10; Table 2.5).

C3 appeared in July 1999 as small juveniles that had been released from C1 females in the spring (juvenile length at release ~4 mm). Juvenile mysids were not collected by the epibenthic sled until they reached a length of ~7 mm. C3 individuals slowly developed sexual characteristics from autumn 1999 through spring and fall 2000 (growth rates 1.3 and 0.29 mm month^{-1}; Table 2.4; Fig. 2.6). Growth of males measured by changes in DM began at a slow rate of 1.9 mg month^{-1}, but increased to 3.7 mg month^{-1} before DM apparently decreased following maturation (Fig. 2.10; Table 2.5). DM growth of females also began slowly at 2.3 mg month^{-1} and increased to 6.8 mg month^{-1} before levelling out at maturity (Fig. 2.10; Table 2.5). A few females completed maturation and spawned early (22 mm body length, March 2000; Figs. 2.7, 2.9), while the majority completed maturation by October 2000 (minimum body length at maturation 29 mm). Thus, following a 5- to 6-month brooding period, the major release of juveniles by this cohort

occurred in April 2001. Within-cohort variation in density and biomass was high in C3 (cohort mean density = 191±300 ind. per 100 m^3; cohort mean biomass = 4.5±6.2 g DM per 100 m^3; Fig. 2.8).

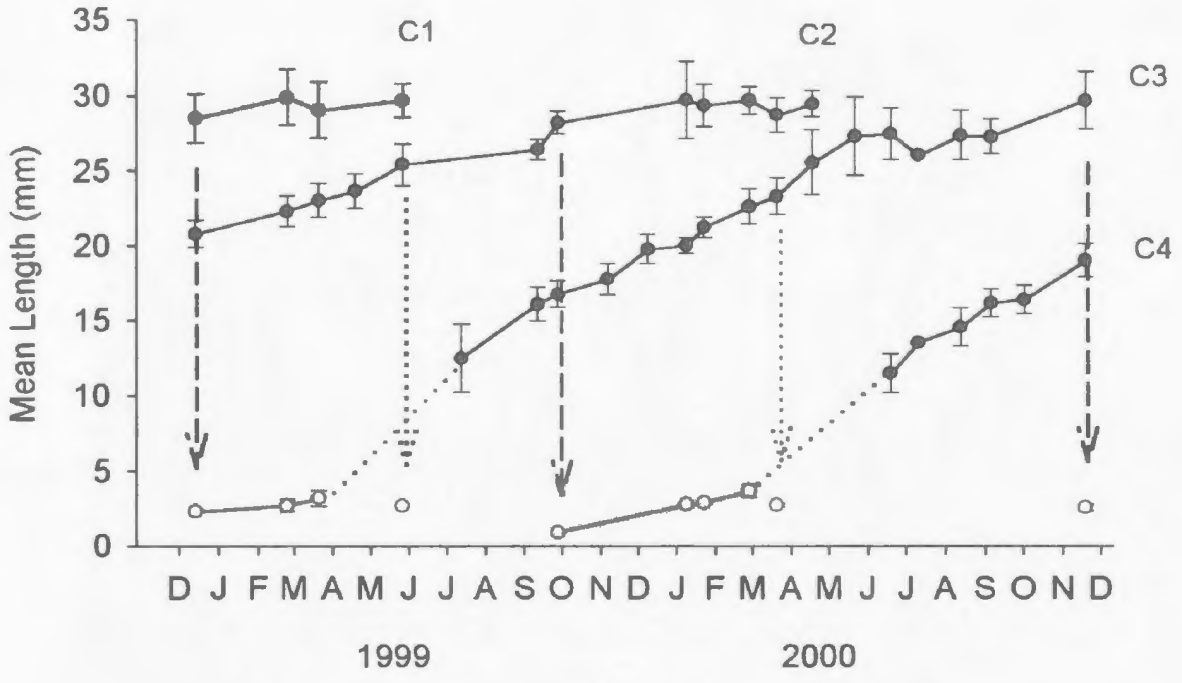

Fig. 2.9 *Mysis mixta.* Increases in body length (L_2, mm) in *C1, C2, C3* and *C4*. Data points <5 mm (*open circles*) represent larvae within marsupia. *Dashed arrows* indicate brood production by 2-year-old females. *Dotted arrows* indicate brood production by small, early spawners. *Dotted lines* represent growth approximations during the post-juvenile release period when juveniles were not collected by the epibenthic sled. *Error bars*: SD around the mean

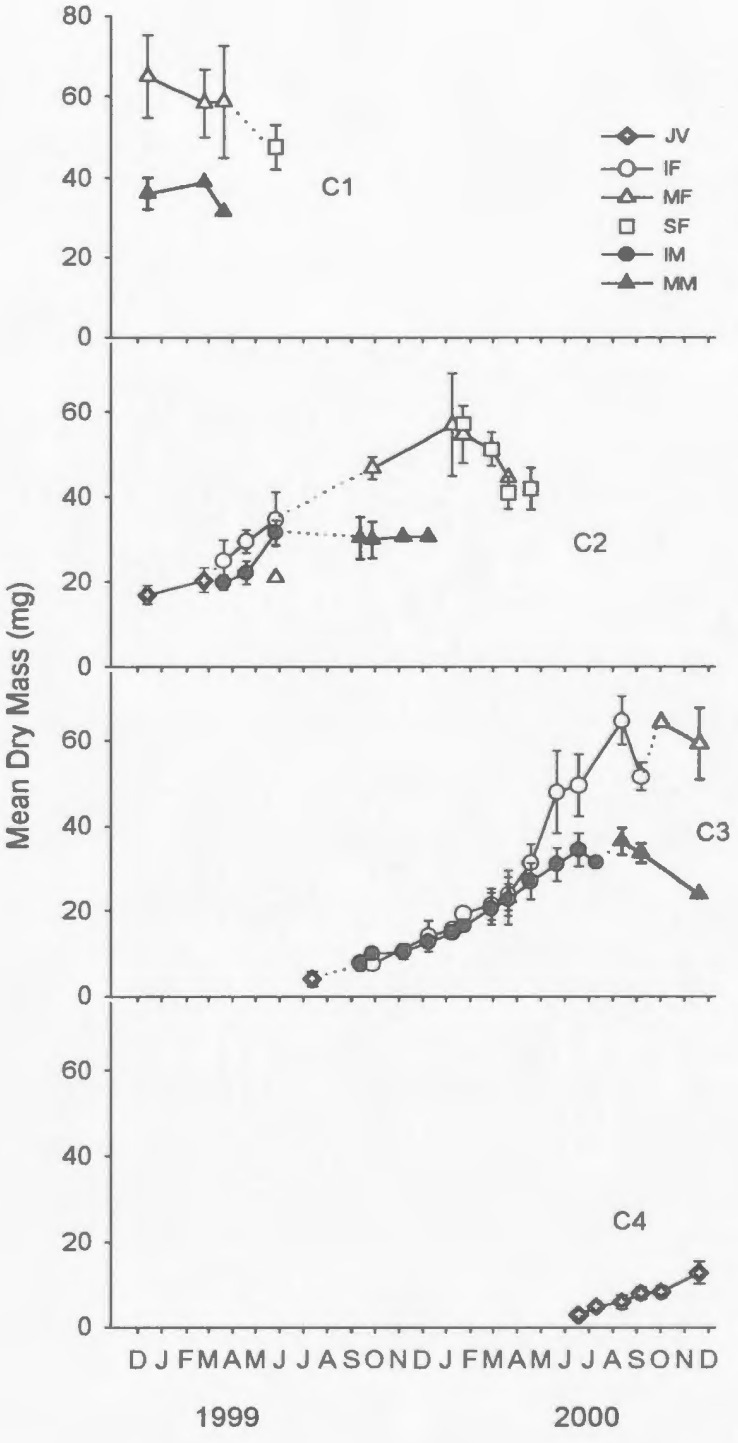

Fig. 2.10 *Mysis mixta.* Changes in dry mass (mg) in *C1, C2, C3* and *C4.* Dry mass of mature females includes their brooded embryos. *Dotted lines* represent transition periods between life-history stages. *Error bars*: SD around the mean

Table 2.4 *Mysis mixta*. Within-cohort (*CO*) changes in body length (L_2, mm; regression analyses of linear sections of untransformed data). Two rates are listed for *C2* and *C3* to reflect different growth periods (*JV* juveniles; *IM* immature males; *IF* immature females; *MM* mature males; *MF* mature females; *SF* spent females). Mysids exhibiting no significant growth ($p > 0.05$) were assigned a rate of 0.00. Significant results denote that growth rates (i.e. slopes) differ from 0.00, and are indicated in only one column but apply to both rates

CO	Stages	Time period	Growth (mm day^{-1})	Growth (mm month^{-1})	r^2	*n*
1	*MM;MF;SF*	Dec 98 – May 99	0.00	0.00	*n/a*	27
2	*JV;IM;MM;IF;MF*	Dec 98 – Jan 00	0.022	0.65****	0.85	111
2	*MF;SF*	Jan 00 – Apr 00	0.00	0.00	*n/a*	29
3	*JV;IM;MM;IF;MF*	July 99 –May 00	0.044	1.3****	0.87	142
3	*IM;MM;IF;MF*	May 00 – Nov 00	0.010	0.29**	0.09	82
4	*JV;IM;IF*	Jun 00 –Nov 00	0.047	1.4****	0.81	80

$^{**}p < 0.01$, $^{****}p < 0.0001$, *n/a* not applicable

C4, consisting of juvenile mysids released from C2 females in April 2000, first appeared in June 2000 and grew at 1.4 mm month^{-1} and 1.9 mg month^{-1} until the last sampling date in November (Figs. 2.7, 2.9, 2.10; Tables 2.4, 2.5). The highest density and biomass of mysids in C4 occurred in September and November, and variation between sampling dates was high (cohort mean density = 755±1201 ind. per 100 m^3; cohort mean biomass = 7.3±11 g DM per 100 m^3; Fig. 2.8).

The ratio of males to females in the population approximated 1:1 throughout the maturation periods. The sex ratio decreased after maturation and spawning, owing to differential mortality of males and females (males tended to die earlier than females).

2.4 Discussion

Smooth cohort transitions and distinct growth patterns provided strong evidence that a single population of *Mysis mixta* was continually sampled throughout this study. This stability may have been partially due to the restrictive sill, weak currents and low turnover rates of deep water masses in Conception Bay (deYoung & Sanderson 1995). Movement of mysids in and out of the bay via advection was thus considered to be negligible.

Table 2.5 *Mysis mixta*. Within-cohort changes in *DM* and organic carbon *(C)* (regression analyses of linear sections of untransformed data). Male and female stages within each cohort *(CO)* were analysed separately *(abbreviations as in Table 2.4)*. Mysids not exhibiting significant growth ($p > 0.05$) were assigned a rate of 0.00. Mass-specific growth rate (calculated as growth rate/mean DM; $month^{-1}$) is provided as a range using initial mean mass – final mean mass of the specified cohort and stages (when growth rate $\neq 0$). Significance is indicated in only one column but applies to all rates

CO	Stages	Time period	Growth (mg DM day^{-1})	Growth (mg DM $month^{-1}$)	Growth (mg C $month^{-1}$)	r^2	*n*	Mass-specific growth ($month^{-1}$)
1	*MF*	Dec 98 –Mar 99	0.00	0.00	0.00	*n a*	19	0.000
1	*MM*	Dec 98 – Mar 99	0.00	0.00	0.00	*n a*	4	0.000
2	*JV;IM;MM*	Dec 98 – May 99	0.071	2.1	0.95****	0.65	71	0.12 – 0.061
2	*IM;MM*	May 99 –Dec 99	0.00	0.00	0.00	*n a*	22	0.000
2	*JV;IF*	Dec 98 –May 99	0.092	2.8	1.3****	0.68	68	0.15 – 0.074
2	*MF*	Sep 99 –Mar 00	0.00	0.00	0.00	*n a*	22	0.000
3	*JV;IM*	July 99 – Jan 00	0.062	1.9	0.84****	0.90	48	0.53 – 0.11
3	*IM;MM*	Jan 00 – Jun 00	0.12	3.7	1.7****	0.73	53	0.25 – 0.12
3	*MM*	Aug 00 – Nov 00	-0.14	-4.3	-2.0****	0.55	23	-0.12 to -0.18
3	*JV;IF*	July 99 – Feb 00	0.076	2.3	1.0****	0.91	58	0.64 – 0.092
3	*IF*	Feb 00 – Aug 00	0.23	6.8	3.8****	0.68	56	0.25 – 0.11
3	*IF;MF*	Aug 00 – Nov 00	0.00	0.00	0.00	*n a*	16	0.000
4	*JV;IM; IF*	Jun 00 –Nov 00	0.064	1.9	0.86****	0.74	80	0.38 – 0.13

**** $p < 0.0001$, *n a* not applicable

M. mixta in Conception Bay falls within the broad category of cold water (0 – 7 C), semelparous epipelagic and coastal mysids (Wittmann 1984). Spawning once during a life cycle is considered advantageous in high latitudes, where the productive season is brief and temperatures remain low year-round. Spawning in this group typically occurs in autumn or winter, and juveniles are released in the spring when food is abundant. According to Wittmann (1984), females usually reach maturity within a year unless extreme arctic and or oligotrophic conditions limit growth, in which case newly released juveniles may require two summers to reach maturity (resulting in a 2-year generation time).

Although the environment in Conception Bay is not as extreme as that in the Arctic, the influence of the Labrador Current and the distinctly seasonal primary production cycle presumably partially determine the life cycle and the ~2-year life span exhibited by *M. mixta* in this region. Life cycle patterns in crustaceans are affected by seasonal variations in food availability (Gage & Tyler 1991). As *M. mixta* is an opportunistic omnivore, it is difficult to predict its seasonal diet in Conception Bay. At different times of the year, mysid stomach contents contained phytoplankton, fragments of small crustaceans, and unidentifiable detritus (qualitative analyses on random sampling dates). Owing to the uncertainty pertaining to seasonal diet and consumption, food quality for *M. mixta* in Conception Bay was estimated from primary productivity and seasonal chl *a* flux. Estimated primary production in Conception Bay ranged from 124 to 137 g C m^{-2} yr^{-1} between 1986 and 1990 (Tian et al. 2003), and estimated production during 1999 and 2000 is assumed to lay somewhere within this range. These values, which represent production for an entire year, include the high production periods or bloom events that are responsible for up to 75% of the annual flux of particulate organic carbon to the hyperbenthos (Redden 1994).

The phytoplankton blooms in the present study (Fig. 2.5C) represent periods of high food quality and availability for *M. mixta* in Conception Bay. Rapid sedimentation of the spring bloom began within 3 weeks of its appearance in 1999 and 2000. The absence of a thermocline and low abundance of zooplankton grazers in the water column allowed the bloom material to reach the benthos in a highly nutritious form (Redden 1994). Flux of particulate organic carbon to the Conception Bay hyperbenthos was 30 - 40% of

estimated primary production in 1988 (Redden 1994). Sub-zero water temperatures in late winter/early spring could decrease bacterial production, allowing benthic and hyperbenthic invertebrates access to high quality organic material (Pomeroy & Deibel 1986). A close relationship between the release of *M. mixta* juveniles and seasonal food availability is apparent using these measurements of chl *a* flux from surface waters.

M. mixta appears to exhibit life-cycle plasticity, similar to its congener *Mysis relicta* (Kjellberg et al. 1991), which has a 1- or 2-year life cycle depending on temperature, food availability and lake productivity (Beeton & Gannon 1991, Chess & Stanford 1998). Numerous transplantations of *M. relicta* among lakes with different environmental conditions have also resulted in rapid changes in life history and growth, which further supports a strong phenotypic component in the life cycle, even in stable and old (8,000 – 10,000 years) populations (Kjellberg et al. 1991). In addition to variations in temperature and food availability, differences in body size probably contribute to life-cycle variations among populations of *M. mixta* in different regions of the world. In general, smaller mysids in warmer waters have shorter life cycles (Mauchline 1980). Similarly, body length of females at maturity is greater in boreal and arctic mysid species than in temperate and tropical species (Wittmann 1984). Body size of *M. mixta* off the east coast of the United States was 5 to 25 mm (L_2, Wigley & Burns 1971), Cape Breton Island coastal mysids were 13 to 24 mm in length (Black 1956) and *M. mixta* living off the coast of Greenland grew as long as 31.2 mm (Hansen 1908 in Black 1956), with males generally smaller than females. Conception Bay mysids were at the larger end of the size spectrum (L_2 range 4 - 32 mm, DM range 0.9 - 69 mg, free-living stages only), whereas Baltic *M. mixta* attained very small maximum sizes (L_2 range 4 - 19 mm, DM range 0.09 - 10 mg; Hansson et al. 1990b) and exhibited correspondingly smaller mean brood sizes (37 embryos brood^{-1}, Shvetsova et al. 1992) than in Conception Bay (79 embryos brood^{-1}, present study) or New Hampshire waters (60 embryos brood^{-1}, Grabe & Hatch 1982). Sexual dimorphism in body length was not observed in Conception Bay mysids, although this population trait has been frequently noted in the mysid literature (e.g. Allen 1982) and may reflect different feeding rates in males and females of similar age (Roa & Ernst 1996). However, a much higher body mass was found in females than in males in

Conception Bay *M. mixta*, and is probably related to reproduction and possibly to lipid accumulation. Mauchline (1980) attributed such differences to the faster decay of growth correlates at successive moults in the male (i.e. growth rates start to decrease earlier and more rapidly in maturing males), or to early maturity in males.

As in Conception Bay, *M. mixta* in the Baltic region breeds in late autumn and males die shortly thereafter (Salemaa et al. 1986, Rudstam & Hansson 1990). However, in contrast to the predominantly biennial life cycle of Conception Bay mysids, *M. mixta* in the Baltic Sea (temperature range 3 – 11°C below the thermocline; primary production 135 - 184 g C m^{-2} yr^{-1} in 1984) generally exhibits an annual life cycle, with a minority of individuals surviving for 2 years (Rudstam et al. 1986, Salemaa et al. 1986). Females brood their young for 4 - 5 months, and juveniles are released in early spring through early summer (peak in March) (Rudstam et al. 1986, Salemaa et al. 1986, Rudstam & Hansson 1990). Most adults then die, and juveniles grow rapidly while food is abundant (Rudstam & Hansson 1990, Gorokhova 1998). In both Baltic Sea and Conception Bay populations, only one generation is produced per year.

The ratio of males to females was close to 1:1 throughout the development of any given *M. mixta* cohort in Conception Bay. This sex ratio did not change until mature males died after December, leaving mature females to dominate. Similarly, the sex ratio in winter *M. mixta* in the Baltic Sea (1984/1985) was 1:1, although fluctuations between 1:3 and 3:1 were found at different stations (Rudstam et al. 1986). Male to female ratios have been reported more disparate than 1:60 in *M. mixta* (Wigley & Burns 1971, Grabe & Hatch 1982), but such extreme ratios appear to occur only late in the life cycle owing to differential mortality.

In general, the body size and life span of *M. mixta* in Conception Bay are more similar to other North American and Greenland populations than to Baltic populations. This trend supports the statement of Mauchline (1980) that: "A species that normally matures to breed at an age of two years may mature and breed at an age of one year in warmer parts of its distribution; individuals of such populations may survive to an age of two years and breed a second time." Since *M. mixta* probably originated in the North Atlantic (Salemaa

et al. 1986), Conception Bay and Greenland mysids may reflect the normal maturation cycle and Baltic mysids the warmer climate maturation cycle.

Increases in body length of *M. mixta* from Conception Bay were greatest in maturing mysids from June to November of both sampling years (~1.4 mm month^{-1}). Variation in body length between males and females of the same age was low within each cohort, allowing pooling of data. Coefficients of variation (CV) of mysid body length at each sampling date were low within the population, including brooded embryos, and ranged from 2.6% in September C2 adults to 18.2% in the smallest C3 juveniles. Differences in mean DM between the sexes increased with age, resulting in significant differences in growth. The CV for mysid DM at each sampling date ranged from 5.7% in mature C2 females to 45.6% in the smallest C3 juveniles. Growth was greatest in maturing females between February and August 2000 (6.8 mg month^{-1}), whereas males tended to grow more slowly, reaching a DM maximum (41 mg) approximately half that of females (79 mg). The differences between body length growth and DM growth in this study demonstrate the potential loss of information when only one growth variable is measured. Determination of a body mass or fatness index can complement length measurements, revealing more details in growth patterns (Lehtonen & Andersin 1998). Mass-specific growth rates of *M. mixta* (range –0.18 to 0.64 month^{-1}) decreased as each cohort matured (Table 2.5). Similarly, estimated mass-specific growth rates of *M. mixta* in the Baltic Sea ranged from 0.42 month^{-1} in subadults to 0.96 month^{-1} in juveniles (Gorokhova 2002).

In general, variations in growth reflect changes in feeding rate, food quality, ambient temperature or metabolic demand. Since male and female mysids in Conception Bay inhabit the same environment, variable feeding rates and metabolic demands are the likely causes of growth differences between sexes. Temperature can be discounted as a causative factor because it remains stable year-round in the Conception Bay hyperbenthos and because mysids are unlikely to migrate to the uppermost zone of the water column, within 50 m of the surface, where temperatures begin to rise above 2°C (Fig. 2.5A). Higher growth rates in females may reflect higher feeding rates necessary to accumulate materials for egg production. Mysid growth also varies seasonally, generally remaining slightly lower in winter months. These seasonal fluctuations indicate that food availability

may regulate the growth, life cycle and population dynamics of *M. mixta* in Conception Bay. Hansson et al. (1990b) found *M. mixta* growth to be limited by food availability in the Baltic Sea. The mean growth rate of juvenile Baltic *M. mixta* in 1984/1985 was 3 mm $month^{-1}$ from June to October (Rudstam et al. 1986), with lower rates in winter. Antarctic mysids showed an even more pronounced decrease in growth rates in winter, as well as rapid growth in summer (Ward 1984). Similarly, juvenile *M. mixta* off the U.S. east coast increased in length at a rate of ~2 mm $month^{-1}$ until winter, when the growth rates decreased appreciably (Wigley & Burns 1971), and growth of females in the Greenland region was ~1 mm $month^{-1}$ during summer and fall, with lower rates in winter (Black 1956). Seasonal changes in growth in Conception Bay mysids were not as pronounced as in other zooplankton populations (e.g. Ward 1984), perhaps as a result of opportunistic feeding and/or seasonal lipid storage and utilisation.

Zero or negative growth in mature mysids reflects the maintenance or loss, respectively, of body mass at the end of the maturation cycle. The levelling out of growth was expected, because as mysids age metabolic losses tend to exceed energy gain (Gorokhova 1998). This energy trade-off, together with the costs of brooding offspring in females, results in less energy available for growth (Hopkins et al. 1984). Furthermore, brooding females do not moult, and thus the opportunity for growth is reduced even further (Beuchel & Lønne 2002). Growth is also reflected in *P/B* ratios; for example, *Mysis relicta* with a 1-year life cycle has a *P/B* ratio of 3, a 2-year population has a *P/B* ratio of 2, and a 4-year population has a *P/B* ratio of 0.5 (Kjellberg et al. 1991 and references therein). *P/B* ratios for Conception Bay *M. mixta* (1.6 and 1.2 in 1999 and 2000, respectively) fall between those of the 2- and 4-year *M. relicta* populations, and below that of Baltic *M. mixta* in 1985/86 (3.5, Shvetsova et al. 1992).

There are few possible explanations for the high degree of within-cohort density and biomass variation in *Mysis mixta* from Conception Bay. Variability in recruitment, mortality or predation are not likely explanations, since recruitment was not continuous, and more older individuals were often observed in a given cohort. It is more likely that mysids were actively swimming into and out of the sampling region between monthly tows. Aggregation and swarming are common traits of mysids (Mauchline 1980, Ritz et

al. 1997), and could readily cause the apparent population fluctuations in *M. mixta*. It has been postulated that *M. mixta* undergoes spawning migrations in some areas, with juveniles moving to deeper, cooler waters in late spring and returning to shallower depths to spawn in the following winter (Grabe & Hatch 1982). Conception Bay mysids may undergo similar spawning migrations, thereby effecting apparent population fluctuations. Higher coefficients of variation from three of the four replicate tow dates may also be explained by aggregation behaviour, although a low CV of 19.5% was obtained from the replicate tows with high densities of individuals in November 2000 (Fig. 2.8). The high degree of motility in mysids also increases the likelihood of their occurrence in the hyperbenthos above the sampling depth of the epibenthic sled, resulting in a significant portion of the population remaining consistently undersampled. Preliminary results in 1996 from hyperbenthic sampling with a larger, two-tiered sled indicated that *M. mixta* was collected more often in the upper net (152 cm above bottom) than the lower net (71 cm above bottom; Mumm et al. unpublished). Furthermore, population density values provided in this study are underestimates, since newly released juveniles were not sampled before they reached a minimum of 7 mm in length. The mesh size of the net (500 μm) was adequate to collect this size group, and copepods and other crustaceans of this size were collected throughout the study. Small, free-living juveniles may live higher within the hyperbenthos than larger mysids, an ontogenetic migration that has been noted in other species (Mauchline 1980). Newly released juveniles may be vulnerable to cannibalism from adults and predation from other hyperbenthic organisms unless they seek spatial or structural refuge (Johnston & Ritz 2001). The synchronous release of juveniles and the migration of juveniles higher into the water column should help to minimize predation pressures (Forward 1987). Differential migratory behaviour and distribution among life-history stages has been observed in other mysid populations, with juveniles commonly found at different depths than adults (Grabe & Hatch 1982, Fosså 1985). Several factors, including light, breeding behaviour, currents, prey distribution and abundance, and distribution of suspended organic material may influence the swimming behaviour that results in such differential distribution (Fosså 1985).

Density and biomass variations between years may result from variability in recruitment. The time when juveniles are first released is critical for semelparous breeders, for if high mortality occurs the population has no potential for further recruitment until the following year. Minor changes in offspring survival rate can alter the abundance of an entire cohort (Lehtonen & Andersin 1998 and references therein). Highly dense cohorts of *M. mixta* may benefit from low predation rates, high food availability or high food quality. For such a species with a biennial, semelparous life cycle, recovery of the population after a low production year will be slow.

Baltic mysids (*Mysis* spp.) occur at densities as high as 300 ind. m^{-3} (Kotta & Simm 1979 in Salemaa et al. 1986). *M. mixta* densities ranged from 22 to 73 ind. per 100 m^3 in open deep areas of the eastern and southeastern Baltic between 1979 and 1990 (Shvetsova et al. 1992), with average densities of 100 ind. per 100 m^3 in more northerly regions (Salemaa et al. 1986, Rudstam & Hansson 1990). Seasonal population fluctuations followed the reproductive cycle, with highest densities in late spring and early summer following recruitment (Rudstam et al. 1986). In the northern Baltic, winter is an unproductive time and the mysid population remains at a minimum (Salemaa et al. 1986, Rudstam & Hansson 1990). Annual mean densities of *M. mixta* in Conception Bay (242 ind. per 100 m^3 in 1998/99 and 544 ind. per 100 m^3 in 2000) exceed mean densities reported from the Baltic Sea.

Estimated secondary production of *M. mixta* in the Baltic during 1984-85 was 0.1 - 0.6 g C m^{-2} yr^{-1} (Rudstam et al. 1986), which is within range of estimates of *M. relicta* production in Lake Michigan (0.09 - 1.12 g C m^{-2} yr^{-1}, Sell 1982). The maximum production estimate of Baltic mysids represents up to 0.33% of annual primary production. Estimated production of *M. mixta* in Conception Bay, integrated over 1 m depth, was a maximum of 0.07 g C m^{-2} in 1999 and 0.21 g C m^{-2} in 2000, representing up to 0.15% of annual primary production. Comparing production among populations provides some indication of the role of a species in the transfer of organic material to other trophic levels. Unfortunately, secondary production estimates have only been determined in one other hyperbenthic invertebrate from Conception Bay, the amphipod *Acanthostepheia malmgreni* (Chapter 3). Further ecological information on the key

species and their trophic interactions within the food web will permit an improved assessment of the relative importance of any one species.

2.5 Summary

Plasticity in the life cycle, growth, motility and diet of *Mysis mixta* creates potential for this species to influence the energetics of other trophic levels within the Conception Bay hyperbenthos. Many of the features of *M. mixta* are typical of those exhibited by arctic, antarctic or boreal crustacean species and can be viewed as adaptations to a seasonally productive and permanently cold environment such as that created by the Labrador Current. Slow and seasonally variable growth rates, seasonal breeding and a lengthy brooding period are the typical responses of populations that are dependent on high primary production in the spring (Ward 1984). The extent of this dependence is not always clear, especially when considering highly motile and omnivorous feeders such as *M. mixta*. The information obtained on the life-history characteristics and population dynamics of *M. mixta* in Conception Bay provides a foundation for further research into the ecological structure of the hyperbenthos.

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Chapter 3. Population biology of *Acanthostepheia malmgreni* (Amphipoda)

3.1 Introduction

The hyperbenthic oedicerotid *Acanthostepheia malmgreni* (Amphipoda, Gammaridea) was sampled in Conception Bay, Newfoundland, for two consecutive years to determine its life-cycle and seasonal population dynamics. *A. malmgreni* is a circumpolar gammarid, with a distribution spanning the Arctic and North Atlantic Oceans, including the Kara, Barents, Bering, Labrador, White, Norwegian and Beaufort Seas, Hudson Bay and Baffin Bay (Shoemaker 1920, 1955, Stephensen 1938, Brandt 1996, 1997, Buhl-Mortensen 1996). In the Gulf of St. Lawrence (Brunel 1978, Sainte-Marie & Brunel 1985) and in Newfoundland coastal waters (Fenwick & Steele 1983, present study), *A. malmgreni* inhabits soft-bottom regions in water <0°C at depths of 10 - 550 m (Stephensen 1938, Shoemaker 1955, Fenwick & Steele 1983). Individuals reach body lengths up to 45 mm (Shoemaker 1955).

Like *Mysis mixta*, *A. malmgreni* is a thoracic brooder, allowing accurate determination of fecundity in brooding females. Many oedicerotid amphipods are detritus feeders that forage on fine-grained, smooth sediment bottoms (Chevrier et al. 1991). *A. malmgreni* is a planktivore that alters its diet at different stages of its life cycle. The adults are primarily carnivores, feeding on small zooplankton such as harpacticoid and calanoid copepods (Sainte-Marie & Brunel 1985, Chevrier et al. 1991), whereas juveniles feed on phytoplankton and/or detritus until they are large enough to capture and ingest zooplankton (Richoux, unpublished; the precise time in the life cycle when the amphipods become more carnivorous is unknown). The large eyes and prehensile gnathopods are probably adaptations for predation on mobile prey. *A. malmgreni* has been found in cod stomachs in the Gulf of St. Lawrence (Sainte-Marie & Brunel 1985), and may be an important component in the diet of other demersal fish and invertebrates. Although they possess a strong swimming ability and are capable of rapid bursts of movement (Sainte-Marie & Brunel 1985), adult *A. malmgreni* maintained in the laboratory for short periods tend to remain partially buried with the eyes and sections of

the dorsal surface protruding from the sediment (Richoux, unpublished). Such burying behaviour occurs in other Oedicerotidae (Beare & Moore 1998b), although *A. malmgreni* was classified by Sainte-Marie & Brunel (1985) as an upper suprabenthic species (defined as species that swim very intensively and regularly just off the bottom), primarily due to its exceptional swimming abilities. There is no information regarding diel vertical migrations by *A. malmgreni* into the pelagic realm of Conception Bay, although individuals commonly occur as far as 100 m off the bottom of upwelling regions in the Gulf of St. Lawrence (Sainte-Marie & Brunel 1985). Further study is necessary to determine the presence and extent of any migratory cycles in the Conception Bay population.

The ecology of *A. malmgreni* is poorly understood because most populations inhabit deep waters that are difficult to sample. This study provides the first detailed information on life-cycle dynamics, density, biomass and production of *A. malmgreni* in relation to seasonal nutrient flux to the hyperbenthos. Comparisons are made with other gammarids, in addition to the sympatric mysid *M. mixta* considered in Chapter 2. As population studies of hyperbenthic species are relatively rare, this study represents an important step towards understanding the organization and dynamics of cold ocean ecosystems.

3.2 Materials and methods

Detailed descriptions of the study site, environmental conditions and sampling methods are available in Chapter 2. Briefly, samples were collected approximately monthly from October 1998 to November 2000 with an epibenthic sled (mouth opening 0.3 m^2 and estimated sampling height 1 m above the seabed) at a site at 240-m depth in Conception Bay, Newfoundland.

Live *Acanthostepheia malmgreni* Goes were categorized into life-history stages (Table 3.1) and counted. Immature individuals (including some mature males) were grouped in a non-sexed category when there was insufficient time to process an entire sample (sex determination of stages other than juveniles and mature females required painstaking microscopic observation and was particularly difficult with preserved specimens). Larval length was the longest axis of egg-like stages and the terminal to frontal tip of hatched

stages (Fig. 3.1). For each free-living stage, the lengths of up to 12 straightened amphipods were measured to the nearest 0.5 mm with a millimetre scale under a stereomicroscope. Two different body-length measurements were recorded: L_1 is the frontal edge of the eyes (as the rostrum was curved) to the uropod tips (Fig. 3.2) and L_2 is the frontal edge of the eyes to the telson tip. L_2 was used for all morphometric relationships due to its prevalence in the amphipod literature, and an equation to convert L_2 to L_1 was derived. From June to November 2000, carbon and nitrogen were determined in 20 individuals (CHN analyser) and protein in 24 others. Methods for determining population structure, dry mass (DM), ash-free dry mass (AFDM), growth, secondary production and biochemical constituents, as well as statistical procedures, are described in Chapter 2.

Table 3.1 *Acanthostepheia malmgreni.* Demographic categories and approximate size ranges (free-living juveniles < 7 mm were not sampled due to a higher depth distribution). Body length = L_2, except for larval stages. Size gap between larval stages III and IV results from uncurling of the embryo once hatched

Life-history stage	Body length (mm)	Dry mass (mg)	Characteristics
Larval stage I	0.82 – 1.06	0.178 – 0.180	Undifferentiated early egg-like embryo within a membrane
Larval stage II	0.98 – 1.23	0.176 – 0.208	Body cleavage apparent in egg-like embryo
Larval stage III	1.16 – 1.28	0.169 – 0.180	Eyespots and thoracic appendages apparent within egg-like embryo
Larval stage IV	3.62 – 4.05	0.149 – 0.179	Embryo is hatched and appears as a miniature adult
Juvenile	7.0 – 23	1.7 – 61	No visible secondary sexual characteristics
Immature	14 – 35	29 – 164	*Female:* has developing oostegites
	17 – 24	23 – 62	*Male:* has developing penes
Mature female	24 – 32	92 – 149	Fully developed marsupium containing embryos
Spent female	28 – 32	74 – 123	Fully developed empty marsupium
Mature male	20 – 33	37 – 162	Well developed penes
Non-sexed	23 – 34	52 – 168	May include immature males, immature females, and/or mature males

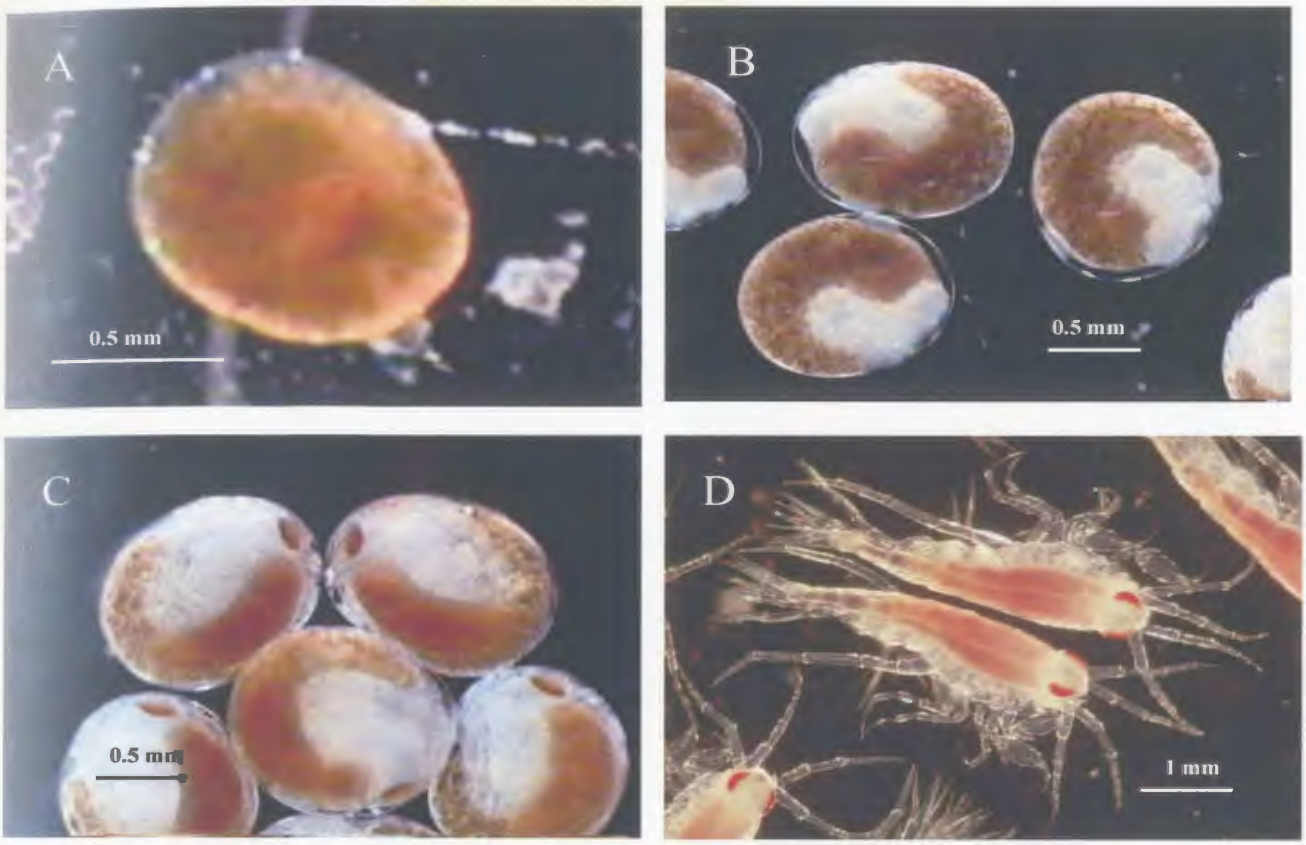

Fig. 3.1 A,B,C,D *Acanthostepheia malmgreni*. Larval stages **A** LI, **B** LII, **C** LIII and **D** LIV within the brood pouch of a female

Fig. 3.2 *Acanthostepheia malmgreni*. Length measurement (L_1) of a juvenile (specimens were straightened for actual measurements; telson is not visible)

3.3 Results and discussion

Four cohorts of *Acanthostepheia malmgreni* were identified during the sampling period (labelled C1 - C4; Figs. 3.3 - 3.7), with two cohorts coexisting during parts of 1999 and 2000. C1 females became sexually mature in December 1998 (mean size of mature females 28.8±1.7 mm), brooded their young for ~5 months, and released fully developed juveniles of ~4 mm body length in April and May 1999 (release indicated by the presence of spent females; Fig. 3.3). Brood release coincided with sedimentation of the spring phytoplankton bloom. Mature females numerically dominated C1 in February, March, and April 1999, whereas spent females dominated in May 1999. Mature C1 males and females disappeared from the hyperbenthos by July 1999, indicating that mature amphipods died shortly after brood release (a frequency histogram for July was not included in Fig. 3.3 because only one juvenile was collected). C2 amphipods first appeared as small juveniles in December 1998, and a few immature individuals were collected sporadically until November 1999 (Fig. 3.3). C3 amphipods, originally released from C1 females in April/May 1999, were not collected until they reached 8.2±1.4 mm (Figs. 3.3, 3.4, 3.5) in September 1999 (two September tows were not included in Fig. 3.3 due to very low amphipod densities). C3 juveniles developed into males and females from May to November 2000 (Fig 3.4; immature males and immature females were grouped together in the non-sexed category in all frequency histograms except July 2000). Sampling was terminated before C3 females completed maturation. C4 juveniles appeared in sled samples beginning in August 2000 (Fig. 3.4), and were probably produced by the few surviving C2 females in spring 2000.

Several oedicerotid amphipods have female-biased sex ratios (Beare & Moore 1998a), although this was observed only in mature *A. malmgreni* in Conception Bay (ratio of C1 males to females 0.14:1 in April, 0.05:1 in May; Fig. 3.3). In contrast, the male to female ratio in C3 was 1.8:1 in July 2000 (Fig. 3.4), when both life-history stages were developing sexual characteristics. The difficulty in easily distinguishing between immature males and immature females, and the resulting necessity to create a non-sexed category, may have obscured further instances of a female- or male-biased sex ratio.

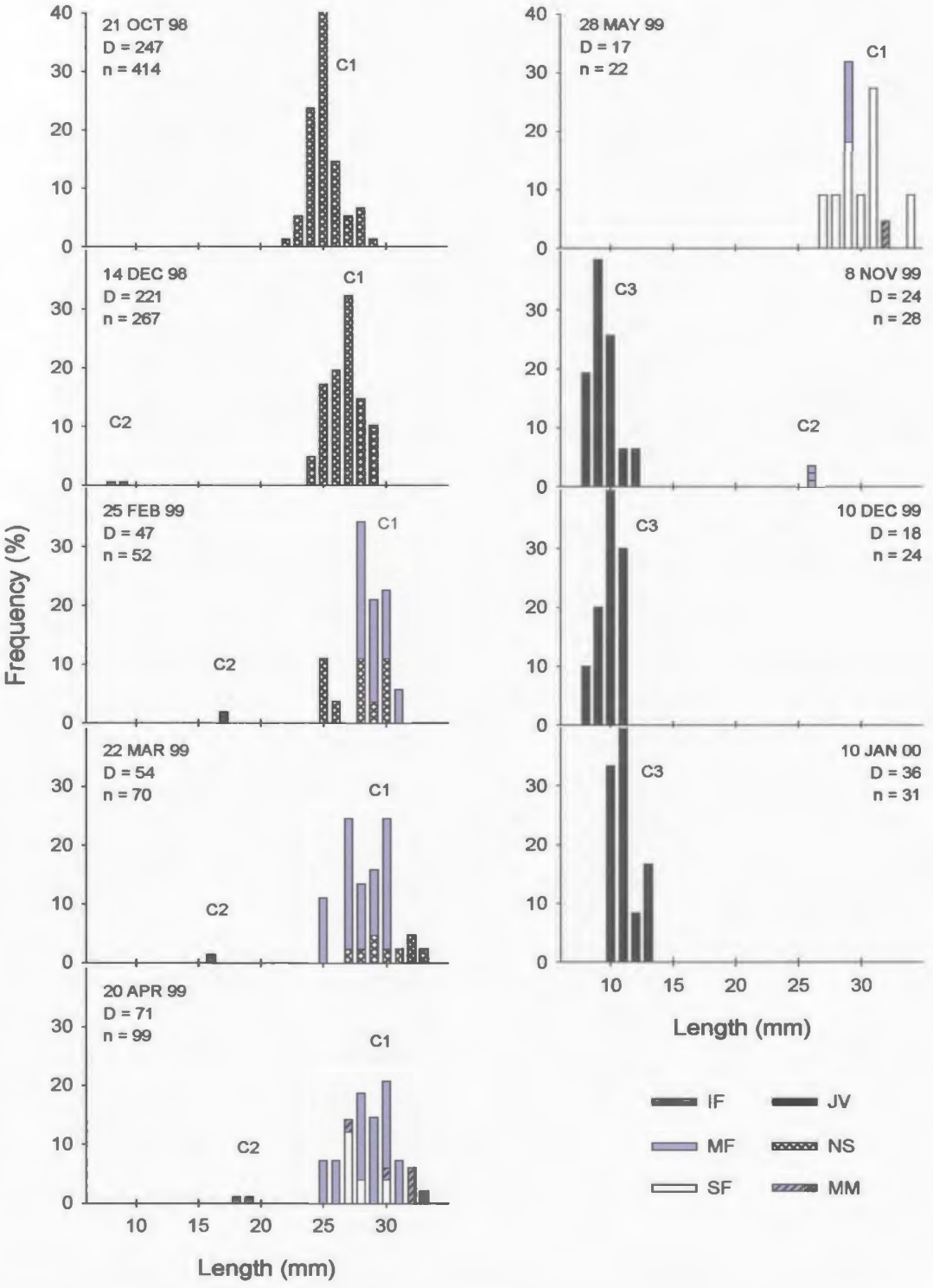

Fig. 3.3 *Acanthostepheia malmgreni.* Length-frequency histograms from 21 Oct 1998 through 10 Jan 2000 (plots for July and Sept 1999 were not included due to the low densities). Labels *C1, C2, C3* and *C4* are cohorts 1-4 [*JV* juveniles; *IM* immature males; *MM* mature males; *IF* immature females; *MF* mature females (brooding females only); *SF* spent females; *NS* non-sexed; *D* density (ind. per 100 m^3); *n* number collected].

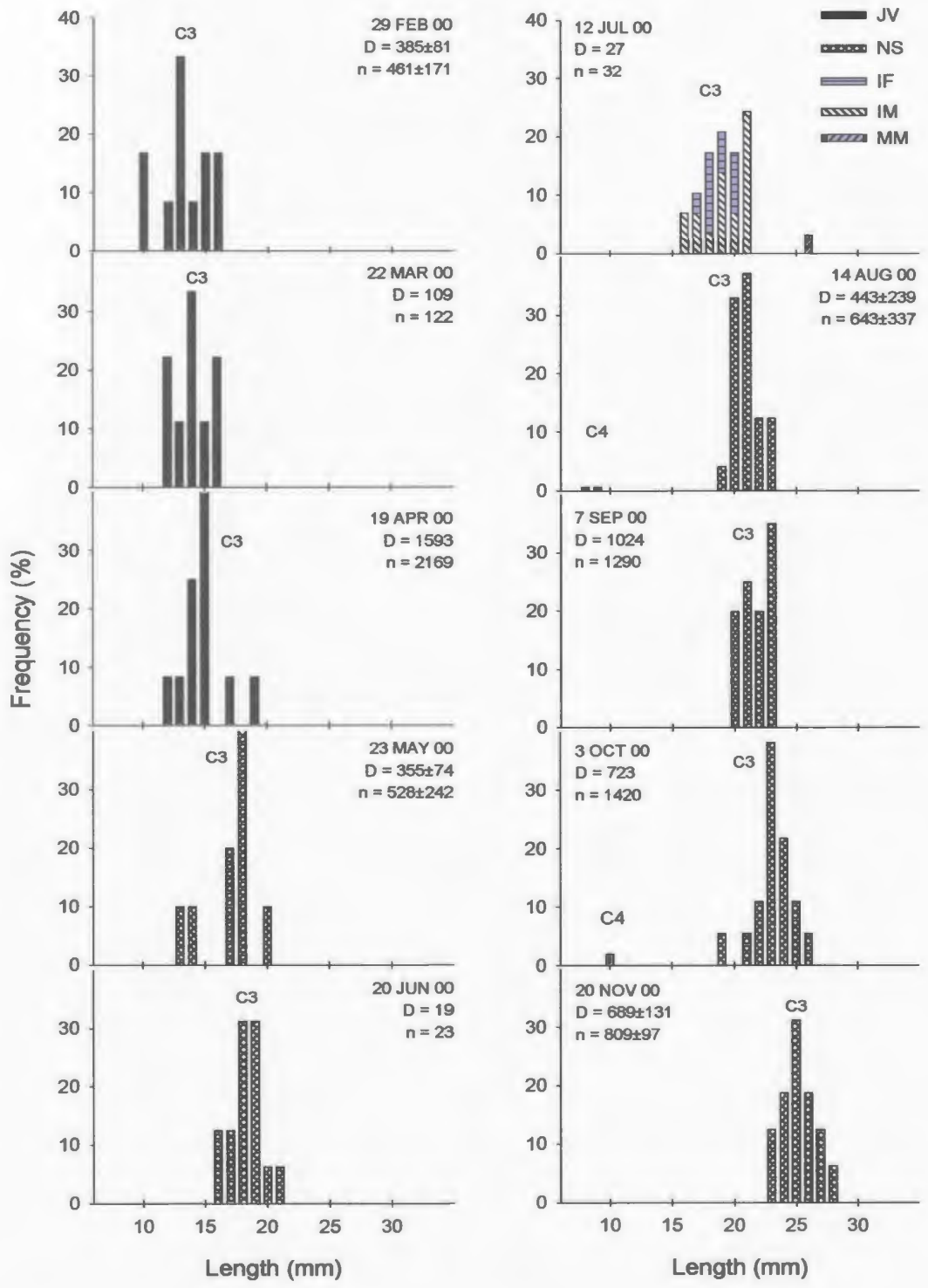

Fig. 3.4 *Acanthostepheia malmgreni.* Length-frequency histograms from 29 Feb through 20 Nov 2000 *(abbreviations as in Fig. 3.3)*. SD around mean density and mean number of amphipods collected in replicate tows in Feb, May, Aug and Nov 2000 are included

When information from all four cohorts was combined, the life span of *A. malmgreni* approximated 2.5 years, and females brooded once for a 5-month period before dying (i.e. were semelparous). Life span in gammarids ranges from only a few months to possibly >10 years (Sainte-Marie 1991). The life cycle exhibited by an amphipod population is largely influenced by reproductive events, which are in turn initiated and/or controlled by photoperiod, temperature or food availability (Beare & Moore 1996 and references therein). Even if *A. malmgreni* regularly migrated vertically, it is unlikely that it would have reached the warm water above 80 m depth (see Fig. 2.5, Chapter 2), therefore temperature effects on the life cycle were assumed to be negligible. The life cycle of *A. malmgreni* was remarkably similar to that of the sympatric mysid *Mysis mixta* (Chapter 2). This similarity between hyperbenthic crustaceans from different taxa emphasizes the influence of environmental factors on population dynamics. As in *M. mixta*, the life-history characteristics of *A. malmgreni*, including the 2+ year life span and the biennial reproductive cycle, are adapted to the high seasonality of primary production in Conception Bay. Tian et al. (2003) estimated primary production in Conception Bay to be 124 - 137 g C m^{-2} yr^{-1}, with most production occurring during the spring bloom. The flux of high-quality detritus in May of each year reached the hyperbenthos rapidly, thereby allowing newly released juvenile amphipods to take advantage of the rich nutrient source. Only when the juveniles grew larger later in the year were they able to capture and consume small prey. Steele & Steele (1975b) concluded that the most important adaptive feature of reproductive cycles in many amphipods is the regulation of juvenile release to coincide with optimal environmental conditions.

The timing of juvenile release is generally regulated by the start and finish times of resting stages in mature females, and by developmental times (Steele & Steele 1975b), which in turn are determined primarily by temperature and body size. Low water temperature and high primary productivity together may result in large amphipods at maturity (Chevrier et al. 1991). The smallest brooding individual of *A. malmgreni* in Conception Bay (25 mm) was similar in size to mature *A. malmgreni* in the Gulf of St. Lawrence (29 mm, Sainte-Marie & Brunel 1985). Gammarids tend to produce one generation per year in cold mesopelagic waters (with female minimum size at maturity

from 9 - 12 mm), whereas larger amphipods in Alaskan waters exhibit greater minimum sizes at maturity (16 - 21 mm) and generation times of 2 years (Yamada & Ikeda 2000). Steele & Steele (1975b) concluded that the large size of northern *Gammarus* spp. is an essential adaptation that allows their success in the Arctic in that it limits juvenile release to the short period of food abundance. High-latitude amphipods are generally characterized by univoltinism (producing one brood per year), large body size, delayed maturity, greater longevity, large embryos and few broods per life cycle (Sainte-Marie 1991). Unfortunately, the relative inaccessibility, longevity and slow growth of many polar invertebrates have resulted in few detailed studies on these cold-water species.

Brood size of *A. malmgreni* ranged from 142 to 339, with an overall mean (±SD) of 221±45 embryos brood^{-1}. All broods appeared intact, and embryo loss was not observed in advanced stages (ANOVA was used to determine whether the brood size differed among broods comprised of the 4 larval stages; $F = 2.179_{3, 24}$, $p = 0.117$). Size ranges of larval stages are listed in Table 3.1. Fecundity increased with female body length (all

Table 3.2 *Acanthostepheia malmgreni*. Relationships describing dry mass (*DM*, mg), carbon (*C*, mg), protein (*P*, mg), fecundity (*Fc*, embryos brood^{-1}) and L_1 (mm) as functions of body length (L_2, mm) (*a, b* regression parameters; r^2 coefficient of determination; *n* sample size; *Eq.* equation number; *JV* juveniles; *IM* immature males; *IF* immature females; *MM* mature males; *MF* mature females; *SF* spent females; *NS* non-sexed)

Model is to predict:	Stages included:	Equation	*a*	*b*	r^2	*n*	*Eq.*
Juveniles and non-sexed	*JV;NS*	$DM = a \times L_2^{\,b}$	0.00673	2.87****	0.97	109	3.1
Males	*JV;IM;MM*	$DM = a \times L_2^{\,b}$	0.00710	2.85****	0.97	81	3.2
Females	*JV;IF;MF*	$DM = a \times L_2^{\,b}$	0.00480	3.01****	0.98	81	3.3
Spent females	*JV;SF*	$DM = a \times L_2^{\,b}$	0.00955	2.73****	0.97	60	3.4
All life stages	*IM;IF;MM*	$C = a \times L_2^{\,b}$	0.00022	3.66****	0.95	20	3.5
All life stages	*JV;IM;IF;MM*	$P = a \times L_2^{\,b}$	0.00023	3.48****	0.75	24	3.6
Fecundity	*MF*	$Fc = b \times L_2 + a$	-83.5	10.4*	0.16	28	3.7
All life stages	*all*	$L_1 = b \times L_2 + a$	-0.116	1.23****	0.99	174	3.8

*$p < 0.05$, ****$p < 0.0001$

larval stages, Eq. 3.7, Table 3.2), and brood DM increased with fecundity: $Br_{DM} = 0.181 \times Fc - 0.340$, where Br_{DM} is brood DM (mg) and Fc is embryos brood^{-1} ($n = 12$, $r^2 = 0.87$, $p < 0.0001$).

As this study provides the first comprehensive information on the life-history and population dynamics of *A. malmgreni*, there is scant research from other regions for comparison. *Gammarus wilkitzkii* in the north-western Atlantic exhibits a similar life cycle to *A. malmgreni* in that females produce a single brood of large eggs in the autumn or winter, and juveniles are released in spring (Steele & Steele 1975a). The smallest *G. wilkitzkii* females carrying broods (96 – 236 embryos brood^{-1}) were 20 mm in length, and the mean length of early embryos was 0.73 mm (range 0.56 - 0.90 mm). Stage I larvae of *A. malmgreni* were similar in size (range 0.82 – 1.06 mm) to those of *G. wilkitzkii*, although brood size range was greater in the former (Table 3.1). Like *A. malmgreni*, *G. wilkitzkii* is a circumpolar amphipod that extends from the Arctic Ocean south to coastal Newfoundland. In contrast, *Monoporeia affinis* matured at a smaller size (9 mm) and averaged 26 embryos brood^{-1} in the warmer waters of the Baltic Sea (Sarvala 1986), although it exhibited a life span (2 years) similar to that of *A. malmgreni*.

Length-DM relationships were significantly different among life-history stages of *A. malmgreni* (ANCOVA: $\log DM = \beta_0 + \beta_1 \cdot sex + \beta_2 \cdot \log L_2 + \beta_3 \cdot sex \cdot \log L_2 + Error$; $F(\text{interaction term}) = 4.606_{1,158}$, $p < 0.05$; Eqs. 3.1 – 3.8, Table 3.2). Predictions of DM for juveniles and non-sexed individuals were obtained from Eq. 3.1, and predictions for males, females and spent females from Eqs. 3.2, 3.3 and 3.4, respectively. To allow comparisons with studies reporting L_1 values, Eq. 3.8 is provided for converting L_2 to L_1 (Table 3.2).

AFDM ranged from 53 to 72% DM (mean±SD, 65±7% DM). Carbon and protein as functions of body length are listed in Table 3.2 (Eqs. 3.5, 3.6). Carbon content was 36±3% DM, and protein content was 21±1% DM. Mean C:N ratio (by mass; all stages included) was 4.6±0.3. Carbon, nitrogen, protein and AFDM were determined primarily to provide a reference for comparative purposes in future studies.

The *A. malmgreni* population alternated in strength between years, with C1 and C3 representing the dominant cohorts, and C2 and C4 the subordinate (Fig. 3.6). This

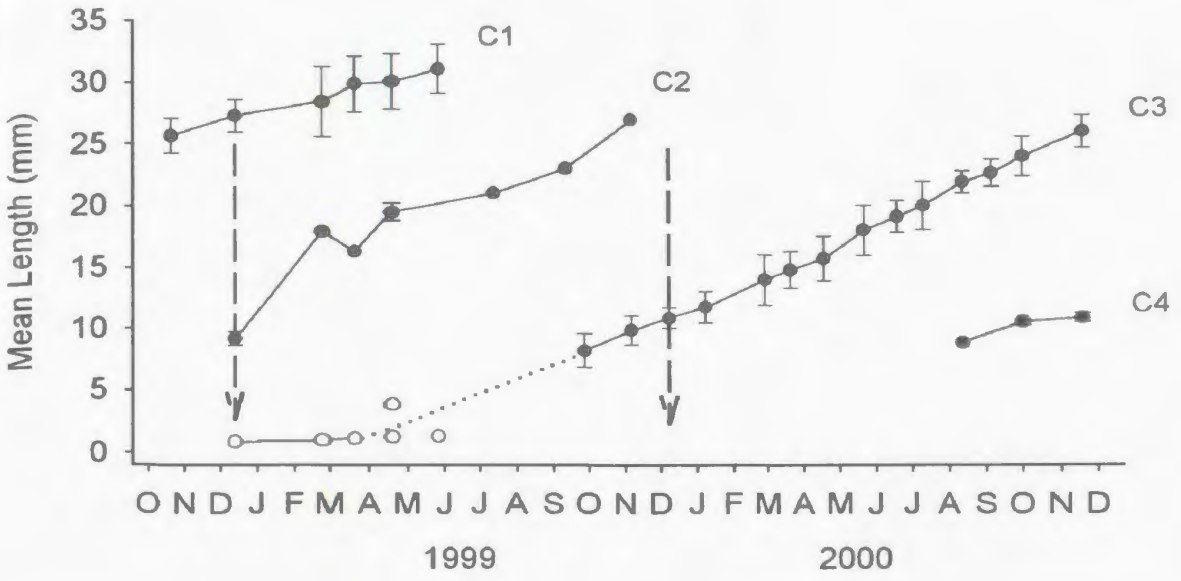

Fig. 3.5 *Acanthostepheia malmgreni.* Increases in body length (L_2, mm) in *C1, C2, C3* and *C4*. Data points <5 mm (*open circles*) represent larvae within marsupia. *Dashed arrows* indicate brood production by 2-year-old females (the *dashed arrow* from *C2* represents a theoretical spawning period since no brooding females were collected). The *dotted line* represents the growth approximation during the post-juvenile release period when juveniles were not collected by the epibenthic sled. *Error bars:* SD around the mean

alternation in cohort dominance resulted in higher amphipod density (490±490 ind. per 100 m^3), biomass (22±24 g DM per 100 m^3) and secondary production (180 – 310 mg C m^{-3}) in 2000 than in 1998/99 (density = 64±87 ind. per 100 m^3, biomass = 5.5±7.2 g DM per 100 m^3, and production = 18 – 44 mg C m^{-3}; Table 3.3). Throughout the study period, mean density of *A. malmgreni* ranged from 1.3 to 388 ind. per 100 m^3, which is comparable to the Gulf of St. Lawrence population (mean density 65 – 102 ind. per 100 m^3 between 1961 and 1969; Sainte-Marie & Brunel 1985). Alternating cohort dominance also coincided with large differences in the *P/B* ratio, with a much lower value in 1998/99 (0.89) than in 2000 (2.3). *P/B* ratios for temperate amphipod communities typically fall between 1 and 5 (Carrasco & Arcos 1984, Collie 1985), with semi-annual species exhibiting higher ratios than annual or biennial species (Collie 1985). *A. malmgreni* fell within the biennial category since the highest *P/B* ratio was <2.5 (Table 3.3). The

secondary production estimates and *P/B* ratios were similar to those for *Mysis mixta* in Conception Bay (Chapter 2), despite the disparity in body-mass maxima in the two species (*A. malmgreni* reaches 164 mg DM, *M. mixta* 79 mg DM). Body mass is the second most important variable in determining somatic production, population biomass being the most important (Tumbiolo & Downing 1994). In the case of the Conception Bay hyperbenthos, differences in diet among species, in addition to biomass, body-mass and reproductive variations, are likely to influence secondary production.

Table 3.3 *Acanthostepheia malmgreni.* Density, biomass and secondary production. [*C1, C2, C3, C4* cohorts 1 - 4; *D* mean density (ind. per 100 m^3, ±SD); *B* mean biomass (lower estimate, ±SD; g DM per 100 m^3); P_{DM} production (minimum estimate – maximum estimate; mg DM m^{-3}); P_C production (min. estimate – max. estimate; mg C m^{-3}); *P/B* ratio of annual production to mean annual biomass (using min. estimates of production and biomass)]

	C1	*C2*	*C3*	*C4*	1998/99	2000
D	110 ± 98	1.3 ± 0.61	390 ± 480	6.1 ± 7.1	64 ± 87	490 ± 490
B	9.9 ± 7.2	0.052 ± 0.043	17 ± 23	0.031 ± 0.042	5.5 ± 7.2	22 ± 24
P_{DM}	47 - 49	1.4 - 1.6	490 – 930	0.43 - 0.79	49 – 120	490 - 860
P_C	17 - 18	0.52 - 0.59	180 - 340	0.23 - 0.31	18 – 44	180 – 310
P/B					0.89	2.3

Several factors may affect the seasonal density of amphipods, including temperature, oxygen concentration, food availability, predation, disease, interspecific competition and horizontal migration (Segerstråle 1969, Sarvala 1986). Density and biomass variation among the cohorts was considerable (highest values in C3; Fig. 3.6; Table 3.3), and mortality, seasonal food availability and/or interspecific competition cannot be discounted as contributory factors. The highest density and biomass of C1 amphipods occurred in October and December 1998, when the population was composed of large individuals (most were placed in the non-sexed category in Fig. 3.3). Cohort density and biomass decreased steadily as C1 matured. C2 density and biomass were very low throughout 1999 (Fig. 3.6; Table 3.3), possibly due to low egg production, poor recruitment or high predation in the previous year. A natural high/low density cycle in alternating years may be a consistent feature of this species' life cycle in Conception Bay. The high overall

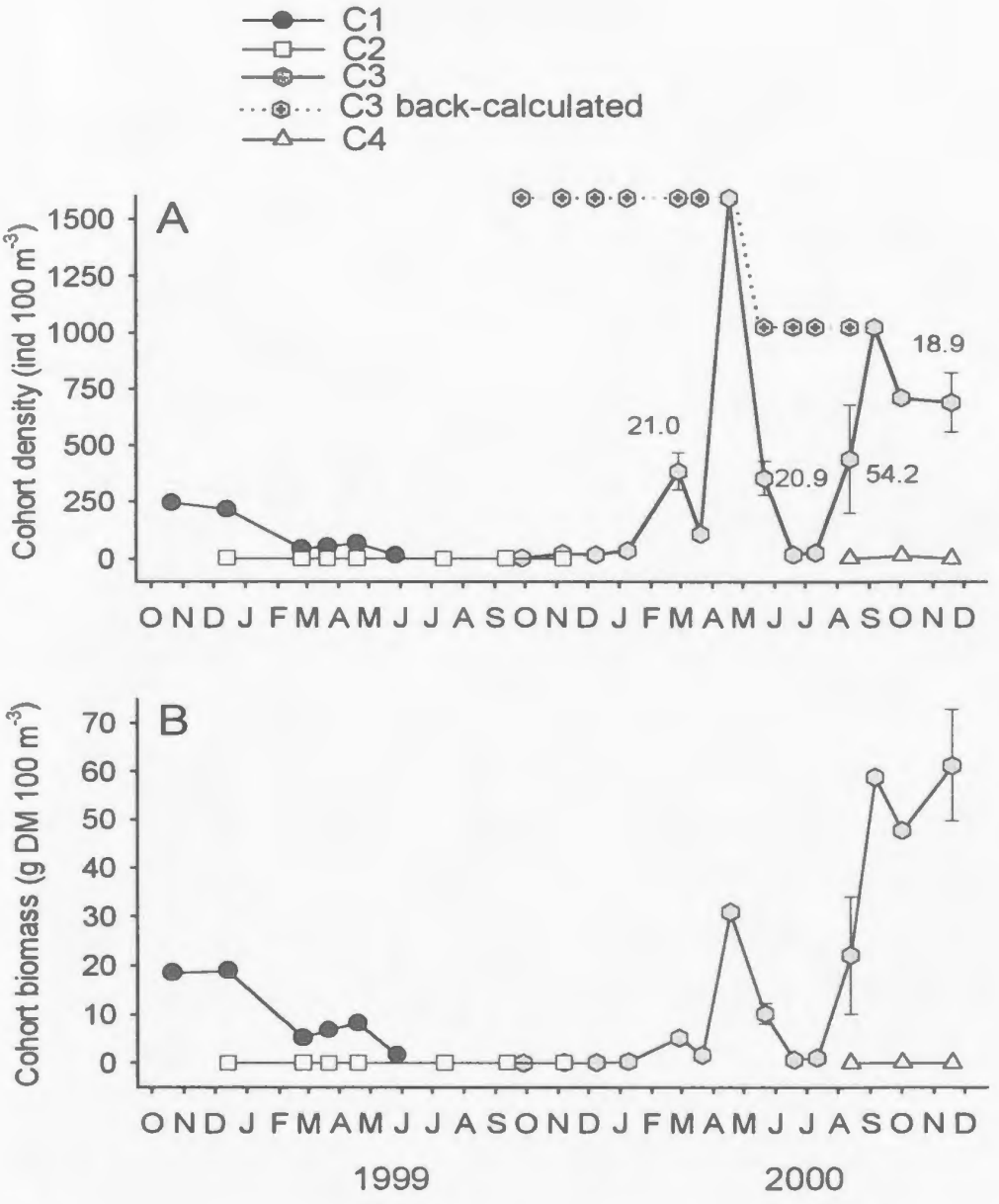

Fig. 3.6 A,B *Acanthostepheia malmgreni.* **A** Cohort density and **B** biomass (*C1, C2, C3, C4* cohorts 1 – 4). Coefficients of variation and error bars representing SD around mean density/biomass for dates having replicate tow data (Feb, May, Aug and Nov 2000) are included. Back-calculated abundance data points for *C3* are included in *panel A* to serve as an example of how back-calculations were done to create maximum production estimates listed in Table 3.3

density and biomass of C3 (Fig. 3.6) resulted from highly successful recruitment by C1. Owing to the low recruitment potential of the small cohort from which it originated, density, biomass and secondary production of C4 remained low until sampling ended in November (Fig. 3.6; Table 3.3). Density values for *A. malmgreni*, like those for *M. mixta*

from Conception Bay, were underestimates, since the youngest free-living juveniles were not sampled from their release at a length of 4 mm in May until they grew to 8 mm. Unlike the adults, small juvenile amphipods observed in the laboratory tended to swim continuously (Richoux, unpublished). Juveniles may have occurred slightly farther from the bottom than the adults, making them difficult to sample until they migrated closer to the benthos. Such behavioural disparity between adults and juveniles occurs in other oedicerotid species (Beare & Moore 1998a).

The fluctuations in within-cohort density and biomass of *A. malmgreni* (Fig. 3.6A; Table 3.3) may be attributable to horizontal movements of amphipods into and out of the sampling zone, in addition to the inherent limitations of sampling one site in the bay. Vertical migration is an improbable explanation because *A. malmgreni* presumably remained closely associated with the benthos during the day, when samples were taken (Brunel 1978). Low collection efficiency for *A. malmgreni* by the sampling net during population explosions of other hyperbenthic species may have contributed to within-cohort variation. Specifically, lower than expected densities of C3 in May, June, and July 2000 corresponded with extremely high densities of cnidarians, chaetognaths and or ctenophores in the hyperbenthic tows (qualitative data not shown). The high within-cohort variation of density and biomass in C1 may be explained by the natural mortality of amphipods at the end of their life cycle (Fig. 3.6). Because only one site in Conception Bay was studied (i.e. sediment-type remained consistent), it is unlikely that seasonal variation occurred in the efficiency with which the sled collected partially buried amphipods. Low coefficients of variation (CV range 18.9 - 21.0%) for cohort density were obtained from triplicate tows in February, May and November, although a slightly higher CV of 54.2% was recorded in August. The low CV values indicate that a single hyperbenthic tow provided a reasonable approximation of amphipod population densities (Rudstam et al. 1986), although without sampling over a wider area there is no measure of the scale-dependent variability in the population estimates.

The marked alternation in dominance of *A. malmgreni* cohorts from year to year clearly illustrates the necessity for ecological studies of this type to span at least 2 years. Periods of recruitment failure have been noted in other deep-water communities, some of

which are caused by biological rather than physical factors. For example, the bivalve *Macoma balthica* exhibited periodic recruitment failure in the Baltic Sea due to the influence of a co-existing amphipod, *Monoporeia affinis* (Segerstrale 1969). *M. affinis* populations, in turn, experienced cyclic fluctuations, possibly due to food shortages, high mortality and low recruitment followed by slow recovery (Sarvala 1986, Lehtonen & Andersin 1998). A similar situation may apply to the *A. malmgreni* population in Conception Bay. Recruitment in the progenitors of C2 and C4 may have decreased for a variety of reasons. For example, the fecundity of brooding females may have been low as a result of slow growth, or the survival of brooding females or newly released juveniles may have been low due to high predation pressure. Because *A. malmgreni* exhibits a 2-year semelparous life cycle, the recovery of the population after a low production year will be slow regardless of the original biological or physical cause(s) of the decrease in recruitment.

A. malmgreni clearly exhibited no sexual dimorphism in body length (Fig. 3.5). Although growth rates differed significantly between the sexes, DM was highly variable and did not exhibit distinctive patterns in the oldest amphipods collected (C1, Fig. 3.7). C3 amphipods did show some signs of sexual dimorphism, with the DM of females slightly greater than that of males. Absence of sexual dimorphism is unusual in gammarids, most species exhibiting some form of size difference between sexes (Beare & Moore 1998a, Yamada & Ikeda 2000). The only clear instance of dimorphism in the current study occurred between brooding and spent females, which is not sexual dimorphism in its strictest sense. Spent females exhibited significantly lower mean DM (93 mg) than brooding females (127 mg) of the same body length due to the release of broods (Fig. 3.7).

Overall, variation in DM at each sampling date was lowest in embryos and in the oldest free-living stages (minimum CV 4.0%), with highest variation in DM occurring in smallest C3 juveniles (maximum CV 46%). Variation in body length at each sampling date increased with maturity, with CV values ranging from 3.2% to 16%. Growth rates were also highly variable among life stages. Adult C1 amphipods grew in body length at 0.75 mm month^{-1} between October 1998 and May 1999 (Fig. 3.5, Table 3.4). Mature

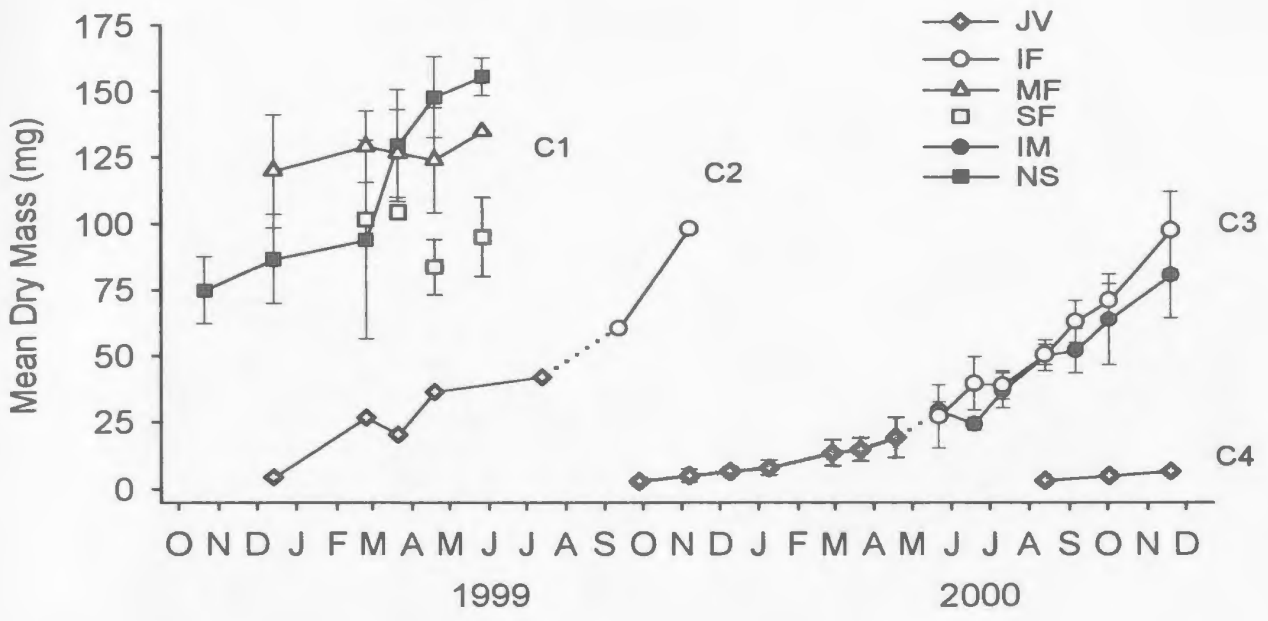

Fig. 3.7 *Acanthostepheia malmgreni.* Changes in dry mass (mg) in *C1, C2, C3* and *C4* (*JV* juveniles; *IM* immature males; *IF* immature females; *MF* mature females; *SF* spent females; *NS* non-sexed). Dry mass of mature females includes brooded embryos. *Dotted lines* represent transition periods between life-history stages. *Error bars:* SD around the mean

females exhibited no significant increase in DM, whereas non-sexed individuals and mature males grew at 10 mg month^{-1} (Fig. 3.7; Table 3.5). C2 amphipods grew at 1.3 mm month^{-1} and 7.5 mg month^{-1} in body length and DM, respectively (Figs. 3.5, 3.7; Tables 3.4, 3.5). The increase in body length within C3 was similar to the increase exhibited by C2 amphipods (1.3 mm month^{-1}; Fig. 3.5; Table 3.4). Growth in DM of juveniles was slow (2.6 mg month^{-1}) from September 1999 to April 2000, increasing to 9.6 and 12 mg month^{-1} in immature males and immature females, respectively, from May to November 2000 (Fig. 3.7; Table 3.5). C4 amphipods grew slowly, relative to the other three cohorts, at 0.55 mm month^{-1} and 1.1 mg month^{-1} (Figs. 3.5, 3.7; Tables 3.4, 3.5).

The slow growth in C1 amphipods was anticipated, since this cohort was composed of large adults nearing the end of their life cycle, but slow growth of the juveniles in C4 was unexpected and possibly reflected a combination of low food availability, low foraging

Table 3.4 *Acanthostepheia malmgreni*. Within-cohort *(CO)* increases in body length (L_2, mm; regression analyses of linear sections of untransformed data) (*JV* juveniles; *IM* immature males; *MM* mature males; *IF* immature females; *MF* mature females; *SF* spent females; *NS* non-sexed). Significance is indicated in only one column but applies to both rates

CO	Stages	Time period	Growth (mm day^{-1})	Growth (mm month^{-1})	r^2	*n*
1	*MF;SF;MM;NS*	Oct 98 – May 99	0.025	0.75****	0.57	185
2	*JV;IF*	Dec 98 – Nov 99	0.045	1.3**	0.89	7
3	*JV;IM;IF*	Sep 99 –Nov 00	0.044	1.3****	0.92	206
4	*JV*	Aug 00 –Nov 00	0.018	0.55*	0.76	7

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$

ability and high competition for food between August and the spring bloom in April. Presumably, growth rate of C4 individuals increased following the termination of sampling in November 2000. Of all the life-history stages of *A. malmgreni*, immature females probably have the best foraging capability, and the start of their rapid growth period coincided with spring bloom sedimentation in May. The combination of all these factors may have caused seasonal fluctuations in growth such as those commonly seen in other amphipod populations (Uitto & Sarvala 1991), but the marked alternation of dominant and subordinate cohorts in the *A. malmgreni* population prevented such patterns from being clearly discerned. Faster growth rates in summer are often attributed to increased water temperature and/or higher food abundance or quality, although temperature remained <0°C below 120 m in Conception Bay throughout the year and was therefore not considered an important growth determinant. Growth in the amphipod *Monoporeia affinis* in the Baltic Sea, for example, is controlled primarily by food availability (Lehtonen & Andersin 1998). In contrast, the oedicerotid *Monoculodes packardi* inhabiting deep waters near Scotland grows at similar rates in both summer and winter, possibly reflecting reduced seasonal variability at 190 m depth (Beare & Moore 1998b).

In *A. malmgreni*, growth in body length (range 0.5 - 1.3 mm month^{-1}) was similar to that exhibited by the sympatric mysid, *M. mixta* (range 0.3 - 1.4 mm month^{-1}; Chapter 2). Growth in DM, however, differed between these two peracarids (range 0 - 12 mg month^{-1}

in *A. malmgreni*, range from −4.3 to 6.8 mg month^{-1} in *M. mixta*). The mass-specific growth rate in *A. malmgreni* ranged from 0.076 to 1.7 month^{-1} (Table 3.5), with younger individuals from each cohort having the highest rates. The higher growth rates in DM attained by the amphipods were unexpected, since adult *M. mixta* are opportunistic omnivores that can take advantage of food from different trophic levels, whereas adult *A. malmgreni* appear limited to a carnivorous diet. As in the *M. mixta* population, the slightly higher growth rates of female *A. malmgreni* compared with other life-history stages may reflect higher consumption rates needed to reach a minimum size required for reproduction. If food consumption was not adequate to enable the amphipods to grow to a size at which they could store lipid at a certain threshold (e.g. ~ 20% DM), they may have been unable to reproduce (Hill et al. 1992). Detailed analyses of the lipid content and composition in the different stages may help to clarify the reproductive requirements in both species (Chapters 4, 5, 6).

3.4 Summary

Many of the population and life-cycle features of *A. malmgreni* in Conception Bay are typical adaptations to a seasonally productive cold-ocean environment. Conception Bay represents an ideal location for the study of the cold-water hyperbenthos due to its accessibility compared with the more remote polar regions. As in the sympatric *M. mixta*, the variable growth rates, seasonal reproduction and relatively slow development times seen in *A. malmgreni* are common in zooplankton living in highly seasonal environments (Ward 1984). There are remarkable similarities in life-cycle and population dynamics between the two species, despite taxonomic and biological differences. This study provides valuable information on the ecology of *A. malmgreni* in Conception Bay, especially concerning temporal variability in the population, laying a solid foundation for continuing population studies within the hyperbenthos and investigations on benthic-pelagic links within the ecosystem.

Table 3.5 *Acanthostepheia malmgreni*. Within-cohort increases in dry mass (DM) and organic carbon (C) (regression analyses of linear sections of untransformed data). Male and female stages within each cohort *(CO)* were analysed separately (*JV* juveniles; *IM* immature males; *MM* mature males; *IF* immature females; *MF* mature females; *NS* non-sexed). Amphipods exhibiting no significant increase ($p > 0.05$) were assigned a rate of 0.00. Mass-specific growth rate (calculated as growth rate/mean DM; month^{-1}) is provided as a range using initial mean mass – final mean mass of the life-history stages during the specified period (when growth rate $\neq 0$). Significance is indicated in only one column but applies to all rates

CO	Stages	Time period	Growth (mg DM day^{-1})	Growth (mg DM month^{-1})	Growth (mg C month^{-1})	r^2	*n*	Mass-specific growth (month^{-1})
1	*MF*	Dec 98 –May 99	0.00	0.00	0.00	*n/a*	29	0.00
1	*MM;NS*	Oct 98 – May 99	0.34	10	3.6****	0.55	143	0.14 – 0.065
2	*JV;IF*	Dec 98 – Nov 99	0.25	7.5	2.7***	0.90	7	1.7 – 0.076
3	*JV*	Sep 99 – Apr 00	0.087	2.6	0.94****	0.59	85	0.88 – 0.090
3	*IF*	May 00 – Nov 00	0.39	12	4.3****	0.84	55	0.44 – 0.12
3	*IM*	May 00 – Nov 00	0.32	9.6	3.5****	0.69	63	0.33 – 0.12
4	*JV*	Aug 00 –Nov 00	0.035	1.1	0.38*	0.78	6	0.32 – 0.16

$^{*}p < 0.05$, $^{***}p < 0.001$, $^{****}p < 0.0001$, *n/a* not applicable

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Chapter 4. Seasonal changes in the lipids of *Mysis mixta*

4.1 Introduction

A knowledge of the lipid content and composition of zooplankton is essential for understanding the importance of lipids to growth and reproduction and the energetic role of zooplankton within marine foodod webs. Conception v. Newfoundland, is an ideal site for the study of cold-water hyperbenthic species owing to the accessibility of the bay and the year-round influence of an inshore branch of the Labrador Current. The life-cycle, growth and reproductive features of some hyperbenthic animals in this region are more typical of polar than temperate populations (Chapters 2, 3, Choe & Deibel 2000). The seasonally productive and perpetually cold environment created by the Labrador Current undoubtedly influences the lipid dynamics of populations in this region.

Mysis mixta exhibits a 2.5-year, semelparous life cycle in the hyperbenthos of Conception Bay (Chapter 2). Two-year-old adults spawn in late autumn, the females carry broods during the winter, and brood release occurs in spring. Mature males disappear from the hyperbenthos earlier than females, presumably because they die following the spawning season (Chapter 2). Juveniles feed on phytoplankton and other small particles (Chapter 2), whereas developing and mature individuals consume rotifers, cladocerans, tintinnids and copepods in addition to detritus and phytoplankton (*M. mixta* from the Baltic Sea; Rudstam et al. 1989, Hansson et al. 1990). Nightly migrations into the water column increase the variety of prey available to these mysids and create a direct trophic path between the hyperbenthic and pelagic regions.

In Conception Bay, most sedimentation occurs following the spring phytoplankton bloom, which provides the benthos with large influxes of fresh particulate organic matter (Redden 1994). During the remainder of the year, sedimentation input is relatively small excepting a secondary pulse in the fall (Tian et al. 2003). This seasonal pattern of primary production and sedimentation creates variation in the availability of high quality food for most benthic and hyperbenthic organisms throughout the year. How much of the material originating during the spring bloom is actually consumed by hyperbenthic organisms, and in what form, remains unclear. Different species are likely to take advantage of this annual influx in different ways, depending on life histories, modes of nutrition and

storage abilities. Of 19 hyperbenthic and benthic macroinvertebrate species in Conception Bay studied during three separate years, *M. mixta* exhibited the second greatest mean lipid level (% wet weight) and the highest proportion of neutral lipid (% total lipid) during and following the spring bloom (Parrish et al. unpublished).

The purpose of this research was to investigate the role of lipids in the growth and life cycle of *M. mixta*, and to establish a trophic connection between the pelagic and benthic regions of a cold-water bay. I hypothesized that lipid content would vary ontogenetically as the mysids matured and reproduced, and that lipid levels in individuals would increase following the spring bloom as a result of the increased availability and quality of food. In addition, areal concentrations of lipid were calculated to determine the degree to which the population accumulates energy from the environment and the quality of the mysids as food for predators. Together with previously reported population dynamics (Chapter 2), the seasonal lipid data augments our knowledge of the ecological and energetic role of hyperbenthic organisms in a cold-ocean ecosystem.

4.2 Materials and methods

4.2.1 Study site and sample collection

Samples of *Mysis mixta* were collected approximately monthly (December 1998 – November 2000) from the depositional zone at 240-m in Conception Bay, Newfoundland. The large bay is 100 km x 30 km wide at the mouth, with a maximum depth of 300 m in the central basin (deYoung et al. 1993). The Labrador Current supplies Conception Bay with deep water ~0°C throughout the year. Detailed sampling methods for both the hyperbenthos and the water column (physical data) are described elsewhere (Chapter 2). Briefly, sampling was done during daytime from a 13-metre boat equipped with an epibenthic sled (mouth area 0.3 m^2), with tows commencing at 47°30.5' N, 53°07.5' W and ending near 47°32.5' N, 53°07.0' W. Organisms on the seafloor and those living within 60 cm of the bottom were collected with a 500-µm mesh net tapered to a closed cod end. Tows lasted 20 to 30 minutes, with the tow distance ranging from 620 to 930 m.

4.2.2 Specimens

Live, depurated mysids were categorized into life-history stages (Table 4.1), and up to

5 straightened, free-living individuals per life stage (depending on availability) were selected randomly and measured to the nearest 0.5 mm under a stereomicroscope (body length measured from the rostrum tip to the telson tip). Larvae were removed from the brood sacs and counted, although each brood was processed as a unit. Mysids and whole broods were rinsed with 1-μm filtered seawater and stored in chloroform under nitrogen at –20°C. Dry mass (DM) of each mysid was calculated from its body length using one of several equations (depending on life-history stage), and DM of whole broods was predicted from brood size (Table 4.2). Calculated DM of each brood was added to the DM of the corresponding brooding female.

Table 4.1 *Mysis mixta*. Life-history stages and approximate size ranges of mysids and broods (free-living juveniles <7 mm were not sampled because they appeared to occur at a different depth)

Life-history stage	Body length (mm) or brood size (embryos brood^{-1})	Dry mass (mg)	Characteristics
Embryos (whole broods)	22 - 111	3.3 - 29	Embryos are contained within a brood sac
Juveniles	7.0 - 15	0.40 – 6.3	No visible sexual characteristics
Immatures	14 - 30	6.1 - 69	*Female:* has developing oostegites
	14 - 30	4.2 - 41	*Male:* has developing penes
Mature females	21 - 32	19 - 79	Fully developed brood sac containing embryos
Spent females	25 - 32	36 - 57	Fully developed brood sac (empty)
Mature males	24 - 27	23 - 41	Well developed penes, and 4th pleopods extend beyond the uropods

Table 4.2 *Mysis mixta*. Equations to calculate dry mass (*DM*, mg) of mysids from body length (*L*, mm) and brood DM (mg) from brood size (*Fc*; embryos brood^{-1}) (Chapter 2)

Eq.	Life-history stage	Equation	r^2	*n*
4.1	Juveniles	$DM = 0.00162 \times L^{3.05}$	0.97	187
4.2	Males (immature and mature)	$DM = 0.00259 \times L^{2.89}$	0.98	102
4.3	Females (immature and mature)	$DM = 0.00140 \times L^{3.11}$	0.98	124
4.4	Spent females	$DM = 0.00282 \times L^{2.86}$	0.99	53
4.5	Broods	$DM = 0.190 \times Fc - 0.137$	0.50	21

4.2.3 Lipid analyses

Lipids were extracted from each sample using a modified Folch procedure (Parrish 1999). Two or three very small juveniles were pooled on a few occasions to obtain an adequate signal. Samples were ground in 2:1 (v/v) chloroform/methanol, 0.5 ml of chloroform-cleaned water added, and the lipid layers removed and combined following each of three chloroform washes. Lipid classes of concentrated extracts were separated by thin-layer chromatography (TLC) on silica gel-coated rods (Chromarods-SIII) and quantified by flame-ionisation detection (FID) using an Iatroscan MK V (Parrish 1987). Up to 11 lipid classes were identified and quantified by comparison with FID responses for the following standards (the lipid class represented follows each compound): n-nonadecane [hydrocarbon (HC)], cholesteryl palmitate [steryl ester (SE)], 3-hexdecanone [ketone (KET)], tripalmitin [triacylglycerol (TAG)], palmitic acid [free fatty acid (FFA)], 1-hexadecanol [alcohol (ALC)], cholesterol [sterol (ST)], 1-monopalmitoyl-rac-glycerol [acetone-mobile polar lipid (AMPL)] and dipalmitoyl DL-α-phosphatidylcholine [phospholipid (PL)] (Sigma-Aldrich Canada Ltd). Methyl esters (ME) were identified by their position between steryl esters/wax esters (SE/WE not separable with this method) and KET, diacylglycerols (DG) were identified by their position following ST, and non-lipid material remained at the origin. ME and DG were quantified using the KET and AMPL calibration equations, respectively. Frequent peak splitting in the TAG and FFA regions made these classes difficult to differentiate. When peak splitting occurred, additional developments using alternative solvent systems separated more saturated from polyunsaturated FFA and TAG (Parrish 1999). These four lipid molecular species were identified by comparison with the standards palmitic acid (C16:0), docosahexaenoic acid (C22:6), tripalmitin (3×C16:0) and triarachidonin (3×C20:4). The TAG to FFA ratio was then applied to the pooled TAG + FFA fraction quantified by conventional TLC-FID. Total lipid (TL) was determined by summing all lipid classes. A lipolysis index [LI (%) ((free fatty acids + alcohols) / (total acyl lipids + alcohols)) × 100, Parrish et al. 1995] was calculated from all samples to assess sample degradation during storage and processing.

4.2.4 Areal concentrations

Following Arts et al. (1992), areal concentrations of reserve (TAG) and total lipid (mg m^{-2}) were estimated from the products of TAG or TL mysid^{-1} and the population density of *Mysis mixta* in the hyperbenthos on each sampling date. Adjusted (i.e. back-calculated) density values for each life-history stage within a cohort were used (Chapter 2) because within-cohort density in later samples was frequently higher than that in earlier samples. Densities, and thus areal lipid concentrations, were under-estimates of the true population values since free-living juveniles (length 4 mm at release) were not collected until they reached 7 mm (Chapter 2).

4.2.5 Statistical analyses

Within-cohort TAG, PL and TL accumulation or utilisation rates were determined from slopes in linear sections of regressions of lipid content per mysid (least squares regression; linear sections were divided according to pre- and post-bloom start periods). The Durbin-Watson statistic was calculated to detect temporal autocorrelation in the regressions, and the Cochrane-Orcutt procedure was used to remove any autocorrelations (Neter et al. 1996). Analysis of covariance (ANCOVA) was used to determine if rates of lipid accumulation or utilisation differed between sexes within a cohort. Changes in minor lipid classes with development, particularly in cohorts 2 and 3, were determined using regressions (spent females were excluded from analyses). Data are reported as means ± one standard deviation (SD, a measure of subsampling and analytical variance).

4.3 Results

4.3.1 Total lipids

Seasonal life cycle, density, biomass and growth of *Mysis mixta* in Conception Bay have been described elsewhere (Chapter 2). Four discrete cohorts (C1, C2, C3 and C4) were identified during the 2-year sampling period (Fig. 4.1). Mysids from C2 collected between December 1998 and February 1999 were not sexed and were pooled in an 'undifferentiated immatures' category. Mysids from C1, C3 and C4 were adequately staged throughout development. Overall, increases in TL mysid^{-1} (mg) with DM (mg) were not significantly different in males and females (ANCOVA, $F = 1.087_{1,136}$, $p > 0.05$,

relationship below was derived using juveniles, immature females, immature males, mature females and mature males pooled from all 4 cohorts):

$$TL = 0.0700 \times DM^{1.218} \qquad (r^2 = 0.85, n = 178, p < 0.0001) \qquad \text{Eq. 4.6}$$

Seasonal changes in lipid content (TL mysid^{-1}) were similar to changes in TL levels (% DM; Fig. 4.2). Mature females in December 1998 contained the highest TL of individuals from C1 (29% DM). Lipids in mature females decreased at a rate of 2.5 mg month^{-1} until broods were released, leaving spent females with a mean lipid level of 15% DM in May 1999 (Table 4.3; Fig. 4.2). Mature males in C1 had low TL (5% DM) between December 1998 and March 1999, and thereafter disappeared from the samples (Table 4.3; Fig. 4.2).

Total lipid in C2 immature mysids collected between December 1998 and March 1999 remained relatively constant, whereas TL increased at a rate of 3.0 mg month^{-1} in immature individuals between March and May 1999 (Table 4.3; Fig. 4.2). The highest lipid level in C2 mysids was recorded in immature females and immature males in May 1999, and in mature females in September 1999 (28% DM; Fig. 4.2). In the predominant group of mature females, TL decreased at a rate of 1.1 mg month^{-1} from September 1999 to March 2000, leaving spent females with low TL (6% DM) in April 2000. Lipid levels in post-spawned males during September 1999 were very low relative to mature females (Fig. 4.2; a lipid utilisation rate was not calculated owing to the dearth of male samples between May and September 1999), and the few other mature males collected between September and December 1999 were damaged, excluding the possibility of lipid analyses. An early-spawning female collected in May 1999 had a very low TL level of 14% DM (Fig. 4.2).

In July 1999, C3 juveniles had a TL level of 9% DM (Fig. 4.2). Immature males and females accumulated lipid at the same rate until March 2000 (0.30 mg month^{-1}; ANCOVA, F(interaction term) = $0.127_{1,48}$, $p > 0.05$; Table 4.3). From March to September 2000, TL accumulation increased to 2 3 mg month^{-1} in the immature mysids, resulting in a maximum mean lipid level (32% DM) in immature females during September 2000. An early-spawning female in March 2000 had a TL level of 8% DM. Total lipid in immature males peaked at 26% DM in June, after which TL (mg mysid^{-1}) remained constant until September 2000 (Table 4.3; Fig 4.2). Total lipid could not be

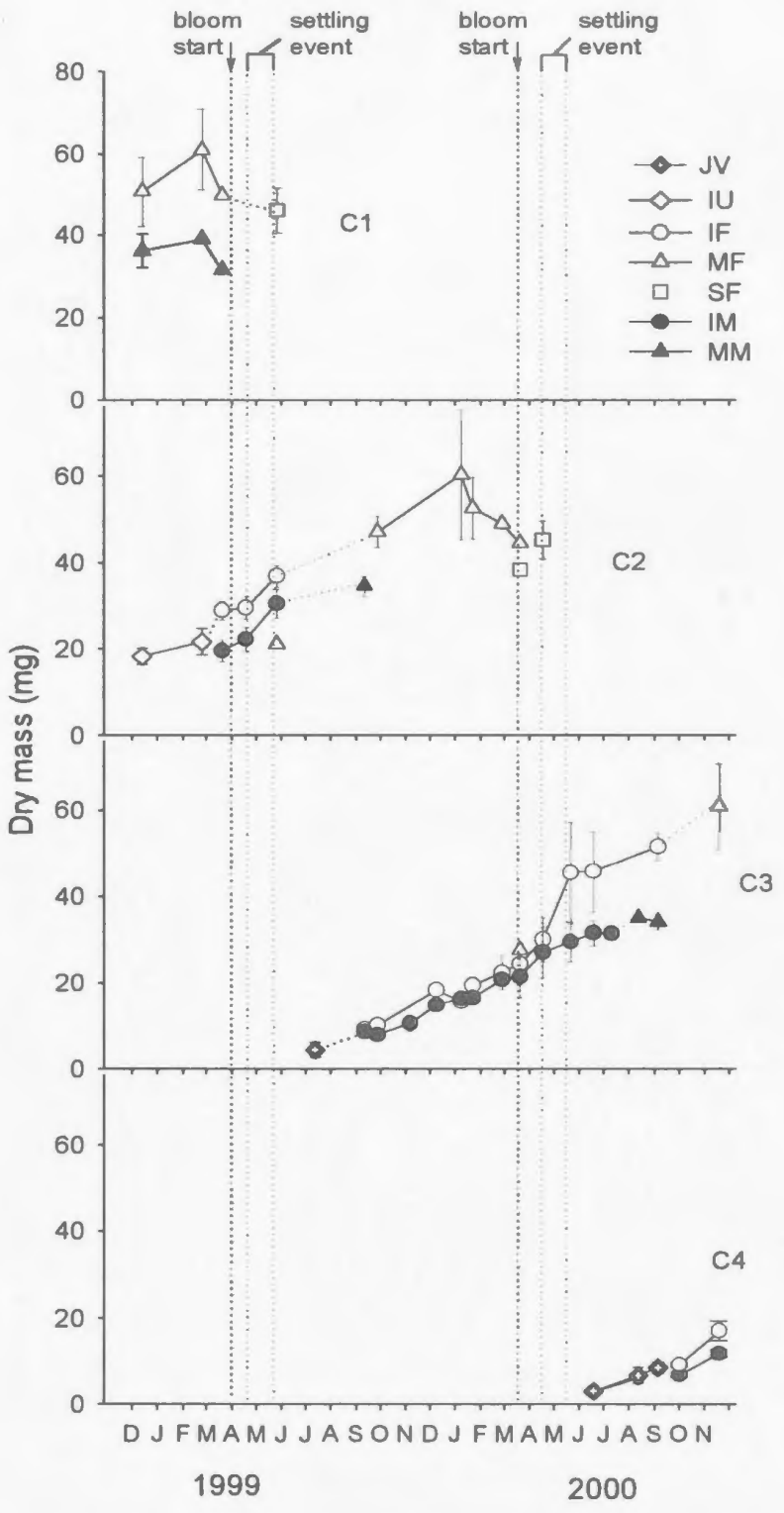

Fig. 4.1 *Mysis mixta.* Calculated dry mass of lipid samples. *C1, C2, C3* and *C4* cohorts 1–4 [*JV* juveniles, *IU* immatures undifferentiated, *IF* immature females, *MF* mature females (includes brooded embryos), *SF* spent females, *IM* immature males, *MM* mature males]. *Dotted lines* within a cohort represent transition periods between stages; *vertical dotted lines* represent bloom start and settling times (Fig. 2.5C). *Error bars*: SD around the mean

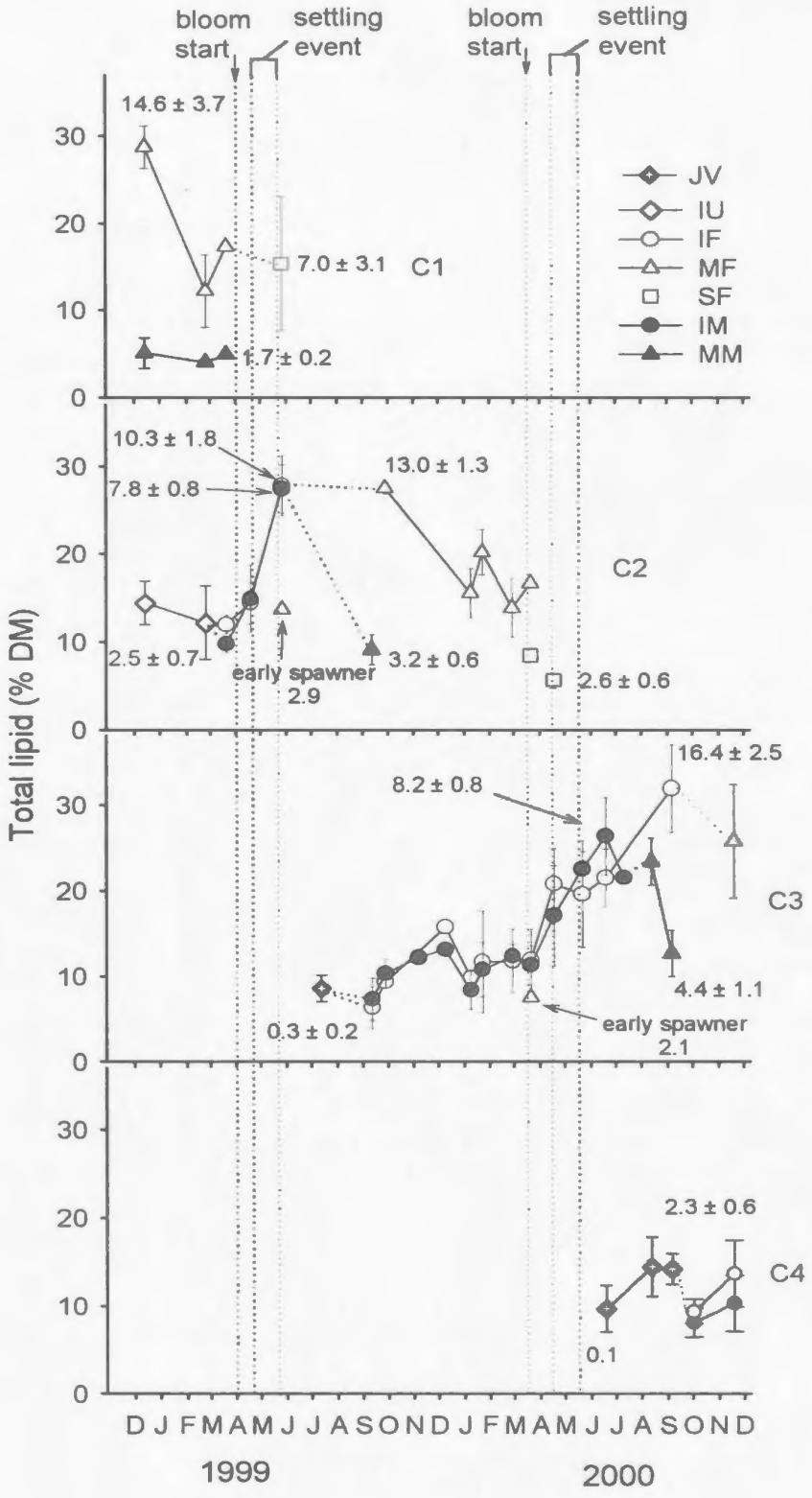

Fig. 4.2 *Mysis mixta.* Seasonal changes in total lipid (% DM). *C1, C2, C3, C4* cohorts 1-4. *Superimposed values* represent maximum or minimum TL content (mg mysid^{-1}) in different stages *(remaining details as in Fig. 4.1)*

determined in damaged mature males collected in November 2000. Total lipid in C4 mysids increased slowly at 0.52 mg•month^{-1} from June to November 2000, when sampling was terminated (Table 4.3; Fig. 4.2).

Table 4.3 *Mysis mixta.* Within-cohort total lipid accumulation or utilisation (rates produced from regression slopes of TL content per mysid). Male and female stages in a cohort were pooled when their rates were not significantly different (ANCOVA, $p > 0.05$). Sub-groups were created to establish rates before (pre) and after (post) the start of the spring bloom, although bloom status was not assigned to mature males/females (*JV* juveniles; *IU* immatures undifferentiated; *IM* immature males; *IF* immature females; *MM* mature males; *MF* mature females). Spent females were not included in any calculations. Mysids that exhibited no change ($p > 0.05$) were assigned a rate of 0.00

Cohort	Stages	Time period	Bloom Status	Accumulation or loss (mg month^{-1})	r^2	*n*
1	*MF*	Dec 98 – Mar 99	*n/a*	-2.5*	0.54	8
1	*MM*	Dec 98 – Mar 99	*n/a*	0.00	*n/a*	4
2[a]	*IU; IF; IM*	Dec 98 – Mar 99	Pre	0.00	*n/a*	26
2[a]	*IF; IM*	Mar 99 – May 99	Post	3.0****	0.72	20
2	*MF*	Sep 99 – Mar 00	*n/a*	-1.1**	0.52	12
3	*JV; IF; IM*	July 99 – Mar 00	Pre	0.30****	0.67	45
3	*IF; IM*	Mar 00 – Sep 00 (Mar to June for *IM*)	Post	2.3****	0.79	39
3[a]	*IM; MM*	June 00 – Aug 00	*n/a*	0.00	*n/a*	12
4[a]	*JV; IF; IM*	June 00 – Nov 00	Post	0.52**	0.37	19

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, *n/a* not applicable
[a]regressions corrected for temporal autocorrelation using Cochrane-Orcutt procedure

In general, mysids were present in the samples as juveniles during July each year, and as they slowly developed sexual characteristics (usually after September) they accumulated small quantities of lipid prior to the spring bloom (TL range 5 – 17% DM), and larger quantities during and following the spring bloom (to a maximum TL of 32% DM in females). Following spawning (main spawning event appears to occur in September or later), TL decreased significantly in mature females.

4.3.2 Lipid class composition

The predominant lipid classes in most stages were TAG and PL, whereas HC, SE/WE, ME, KET, FFA, ALC, ST, DG and AMPL were minor classes.

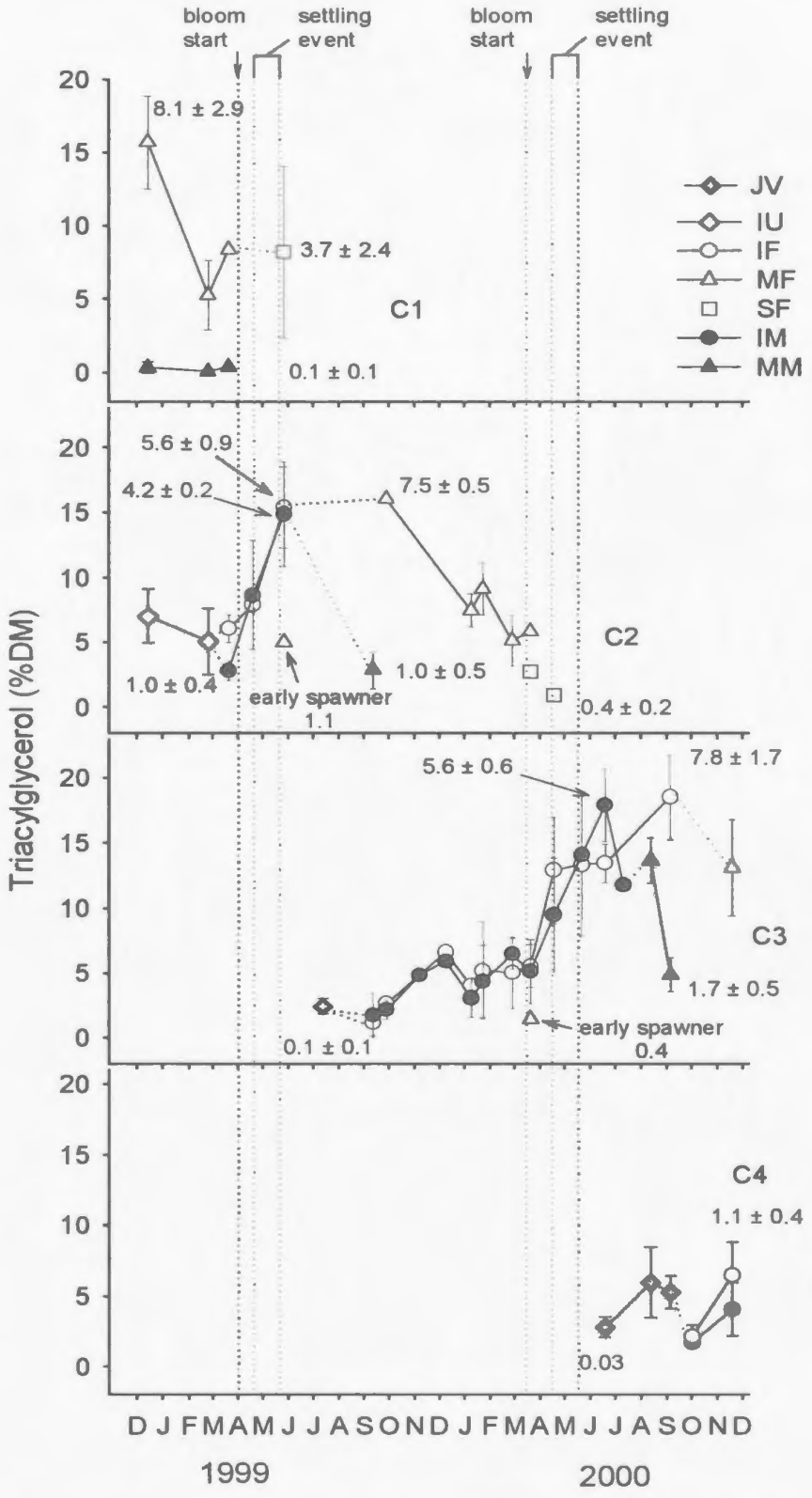

Fig. 4.3 *Mysis mixta.* Seasonal changes in triacylglycerol (% DM). *C1, C2, C3, C4* cohorts 1 - 4. *Superimposed values* represent max. or min. TAG content (mg mysid^{-1}) for different stages *(remaining details as in Fig. 4.1)*

4.3.2.1 Females

Highest TAG levels of all life-history stages were recorded in mature females early in the brooding period (up to 16% DM) and in immature females (and immature males) just prior to full maturity (15 – 19% DM; Fig. 4.3). To reach these maximum levels, growing C2 and C3 females accumulated TAG at 1.4 - 1.7 mg month^{-1} (Table 4.4; Fig. 4.3) from the start of the spring bloom in March. Before the bloom, maturing mysids accumulated TAG at slower rates of 0.15 and 0.22 mg month^{-1} (C3 and C4, respectively) or showed no change (C2) (Table 4.4).

The lowest TAG level in mature females was recorded in early spawners from May 1999 and March 2000 (5.0% DM and 1.5% DM, respectively; Fig. 4.3). These early spawners had lower levels of both neutral and polar lipids than the dominant group of mature females spawning in September or later (Figs. 4.2, 4.3, 4.4). Following spawning, the dominant groups of females utilised TAG at 1.7 mg month^{-1} (C1) or 0.94 mg month^{-1} (C2; Table 4.4; Fig. 4.3). Mean TAG levels in spent females ranged from 1.0 to 8.2% DM

Table 4.4 *Mysis mixta*. Within-cohort triacylglycerol accumulation or utilisation (rates produced from regression slopes of TAG per individual). Males and females were pooled when their rates were not significantly different (ANCOVA, $p > 0.05$). Sub-groups were created to establish rates before (pre) and after (post) the start of the spring bloom, although bloom status was not assigned to mature males/females. Spent females were not included in any calculations. Mysids that exhibited no change ($p > 0.05$) were assigned a rate of 0.00 *(abbreviations as in Table 4.3)*

Cohort	Stages	Time period	Bloom status	Accumulation or loss (mg month^{-1})	r^2	n
1	*MF*	Dec 98 – Mar 99	*n/a*	-1.7*	0.56	8
1	*MM*	Dec 98 – Mar 99	*n/a*	0.00	*n/a*	4
2[a]	*IU; IF; IM*	Dec 98 – Mar 99	Pre	0.00	*n/a*	26
2[a]	*IF; IM*	Mar 99 – May 99	Post	1.7***	0.73	20
2	*MF*	Sep 99 – Mar 00	*n/a*	-0.94***	0.74	12
3	*JV; IF; IM*	July 99 – Mar 00	Pre	0.15****	0.59	45
3	*IF; IM*	Mar 00 – Sep 00 (Mar – June for *IM*)	Post	1.4****	0.76	39
3[a]	*IM; MM*	June 00 – Sep 00	*n/a*	-1.6**	0.52	12
4[a]	*JV; IF; IM*	June 00 – Nov 00	Post	0.22*	0.38	19

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, *n/a* not applicable

[a]regressions corrected for temporal autocorrelation using Cochrane-Orcutt procedure

(March April, Fig. 4.3). Triacylglycerol in C2 spent females was lower than in C1 spent females, resulting in lower TL quantities overall.

Females maturing through summer accumulated some PL (up to 0.32 mg month^{-1}, Table 4.5), and had maximum PL levels of 6% DM once they became fully mature in autumn (Fig. 4.4). Otherwise, PL levels fluctuated around 4% DM, with seasonal patterns less pronounced than those of TAG. The PL levels in mature females remained consistently high throughout the brooding period until the females died following the release of broods in April or May (Table 4.5, Fig. 4.4).

The predominant groups of mature females in C1, C2 and C3 contained similar levels of all lipid classes and had higher levels of each lipid class than did spent females and mature males. Grand mean lipid levels are provided for life-history stages collected on more than one sampling date (Tables 4.6, 4.7, 4.8). The early-spawning females in C2 and C3 had lower amounts of all major lipid classes, presumably because they lacked the time and the resources to build up the higher levels seen in females spawning later in the year. Following release of the broods, females were left with lipid class profiles similar to those in mature males.

4.3.2.2 Males

Most mature, post-spawned males had very low levels of TAG (mean range 0.07 - 14% DM, Fig. 4.3). The lipid pool of age 2+ males in C1 was not dominated by TAG, unlike that of all other life-history stages including younger mature males in C2 and C3. Low TAG in C1 mature males resulted in consistently higher values for ST and FFA than for any other neutral lipid (Table 4.6). High TAG accumulation rates in maturing males began in March and continued for several months (1.4 - 1.7 mg month^{-1}, Table 4.4), indicating that males required significant energy reserves for reproduction, as did females. It is unlikely that these energy reserves were utilised for overwintering since lipid levels dropped during the spawning period in autumn. Like females, maturing males accumulated TAG at slow rates prior to the initiation of the spring bloom (Table 4.4; Fig. 4.3). Highest TAG levels in males occurred following spring bloom settlement in May of both years (15 - 18% DM, Fig. 4.3). In general, PL levels remained fairly consistent throughout development (Tables 4.6, 4.7, 4.8, 4.9). Small increases in PL were observed

in juveniles and immature males prior to the spring bloom (0.091 – 0.11 mg•month^{-1}; Table 4.4), and PL accumulation in C3 immatures increased to 0.32 mg•month^{-1} following bloom settlement (no significant change in C2 immatures).

Table 4.5 *Mysis mixta.* Within-cohort phospholipid accumulation (rates produced from regression slopes of PL per mysid). Males and females were pooled when their rates were not significantly different (ANCOVA, $p > 0.05$). Sub-groups were created to establish rates before (pre) and after (post) the start of the spring bloom, although bloom status was not assigned to mature males/females. Spent females were not included in any calculations. Mysids that exhibited no changes ($p > 0.05$) were assigned a rate of 0.00 *(abbreviations as in Table 4.3)*

Cohort	Stages	Time period	Bloom status	Accumulation (mg month^{-1})	r^2	n
1	*MF*	Dec 98 – Mar 99	*n/a*	0.00	*n/a*	8
1	*MM*	Dec 98 – Mar 99	*n/a*	0.00	*n/a*	4
2[a]	*IU; IF; IM*	Dec 98 – Mar 99	Pre	0.11**	0.34	26
2[a]	*IF; IM*	Mar 99 – May 99	Post	0.00	*n/a*	20
2	*MF*	Sep 99 – Mar 00	*n/a*	0.00	*n/a*	12
3	*JV; IF; IM*	July 99 – Mar 00	Pre	0.091****	0.75	45
3	*IF; IM*	Mar 00 – Sep 00 (Mar – June for *IM*)	Post	0.32****	0.63	39
3[a]	*IM; MM*	June 00 – Sep 00	*n/a*	0.00	*n/a*	12
4[a]	*JV; IF; IM*	June 00 – Nov 00	Post	0.093****	0.68	19

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, *n/a* not applicable
[a]regressions corrected for temporal autocorrelation using Cochrane-Orcutt procedure

4.3.2.3 Minor classes and lipolysis index

Hydrocarbons, KET, ME and SE/WE all increased with maturation and reached highest levels in mature females and males (Tables 4.6, 4.7, 4.8, 4.9). Free fatty acids either remained constant throughout a cohort (C2) or decreased during maturation (C3). Alcohol levels did not change in a predictable manner within cohorts, although they varied among cohorts and were highest in C2 and C3 mature females but absent in adult C1 mysids. Sterol, AMPL and DG levels remained constant throughout maturation (Tables 4.6, 4.7, 4.8, 4.9; changes in lipid content per mysid were determined using linear regressions, significance level 0.05).

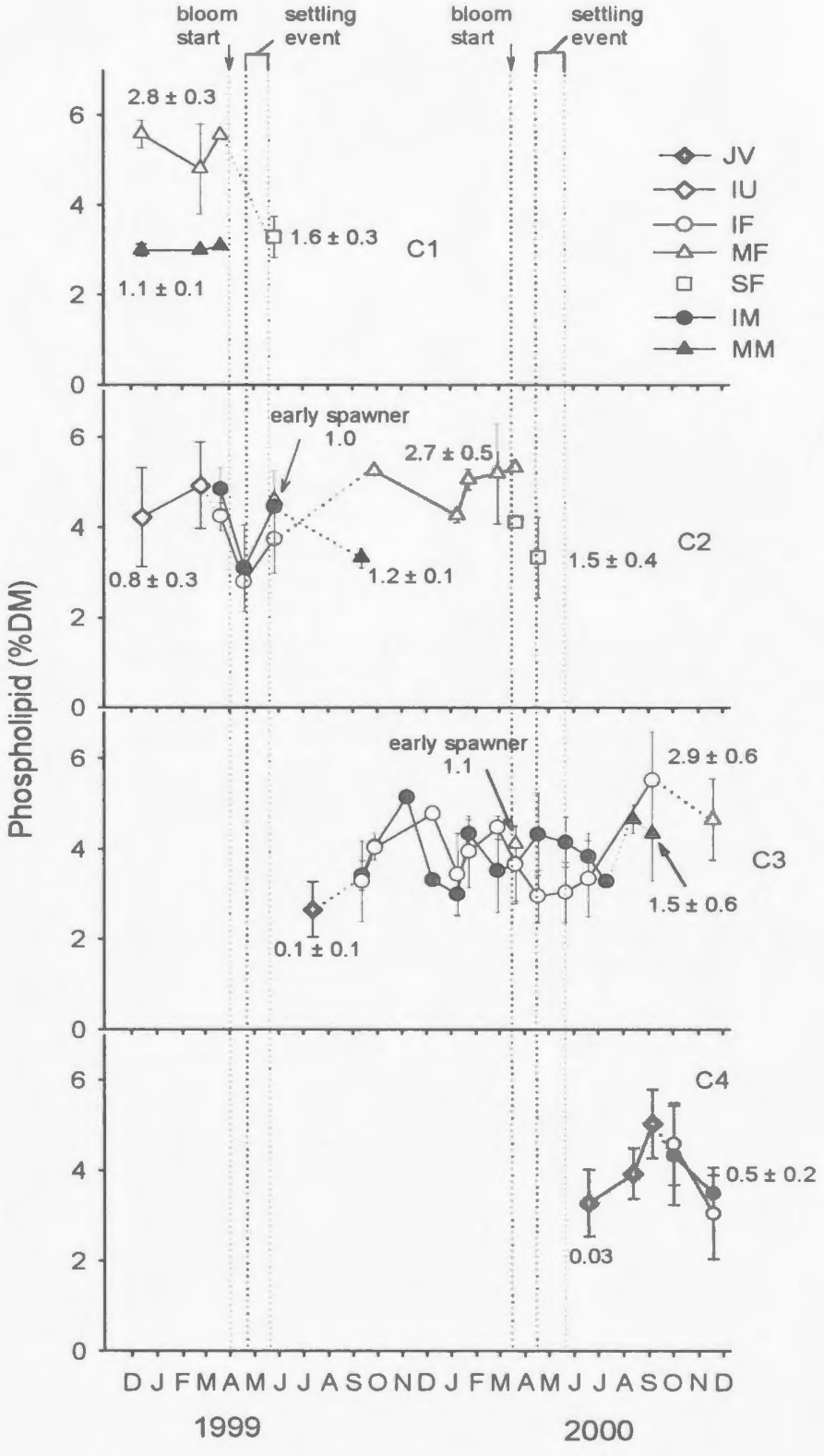

Fig. 4.4 *Mysis mixta.* Seasonal changes in phospholipid (% DM). *C1, C2, C3, C4* cohorts 1 - 4. *Superimposed values* represent max. or min. PL content (mg mysid^{-1}) for different stages *(remaining details as in Fig. 4.1)*

Small juveniles in C3 had unusually high lipolysis index (LI) values, ranging from 10 to 30%, whereas LI values for all other mysids remained below 15%. These low LI values indicate that the integrity of the samples was maintained during storage and analysis, and the consistently high LI values in C3 juveniles (20.1±7.6%) probably indicate a physiological change related to rapid allocation of dietary lipid to growth in this life stage, rather than sample degradation.

4.3.3 Areal concentrations

Areal concentrations of TAG and TL varied both seasonally and inter-annually (Fig. 4.5). Maxima occurred in February 1999 (25 mg TL m^{-2}, 10 mg TAG m^{-2}), and June 2000 (55 mg TL m^{-2}, 39 mg TAG m^{-2}).

Table 4.6 *Mysis mixta.* Lipid class composition of cohort 1 (major classes and totals are highlighted in grey). Data are grand means ±SD for stages collected on more than one sampling date (*HC* hydrocarbon; *SE/WE* steryl ester/wax ester; *ME* methyl ester; *KET* ketone; *TAG* triacylglycerol; *FFA* free fatty acid; *ALC* alcohol; *ST* sterol; *DG* diacylglycerol; *Neutral* sum of neutral lipids; *AMPL* acetone-mobile polar lipid; *PL* phospholipid; *Polar* sum of polar lipids; *TL* total lipid; *DM* dry mass)

Class	Mature females Dec. 1998 – Mar. 1999		Spent females May 1999		Mature males Dec. 1998 – Mar. 1999	
	% DM	% TL	% DM	% TL	% DM	% TL
HC	0.18±0.12	0.87±0.21	0.21	1.28	0.00	0.04±0.07
SE/WE	0.83±0.84	3.51±2.48	0.24	1.53	0.14±0.18	2.41±2.89
ME	0.85±1.29	3.21±4.34	0.37	2.14	0.03±0.06	0.54±0.93
KET	0.13±0.11	0.68±0.68	0.09	0.39	0.00	0.00
TAG	9.78±5.36	48.10±6.25	8.20	47.85	0.27±0.17	5.15±3.11
FFA	0.33±0.10	1.93±0.96	1.23	7.93	0.48±0.11	9.74±0.61
ALC	0.00	0.00	0.27	1.22	0.00	0.00
ST	1.09±0.45	6.19±2.80	0.65	4.64	0.49±0.05	10.61±0.89
DG	0.12±0.07	0.64±0.49	0.24	1.71	0.06±0.05	1.10±0.95
Neutral	13.30±7.53	65.13±10.07	11.49	68.69	1.47±0.54	29.60±6.65
AMPL	0.85±0.61	4.19±1.37	0.57	3.63	0.15±0.06	3.03±1.36
PL	5.32±0.44	30.68±10.57	3.30	27.68	3.04±0.06	67.37±6.45
Polar	6.16±0.91	34.87±10.07	3.87	31.31	3.18±0.11	70.40±6.65
TL	19.47±8.44		15.36		4.65±0.56	
TL ind^{-1} *(mg)*	10.24±3.86		7.02		1.65±0.18	

Table 4.7 *Mysis mixta.* Lipid class composition of cohort 2. Data are grand means ±SD for stages collected on more than one sampling date. Values in *square parentheses* represent an early spawning female in May 1999 *(abbreviations as in Table 4.6, Table 4.7 continues below)*

Class	Immatures undifferentiated Dec. 1998 – Feb. 1999		Immature females Mar. 1999 – May 2000		Immature males Mar. 1999 – May 2000	
	% DM	% TL	% DM	% TL	% DM	% TL
HC	0.06±0.05	0.49±0.31	0.08±0.07	0.40±0.34	0.13±0.15	0.51±0.43
SE/WE	0.48±0.12	3.44±0.57	0.22±0.13	1.30±0.33	0.34±0.31	1.65±0.72
ME	0.10±0.04	0.82±0.38	0.23±0.15	1.20±0.38	0.39±0.52	1.44±1.52
KET	0.23±0.18	1.50±1.12	0.14±0.22	0.97±1.52	0.08±0.05	0.45±0.20
TAG	6.05±1.37	43.31±6.33	9.79±4.94	55.66±5.97	8.79±6.05	48.03±17.26
FFA	0.50±0.15	3.85±0.66	0.75±0.36	4.28±0.75	1.11±0.45	6.79±2.34
ALC	0.00	0.00	0.49±0.84	1.97±3.41	0.25±0.43	0.96±1.67
ST	0.72±0.12	5.59±0.01	0.65±0.40	4.52±3.14	0.85±0.31	5.27±1.50
DG	0.18±0.01	1.31±0.09	0.26±0.12	1.48±0.16	0.19±0.14	1.02±0.19
Neutral	8.32±2.02	60.31±9.27	12.61±6.26	71.77±8.72	12.13±8.21	66.12±17.06
AMPL	0.45±0.06	3.34±0.30	0.91±0.60	4.81±2.33	0.76±0.40	4.50±2.19
PL	4.57±0.50	36.36±9.56	3.59±0.73	23.42±10.81	4.18±0.95	29.37±17.89
Polar	5.03±0.43	39.69±9.27	4.51±0.69	28.23±8.72	4.94±0.73	33.88±17.06
TL	13.34±1.59		17.11±6.77		18.18±10.37	
TL ind^{-1} *(mg)*	2.60±0.13		5.62±3.01		4.61±3.45	

Table 4.7 *(concluded)*

Class	Mature females Sept. 1999 – Mar. 2000 [May 1999]		Spent females Mar. 2000 – Apr. 2000		Mature males Sept. 1999	
	% DM	% TL	% DM	% TL	% DM	%TL
HC	0.18±0.04 [0.04]	0.97±0.19 [0.28]	0.01±0.00	0.20±0.13	0.14	1.45
SE/WE	0.74±0.18 [0.30]	4.03±0.80 [2.20]	0.10±0.09	1.33±0.92	0.84	9.21
ME	0.74±0.72 [0.13]	3.79±4.09 [0.93]	0.00	0.00	0.34	3.70
KET	0.36±0.36 [0.15]	2.00±1.74 [1.05]	0.12±0.00	1.67±0.33	0.04	0.47
TAG	8.74±4.37 [4.99]	44.47±9.61 [36.34]	1.85±1.28	24.32±11.51	2.85	29.69
FFA	0.47±0.34 [0.86]	2.54±2.03 [6.24]	0.35±0.09	4.70±0.20	0.70	7.80
ALC	0.96±0.48 [0.68]	5.00±1.48 [4.93]	0.02±0.00	0.28±0.11	0.09	0.89
ST	0.61±0.13 [0.58]	3.46±1.02 [4.19]	0.46±0.07	6.66±0.95	0.59	6.59
DG	0.20±0.10 [0.18]	1.15±0.54 [1.30]	0.11±0.10	1.41±1.05	0.06	0.71
Neutral	12.99±5.03 [7.90]	67.40±7.19 [57.47]	3.03±1.63	40.57±12.16	5.64	60.51
AMPL	0.77±0.36 [1.24]	4.14±1.54 [9.05]	0.33±0.18	4.90±3.56	0.19	2.12
PL	5.02±0.43 [4.60]	28.46±7.20 [33.49]	3.72±0.56	54.53±8.60	3.32	37.36
Polar	5.79±0.56 [5.85]	32.60±7.19 [42.54]	4.05±0.38	59.43±12.16	3.51	39.49
TL	18.78±5.39 [13.74]		7.07±2.00		9.15	
TL ind^{-1} *(mg)*	9.49±2.50 [2.89]		2.92±0.47		3.16	

Table 4.8 *Mysis mixta.* Lipid class composition of cohort 3. Data are grand means ±SD for stages collected on more than one sampling date. Values in *square parentheses* represent an early spawning female in March 2000 *(abbreviations as in Table 4.6)*

Class	Juveniles July 1999		Immature females Sept. 1999 – Sept. 2000		Immature males Sept. 1999 – July 2000		Mature females Nov. 2000 [Mar. 2000]		Mature males Aug. 2000 – Sept. 2000	
	% DM	% TL	% DM	% TL	% DM	% TL	% DM	% TL	% DM	% TL
HC	0.01	0.11	0.14±0.20	0.61±0.71	0.09±0.10	0.51±0.46	0.20 [0.05]	0.82 [0.64]	0.28±0.08	1.51±0.11
SE/WE	0.17	1.79	0.46±0.49	2.54±1.74	0.54±0.46	3.65±2.18	1.76 [0.03]	6.88 [0.35]	1.55±0.42	8.82±1.49
ME	0.06	0.66	0.57±0.73	2.92±2.26	0.22±0.29	1.39±2.11	1.03 [0.00]	4.52 [0.00]	0.41±0.32	1.93±0.80
KET	0.02	0.20	0.34±0.43	1.57±1.80	0.38±0.57	2.16±2.51	1.70 [0.00]	5.87 [0.00]	0.55±0.28	3.00±0.16
TAG	2.43	28.34	8.06±5.55	45.37±15.51	7.28±5.05	44.69±15.01	13.11 [1.45]	50.64 [19.05]	9.28±6.20	48.37±13.87
FFA	1.60	17.70	0.47±0.17	3.93±2.95	0.75±0.25	6.00±3.06	0.09 [0.09]	0.37 [1.18]	0.56±0.15	3.74±2.53
ALC	0.05	0.60	0.24±0.22	1.85±2.01	0.05±0.09	0.37±0.66	1.65 [0.31]	6.07 [4.07]	0.02±0.03	0.10±0.15
ST	0.77	9.01	0.52±0.10	4.30±2.53	0.57±0.11	4.51±1.89	0.38 [0.45]	1.55 [5.91]	0.52±0.01	3.20±1.32
DG	0.12	1.37	0.13±0.05	0.91±0.45	0.12±0.04	0.94±0.33	0.05 [0.09]	0.21 [1.14]	0.10±0.06	0.55±0.15
Neutral	5.22	53.25	10.94±6.96	63.97±13.82	10.01±5.83	64.22±12.14	19.97 [2.45]	76.94 [32.33]	13.27±7.25	71.22±9.68
AMPL	0.75	8.81	0.76±0.47	5.29±2.63	0.61±0.44	4.65±3.34	1.15 [1.01]	4.74 [13.27]	0.33±0.13	1.90±0.14
PL	2.66	31.41	3.87±0.80	30.74±13.28	3.85±0.60	31.12±11.38	4.67 [4.13]	18.32 [54.40]	4.52±0.23	26.87±9.54
Polar	3.41	40.22	4.63±0.99	36.03±13.82	4.46±0.69	35.78±12.14	5.82 [5.14]	23.06[67.67]	4.85±0.35	28.78±9.68
TL	8.62		15.56±7.38		14.51±6.06		25.79 [7.59]		18.11±7.61	
TL ind^{-1} (mg)	0.36		4.95±4.91		3.24±2.62		15.38 [2.09]		6.29±2.73	

Table 4.9 *Mysis mixta.* Lipid class composition of cohort 4. Data are grand means ±SD for stages collected on several sampling dates *(abbreviations as in Table 4.6)*

Class	Juveniles June 2000 – Sept. 2000		Immature females Oct. 2000 – Nov. 2000		Immature males Oct. 2000 – Nov. 2000	
	% DM	% TL	% DM	% TL	% DM	% TL
HC	0.11±0.10	0.85±0.86	0.05±0.06	0.43±0.32	0.02±0.02	0.16±0.15
SE/WE	0.76±0.36	5.46±2.42	0.86±0.80	6.91±3.87	0.49±0.39	4.76±2.22
ME	0.47±0.50	3.49±3.56	0.49±0.71	3.70±4.22	0.26±0.29	2.33±2.08
KET	0.36±0.52	2.41±3.11	0.18±0.33	1.54±2.60	0.04±0.04	0.49±0.55
TAG	4.94±1.99	36.24±6.78	4.04±2.71	33.30±14.02	3.16±1.85	31.81±10.95
FFA	1.04±0.38	8.70±5.15	0.65±0.21	6.06±2.23	0.77±0.26	8.75±4.08
ALC	0.02±0.03	0.15±0.22	0.02±0.06	0.17±0.46	0.00±0.01	0.05±0.11
ST	0.56±0.12	4.46±1.24	0.40±0.04	3.80±0.92	0.39±0.06	4.41±1.50
DG	0.11±0.05	0.88±0.47	0.06±0.02	0.58±0.19	0.06±0.04	0.56±0.22
Neutral	8.37±2.67	62.65±6.30	6.75±4.07	56.49±17.21	5.18±2.50	53.31±11.36
AMPL	0.63±0.26	4.74±1.92	0.54±0.20	4.90±1.49	0.43±0.19	4.60±1.73
PL	4.19±0.95	32.61±6.67	3.93±1.33	38.60±17.41	3.84±1.05	42.10±11.98
Polar	4.81±0.98	37.35±6.30	4.48±1.17	43.51±17.21	4.27±1.02	46.69±11.36
TL	13.19±3.13		11.23±3.31		9.45±2.70	
TL•ind^{-1} (mg)	0.33±0.21		1.48±0.85		0.94±0.43	

4.4 Discussion

This study demonstrates a close relationship between lipid and reproductive dynamics in *Mysis mixta* and seasonal production in the water column. Lipid content and composition indicate that *M. mixta* in Conception Bay accumulates lipid primarily for reproduction rather than growth, is a lipid-rich food source for predators, does not experience prolonged periods of starvation in Conception Bay, and secures what may be a significant amount of lipid energy from the environment.

The spring phytoplankton bloom in Conception Bay began in early March in 1999 (bloom start defined as chl *a* > 1 μg l^{-1}), with a chl *a* maximum occurring in late April (Chapter 2). In 2000, the bloom began in late March and the chl *a* maximum was reached in mid-May. Rapid settlement of this fresh and highly nutritive material to the hyperbenthos was evident in April and May each year (Chapter 2). A smaller, secondary

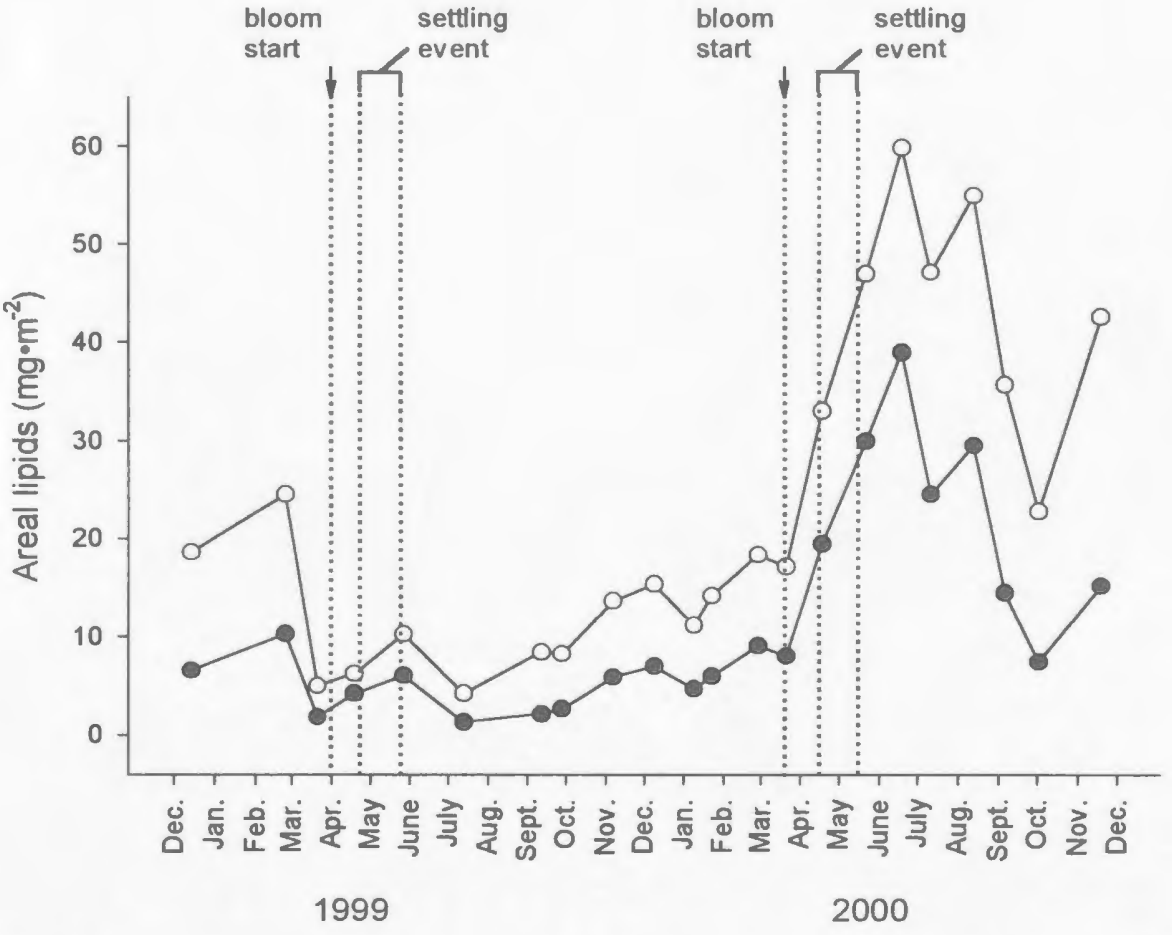

Fig. 4.5 *Mysis mixta*. Areal concentrations of total lipid (open circles) and triacylglycerol (filled circles). *Vertical dotted lines* represent spring bloom start and settling times

peak of phytoplankton biomass occurred in July 1999, but no secondary bloom was apparent in 2000. Temperature scarcely deviated from -0.5°C below 200 m, although surface waters showed a marked seasonal temperature cycle, with values exceeding 14°C in September each year. Hyperbenthic mysids would have had to migrate within 120 m of the surface to experience temperatures >0°C. Salinity in the hyperbenthic region remained between 32.0 and 34.0 psu in both years (Chapter 2).

Tian et al. (2003) predicted primary production values of 124 – 137 g C m^{-2} yr^{-1} from a model of phytoplankton dynamics in Conception Bay, and Redden (1994) recorded a maximum flux of 600 mg C $m^{-2}d^{-1}$ during bloom sedimentation to the benthos in May. Parrish (1998) obtained a range of organic carbon flux [24 – 1361 mg C $m^{-2}d^{-1}$; derived from nearshore (224 m) and offshore (363 m) stations during spring, summer, autumn and

winter between 1994 and 1997] from Trinity Bay, a nearby fjord, that encompassed the value derived from Conception Bay. Values of primary production in both Newfoundland bays are consistent with total annual phytoplankton production reported for fjords in the Northern Hemisphere (49°N – 77°N), which ranges from 100 to 150 g C m^{-2} yr^{-1} (Falk-Petersen et al. 1990). The proportion of fixed carbon recovered as TL in seston is normally between 10 and 20% (Sargent et al. 1985). The limited information available on the changes in seston lipids within Conception Bay (Ramos et al. 2003, Parrish et al. unpublished) indicate similarities to seasonal conditions in Trinity Bay (Parrish 1998). In Trinity Bay, TAG levels in diatoms increase substantially during the peak of the spring bloom, particularly as nitrate and silicate are exhausted (Parrish 1998). The TAG increase is followed by increases in PL coinciding with the growth of dinoflagellate and zooplankton populations. Following the bloom, rapid flux results in the arrival of high quality seston to the benthos of Trinity Bay and Conception Bay, where the fresh organic material is consumed. Up to 70% of the total annual primary production in ice-free temperate and polar regions occurs during the spring phytoplankton bloom (Falk-Petersen et al. 1990), and the spring bloom produces 38 - 75% of the annual flux of particulate organic carbon to the hyperbenthos of Conception Bay (Redden 1994, Tian et al. 2003).

Choe et al. (2003) determined the seasonal abundance of stage VI female copepods (*Temora* spp., *Metridia* spp., *Pseudocalanus* spp. and *Calanus* spp.) in Conception Bay during 1997 and 1998. High abundance of copepods, important prey of immature and mature mysids (Hansson et al. 1990), coincided with the primary production cycle, albeit with a 3-week time lag similar to the zooplankton increase observed in Trinity Bay (Parrish 1998). Maximum numbers of mature copepods were therefore assumed to occur in early or late May in Conception Bay during the present study, with maximum numbers of copepodite stages occurring in late summer and early fall (Davis 1982).

Given high chl *a* concentrations, copepod abundance and lipid content in seston associated with the spring bloom, it is likely that lipids in hyperbenthic zooplankton such as *M. mixta* correspond in some predictable way to bloom periods. Although not strictly herbivorous, *M. mixta* can rapidly exploit the high quality organic material produced during the bloom, presumably in part owing to the vertical migrations that bring it into the

water column each night (Rudstam et al. 1989). This diel migration provides the mysids with early access to freshly formed aggregates produced in shallower water, whereas non-migratory hyperbenthic organisms must wait until the material has settled. The commencement of the spring phytoplankton bloom coincides with both the release of broods from mature females (Chapter 2) and the rapid accumulation of lipid in maturing mysids. Lipid-rich mysids are high quality prey for demersal and pelagic predators, and organic material consumed by the mysids is repackaged and redistributed through fecal pellet production during diel vertical migrations. The continual process of *M. mixta* "drawing down" organic material within the water column and the seasonal release of juveniles into the hyperbenthos probably have a large influence on the energetics of the hyperbenthos and on the coupling of the benthic and pelagic regions in Conception Bay.

Within-cohort TL in *M. mixta* varied substantially throughout the study period. Some differences were observed among the four cohorts, possibly owing to individual variability and to inter-annual variation in environmental factors, and general patterns reflected both seasonal food availability and the reproductive cycle. The smallest free-living mysids contained small quantities of TL, and lipid was initally accumulated slowly. Rapid lipid accumulation commenced in maturing mysids concomitantly with the start of the spring phytoplankton bloom in April and continued through the periods of maximum abundance of adult copepods and copepodite stages in summer and autumn. Slower accumulation or rapid utilitisation of lipid, depending on the life-history stage, occurred at other times of the year. Specifically, once females were fully mature and bearing broods they began to utilise their lipid stores rapidly, in a manner similar to that of post-spawned males. The apparently comparable net loss of lipid in some groups of mature females and mature males (significant utilisation of lipids by C1 and C2 males was assumed owing to the very low lipid content in post-spawned males compared with immature males or mature females) suggests that the process of maintaining broods may not require exceptionally high energy stores. Detailed examination of brood lipids in relation to those of the parent will help to elucidate the energy changes in each constituent.

With the exceptions of spent females, post-spawned males and late-brood stage females, larger individuals of *M. mixta* generally had higher quantities of lipid than

smaller ones, both on a TL mysid^{-1} and a TL DM^{-1} basis. The smallest juveniles (starting at 0.4 mg DM) had as little as 0.04 mg of lipid (5 – 10% DM), whereas the largest females (up to 71 mg DM) had 19 mg of lipid (24 – 38% DM). Similarly, TL level in krill *Euphausia superba* from the Antarctic increased with body size (krill up to 30 mg DM had 16% lipid•DM^{-1}, whereas krill between 200 and 300 mg had 35% lipid•DM^{-1}; Ferguson & Raymont 1974). It is common for crustaceans to accumulate lipid in this manner (Adare & Lasenby 1994), as younger stages often invest dietary energy in growth and development rather than in the accumulation of energy reserves (Ouellet et al. 1992, Adare & Lasenby 1994, Kattner et al. 1994). In *M. mixta*, increased opportunities to feed on small zooplankton in addition to seston as the mysids matured, and increased access to a greater variety of prey items throughout the water column owing to increased swimming ability and distance travelled during vertical migrations, may have contributed to higher food availability in larger animals. On the other hand, immature antarctic *E. superba* (Pond et al. 1995) and juvenile *Mysis relicta* (Chess & Stanford 1998) contain higher lipid per DM than older and larger stages. These contrasting results emphasize that lipid storage patterns in crustaceans are highly variable, even within the same species, and that it is advantageous to study the general biology concurrently with the lipid dynamics in a population.

Seasonal variability in the lipids of *M. mixta* is similar to that in some deep-water and/or high latitude zooplankton species. In general, species whose food is unpredictable or seasonally variable are more likely to store lipids for use when food is scarce (Clarke 1977). Alternatively, an absence of substantial seasonal fluctuations in zooplankton lipid suggests that food availability and quality are consistent year-round. Species that are opportunistic omnivores and that undergo diel vertical migrations may have access to ample food sources in both the hyperbenthic and pelagic regions throughout the year (Adare & Lasenby 1994), negating any necessity for seasonal storage of energy reserves. Since protein usually does not have a storage function in zooplankton, and carbohydrate levels remain low year-round, species with low lipid levels probably feed consistently throughout the year to survive and reproduce (Clarke 1977, Percy 1979).

Mysis mixta in Conception Bay accumulates substantial amounts of lipid despite its flexible feeding habits and its apparent year-round access to food in both the pelagic and hyperbenthic realms. This population has probably evolved to mature and spawn using accumulated reserves, and to release juveniles during the period of greatest food abundance. The primary storage lipid of *M. mixta* is TAG, which is commonly accumulated in zooplankton species within 1 or 2 months of the spring bloom in preparation for gametogenesis and brooding (Clarke 1983, Gardner et al. 1985, Hill et al. 1992, Hopkins et al. 1993). Triacylglycerol is produced in great amounts by diatoms and flagellates (Parrish 1988), and is more prevalent in the water column during phytoplankton blooms. In *M. mixta*, the most rapid accumulation of TAG (up to 1.7 mg month^{-1} in maturing mysids) occurred during the months following the start of the phytoplankton bloom in March 1999 and 2000. As in the decapod *Pandalus borealis* (Hopkins et al. 1993), TAG levels increased from 20% of TL in smallest juvenile *M. mixta* to 74% of TL in female mysids nearing full maturity (~20% DM in some individuals). Triacylglycerol is mobilised faster than WE, and Sargent & Henderson (1986) noted that copepods not experiencing prolonged periods of starvation deposit storage lipids in the form of TAG rather than the more common WE. *Mysis mixta* may store easily-mobilised TAG to take advantage of the higher food availability and quality during the spring bloom, but it may not necessarily require lipid stores to survive and reproduce, as evidenced by low lipid quantities in early-spawning females. The few early-spawners were much smaller and produced smaller broods (22 – 56 larvae brood^{-1}) than the dominant group of large mature females spawning later in the year (up to 111 larvae brood^{-1}). Although brood sizes differed, larvae from early-spawning and late-spawning females had similar quantities of TL (~18 µg TL larva^{-1} and 17 – 54 µg TL larva^{-1} respectively). Since most females postpone spawning until the second year, following accumulation of substantial lipid reserves, there must be a reproductive advantage for individuals that are lipid-rich prior to the production and release of larvae.

In contrast to TAG, PL levels remained relatively low and stable, with very low rates of accumulation occurring in maturing mysids (up to 0.32 mg month^{-1} in the spring). Highest PL levels were observed in immature and mature females (up to 6.5% DM), and

levels in immature males remained below 5% DM. Likewise, PL quantities (mg per individual) in mature males were consistently low relative to those in mature females. The disparity between males and females may have been attributable to the requirement by females to produce egg membranes, as evidenced by low quantities of PL in most spent females. It also suggests that mature males invest much of their PL in the production of sperm, and accordingly their PL content was greatly reduced following spawning in autumn. If ingested lipid had been intended solely for growth in *M. mixta*, PL levels would have increased at higher rates during the growth phase of males and females. Increases in PL are normally associated with growth because they are major structural and functional components of membranes and organelles (Vanderploeg et al. 1992, Arts 1999). In addition, of the extra ~20 mg of body mass in immature or mature females compared with immature or mature males of similar body length (Chapter 2), up to 40% is composed of lipid, of which 70 – 80 % is TAG and 20 - 30% is PL. This trend provides additional evidence that males and females have different energy requirements for reproductive processes.

Seasonal changes in the availability of essential fatty acids may provide a dietary rather than a reproductive influence on seasonal lipid patterns in *M. mixta*, whereby rapid storage of lipid occurs during periods of high abundance of specific fatty acids. Throughout the 1996 bloom in Conception Bay, Parrish et al. (unpublished) observed high levels (up to 35% of total fatty acids) of polyunsaturated fatty acids even in sediment trap material collected at 220 m. Polyunsaturated fatty acid levels were lower (~ 20% of total fatty acids) both before and after the spring bloom (Parrish et al., unpublished). Variation in the swimming activity and daily migrations of the mysids, resulting in higher metabolic requirements at certain times of the year, may also contribute to seasonal variation in lipids. Less active hyperbenthic organisms living in the same region but exhibiting a similar biennial life cycle may have access to fewer resources year-round, and may also have lower and less variable metabolic demands. Such populations may accumulate lower and less variable amounts of lipid seasonally, as in the sympatric amphipod *Acanthostepheia malmgreni,* which has lipid levels as high as 15% DM and relatively slow lipid accumulation rates (Chapter 5). Other than *M. mixta* and *A.*

malmgrem, the chaetognath *Parasagitta elegans* is the only hyperbenthic species in Conception Bay for which lipid dynamics have been determined in relation to the reproductive cycle (Choe et al. 2003). The TL content in *P. elegans*, a highly motile and carnivorous species, shows small increases in maturing individuals, with maximum lipid levels reaching 16% DM (Choe et al. 2003).

Female *M. mixta* accumulated higher quantities of lipid than all other life-history stages (up to 38% DM or 19 mg mysid^{-1}). High levels of reserves in females are common in brooding zooplankton (Adare & Lasenby 1994) and reflect the energy requirements of females for brood production and possibly protection. Males accumulated lipid at the same rates but never achieved the high quantities observed in age 2+ mature females (maximum TL in males was 30% DM or 9 mg mysid^{-1}). With few exceptions, TAG was the most abundant lipid class in *M. mixta* throughout the study period, whereas in other zooplankton species TAG is predominant only during and following the spring bloom (Gardner et al. 1985, Hill et al. 1992, Hopkins et al. 1993). Phospholipid was most abundant in those few instances when TAG was not the major lipid class in *M. mixta*.

Total lipid decreased in mature females, primarily as a result of TAG loss concomitant with larval development from utilisation of stored yolk. Females fed while brooding (personal observation), so loss of lipid for maintenance was not the likely cause of the observed decrease. The presence of one-year-old spawners in both C2 and C3 demonstrates that reproduction is possible in females with lipid concentrations as low as 8% DM, although nothing is known of the survival of early-spawned juveniles. Hill et al. (1992) have hypothesized that amphipods not able to store lipids at a level of 20% DM may have to postpone reproduction for an entire year. In *Mysis relicta*, it appears that body size and TL content, rather than lipid level, are the most important factors determining time of reproduction (Adare & Lasenby 1994). Female *M. relicta* studied in two different lakes required 1.4 mg TL before they produced broods (Adare & Lasenby 1994). In contrast, early-spawning female *M. mixta* in Conception Bay contained 2 - 3 mg TL, compared with the 7 - 19 mg in two-year-old females. Release time of larvae by early spawners was estimated as late summer or early autumn, perhaps when the abundance of predators was low.

At the population level, areal concentrations of TL and TAG indicate that *M. mixta* inhabiting the hyperbenthos of Conception Bay can rapidly appropriate organic material produced during a spring phytoplankton bloom. Accumulation of reserves by the population on an areal basis began in March of both years, although markedly higher concentrations occurred during the bloom in 2000. The magnitude, estimated from chl *a* concentrations, and duration of the bloom in 2000 were notably greater than in 1999 (Chapter 2). Maximum areal concentration of TL in 2000 (55 mg m^{-2}) corresponded to 3% of the integrated input of TL to the hyperbenthos, as measured in 220-m sediment trap material collected throughout the 1996 spring bloom in Conception Bay (1750 g m^{-2}; Ramos et al. 2003). Unfortunately, similar calculations of areal lipid concentration as a proportion of integrated flux in organisms from other systems are not available for comparison. However, Arts et al. (1992) reported seasonal variation in areal TAG reserves in several small zooplankton species (cladocerans and copepods) from a hyper-eutrophic lake in Saskatchewan, Canada. Areal lipid energy in the freshwater zooplankton increased 1 month after significant increases in bacterial or algal populations, with maximum concentrations (up to 1200 mg m^{-2} in dominant populations) reflecting the high productivity of the ecosystem (Arts et al. 1992). In addition to food availability, areal concentrations of lipids can reflect interactions among numerous factors including competition, predation, life cycle and diet (Arts et al. 1992).

4.5 Summary

Lipid content and composition of zooplankton are influenced by various factors including location, season, temperature, salinity, food availability, diet, body size, physiology and reproductive condition. The interactions among these factors can result in substantial and predictable seasonal patterns in lipids, and these patterns can greatly influence the flow of energy through an ecosystem. Unfortunately, environmental influences are not easily distinguished from biological effects on the seasonal lipids of an organism. As a hyperbenthic species rich in lipid reserves, *M. mixta* probably serves an important role as an intermediary trophic link in the ecosystem of Conception Bay and the

dependence of *M. mixta* on the seasonal phytoplankton bloom is reflected in a strong seasonality in reproduction and lipid accumulation.

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Chapter 5. Seasonal changes in the lipids of *Acanthostepheia malmgreni*

5.1 Introduction

The hyperbenthos is a poorly understood but highly productive oceanic realm that is difficult to sample, resulting in relatively few studies on hyperbenthic ecology. However, the information available suggests that hyperbenthic zooplankton successfully exploit a variety of energy sources and are important intermediate trophic links between small (bacteria, phytoplankton, detritus and meiofauna) and large (fish and predatory invertebrates) size fractions in the marine food web (e.g. Mees et al. 1995, Mees & Jones 1997). Zooplankton commonly rely on stored lipids to reproduce or to survive through periods of low food availability, and determination of seasonal lipid content with respect to life cycle can be critical for an understanding of the energetic role of a species within a food web. Seasonal lipid patterns in keystone species, particularly in the energy storage lipid classes, can be used to deduce the critical periods of energy transfer from one trophic level to another (Arts et al. 1992). Sympatric species with dissimilar life styles and nutritional requirements may have different critical periods of energy accumulation and utilisation.

Acanthostepheia malmgreni Goës, a semelparous breeder with a 2.5-yr life span (Chapter 3), was the most common and abundant amphipod in the hyperbenthos of Conception Bay, Newfoundland, from April 1997 to June 1998 (Deibel et al. unpublished). Brooding requires a 5-month period, and juveniles are released into the hyperbenthos during April and May. Juveniles feed on small particles, including phytoplankton and detritus (Richoux, unpublished), whereas older stages prey on copepods and other small invertebrates (Sainte-Marie & Brunel 1985). *A. malmgreni* is capable of rapid bursts of swimming (Sainte-Marie & Brunel 1985), although in the laboratory individuals often remain partially buried in the sediment (Richoux, unpublished). There have been no investigations to determine whether the population migrates vertically within Conception Bay.

The aim of the present study was to examine the seasonal energy storage in *A.*

malmgreni, with particular emphasis on the relationships among lipid content and composition, the reproductive cycle and the timing of spring bloom sedimentation. If the population depends on the annual spring bloom to provide sufficient energy for reproduction and survival, lipid levels should rise shortly after the fallout of the bloom. The following questions were addressed: (1) does lipid storage vary according to the reproductive cycle, seasonal food availability, or both? (2) if lipids are stored seasonally, in what form are they stored? (3) when are the lipid maxima and minima and what is their nature? (4) how do areal lipid concentrations in the population change over a 2-year period? (5) how is lipid content affected during periods of starvation?, and (6) how does the seasonal energy cycle in *A. malmgreni* compare with *Mysis mixta* inhabiting the same region?

5.2 Material and methods

5.2.1 Study site and sample collection

Samples of *Acanthostepheia malmgreni* were collected approximately monthly from October 1998 to November 2000 from 240-m depth in the hyperbenthos of Conception Bay. Detailed sampling methods for both the hyperbenthos and the water column are presented in Chapter 2.

5.2.2 Specimens

Live, depurated amphipods (*Acanthostepheia malmgreni*) were sorted into life-history stages (Table 5.1). The body lengths of 1 to 5 straightened individuals from each free-living stage (sample size dependent on availability) were measured to the nearest 0.5 mm (frontal edge of the eyes to the telson tip). Embryos were removed from brood sacs for counting, although each brood was maintained as a unit for lipid analysis. Each sample was rinsed with 1-μm filtered seawater and stored in chloroform under nitrogen at –20°C. Dry mass (DM) of each amphipod was calculated from body length using an equation derived for the appropriate life-history stage (4 equations in total), and DM of each brood was calculated from brood size (Table 5.2; Chapter 3). Approximately 200 live amphipods collected on 14 August 2000 were placed in several 18-litre containers of

1-μm filtered, aerated seawater at ~2°C (~30 individuals in each container). The amphipods were maintained without food, light or sediment for 2.5 months to examine the changes in lipids in response to starvation. Exuviae and dead amphipods were removed daily, and the filtered seawater replaced every few days. Triplicate samples (sexes not separated) for lipid or DM analyses were collected every 3 to 14 days until 1 November 2000, when daily mortality was ~50%.

Table 5.1 *Acanthostepheia malmgreni.* Life-history stages and approximate size ranges of amphipods (L_2) and entire broods (embryos brood^{-1}). Free-living juveniles <7 mm were not sampled because they appear to occur at a different depth

Life-history stage	Body length (mm) or brood size (embryos brood^{-1})	Dry mass (mg)	Characteristics
Embryos (whole broods)	142 – 339	21 – 61	Embryos are within a brood sac
Juveniles	7.0 – 23	1.7 – 42	No visible secondary sexual characteristics
Immatures	14 – 35	29 – 164	*Female*: has developing oostegites
	17 – 24	23 – 62	*Male*: has developing penes
Mature females	24 – 32	92 – 149	Fully developed brood sac with embryos
Spent females	28 – 32	74 – 123	Fully developed empty brood sac
Mature males	20 – 33	37 – 162	Well developed penes
Non-sexed individuals	23 – 34	52 – 168	May include immature males, immature females, and mature males

Table 5.2 *Acanthostepheia malmgreni.* Equations used to calculate dry mass (DM, mg) of amphipods from body length (L, mm) and brood DM (mg) from brood size (Fc, embryos brood^{-1}) (Chapter 3)

Eq.	Life-history stage	Equation	r^2	n
5.1	Juveniles and non-sexed	$DM = 0.00673 \times L^{2.87}$	0.97	109
5.2	Males (immature and mature)	$DM = 0.00710 \times L^{2.85}$	0.97	81
5.3	Females (immature and brooding)	$DM = 0.00480 \times L^{3.01}$	0.98	81
5.4	Spent females	$DM = 0.00955 \times L^{2.73}$	0.97	60
5.5	Broods	$DM = 0.181 \times Fc - 0.340$	0.87	12

5.2.3 Lipid analyses, areal concentrations and statistics

Lipids were extracted from amphipods and broods and analyzed using the methods described in Chapter 4. Likewise, calculations of areal concentrations of total lipid (TL) and triacylglycerol (TAG) (mg m^{-2}) and statistical analyses follow Chapter 4. All mean values are reported ± one standard deviation (SD).

5.3 Results

Seasonal life cycle, density, biomass, growth and production of *Acanthostepheia malmgreni* in Conception Bay were described in Chapter 3. Amphipods from four cohorts (C1, C2, C3 and C4) were sampled between October 1998 and November 2000 (Fig. 5.1A), although only one C4 amphipod was available for lipid analysis, and C2 was a minor cohort with only a few representatives. The dominant lipid classes were TAG and PL, with ST, AMPL, DG, SE/WE, FFA, HC, KET, ME and ALC representing the minor classes.

The highest TL (13% DM) and TAG (7% DM) levels occurred in large adults from C1 and immature individuals from C2 and C3 (Figs. 5.1B, C, Figs. 5.2B, C), whereas highest PL levels (4% DM) occurred in C1 mature females early in the brooding period (Fig. 5.3). TL content in mature females (mg per individual) decreased at 1.4 mg $month^{-1}$ during brooding (Table 5.3), primarily due to decreases in PL rather than TAG (Tables 5.4, 5.5). Females released their broods in April and May, leaving spent females with low quantities of TL (May 1999, 2% DM). TL concentrations in large non-sexed amphipods in C1 averaged 9% DM from October 1998 to May 1999, after which this group disappeared from the hyperbenthos, together with the mature and spent females. The mixed group of amphipods showed a significant accumulation of TL (1.4 mg $month^{-1}$, Table 5.3), resulting primarily from increases in TAG rather than PL (Tables 5.4, 5.5).

TL in C2 juveniles averaged 4% DM from February to November 1999. C2 amphipods accumulated TAG at 0.78 mg $month^{-1}$ following spring bloom sedimentation (Table 5.4), although no significant accumulation of PL was detected (Table 5.5). Very few C2 samples were collected, so it was not possible to determine conclusively seasonal or developmental changes in the lipids of this cohort.

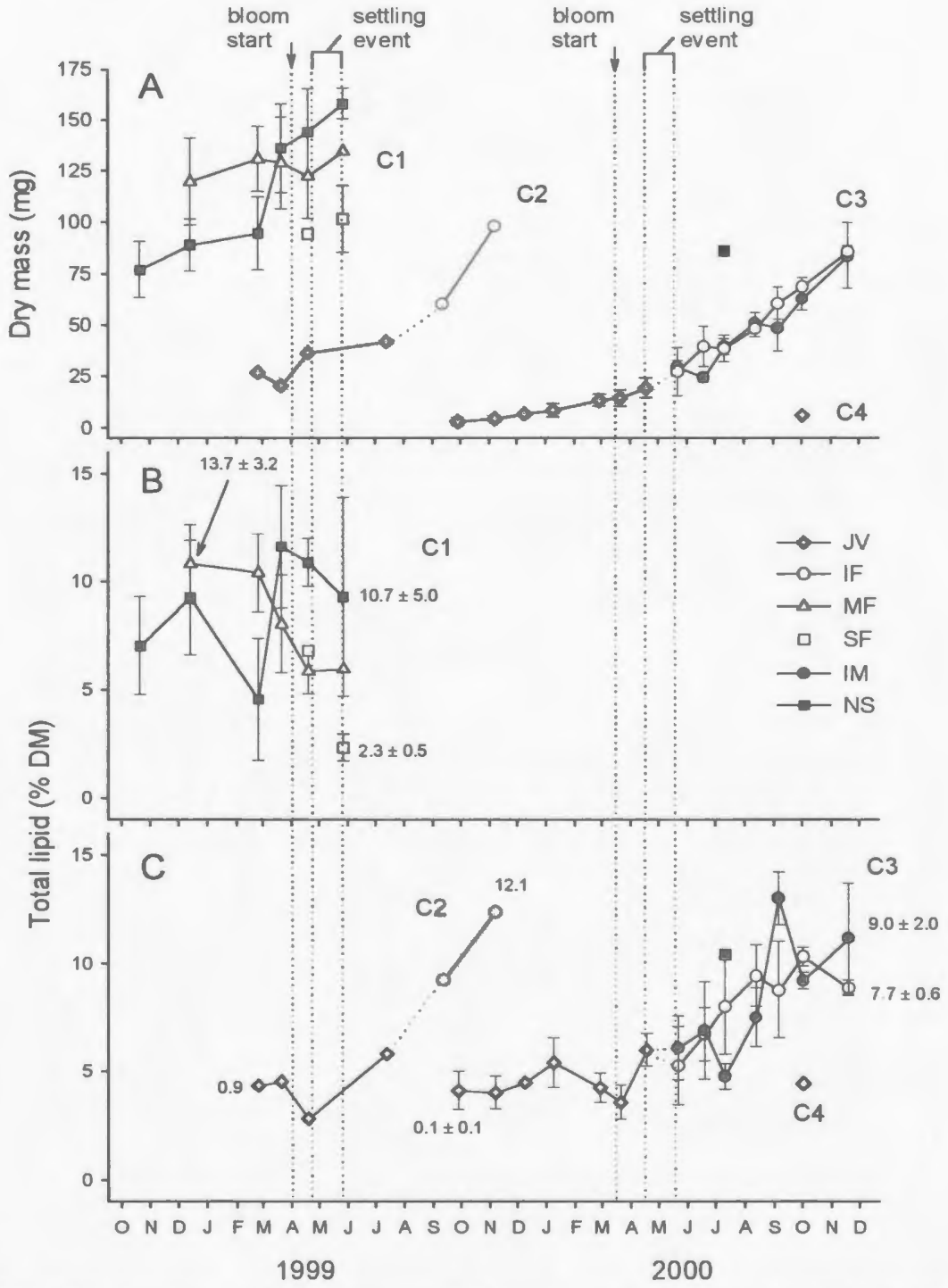

Fig. 5.1 A,B,C *Acanthostepheia malmgreni.* **A** Changes in dry mass (mg) in lipid samples, and **B** changes in total lipid (% DM) in cohort 1 *(C1)* and **C** cohorts 2 and 3 *(C2, C3)* [only one *C4* sample was available for lipid analysis; *JV* juveniles; *IF* immature females; *MF* mature females (includes brooded embryos); *SF* spent females; *IM* immature males; *NS* non-sexed]. *Superimposed values* represent max. or min. lipid content (mg ind^{-1}); *dotted lines* within a cohort represent transition periods between stages; *vertical dotted lines* represent bloom start and settling times. *Error bars*: SD around the mean

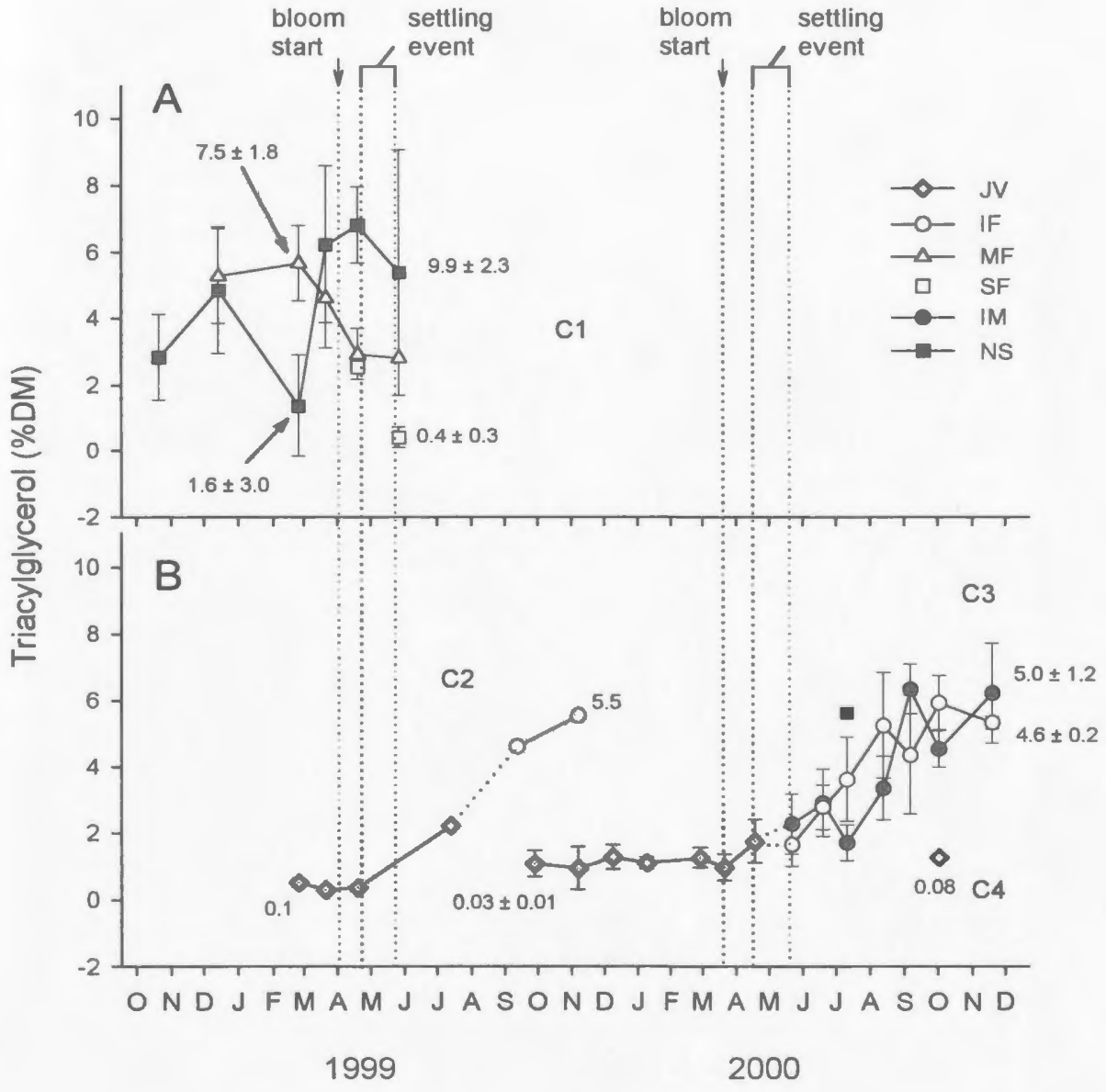

Fig. 5.2 A,B *Acanthostepheia malmgreni*. Changes in triacylglycerol (% DM) in **A** cohort 1 *(C1)* and **B** cohorts 2 and 3 *(C2, C3)*. *Superimposed values* represent max. or min. TAG content (mg ind^{-1}); *dotted lines* within a cohort represent transition periods between stages; *vertical dotted lines* represent bloom start and settling times. *Error bars*: SD around the mean *(abbreviations as in Fig. 5.1)*

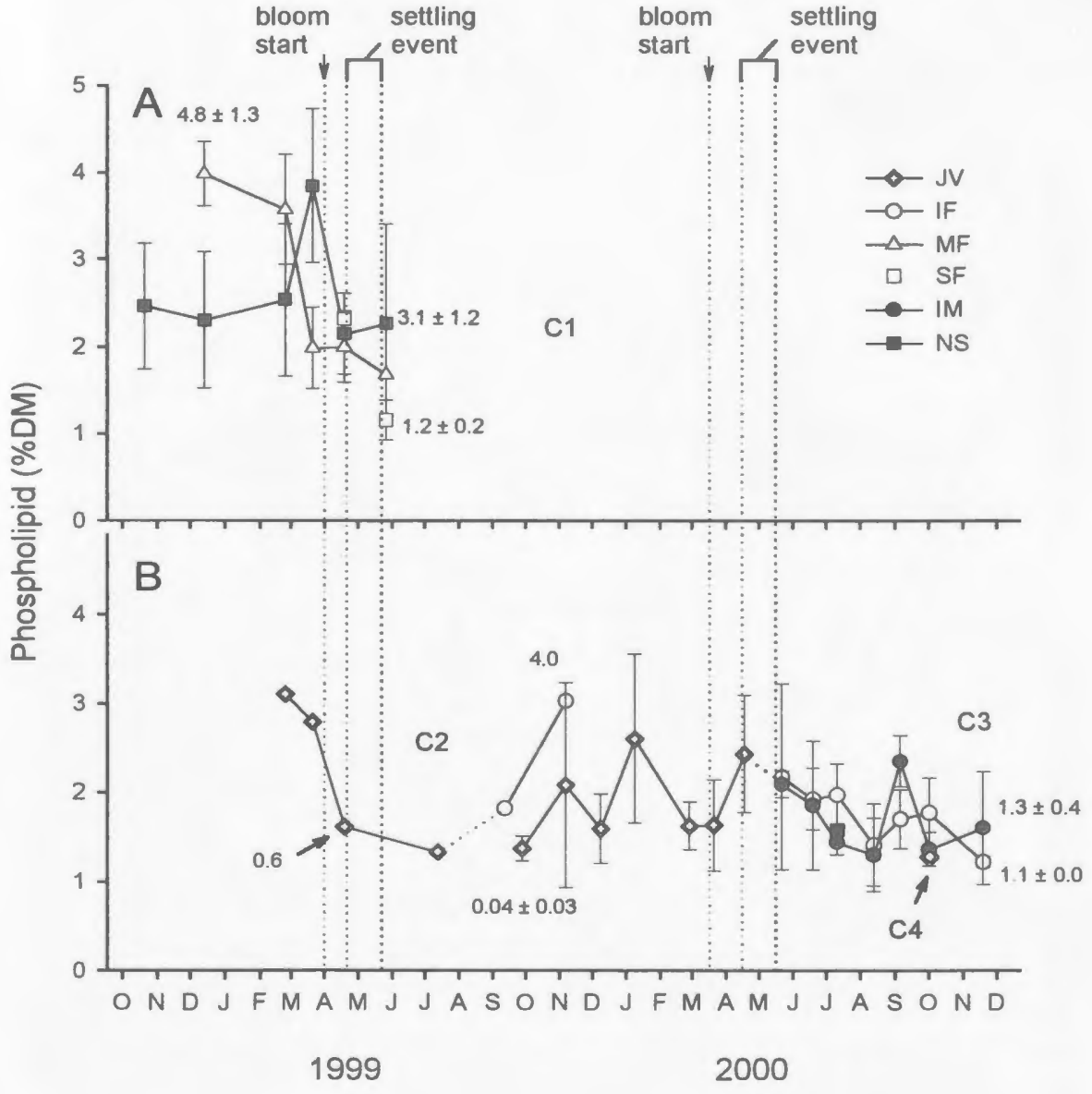

Fig. 5.3 A,B *Acanthostepheia malmgreni*. Changes in phospholipid (% DM) in **A** cohort 1 (C1) and **B** cohorts 2 and 3 (C2, C3). *Superimposed values* represent max. or min. PL content (mg ind^{-1}); *dotted lines* within a cohort represent transition periods between stages; *vertical dotted lines* represent bloom start and settling times. *Error bars*: SD around the mean *(abbreviations as in Fig. 5.1)*

The larger cohort C3 was the longest continually-sampled cohort of *A. malmgreni*, although it contained no mature males or mature females. Immature males and immature females accumulated lipid at the same rate (ANCOVA, F(interaction term) = $1.040_{1,49}$, $p > 0.05$). C3 accumulated TL at low rates prior to bloom sedimentation (0.082–0.55 mg month^{-1}), and then increased accumulation to 1.4 mg month^{-1} until sampling was terminated in November 2000 (Fig. 5.1C, Table 5.3). Changes in TL within C3 resulted primarily from increases in TAG (up to 0.81 mg month^{-1}) rather than PL (up to 0.11 mg month^{-1}; Tables 5.4, 5.5). Highest TL (13% DM) occurred in immature males in September 2000. TAG levels peaked in October/November 2000 in developing females (~6% DM), and PL in January 2000 in juveniles (~3% DM) (Figs. 5.2B, 5.3B). One amphipod collected in June 2000 was over twice the size of the predominant group of immature individuals from C3 (Fig. 5.1A). Despite its large size, the TL, TAG and PL levels in this individual were similar to those in the smaller amphipods (Figs. 5.1C, 5.2B, 5.3B). The single C4 juvenile was approximately the same size as small C3 juveniles, with similar lipid class levels.

Table 5.3 *Acanthostepheia malmgreni*. Within-cohort total lipid accumulation or utilisation rates (rates produced from regression slopes of TL per individual). Males and females were pooled when their rates were not significantly different (ANCOVA, $p > 0.05$). Sub-groups were created to establish rates before (pre) and after (post) the start of the spring bloom, although bloom status was not assigned to mature males/females and non-sexed adults, and 2 post-bloom rates were calculated for cohort 3 (*JV* juveniles; *IM* immature males; *IF* immature females; *MM* mature males; *MF* mature females; *NS* non-sexed). Spent females were not included in calculations. Amphipods that exhibited no significant change ($p > 0.05$) were assigned a rate of 0.00

Cohort	Stages	Time period	Bloom status	Accumulation or loss (mg month^{-1})	r^2	n
1	*MF*	Dec 98 – May 99	*n/a*	-1.4*	0.34	16
1[a]	*NS; MM*	Oct 98 – May 99	*n/a*	1.4**	0.32	32
2	*JV*	Feb 99 – Apr 99	Pre	0.00	*n/a*	3
2	*JV; IF*	Apr 99 – Nov 99	Post	0.00	*n/a*	4
3	*JV*	Sep 99 – Mar 00	Pre	0.082****	0.75	22
3[a]	*JV; IM; IF*	Mar 00 – Jul 00	Post	0.55****	0.41	27
3	*IM; IF*	Jul 00 – Nov 00	Post	1.4****	0.80	33

$^{*}p < 0.05$, $^{****}p < 0.0001$, *n/a* not applicable

[a]regressions corrected for temporal autocorrelation using Cochrane-Orcutt procedure

Table 5.4 *Acanthostepheia malmgreni*. Within-cohort triacylglycerol accumulation rates (rates produced from regression slopes of TAG content per individual). Males and females were pooled when their rates were not significantly different (ANCOVA, $p > 0.05$). Amphipods that exhibited no change ($p > 0.05$) were assigned a rate of 0.00 *(bloom status and abbreviations as in Table 5.3)*

Cohort	Stages	Time period	Bloom status	Accumulation (mg month^{-1})	r^2	n
1	*MF*	Dec 98 – May 99	*n/a*	0.00	*n/a*	16
1[a]	*NS; MM*	Oct 98 – May 99	*n/a*	0.80**	0.23	32
2	*JV*	Feb 99 – Apr 99	Pre	0.00	*n/a*	3
2	*JV; IF*	Apr 99 – Nov 99	Post	0.78*	0.90	4
3	*JV*	Sep 99 – Mar 00	Pre	0.023****	0.63	22
3	*JV; IM; IF*	Mar 00 – Jul 00	Post	0.26****	0.49	28
3	*IM; IF*	Jul 00 – Nov 00	Post	0.81****	0.78	33

*$p < 0.05$, ****$p < 0.0001$, *n/a* not applicable
[a]regression corrected for temporal autocorrelation using Cochrane-Orcutt procedure

Table 5.5 *Acanthostepheia malmgreni*. Within-cohort phospholipid accumulation or utilisation rates (rates produced from regression slopes of PL content per individual). Males and females were pooled when their rates were not significantly different (ANCOVA, $p > 0.05$). Sub-groups were created to establish rates before (pre) and after (post) the start of the bloom, although bloom status was not assigned to mature males/females and non-sexed adults. Amphipods that exhibited no significant change ($p > 0.05$) were assigned a rate of 0.00 *(abbreviations as in Table 5.3)*

Cohort	Stages	Time period	Bloom status	Accumulation or loss (mg month^{-1})	r^2	n
1[a]	*MF*	Dec 98 – May 99	*n/a*	-0.83**	0.42	15
1	*NS; MM*	Oct 98 – May 99	*n/a*	0.29**	0.24	33
2	*JV*	Feb 99 – Apr 99	Pre	0.00	*n/a*	4
2	*JV; IF*	Apr 99 – Nov 99	Post	0.00	*n/a*	3
3[a]	*JV*	Sep 99 – Apr 00	Pre	0.054****	0.49	25
3	*JV; IM; IF*	Apr 00 – Nov 00	Post	0.11****	0.47	49

**$p < 0.01$, ****$p < 0.0001$, *n/a* not applicable
[a]regressions corrected for temporal autocorrelation using Cochrane-Orcutt procedure

TAG was the predominant lipid class in immature and mature males and females, whereas PL was dominant in juveniles and spent females (Tables 5.6, 5.7, 5.8). Of the less prominent lipid classes, KET, FFA and DG increased as amphipods grew and matured (least squares regression analyses of C3, $p < 0.05$). All other minor classes remained constant throughout development ($p > 0.05$). ST was among the four most

dominant lipid classes (along with TAG, PL and FFA). For the entire population, the relationship between TL ind^{-1} (mg) and DM (mg) did not differ significantly between males and females (ANCOVA, $F = 3.924_{1,67}$, $p > 0.05$), and is given by:

$$TL = 0.0284 \times DM^{1.2317} \qquad (r^2 = 0.91, n = 126, p < 0.0001) \qquad \text{Eq. 5.6}$$

Areal concentrations of TL and TAG in the hyperbenthos were highest in November 2000 (maxima 67 and 33 mg m^{-2}, respectively; Fig. 5.4). Areal lipid concentrations remained low throughout 1999 following maximum areal TL and TAG concentrations in December 1998 (24 and 11 mg m^{-2}, respectively).

Table 5.6 *Acanthostepheia malmgreni*. Lipid class composition of cohort 1. Data are grand means ±SD calculated for stages collected on more than one sampling date (*HC* hydrocarbon; *SE/WE* steryl ester/wax ester; *ME* methyl ester; *KET* ketone; *TAG* triacylglycerol; *FFA* free fatty acids; *ALC* alcohol; *ST* sterol; *DG* diacylglycerol; *Neutral* sum of neutral lipids; *AMPL* acetone-mobile polar lipid; *PL* phospholipid; *Polar* sum of polar lipids; *TL* total lipid; *DM* dry mass)

Class	Mature females Dec. 1998 – May 1999		Spent females Apr. 1999 – May 1999		Non-sexed adults Oct. 1998 – May 1999	
	% DM	% TL	% DM	% TL	% DM	% TL
HC	0.06±0.03	0.64±0.27	0.00	0.14±0.20	0.04±0.02	0.41±0.16
SE/WE	0.02±0.01	0.17±0.17	0.01±0.01	0.29±0.41	0.11±0.12	1.12±1.39
ME	0.00	0.02±0.04	0.01±0.01	0.21±0.30	0.01±0.02	0.07±0.16
KET	0.02±0.02	0.31±0.20	0.00	0.00	0.02±0.01	0.31±0.22
TAG	4.27±1.32	51.44±4.15	1.50±1.51	27.11±14.68	4.59±2.08	45.80±16.80
FFA	0.32±0.21	4.47±3.47	0.68±0.56	13.97±2.49	0.46±0.30	5.58±2.71
ALC	0.00	0.00	0.00	0.12±0.17	0.02±0.03	0.24±0.40
ST	0.55±0.11	6.79±1.16	0.44±0.14	11.55±5.28	0.50±0.09	6.57±2.36
DG	0.03±0.02	0.43±0.30	0.04±0.04	0.66±0.40	0.08±0.06	0.94±0.34
Neutral	5.26±1.33	64.18±5.15	2.67±2.22	54.06±11.22	5.81±2.40	61.06±16.25
AMPL	0.33±0.15	3.97±1.28	0.18±0.16	3.62±0.86	0.38±0.22	4.23±2.78
PL	2.64±1.06	31.85±5.20	1.73±0.82	42.33±12.08	2.59±0.63	34.70±16.81
Polar	2.96±1.15	35.82±5.15	1.91±0.98	45.94±11.22	2.97±0.77	38.94±16.25
TL	8.22±2.35		4.57±3.19		8.78±2.60	
TL ind^{-1} (mg)	10.38±2.87		4.37±2.92		10.74±4.99	

Table 5.7 *Acanthostepheia malmgreni*. Lipid class composition of cohort 2. Data are grand means ±SD calculated for stages collected on more than one sampling date *(abbreviations as in Table 5.6)*

Class	Juveniles Feb. 1999 – July 1999		Immature females Sept. 1999 – Nov. 1999	
	% DM	% TL	% DM	% TL
HC	0.00	0.00	0.01±0.01	0.09±0.13
SE/WE	0.02±0.04	0.35±0.70	0.05±0.08	0.58±0.82
ME	0.02±0.03	0.30±0.59	0.03±0.04	0.24±0.34
KET	0.01±0.02	0.20±0.41	0.00	0.00
TAG	0.86±0.93	17.56±14.28	5.09±0.65	47.48±3.57
FFA	0.50±0.28	11.20±4.53	0.71±0.71	7.35±8.10
ALC	0.05±0.10	0.87±1.75	0.39±0.27	3.44±1.83
ST	0.49±0.23	11.68±5.05	0.59±0.14	5.40±0.21
DG	0.02±0.03	0.44±0.54	0.04±0.02	0.44±0.30
Neutral	1.97±1.33	42.60±17.25	6.91±0.28	65.02±10.54
AMPL	0.20±0.25	4.11±4.06	0.47±0.37	4.79±4.40
PL	2.21±0.87	53.29±21.15	3.43±2.28	30.19±14.94
Polar	2.40±0.69	57.40±17.25	3.90±1.90	34.98±10.54
TL	4.37±1.22		10.81±2.19	
TL ind^{-1} *(mg)*		1.39±0.71		8.86±4.61

Table 5.8 *Acanthostepheia malmgreni*. Lipid class composition of cohort 3. Data are grand means ±SD calculated for stages collected on more than one sampling date *(abbreviations as in Table 5.6)*

Class	Juveniles Sept. 1999 – Apr. 2000		Immature females May 2000 – Nov. 2000		Immature males May 2000 – Nov. 2000	
	% DM	% TL	% DM	% TL	% DM	% TL
HC	0.02±0.03	0.34±0.48	0.04±0.02	0.54±0.20	0.05±0.03	0.51±0.25
SE/WE	0.11±0.11	2.39±2.32	0.36±0.19	4.22±1.96	0.40±0.33	4.52±3.15
ME	0.02±0.02	0.40±0.49	0.06±0.05	0.65±0.48	0.17±0.23	1.65±1.80
KET	0.05±0.03	0.99±0.67	0.24±0.09	2.90±1.05	0.27±0.10	3.41±1.38
TAG	1.10±0.38	24.09±6.10	4.14±1.54	48.32±10.20	3.92±1.85	44.90±7.21
FFA	0.44±0.24	9.34±3.60	0.84±0.15	10.50±1.54	0.93±0.35	11.38±2.23
ALC	0.01±0.02	0.33±0.46	0.02±0.02	0.22±0.31	0.03±0.03	0.36±0.39
ST	0.42±0.06	9.36±0.98	0.39±0.04	5.08±1.19	0.42±0.07	5.41±1.33
DG	0.03±0.02	0.57±0.34	0.07±0.02	0.90±0.23	0.11±0.05	1.19±0.33
Neutral	2.35±0.55	47.82±10.37	6.17±1.88	73.31±9.56	6.30±2.66	73.33±8.51
AMPL	0.26±0.14	5.74±3.05	0.30±0.09	3.70±0.50	0.36±0.19	4.20±1.29
PL	2.11±0.63	46.43±12.52	1.74±033	22.99±9.54	1.71±0.40	22.47±8.47
Polar	2.19±0.47	52.18±10.37	2.04±0.27	26.69±9.56	2.08±0.46	26.67±8.51
TL	4.55±0.85		8.21±1.71		8.37±2.91	
TL ind^{-1} *(mg)*	0.44±0.31		4.50±2.32		4.33±2.81	

Specimens from C3 collected on 14 August 2000 were starved in the laboratory, resulting in curtailed growth (DM decreased from 50 ± 4.4 mg to 43 ± 7.6 mg, 14 August–1 November), whereas field amphipods from the same cohort grew to 85 mg DM by November 2000. Maturing males and females from C3 were present in Conception Bay during and following the spring diatom bloom of 2000. When collected, the amphipods contained 4 mg TL (8% DM), and after utilizing TL at a rate of 1.2 mg month^{-1} for 2.5 months while starved in the laboratory, they contained only 1.4 mg TL (3% DM) (Fig. 5.5). In contrast, amphipods collected from the field in November showed an increase in TL content (up to 8 mg per individual). The lipid composition in starved amphipods began to change within the first few days without food, and a marked increase was observed in the level of AMPL (maximum 9% DM on 23 August, Fig. 5.6A). TAG content decreased at a rate of 0.54 mg month^{-1} throughout the starvation period, while PL content remained constant (Fig. 5.6A). Individual variability in most lipid classes was extremely high throughout the 2.5-month period (Fig. 5.6). Except for SE:WE, which decreased at rate of 0.10 mg month^{-1}, lipid classes FFA and ST remained constant, although an unexpected peak in FFA occurred in early October (Fig. 5.6B).

Lipolysis index (LI) values (Parrish 1998) remained below 15% in most of the samples, indicating that their integrity was maintained during storage and analysis. Maximum LI values of 19% were calculated in only a few juveniles from C2 and C3.

5.4 Discussion

Lipid content, level and composition in zooplankton are affected by numerous factors, including geographical location, temperature, season, food availability, food quality and quantity, body size, salinity, physiology and life cycle. Temporal changes in lipids result from interactions among these external and internal factors, and lipid dynamics in one species have the potential to affect the energetics of an entire ecosystem (Arts 1991). Hyperbenthic organisms living in deep areas of Conception Bay experience temperatures consistently < 0°C (Chapter 2) owing to the year-round influence of the Labrador Current. Deep areas of Conception Bay represent a typical subarctic marine environment owing to

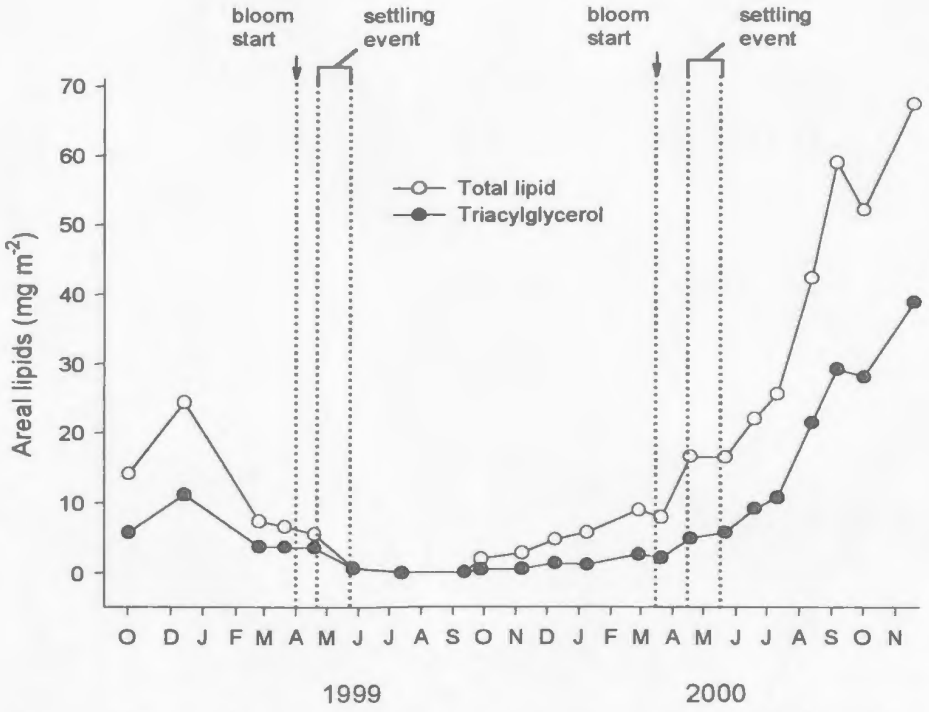

Fig. 5.4 *Acanthostepheia malmgreni*. Areal concentrations of total lipid and triacylglycerol. *Vertical dotted lines* represent spring bloom start and settling times

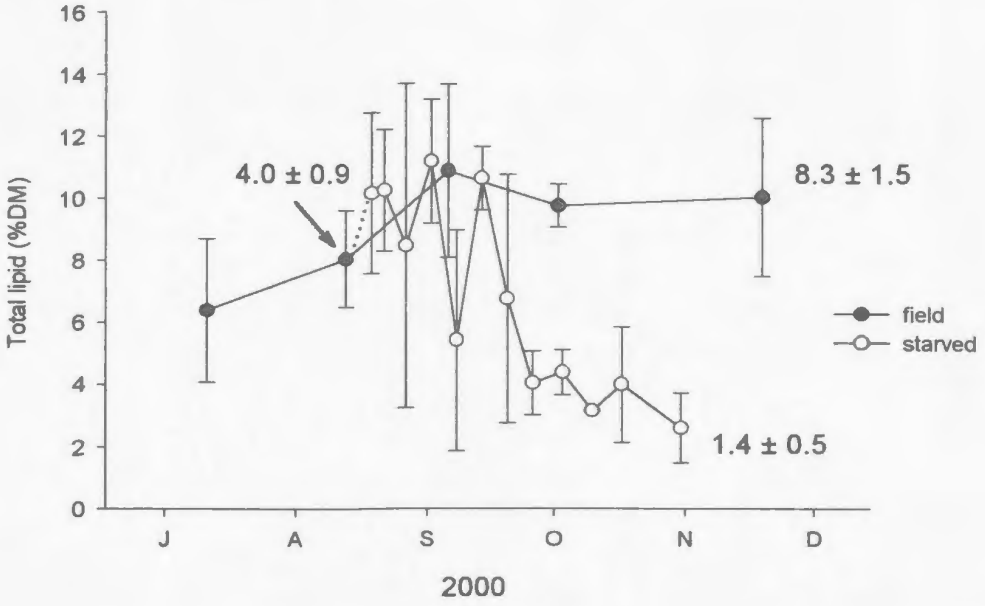

Fig. 5.5 *Acanthostepheia malmgreni*. Total lipid (% DM) in C3 amphipods starved in the laboratory, compared with those collected in the field from August to November 2000. Males and females were pooled. *Superimposed values* represent initial and final values of TL content (mg ind^{-1}). *Error bars*: SD around the mean

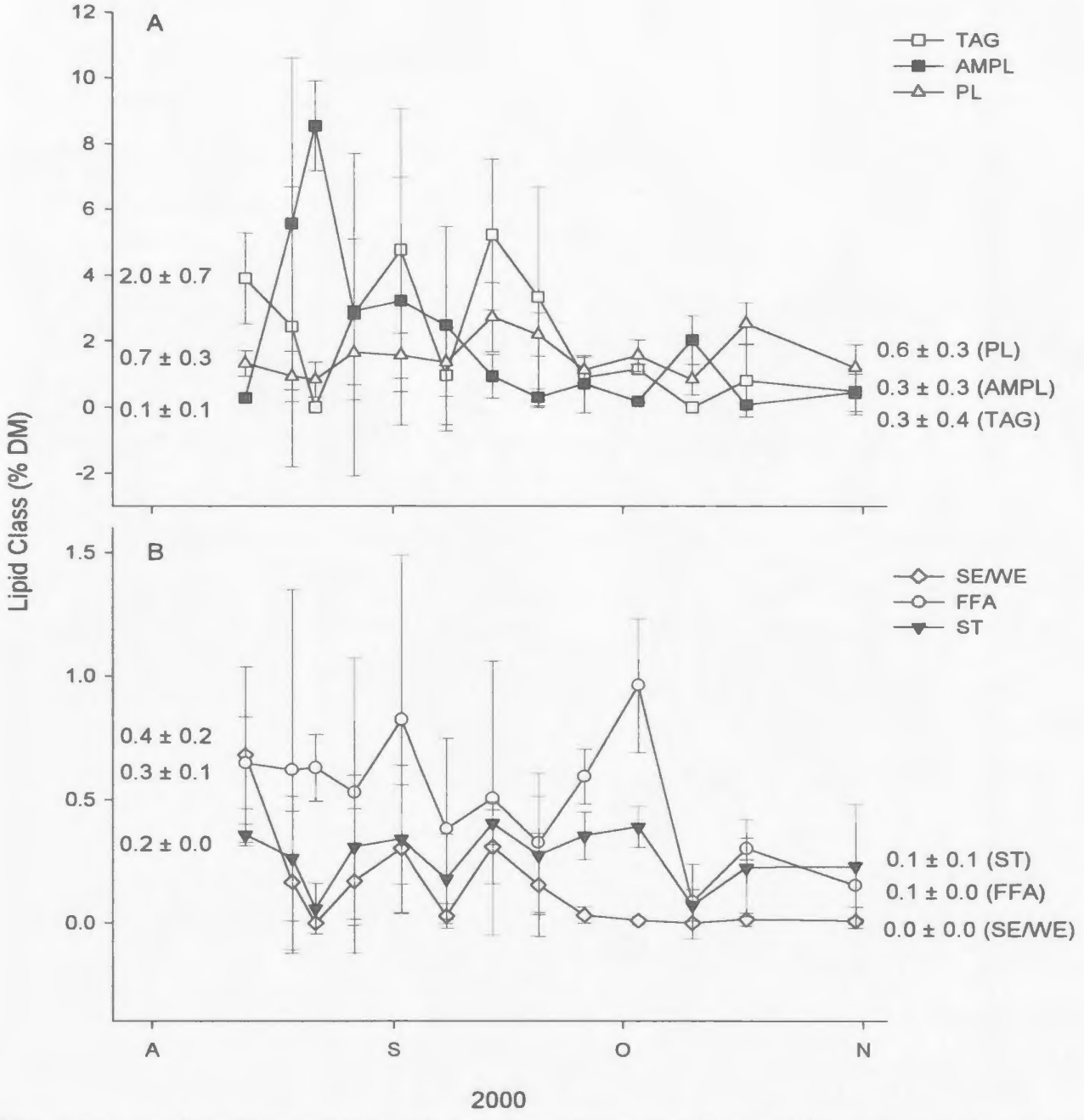

Fig. 5.6 A,B *Acanthostepheia malmgreni*. **A** Major and **B** minor lipid classes (% TL) in C3 amphipods starved in the laboratory from 14 August to 1 November 2000. Males and females were pooled (*TAG* triacylglycerol; *AMPL* acetone-mobile polar lipid; *PL* phospholipid; *SE/WE* steryl/wax ester; *FFA* free fatty acids; *ALC* alcohol; *ST* sterol). *Superimposed values* represent initial and final values of lipid class content (mg ind^{-1}). *Error bars*: SD around the mean

the perpetually low temperatures and the influence of a seasonally productive euphotic zone. Even highly motile hyperbenthic zooplankton that undergo diel vertical migrations are unlikely to reach shallow water (< 50 m) characterized by temperatures < 2°C. Similarly, salinity remains between 32.0 and 34.0 psu below 50 m depth (Chapter 2). Food availability and quality (related to season, location and diet), in addition to the reproductive cycle (related to body size, physiology and life-cycle stage), are probably key regulators of lipid content and composition in *Acanthostepheia malmgreni* inhabiting Conception Bay.

Seasonal lipid data have been documented for only a few invertebrate species living in the hyperbenthos of Conception Bay and similar cold-ocean regions. In addition to the lipids of *A. malmgreni*, those of both the mysid *Mysis mixta* (Chapter 4) and the chaetognath *Parasagitta elegans* (Choe et al. 2003) vary with the cycles of reproduction and local pelagic production. As in *M. mixta* and *P. elegans*, total lipid levels in *A. malmgreni* increase with maturity stage. Lipid levels in *P. elegans* are highest in spring and summer, when there is an abundance of copepods rich in energy derived from the spring bloom (Choe et al. 2003). The *M. mixta* population exhibits a particularly striking response, and rapid accumulation of lipid occurs in developing mysids as soon as the spring diatom bloom begins (Chapter 4). In contrast, lipid accumulation in *A. malmgreni* occurs following bloom settlement and is not as pronounced as the response in *M. mixta*. Gelatinous zooplankton are protein-rich rather than lipid-rich, and in the study of Choe et al. (2003) the TL levels in *P. elegans* ranged from 9 to 16% DM, with lowest values occurring in winter months. Seasonal lipid variation in the two brooding crustaceans was greater and ranged from 4 to 32% DM in *M. mixta* (Chapter 4) and from 3 to 13% DM in *A. malmgreni*. Maximum lipid concentrations were observed in individuals nearing full maturity in each species, although the maximum value for *M. mixta* was more than twice that of *A. malmgreni* (Chapter 4). In contrast, maximum lipid content per individual was approximately equivalent (amphipods 14 mg; mysids 16 mg), owing in part to the relatively heavier carapace of *A. malmgreni*. Calculated as proportions of the digestible portion of dry mass (% ash-free dry mass, AFDM), maximum TL levels in the two species were slightly less disparate (amphipods 20% AFDM; mysids 38% AFDM).

The lipid content maxima are consistent with observed lipid accumulation rates in the two crustacean species. The highest rate of lipid accumulation in *M. mixta* was 2.7 mg month^{-1}, whereas *A. malmgreni* accumulated TL at a maximum of 1.4 mg month^{-1}. Rapid lipid accumulation in maturing mysids occurred between March and November in 1999 and 2000 (Chapter 4). Maturing *A. malmgreni*, on the other hand, accumulated significant amounts of lipid during 2000 only (C3). The 1999 cohort (C2) was probably too sparse and short-lived to provide sufficient information on lipid dynamics in the population, and C1 consisted of larger age-2+ amphipods nearing the end of their life span. As mentioned above, C1 mature amphipods contained the highest quantities of lipid. Unfortunately, this study did not encompass the critical period of maturation and lipid accumulation for C1 (summer and autumn 1998). In view of the 2.5-year life span and the apparent maintenance of low lipid levels during the first year, maturing female *A. malmgreni* in C1 must have accumulated lipid at ~1.2 mg month^{-1} for 12 months to reach the 14 mg peak in TL in February 1999. One-year-old amphipods in C3 accumulated lipid at a similar rate of 1.4 mg month^{-1}, and mean lipid content in maturing individuals in November 2000 was 8 mg in females and 9 mg in males. To obtain the maximum lipid content achieved by their progenitors (C1 females, 14 mg), C3 females would have had to increase lipid accumulation to a rate of 3.2 mg month^{-1} from December 2000 to February 2001, a period when food quantity and quality were probably low. It is more likely that C3 amphipods continued to accumulate lipid at a rate closer to 1.4 mg month^{-1}, resulting in a lipid content of less than 10 mg by February 2001. Alternatively, additional lipid could have been synthesised from protein or carbohydrate, which is possible since 2-year-old C3 amphipods in November 2000 were as large as 2-year-old C1 amphipods (non-brooding) in November 1998. On the other hand, conversion of protein into lipid by C3 amphipods was unlikely because protein levels remained constant at 21±1.0% DM from June to November 2000 (Chapter 3). Carbohydrate was not measured, since it is assumed that it plays a minor role in the seasonal energy cycle of the amphipod population (Nair & Anger 1980). The ecological implications of C3 *A. malmgreni* storing less than the observed maximum lipid content exhibited by C1 females are probably minor and reflect typical year-to-year variation in this species.

Between-year differences in the lipid content, level and composition of *T. malmgreni* probably reflected interannual variation in pelagic productivity. The crucial period for accumulating lipids in developing C1 females (spring 1998) coincided with the highest chl *a* concentrations observed between February 1998 and December 2000. The 1998 bloom, like those of 1999 and 2000, started in March (bloom start defined as chl *a* = 1 µg l^{-1}), but its magnitude was greater and its duration longer (Chapter 2). The chl *a* maximum was highest in 1998 (5.27 µg l^{-1}), and high chl *a* levels (up to 3.00 µg l^{-1}) persisted from mid-March to late May. In contrast, chl *a* maxima were 2.22 and 3.64 µg l^{-1} in 1999 and 2000, respectively, with high concentrations occurring only briefly in mid-April 1999 and throughout May 2000 (Chapter 2). There was also evidence of an early settling event in March 1998, in addition to the usual sedimentation period in May. Total primary production (g C $m^{-2}yr^{-1}$) was not measured, although values are available for the preceding years (1986 – 1990, 124 – 137 g C $m^{-2}yr^{-1}$; Tian et al. 2003).

Inter-annual variation in lipid accumulation and maximum lipid level was not as pronounced in the sympatric *M. mixta* (e.g. maximum lipid content in mature females was 13 to 15 mg in cohorts 1, 2 and 3, Chapter 4), which suggests that the mysids are better able to compensate for fluctuations in food availability and or quality, owing to a high degree of motility, opportunistic feeding and the potential for diet switching. The amphipod *T. malmgreni* may possess few or none of these compensatory mechanisms, and thus may have no means to adapt rapidly to inter-annual fluctuations in its food supply. The superior adaptation of *M. mixta* relative to *T. malmgreni* is also reflected in the timing of peaks in areal concentrations of total and reserve lipid (as in Arts et al. 1992). Comparable maximum values of areal concentrations of TL were observed in both species in 2000 (67 mg m^{-2} in *T. malmgreni*, 55 mg m^{-2} in *M. mixta*, Chapter 4), but peak concentrations in the amphipod population occurred several months later than in the mysid population. Maximum areal concentrations of lipids in *T. malmgreni* represent 3.8% of the integrated input of seston lipids to the hyperbenthos of Conception Bay (1.75 g m^{-2}, 1996 bloom, Ramos et al. 2003). Throughout most of 1999, areal concentrations of total and reserve lipid in *T. malmgreni* were extremely low, probably as a result of the lower quality and shorter duration of the spring bloom. Unlike the mysid population, *T.*

malmgremi exhibited decreases in areal lipid concentrations during the spring bloom of 1999, indicating severe limitations in the population's ability to sequester organic material during this period.

The changes in TL in both male and female *A. malmgremi* were due primarily to increases or decreases in TAG content, presumably to fuel gametogenesis. The reproductive cycle appeared to centre around food availability for newly released offspring, the release period coinciding with spring bloom sedimentation (Chapter 3). When data from all life-history stages were combined, TL individual^{-1} increased with dry mass. Accumulation of lipid during growth is common in zooplankton (e.g. Ouellet et al. 1992, Kattner et al. 1994), the energy reserve component (TAG or WE) being the main source of seasonal variation (Arts 1999). In contrast, PL content and concentration in *A. malmgremi* remained relatively constant year-round, with highest levels occurring in mature females. One developing female from C2 (November 1999) contained more PL (4 mg) than females of the same age from C3 (1 mg). This discrepancy may reflect inter-annual variation caused by differences in food quality or availability, although unequivocal conclusions are not possible with data available from only one C2 amphipod. Because PL are a major component of membranes, they tend to be more stable than TAG (Arts 1991), and increased incorporation of PL typically reflects periods of growth or membrane production.

Increases of lipids in hyperbenthic zooplankton during or following the spring phytoplankton bloom in Conception Bay are similar to patterns observed in zooplankton from other environments (e.g. Gardner et al. 1985, Hill et al. 1992). The accumulation of large energy reserves is generally associated with organisms exposed to an abundant food supply for only brief periods. When nutrients become limiting, such organisms must utilise their lipid stores to survive and/or reproduce. Nair & Anger (1980) found high lipid reserves in the shallow water amphipod *Jassa falcata* when food was abundant in the North Sea. Lipid dynamics in *J. falcata*, unlike those of *A. malmgremi*, are not related to the reproductive cycle (Nair & Anger 1980). Furthermore, amphipod populations living in habitats where food is available year-round do not accumulate large lipid stores

(Percy 1979, Napolitano & Ackman 1989) or exhibit little seasonal variation in lipid content (Moore 1976)

Unlike the sympatric *M. mixta*, early-spawning females were not found in the *A. malmgreni* population, suggesting that a threshold lipid level may be required for brood synthesis. Hill et al. (1992) postulated such a threshold for the amphipods *Monoporeia affinis* and *Pontoporeia femorata*, in which reproduction was postponed for a year in females that did not store lipids at a level of 20% DM. The benthic amphipod *Diporeia hoyi* (formerly *Pontoporeia hoyi*) in Lake Michigan accumulated TAG (>60% TL) in response to the increase in the quality of detritus following the spring diatom bloom (Gardner et al. 1985). These levels of TAG are similar to those in mature female *A. malmgreni* before and during the 1999 spring bloom in Conception Bay (63 – 71% of TL). Similarly, TAG reserves in the benthic amphipods *M. affinis* and *P. femorata* in the Baltic Sea increased (up to 44% DM in *M. affinis*) during and immediately after the bloom (Hill et al. 1992, Lehtonen 1996).

While large adult *A. malmgreni* continued to accumulate lipid, mature females utilised significant amounts of PL before releasing their broods in April or May. The use of PL in this instance was unexpected, since one would expect that any energy requirements for brood irrigation and protection would have been fueled by reserve lipids (although some krill species utilize PL as storage lipid; Falk-Petersen et al. 2000). It is unlikely that lipid utilisation was due to a decrease or cessation in feeding because it was not accompanied by a decrease in dry mass (~125 mg DM throughout brooding period). Most of the lipid was transferred from the females to their broods, as evidenced by markedly lower lipid content in spent females.

The energetic cost of reproduction in males is assumed to be minor in non-broadcast spawning species (Clarke & Morris 1983). Although male *A. malmgreni* presumably transfer sperm directly to the females during copulation, it is difficult to estimate the cost of sperm production because all C1 adults without brood sacs were included in a non-sexed category. It was assumed that most of the individuals in this mixed category were male, although a significant proportion may have been females unsuccessful at producing broods. Why the large post-spawned or non-reproductive adults increased their lipid

stores until they died remains unknown, although the very low lipid levels in adults particularly during February 1999, may result from the prevalence of post-spawned males. More information is needed to estimate accurately the cost of reproduction in male *A. malmgreni*, although rapid accumulation of lipid in developing males, as in females, suggests that energetic costs may be significant.

Cessation of growth was observed in starved *A. malmgreni*, although the body shrinkage commonly seen in other zooplankton (e.g. Virtue et al. 1997, Stübing et al. 2003) was not. Lipids in *A. malmgreni* were mobilized within the first few days of exposure to starvation, and general stress probably contributed to the rapid production of AMPI (Parrish et al. 1998) and the high individual variation in lipid class levels throughout the starvation period. As expected, amphipods utilised TAG stores while conserving PL as long as possible. Unlike other species that appear more able to tolerate long periods of starvation (e.g. antarctic *Euphausia superba*, Virtue et al. 1997), starved *A. malmgreni* did not maintain TL relative to body mass or relative proportions of the major lipid classes. Given the increased mortality and low lipid levels after only 2.5 months starvation, it is very unlikely that a field population thus starved would have the ability to recover sufficiently to reproduce. In comparison, the carnivorous amphipod *Themisto libellula* living in arctic waters (sample group consisted of immature amphipods collected in autumn) can withstand starvation for over 5 months (Percy 1993). In *A. malmgreni*, variation in the ecdysis cycle may have contributed to the high variation in lipid composition throughout the starvation period. Nicol et al. (1992) found only weak fluctuations in lipids during the moult cycle of the euphausiid *E. superba*, although responses in stressed amphipods may be considerably different from those of relatively unstressed individuals in the field.

5.5 Summary

The present work represents the first documentation of the seasonal lipid cycle in *A. malmgreni*, a poorly known but abundant species living in the hyperbenthos of Conception Bay and other cold-ocean regions. The importance of the annual phytoplankton bloom to this hyperbenthic population is revealed by seasonality in

development and lipid dynamics. Inter-annual differences in the lipids of *T. malmgreni* are probably caused by variation in production in the upper water column, whereas seasonal lipid variation is closely related to the reproductive cycle. Lipid dynamics in starved *A. malmgreni* indicate that age-1+ individuals lack sufficient reserves to survive and reproduce through the winter in the absence of food. Divergent lipid dynamics among sympatric species inhabiting Conception Bay undoubtedly reflect taxonomy, life style, diet and migratory behaviour.

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Chapter 6. Seasonal and ontogenetic variation in the fatty acid composition of *Mysis mixta* and *Acanthostepheia malmgreni*

6.1 Introduction

Previous examination of population and lipid class dynamics in *Mysis mixta* and *Acanthostepheia malmgreni* indicated a close coupling between the species' life-history and energy cycles and seasonal production in the euphotic zone of Conception Bay, Newfoundland (Chapters 2 – 5). Despite the important role of hyperbenthic populations in the energetics of marine systems (Mees & Jones 1997), little is known about any zooplankton species inhabiting the hyperbenthos. Secondary production and areal concentrations of lipid estimates in *M. mixta* and *A. malmgreni* indicated that both species may be important ecologically and energetically in the Conception Bay ecosystem (Chapters 2 – 5). The aim of this chapter was to determine the seasonal fatty acid composition of *M. mixta* and *A. malmgreni*, particularly in relation to the reproductive cycles, foraging strategies and diet changes resulting from variations in primary productivity within the euphotic zone. It is generally known that lipid composition of consumers can vary quantitatively and qualitatively with the lipid composition of their food (Sargent & Falk-Petersen 1988). Information on the accumulation of specific fatty acid markers will indicate when and how successfully each species sequesters organic material produced during and following the spring phytoplankton bloom.

Mysis mixta Lilljeborg (Mysidacea) and *Acanthostepheia malmgreni* Goes (Amphipoda) are abundant, lipid-rich crustaceans living in the hyperbenthos of Conception Bay (Chapters 2 – 5). Both species protect spawned eggs within a brood pouch following fertilization. Larvae are brooded for ~5 months, and brood release occurs in April and May each year while the spring bloom material settles to the hyperbenthos (Chapters 2, 3). In Conception Bay, life span of females is 2.5 years from spawning to death, and 2 years of development are usually required before reproduction can occur (Chapters 2, 3). *M. mixta* is an opportunistic omnivore that undergoes ontogenetic shifts in its diet (Chapter 2, Viherluoto & Viitasalo 2001). *A. malmgreni* appears more limited to a carnivorous feeding mode, although juveniles feed on small particles including

phytoplankton and detritus (Chapter 3; Sainte-Marie & Brunel 1985). In turn, both species are potentially important links to higher trophic levels as they are prey for fish and a variety of invertebrate species (Mauchline 1980; Sainte-Marie & Brunel 1985). Vertical migrations into shallower depths bring a portion of the *M. mixta* population out of the hyperbenthos every night (Rudstam et al. 1989); however, there is no evidence that *A. malmgrem* undergoes diel migrations in Conception Bay. Observations in the laboratory indicate that *A. malmgrem*, although capable of rapid bursts of swimming to capture prey, is generally a less motile species than is *M. mixta* (Richoux, unpublished). The differences in behaviour, diet and motility in the two zooplankton populations are likely to be reflected in the seasonal ontogenetic fatty acid composition.

6.2 Materials and methods

6.2.1 Study site and sample collection

Hyperbenthic zooplankton were collected monthly between October 1998 and November 2000 from a 240-m depositional site in Conception Bay, Newfoundland, with an epibenthic sled. Detailed information on sampling methodology in the hyperbenthos is presented elsewhere (Chapter 2).

6.2.2 Specimens

Depending on availability, 1 - 5 replicates of all life-history stages of *A. malmgrem* and *M. mixta* were analysed following each sampling day. Life-stage categories and measurements used, in addition to sample storage protocol, are described in Chapters 4 and 5. The dry mass (DM) of each sample was calculated from body length, and the DM of entire broods was calculated from brood size (Chapters 4, 5). Lipids were extracted from each sample using a modified Folch procedure (Parrish 1999), and total lipid (TL) was determined by summing all lipid classes following thin-layer chromatography and flame-ionisation detection (Parrish 1987, Chapter 4). Fatty acid composition of each extract was determined by gas chromatography (GC) analysis (Omegawax column, Varian Model 3400) of fatty acid methyl esters prepared by transmethylation in the presence of boron trifluoride (85°C for 1 hr; Budge & Parrish 1998). Fatty acid methyl

esters were identified by comparison of retention times with known external standards (Supelco; identifications confirmed using a Varian 2000 GC mass spectrometer), and quantified by comparing peak areas with the area under an internal standard [23:0, at a concentration of ~10% total fatty acids (TFA)]. Integrity of the samples was confirmed with a lipolysis index that quantified the free fatty acid + alcohol proportions relative to other lipid classes (Parrish 1988).

6.2.3 Calculations and statistics

Each major fatty acid (μg individual^{-1}, defined as >1% TFA) was regressed against TL per individual, regardless of sex, season, stage or cohort, to determine its relative contribution to lipid accumulation (Hagen & Kattner 1998, Kattner & Hagen 1998). Based on these initial regressions, accumulation rates for the six most important fatty acids and the four fatty acid groups were estimated from slopes of linear sections of fatty acid per individual over time [μg month^{-1}; cohorts and life-history stages were regressed separately and divided into pre- and post-bloom periods (*M. mixta*) or according to development stage (*A. malmgreni*)]. The Durbin-Watson statistic was calculated to detect temporal autocorrelation in the regressions, and an Cochrane-Orcutt procedure was used to remove any autocorrelations (Neter et al. 1996). Biomarker ratios (16:1ω7 + 16:1ω5)/16:0, 22:6ω3/20:5ω3, sum polyunsaturates/sum saturates, sum C16/sum C18 and 18:1ω9/18:1ω7 were calculated in all cohorts. Data are reported as means ± one standard deviation (SD). The functional relationship between 16:1ω7 and 18:1ω7 was assessed with regression analysis. Areal concentrations of polyunsaturated fatty acids (PUFA) in each species were estimated from population density values (Chapters 2, 3) multiplied by mean PUFA content per individual on each sampling date (Arts et al. 1992).

6.3 Results

6.3.1 Fatty acids in Mysis mixta

Forty-three fatty acids were identified in *Mysis mixta* (Tables 6.1, 6.2). Major saturated fatty acids (SFA) in all life-history stages within the population were 14:0 and 16:0. Principal monounsaturated fatty acids (MUFA) were 16:1ω7, 18:1ω9, 18:1ω7.

20:1ω9(11) and 22:1ω11, and principal PUFA included 18:2ω6, 18:4ω3, 20:4ω6, 20:5ω3 and 22:6ω3.

In general, the fatty acids in juveniles were dominated by 20:5ω3, 22:6ω3, 16:0 and 18:1ω9, typical components of phospholipids (PL) (Fraser et al. 1989, Hagen et al. 2001). As the juveniles showed increases in their lipid stores while developing into mature mysids (Chapter 4), concentrations of these four fatty acids remained high and 16:1ω7 and 14:0 increased in importance (Tables 6.1, 6.2). Of all the major fatty acids (μg ind^{-1}) regressed against TL (μg ind^{-1}), these six fatty acids (in decreasing order of importance 20:5ω3, 18:1ω9, 16:1ω7, 16:0, 14:0 and 22:6ω3) had the greatest slopes, confirming their overall importance to lipid accumulation in *M. mixta* (see also Table 6.3).

Four cohorts (C1, C2, C3 and C4) were distinguished during the 2-year sampling period. Mysids from C2 collected between December 1998 and February 1999 were not sexed and were pooled in an 'undifferentiated immatures' category. Mysids from C1, C3 and C4 were adequately staged throughout development. The concentrations (% DM) of the three fatty acid types (SFA, MUFA and PUFA) varied within and among the 4 cohorts (Tables 6.1, 6.2). PUFA, dominated by 20:5ω3 and 22:6ω3, were generally the most prevalent fatty acids in most juveniles, immatures, spent females and mature males, whereas MUFA dominated in most mature females (Tables 6.1, 6.2). SFA concentrations varied, but were commonly less than those of PUFA and MUFA.

The highest concentrations of PUFA occurred in immature females from C2 (8% DM, Fig. 6.1). No change, or slow accumulation, of PUFA was observed in immature mysids prior to the spring bloom. Rapid rates of PUFA accumulation, primarily due to increases in 20:5ω3, occurred once the bloom started in March, particularly in immature females (Fig. 6.1, Table 6.3). Throughout development from juveniles to mature males/females, mysids were characterized by increases in SFA and MUFA concentrations that were similar to the changes observed in PUFA concentrations, and accumulation was often more pronounced in females than in males (Tables 6.1, 6.2, 6.3). Significant amounts of PUFA, SFA and MUFA were utilised by C2 mature females during the 5-month brooding period (no significant utilisation of PUFA by C1 mature females, Table 6.3), resulting in low levels of all fatty acids in spent females (Table 6.1). Mature females utilised MUFA

Table 6.1 *Mysis mixta.* Major fatty acids (>1% TFA; % DM±SD; *values in brackets* % TFA), dry mass (mg±SD) and total lipid (mg±SD) in mature males and females from *C1, C2* and *C3*. Data are means for life-history stages collected within the indicated periods [*SFA* sum saturated acids, *MUFA* sum monounsaturated acids, *PUFA* sum polyunsaturated acids, *ω3* sum ω3 fatty acids, *TFA* total fatty acids, *Bacterial* sum odd chain acids (iso and ante-iso forms), *Copepod* sum 22:1 + 20:1, *Terrestrial* sum 18:2ω6 + 18:3ω3, *n* sample size]

	C1	C2	C3	C3	C1	C2	C3	C1	C2
	Mature females Dec 1998 – Mar 1999	**Mature females** Sep 1999 – Mar 2000	**Mature female** (early-spawner) Mar 2000	**Mature females** Nov 2000	**Males** Dec 1998 – Mar 1999	**Males** May – Sep 1999	**Males** Aug – Sep 2000	**Spent females** May 1999	**Spent females** Mar – Apr 2000
14:0	0.97±0.76	0.88±0.51	0.26	0.94±0.30	0.06±0.02	0.36±0.25	0.63±0.33	0.95±0.21	0.24±0.14
16:0	1.41±0.63	1.52±0.39	0.98	1.47±0.42	0.34±0.09	0.93±0.37	1.11±0.32	1.81±0.85	0.70±0.22
SFA	2.51±1.41 (22)	2.51±0.92 (27)	1.33 (66)	2.52±0.76 (22)	0.43±0.12(19)	1.37±0.66(24)	1.82±0.67 (19)	2.93±1.13(24)	1.01±0.38(44)
16:1ω7	1.29±1.02	0.98±1.00	0.03	1.42±0.50	0.06±0.05	0.63±0.57	1.42±0.79	1.64±0.28	0.17±0.12
18:1ω9	1.60±1.10	1.31±0.70	0.16	1.88±1.02	0.27±0.03	0.74±0.31	1.01±0.32	1.34±0.63	0.25±0.19
18:1ω7	0.32±0.16	0.22±0.14	0.02	0.30±0.11	0.05±0.01	0.15±0.07	0.24±0.07	0.31±0.14	0.04±0.03
20:1ω9,11	0.67±0.48	0.56±0.39	0.05	0.75±0.27	0.11±0.05	0.35±0.11	0.60±0.27	0.43±0.04	0.07±0.05
22:1ω11	0.34±0.32	0.30±0.23	0.02	0.38±0.14	0.05±0.04	0.18±0.06	0.33±0.17	0.14±0.15	0.03±0.02
MUFA	4.43±3.20 (39)	3.52±2.46 (38)	0.29 (15)	4.93±1.67 (42)	0.59±0.17(26)	2.13±0.95(35)	3.76±1.63 (40)	4.01±0.99(33)	0.60±0.37(26)
18:2ω6	0.18±0.12	0.16±0.12	0.02	0.22±0.07	0.02±0.01	0.11±0.07	0.24±0.10	0.27±0.03	0.03±0.02
18:4ω3	0.26±0.18	0.22±0.18	0.01	0.31±0.15	0.01±0.00	0.07±0.07	0.18±0.12	0.03±0.02	0.04±0.03
20:4ω6	0.05±0.02	0.04±0.01	0.01	0.05±0.02	0.05±0.02	0.07±0.03	0.10±0.02	0.07±0.03	0.01±0.01
20:5ω3	1.72±0.72	1.32±0.78	0.12	1.90±0.78	0.46±0.11	1.17±0.73	1.51±0.48	2.41±1.32	0.28±0.21
22:6ω3	1.30±0.47	0.92±0.33	0.10	1.02±0.51	0.59±0.17	0.89±0.42	1.19±0.16	1.33±1.26	0.17±0.18
PUFA	4.09±1.79 (36)	3.15±1.75 (34)	0.31 (16)	4.15±1.74 (35)	1.20±0.31(53)	2.61±1.46(39)	3.66±1.11 (39)	4.96±2.78(41)	0.67±0.48(29)
ω3	3.54±1.46 (31)	2.69±1.38 (29)	0.26 (13)	3.52±1.54 (30)	1.09±0.28(48)	2.25±1.21(34)	3.03±0.82 (32)	4.10±2.63(34)	0.53±0.42(23)
TFA	11.24±6.38	9.33±5.15	1.99	11.74±3.84	2.26±0.56	6.20±3.09	9.40±3.43	12.12±4.83	2.32±1.11
Bacterial	0.28±0.11 (3)	0.20±0.12 (2)	0.11 (6)	0.19±0.07 (2)	0.06±0.01(3)	0.13±0.09(2)	0.20±0.08 (2)	0.27±0.13(2)	0.07±0.06(3)
Copepod	1.08±0.84 (9)	0.91±0.65 (9)	0.08 (4)	1.16±0.43 (10)	0.17±0.09(7)	0.56±0.07(11)	0.98±0.47 (10)	0.62±0.17(6)	0.11±0.08(4)
Terrestrial	0.26±0.16 (2)	0.22±0.15 (2)	0.02 (1)	0.33±0.11 (3)	0.03±0.01(1)	0.12±0.08(2)	0.27±0.12 (3)	0.31±0.04(3)	0.04±0.03(2)
Dry mass	56.95±9.85	51.10±7.45	27.57	60.80±10.00	35.60±3.88	34.59±2.31	34.56±1.28	47.33±5.17	43.52±5.04
Total lipid	19.99±5.97	19.14±5.78	4.40	31.32±4.62	1.70±0.51	3.16±0.60	6.29±2.26	7.02±3.10	2.75±0.60
N	8	13	1	4	4	6	8	3	4

fatty acids found in trace amounts: 15:0, 15:0i, 15:0ai,16:0i, 16:0ai, 17:0, 17:0i, 17:0ai, 18:0, 16:1ω5, 18:1ω5, 20:1ω7, 22:1ω9, 24:1, 16:2ω4, 18:2ω4, 20:2ω6, 16:3ω4, 18:3ω6, 18:3ω4, 18:3ω3, 20:3ω6, 20:3ω3, 16:4ω3, 16:4ω1, 18:4ω1, 20:4ω3, 22:4ω6, 21:5ω3, 22:5ω6, 22:5ω3

Table 6.2 *Mysis mixta.* Major fatty acids (% DM±SD; *values in brackets* % TFA), DM (mg±SD) and TL (mg±SD) in juveniles and immatures from *C2 - C4.* Data are means for stages collected within the indicated periods. Immatures from *C2* were pooled, but *C3* males and females were separated owing to their divergent profiles. *C2* and *C3* immatures were divided into high lipid versus low lipid periods roughly corresponding to pre-bloom and post-bloom start periods *(abbreviations follow Table 6.1)*

	C2	C3	C4	C2	C2	C3	C3	C3	C3
	Juveniles	**Juveniles**	**Juveniles**	**Immature: females + males**	**Immature: females + males**	**Immature females**	**Immature females**	**Immature males**	**Immature males**
	Dec 1998 – Feb 1999	Jul 1999	Jun – Nov 2000	Mar – Apr 1999	May 1999	Sep 1999 - Feb 2000	Mar – Sep 2000	Sep 1999- Feb 2000	Mar – Jul 2000
14:0	0.42±0.20	0.17±0.06	0.47±0.24	0.44±0.13	1.35±0.39	0.41±0.27	0.94±0.34	0.39±0.15	1.00±0.35
16:0	1.14±0.45	0.78±0.16	1.15±0.26	1.06±0.22	2.55±0.78	0.91±0.31	1.47±0.41	1.03±0.24	1.59±0.40
SFA	1.67±0.63 (22)	1.04±0.23 (57)	1.74±0.49 (23)	1.59±0.34 (22)	4.11±1.15 (23)	1.41±0.58 (45)	2.52±0.77 (23)	1.51±0.56 (25)	2.71±0.77 (22)
16:1ω7	0.44±0.22	0.08±0.09	0.55±0.30	0.49±0.17	2.53±0.76	0.23±0.25	1.81±0.82	0.40±0.21	1.99±0.85
18:1ω9	0.95±0.36	0.15±0.16	0.88±0.30	0.84±0.22	1.84±0.58	0.31±0.32	1.20±0.43	0.77±0.33	1.22±0.28
18:1ω7	0.18±0.07	0.05±0.05	0.23±0.08	0.16±0.04	0.40±0.12	0.05±0.06	0.27±0.11	0.15±0.07	0.26±0.06
20:1ω9,11	0.23±0.10	0.05±0.04	0.56±0.43	0.19±0.05	0.47±0.17	0.12±0.16	0.40±0.20	0.24±0.47	0.41±0.13
22:1ω11	0.08±0.04	0.01±0.01	0.31±0.30	0.07±0.04	0.19±0.07	0.05±0.06	0.19±0.11	0.10±0.08	0.20±0.08
MUFA	1.99±0.80 (26)	0.35±0.35 (19)	2.67±0.99 (35)	1.85±0.43 (26)	5.65±1.57 (32)	0.80±0.89 (26)	4.01±1.59 (35)	1.76±1.12 (28)	4.24±1.30 (35)
18:2ω6	0.10±0.04	0.02±0.02	0.13±0.06	0.11±0.03	0.39±0.11	0.04±0.05	0.26±0.09	0.09±0.04	0.29±0.11
18:4ω3	0.15±0.09	0.02±0.04	0.17±0.10	0.11±0.05	0.32±0.10	0.07±0.08	0.32±0.16	0.12±0.08	0.34±0.11
20:4ω6	0.07±0.03	0.06±0.12	0.06±0.02	0.07±0.03	0.10±0.04	0.04±0.08	0.05±0.01	0.06±0.48	0.07±0.02
20:5ω3	1.47±0.58	0.10±0.13	1.13±0.43	1.66±0.34	3.82±1.35	0.24±0.29	2.12±0.73	1.04±0.84	2.21±0.51
22:6ω3	1.46±0.59	0.07±0.09	1.13±0.40	1.20±0.35	2.09±0.95	0.21±0.23	1.07±0.36	1.15±0.86	1.15±0.37
PUFA	3.63±1.38 (49)	0.36±0.29 (20)	3.00±0.81 (40)	3.53±0.79 (49)	7.78±2.70 (43)	0.79±0.83 (25)	4.59±1.52 (41)	2.75±2.84 (45)	4.89±1.11 (41)
ω3	3.27±1.27 (44)	0.23±0.27 (13)	2.61±0.78 (35)	3.12±0.71 (44)	6.50±2.38 (36)	0.63±0.68 (20)	3.71±1.23 (33)	2.46±1.99 (39)	3.92±0.88 (33)
TFA	7.49±2.82	1.83±0.84	7.56±2.00	7.15±1.55	17.80±5.30	3.12±2.26	11.29±3.84	6.16±4.52	12.04±2.99
Bacterial	0.24±0.09 (3)	0.12±0.04 (7)	0.21±0.07 (3)	0.21±0.06 (3)	0.35±0.09 (2)	0.13±0.06 (5)	0.21±0.06 (2)	0.18±0.18 (3)	0.25±0.06 (2)
Copepod	0.34±0.16 (5)	0.06±0.05 (3)	0.90±0.72 (11)	0.29±0.10 (4)	0.75±0.29 (4)	0.19±0.24 (5)	0.62±0.32 (5)	0.36±0.88 (6)	0.64±0.23 (5)
Terrestrial	0.16±0.07 (2)	0.03±0.03 (1)	0.19±0.08 (3)	0.14±0.04 (2)	0.44±0.12 (3)	0.07±0.07 (2)	0.31±0.11 (3)	0.14±0.06 (2)	0.34±0.11 (3)
Dry mass	20.19±2.73	4.30±1.70	9.39±4.17	25.68±4.81	32.74±5.03	16.32±5.10	38.97±12.54	14.49±5.73	26.97±6.39
Total lipid	2.58±0.59	0.36±0.15	1.11±0.66	3.28±0.96	8.78±1.41	1.80±1.00	8.32±4.43	1.58±0.81	5.54±2.42
n	16	5	20	11	6	13	20	15	17

fatty acids found in trace amounts: 15:0, 15:0i, 15:0ai,16:0i, 16:0ai, 17:0, 17:0i, 17:0ai, 18:0, 16:1ω5, 18:1ω5, 20:1ω7, 22:1ω9, 24:1, 16:2ω4, 18:2ω4, 20:2ω6, 16:3ω4, 18:3ω6, 18:3ω4, 18:3ω3, 20:3ω6, 20:3ω3, 16:4ω3, 16:4ω1, 18:4ω1, 20:4ω3, 22:4ω6, 21:5ω3, 22:5ω6, 22:5ω3

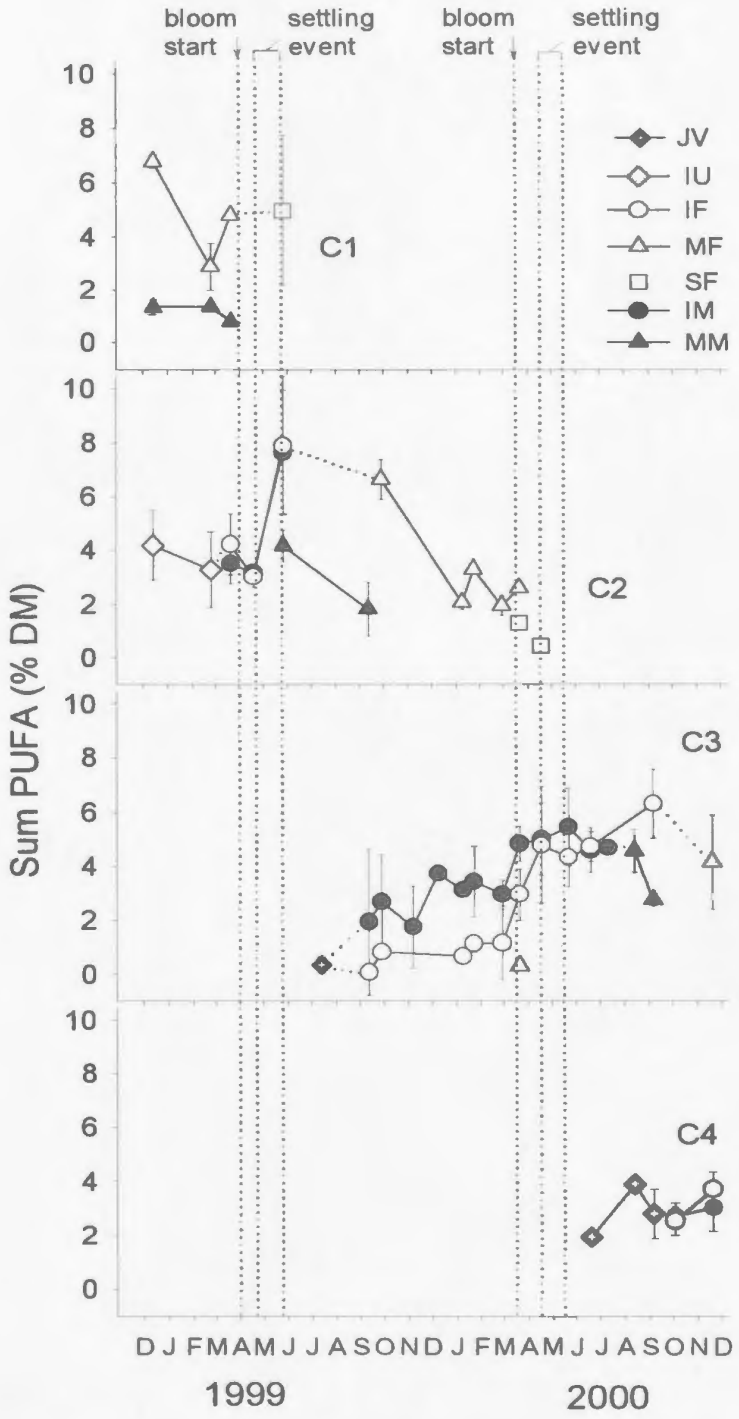

Fig. 6.1 *Mysis mixta*. Changes in the sum of polyunsaturated fatty acids (% DM) in cohorts 1 - 4 *(C1, C2, C3, C4)* [JV, juveniles; IU, immature undifferentiated; IF, immature females; MF, mature females (includes brooded embryos); SF, spent females; IM, immature males; MM, mature males]. *Dotted lines* within a cohort represent transition periods between stages; *vertical dotted lines* are bloom start and settling times. *Error bars:* SD around the mean

Table 6.3 *Mysis mixta, Acanthostepheia malmgreni.* Within-cohort *(CO)* rates of accumulation or utilisation of major fatty acids and sums of *PUFA*, *SFA*, *MUFA* and copepod marker 20:1 + 22:1 (μg month^{-1}; rates produced from regression slopes). Sub-groups were created to establish rates before (pre) and after (post) the start of the spring bloom, although bloom status was not assigned to mature males/females [*JV* juveniles; *IM* immature males; *IF* immature females; *MM* mature males; *MF* mature females; *NS* non-sexed (includes mature male and non-reproductive female amphipods)]. Spent females were not included in the analyses. A rate of 0.00 μg month^{-1} was assigned when a regression was not significant ($p > 0.05$)

CO	Stages	Time period	Bloom status	20:5ω3	22:6ω3	PUFA	14:0	16:0	SFA	18:1ω9	16:1ω7	20:1 + 22:1	MUFA	n
M. mixta														
1[a]	MF	Dec 98 – Mar 99	*n/a*	0.00	0.00	[illegible]	-305**	0.00	[illegible]*	-429**	-417***	-339**	[illegible]**	8
1	MM	Dec 98 – Mar 99	*n a*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4
2[a]	IU;IM;IF	Dec 98 – Mar 99	Pre	0.00	0.00	0.00	0.00	0.00	0.00	0 00	0.00	0.00	0.00	21
2[a]	IM	Mar 99 – May 99	Post	349**	0.00	710**	152**	251**	422**	180**	313**	-89.0**	634**	9
2	IF	Mar 99 – May 99	Post	397*	0.00	762*	147*	257*	424*	0.00	337**	-61.7*	607*	8
2	MM	May 99 – Sep 99	*n a*	0.00	0.00	0.00	-40.5*	-60.6*	-107*	-107*	0.00	0.00	0.00	6
2	MF	Sep 99 – Mar 00	*n a*	-174***	-63.5**	-391****	-109***	-73.4***	-189***	-143**	-174**	-123**	-541****	12
3[a]	JV;IM	Jul 99 – Mar 00	Pre	39.9***	32.5****	103****	17.2****	26.5****	43.7****	25.5****	22.3**	12.3****	60.4****	24
3[a]	JV;IF	Jul 99 – Mar 00	Pre	19.5**	0.00	0.00	16.7****	26.3****	45.1****	14.9***	13.5*	9.51**	41.4**	21
3[a]	IM	Mar 00 – Jul 00	Post	80.1*	0.00	0.00	0.00	0.00	0.00	43.0*	169***	27.7**	237**	16
3	IF	Mar 00 – Sep 00	Post	215****	119****	444****	75.7****	107****	190****	127****	141**	87.9****	425****	20
3	MM	Aug 00 – Sep 00	*n a*	-355**	-124**	-829**	-229*	-223*	-473**	-202*	-512*	0.00	-934*	8
4[a]	JV;IM;IF	Jun 00 – Nov 00	Post	27.0*	43.4*	79.3***	23.2*	32.2**	58.6*	26.9**	0.00	43.3**	88.2***	20

A. malmgreni				20:5ω3	22:6ω3	PUFA	16:0	SFA	18:1ω9	18:1ω7	16:1ω7	20:1 + 22:1	MUFA	
1	MF	Dec 98 – May 99	*n/a*	0.00	0.00	[illegible]	-126*	[illegible]*	-173*	-115**	-76.5*	-92.3**	[illegible]**	16
1[a]	NS	Oct 98 – May 99	*n a*	269**	0.00	0.00	112*	143*	155*	70.2*	0.00	0.00	315*	20
2	JV	Feb 99 – Jul 99	*n a*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4
2	JV;IF	Jul 99 – Nov 99	*n a*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3
3	JV	Sep 99 – Apr 00	*n a*	24.9****	18.8****	52.0****	8.53****	11.4****	9.44****	6.49****	2.27***	4.52***	24.0****	27
3	IM	May 00 – Nov 00	*n a*	41.7**	25.1**	97.8***	22.5**	39.5**	24.1***	18.9**	23.2**	21.9**	97.1**	22
3	IF	May 00 – Nov 00	*n a*	50.8****	26.1****	[illegible]****	22.8****	[illegible]****	31.6****	21.6****	22.3****	22..4****	[illegible]****	23

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$, *n/a* not applicable

[a]some regressions in these sub-groups were corrected for temporal autocorrelation using Cochrane-Orcutt procedure

at the fastest rates, and PUFA and SFA at lower rates (Table 6.3). An early-spawning female from C3 had very low concentrations of all fatty acids (Table 6.1). Mature males showed either no change or significant decreases in fatty acid concentrations before death (Fig. 6.1; Table 6.3). Juveniles from C4 exhibited fatty acid accumulation rates similar to those in C3 juveniles, although there were no post-bloom data available for comparison because C4 juveniles were released during the spring bloom in 2000 (Fig. 6.1, Table 6.3).

6.3.1.1 Dietary markers

The diatom marker ratio (16:1ω7 + 16:1ω5)/16:0 increased rapidly in immature mysids beginning in March each year, and reached 1.5 after the spring bloom had settled to the hyperbenthos (Fig. 6.2). At other times of the year the ratio remained below 1.0 except in some C1 females. The trend in the dinoflagellate marker ratio 22:6ω3/20:5ω3 was opposite to that of the diatom marker ratio, with values fluctuating near 1.0 during non-bloom times and then dropping rapidly once the bloom had started (Fig. 6.2). PUFA:SFA ratios (using absolute PUFA and SFA content, proposed as a carnivory index by Cripps & Atkinson 2000) increased with development in C3 mysids, and with the exception of C3 immature females that showed a rapid increase in PUFA/SFA at the start of the bloom, the ratio fluctuated around 2.0 and appeared to be largely independent of bloom start and settling times (Fig. 6.3). A gradual decline in PUFA:SFA to 1.0 was apparent in mature females from C2. In both C2 and C3 mysids, the ratio 18:1ω9/18:1ω7 (carnivory marker, Graeve et al. 1997) peaked at 8.0 and then returned to ~5.0 prior to the spring bloom (Fig. 6.3). Ratios of C16:C18 were very irregular and exhibited no obvious patterns (data not shown, ratio indicates dominance of diatoms or dinoflagellates, Claustre et al. 1989). The fatty acid 18:1ω9 (% DM; associated with animals) and the sum of 20:1 + 22:1 fatty acids (% DM, trophic markers of calanoid copepods, Sargent & Falk-Petersen 1988) increased in developing mysids with the onset of the spring bloom, similar to the changes in total PUFA (Figs. 6.1, 6.4). Unlike PUFA levels, concentrations of the monoenes 18:1ω9 and 20:1 + 22:1 continued to increase after the spring bloom had settled to the hyperbenthos. As a result, mature females early in their brooding period had high concentrations of both markers in mid-winter (Fig. 6.4). Bacterial (~7% FA,

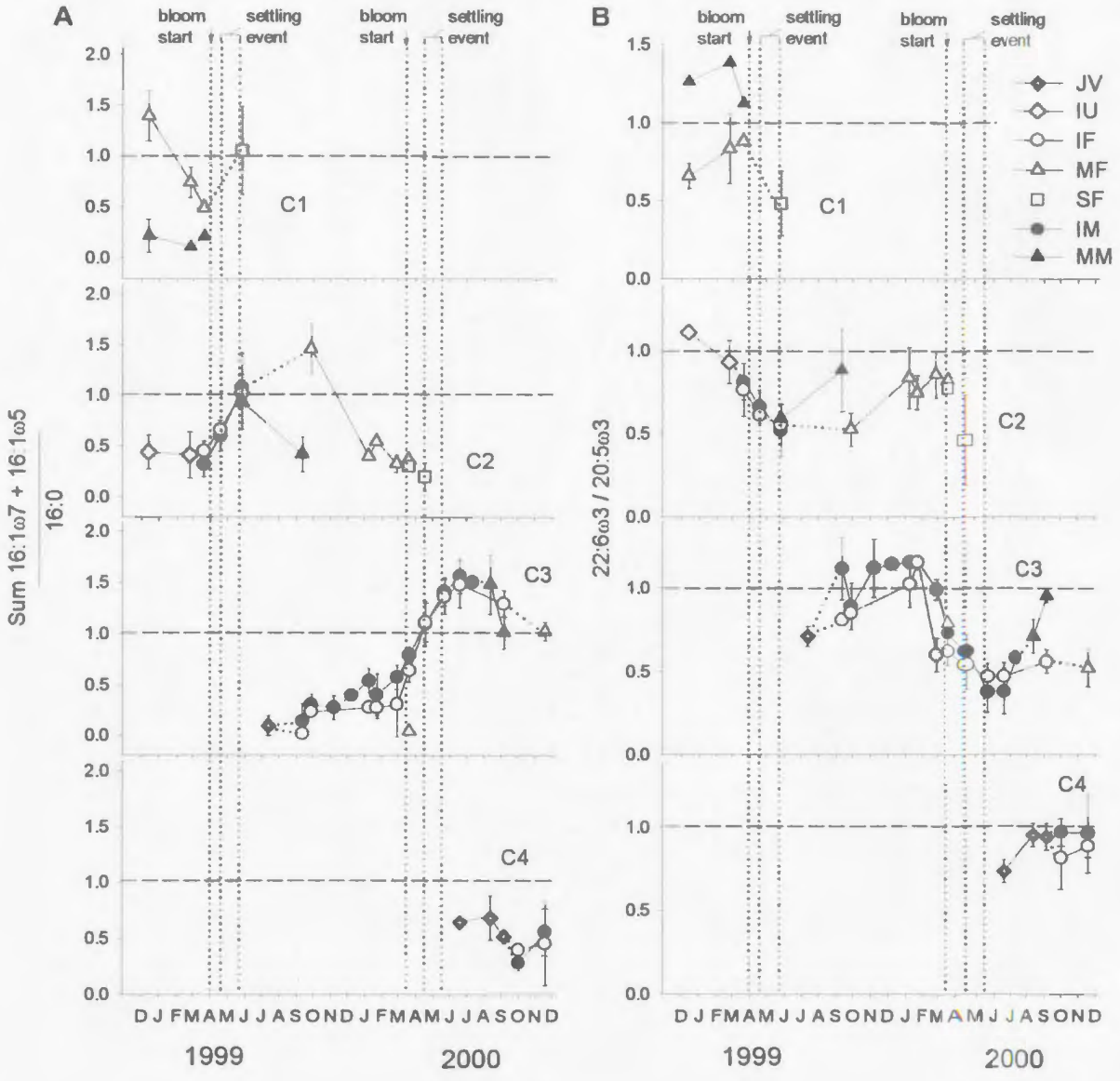

Fig. 6.2 *Mysis mixta*. Changes in (A) the (16:1ω7 + 16:1ω5)/16:0 ratio (diatom marker) and (B) the 22:6ω3/20:5ω3 ratio (dinoflagellate marker) in cohorts 1 - 4 *(C1, C2, C3, C4)*. *Dashed horizontal lines* are reference lines for comparisons among vertical panels (*additional details as in Fig. 6.1)*

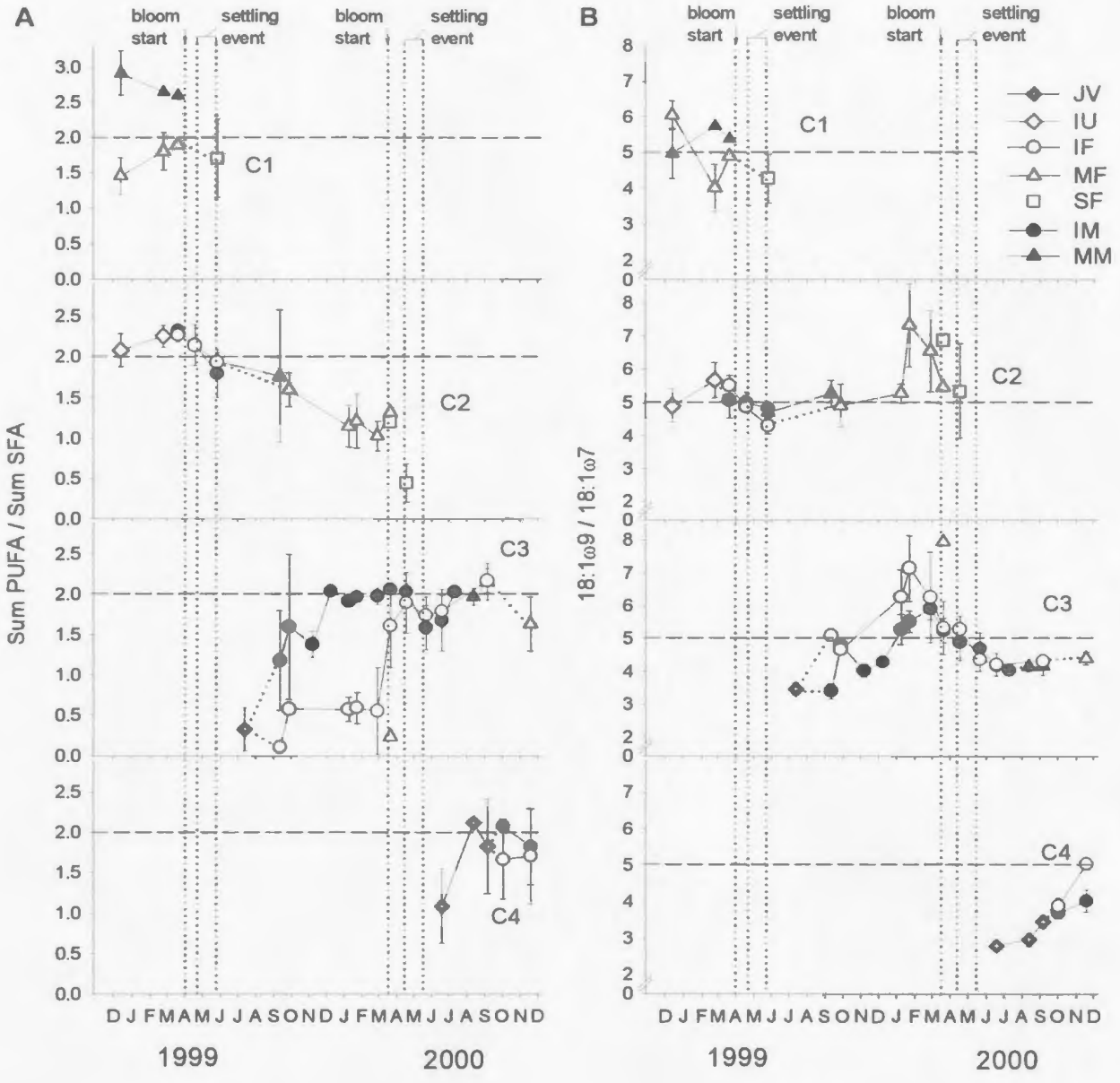

Fig. 6.3 *Mysis mixta*. Changes in (A) the sum PUFA/sum SFA ratio and (B) the 18:1ω9/18:1ω7 ratio (omnivory/carnivory markers) in cohorts 1 - 4 *(C1, C2, C3, C4)*. *Dashed horizontal lines* represent reference lines for comparisons among vertical panels *(additional details as in Fig. 6.1)*

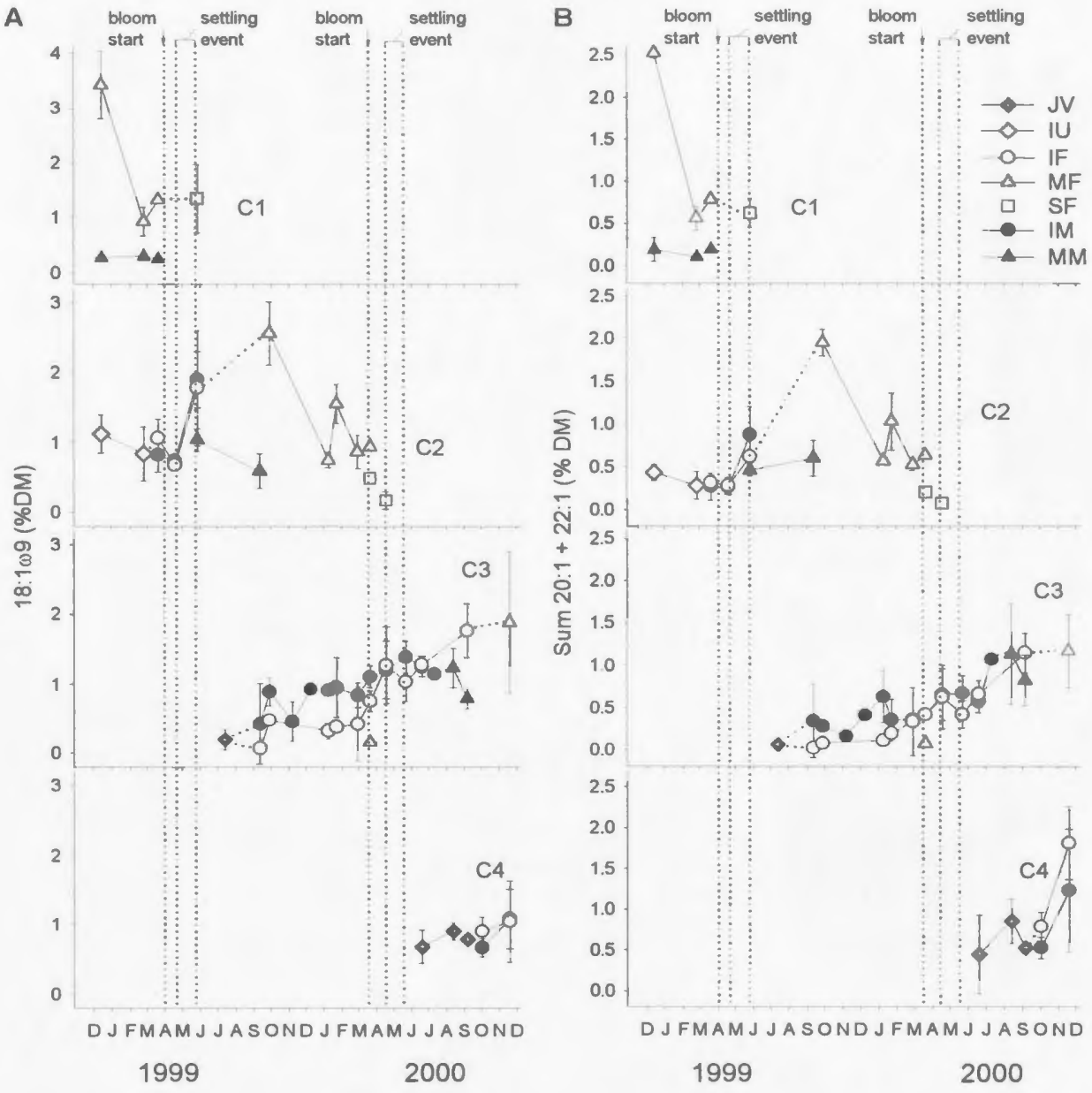

Fig. 6.4 *Mysis mixta*. Changes in (A) 18:1ω9 (%DM; carnivory marker) and (B) 20:1 + 22:1 fatty acids (% DM; copepod marker) in cohorts 1 - 4 *(C1, C2, C3, C4; additional details as in Fig. 6.1)*

defined as odd and branched chain fatty acids; Harvey 1994; Parrish et al. 2000) and terrestrial plant (<3% TFA; defined as the sum of 18:2ω6 and 18:3ω3 in coastal Newfoundland regions; Budge & Parrish 1998) fatty acid marker proportions were

relatively low; therefore, bacteria and terrestrial plants were not considered important sources of nutrition for *M. mixta*.

6.3.2 Fatty acids in Acanthostepheia malmgreni

Forty-three fatty acids were identified in *A. malmgreni* (Table 6.4). Fatty acids 14:0, 16:0 and 18:0 were the major SFA in all life-history stages within the population. Fatty acids 16:1ω7, 18:1ω9, 18:1ω7, 18:1ω5, 20:1ω9(11) and 20:1ω7 dominated the MUFA fraction, and 18:2ω6, 20:4ω6, 20:5ω3, 22:5ω3 and 22:6ω3 dominated the PUFA fraction.

The most abundant fatty acids in juveniles were 20:5ω3, 22:6ω3, 16:0, 18:1ω7 and 18:1ω9. These 5 fatty acids remained dominant while *A. malmgreni* matured and accumulated lipid stores (Chapter 5, Table 6.4), and quantities of 16:1ω7 increased with developmental stage. Of those regressed against TL (μg ind^{-1}), fatty acids 20:5ω3, 18:1ω9, 22:6ω3, 16:0, 18:1ω7 and 16:1ω7 (in order of decreasing importance) had the greatest slopes and were most important to lipid accumulation in *A. malmgreni*.

The concentrations (% DM) of the three acid types (SFA, MUFA and PUFA) remained relatively consistent among different life-history stages in the 4 cohorts (C1, C2, C3, C4) of *A. malmgreni* (Table 6.4). PUFA, consisting primarily of 20:5ω3 and 22:6ω3, were the predominant fatty acids in all stages, followed by MUFA and then SFA (Table 6.4).

Maximum concentrations of PUFA within the population occurred in immature males from C3 (3.4±1.0% DM, Fig. 6.5). PUFA accumulation rates in immature amphipods from C3 were slow prior to the spring bloom but increased once the bloom had settled in April. Accumulation rates were similar in immature males and females (Fig. 6.5, Table 6.3). Only a few amphipods from C2 were collected, therefore data from this cohort are less informative than are data derived from the larger cohorts. Immature mysids from C3 accumulated MUFA at rates similar to PUFA accumulation rates, whereas SFA accumulation was generally much lower (Table 6.3). Mature females utilised significant amounts of SFA and MUFA during the brooding period, although PUFA concentrations remained relatively level at ~2% DM (Table 6.3). Spent females contained half the amount of PUFA, SFA and MUFA as did mature females (Table 6.4). The non-sexed group in C1, consisting of mature males and large non-reproductive females, showed

Table 6.4 *Acanthostepheia malmgreni.* Major fatty acids (% DM±SD; *values in brackets* % TFA), dry mass (mg±SD) and total lipid (mg±SD) in *C1 - C4.* Data are means for stages collected within the indicated periods (Non-sexed *C1* group includes mature males and some large immature or non-reproductive females) *(abbreviations as in Table 6.1)*

	C1	C1	C1	C2	C3	C4	C2	C3	C3
	Mature females Dec 1998 – May 1999	**Spent females** Apr – May 2000	**Non-sexed** Oct 1998 – May 1999	**Juveniles** Feb – Jul 1999	**Juveniles** Sep 1999 – Apr 2000	**Juvenile** Sep 2000	**Immature females** Sep – Nov 1999	**Immature females** May – Nov 2000	**Immature males** May - Nov 2000
14:0	0.10±0.07	0.03±0.04	0.10±0.07	0.05±0.04	0.05±0.03	0.08	0.08±0.02	0.17±0.08	0.21±0.11
16:0	0.61±0.29	0.29±0.26	0.64±0.41	0.32±0.15	0.42±0.16	0.35	0.47±0.10	0.63±0.20	0.71±0.31
18:0	0.06±0.03	0.02±0.02	0.05±0.03	0.04±0.02	0.05±0.02	0.03	0.05±0.00	0.06±0.02	0.07±0.04
SFA	0.85±0.43 (17)	0.38±0.36 (16)	0.86±0.53 (18)	0.44±0.23 (21)	0.58±0.22 (18)	0.50 (18)	0.65±0.13 (15)	0.93±0.31 (17)	1.06±0.49 (18)
16:1ω7	0.26±0.17	0.09±0.13	0.24±0.21	0.06±0.04	0.08±0.05	0.28	0.18±0.05	0.49±0.23	0.58±0.33
18:1ω9	0.80±0.36	0.34±0.26	0.71±0.50	0.24±0.05	0.39±0.15	0.30	0.58±0.26	0.58±0.26	0.60±0.27
18:1ω7	0.45±0.23	0.20±0.19	0.39±0.22	0.16±0.03	0.28±0.11	0.17	0.34±0.13	0.42±0.17	0.46±0.22
18:1ω5	0.07±0.03	0.03±0.03	0.06±0.04	0.02±0.00	0.04±0.02	0.02	0.05±0.02	0.06±0.03	0.07±0.04
20:1ω9,11	0.18±0.10	0.07±0.09	0.14±0.10	0.05±0.02	0.10±0.05	0.05	0.14±0.05	0.19±0.10	0.23±0.12
20:1ω7	0.09±0.06	0.04±0.05	0.09±0.05	0.03±0.01	0.06±0.03	0.02	0.07±0.03	0.11±0.05	0.13±0.08
MUFA	1.90±0.94 (38)	0.81±0.77 (33)	1.68±1.10 (36)	0.57±0.04 (28)	0.99±0.41 (31)	0.86 (31)	1.40±0.52 (33)	1.94±0.85 (36)	2.20±1.11 (37)
18:2ω6	0.10±0.04	0.05±0.05	0.07±0.04	0.03±0.00	0.05±0.02	0.05	0.06±0.02	0.13±0.05	0.14±0.07
20:4ω6	0.11±0.04	0.08±0.05	0.11±0.08	0.06±0.03	0.08±0.04	0.06	0.10±0.02	0.10±0.03	0.11±0.05
20:5ω3	1.01±0.39	0.54±0.43	0.93±0.65	0.46±0.20	0.73±0.33	0.75	1.06±0.41	1.17±0.40	1.22±0.50
22:5ω3	0.07±0.03	0.04±0.04	0.07±0.06	0.03±0.01	0.05±0.02	0.03	0.07±0.03	0.08±0.03	0.09±0.06
22:6ω3	0.64±0.20	0.40±0.22	0.69±0.53	0.34±0.20	0.52±0.27	0.37	0.66±0.20	0.73±0.20	0.78±0.30
PUFA	2.13±0.75 (43)	1.22±0.87 (50)	2.06±1.41 (44)	0.99±0.44 (48)	1.59±0.68 (50)	1.39 (50)	2.12±0.74 (50)	2.46±0.80 (46)	2.63±1.09 (44)
ω3	1.81±0.64 (36)	1.03±0.73 (42)	1.77±1.25 (38)	0.85±0.40 (41)	1.36±0.62 (43)	1.20 (43)	1.86±0.66 (43)	2.09±0.67 (39)	2.22±0.91 (37)
TFA	4.97±2.01	2.45±2.02	4.72±2.83	2.05±0.23	3.20±1.18	2.77	4.28±1.35	5.39±1.97	5.97±2.69
Bacterial	0.16±0.10 (3)	0.08±0.06 (3)	0.18±0.11 (4)	0.08±0.02 (4)	0.09±0.04 (3)	0.05 (2)	0.16±0.03 (4)	0.12±0.05 (2)	0.14±0.07 (2)
Copepod	0.31±0.17 (6)	0.13±0.16 (4)	0.27±0.17 (6)	0.09±0.04 (5)	0.18±0.09 (6)	0.10 (4)	0.25±0.07 (6)	0.37±0.18 (7)	0.46±0.26 (8)
Terrestrial	0.12±0.05 (2)	0.06±0.06 (2)	0.09±0.06 (2)	0.04±0.01 (2)	0.06±0.03 (2)	0.05 (2)	0.07±0.02 (2)	0.15±0.07 (3)	0.17±0.09 (3)
Dry mass	127.23±17.60	100.21±14.39	108.96±31.26	31.44±9.67	5.08±5.46	6.03	79.31±26.57	51.49±20.31	49.29±19.27
Total lipid	28.79±8.58	3.14±1.89	9.86±5.33	1.39±0.71	0.45±0.31	0.27	8.86±4.61	4.36±2.30	4.30±2.67
n	16	4	22	4	37	1	2	16	17

fatty acids found in trace amounts: 15:0, 15:0i, 15:0ai,16:0i, 16:0ai, 17:0, 17:0i, 17:0ai, 16:1ω5, 18:1ω5, 22:1ω9, 22:1ω11, 24:1, 16:2ω4, 18:2ω4, 20:2ω6, 16:3ω4, 18:3ω6, 18:3ω4, 18:3ω3, 20:3ω6, 20:3ω3, 16:4ω3, 16:4ω3, 20:4ω3, 22:4ω6, 21:5ω3, 22:5ω6

significant increases in all three fatty acid types before it disappeared after May 1999 (Fig. 6.5, Table 6.3). Variation in fatty acid composition was high within this group, presumably because it was composed of more than one life-history stage.

6.3.2.1 Dietary markers

The diatom marker ratio (16:1ω7 + 16:1ω5) 16:0 was normally below 0.6 and increased rapidly to 1.0 only in immature mysids from C3 after the settlement of spring bloom material (Fig. 6.6). Changes in the C16:C18 ratios (data not shown) mirrored the pattern observed in the 16:1 16:0 ratio. The dinoflagellate marker ratio 22:6ω3 20:5ω3 was extremely variable within each cohort, with values approaching 1.0 prior to and during the spring bloom and then decreasing to 0.6 or lower following bloom sedimentation (Fig. 6.6). Ratios of PUFA SFA increased prior to the spring bloom in C1, C2 and C3 (fatty acid analysis was done on only one sample from C4), peaked above 4.0 in March or April, and then decreased to ~3.0 in the summer (Fig. 6.7). The ratio 18:1ω9 18:1ω7 did not fluctuate far from 1.4 in C2 and C3, although ratios in the large amphipods from C1 varied considerably and exceeded 2.2 in the spring (Fig. 6.7). Trends in the fatty acid 18:1ω9 (% DM) and the sum of 20:1 + 22:1 fatty acids (% DM) were generally similar to changes observed in PUFA concentrations (i.e. the fatty acid markers increased in developing amphipods following sedimentation of bloom material, decreased in mature females during brooding, and varied substantially within C1, Figs. 6.5, 6.8). Highest concentrations (% DM) of 18:1ω9, 20:1 + 22:1 and PUFA were reached during winter in amphipods from C1, C2 and C3 (Figs. 6.5, 6.8). As in *M. mixta*, storage of bacterial and terrestrial plant fatty acid markers was minimal (Table 6.4).

6.3.3 Areal PUFA calculations

Maximum areal concentrations of PUFA in the hyperbenthic populations of *M. mixta* (14.6 mg m^{-2}) and *A. malmgreni* (5.77 mg m^{-2}) occurred in August 2000 (Fig. 6.9). Areal concentrations in *M. mixta* during 1999 were less than half those observed in 2000 (up to 6.36 mg m^{-2} in 1999), with lowest concentrations occurring in June (Fig. 6.9). *A. malmgreni* exhibited similar maximum areal concentrations of PUFA in both years.

(maximum in 1999 was 5.57mg m^{-2}), although a prolonged period of low concentrations was apparent throughout 1999 (Fig. 6.9).

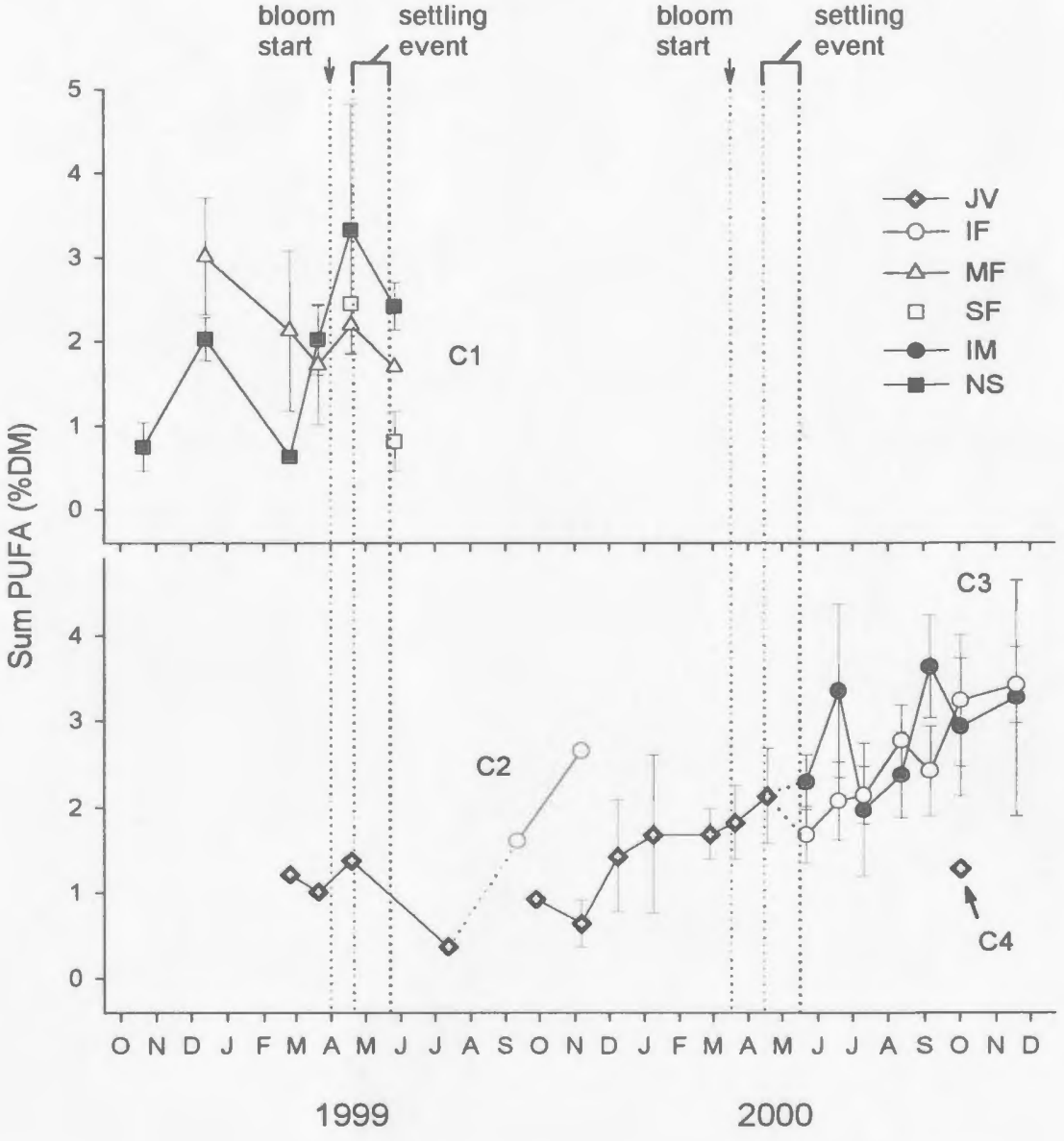

Fig. 6.5 *Acanthostepheia malmgreni*. Changes in polyunsaturated fatty acids (PUFA; % DM) in cohorts 1 - 4 (*C1, C2, C3, C4*). *Dotted lines* within a cohort represent transition periods between stages; *vertical dotted lines* represent bloom start and settling times. *Error bars:* SD around the mean

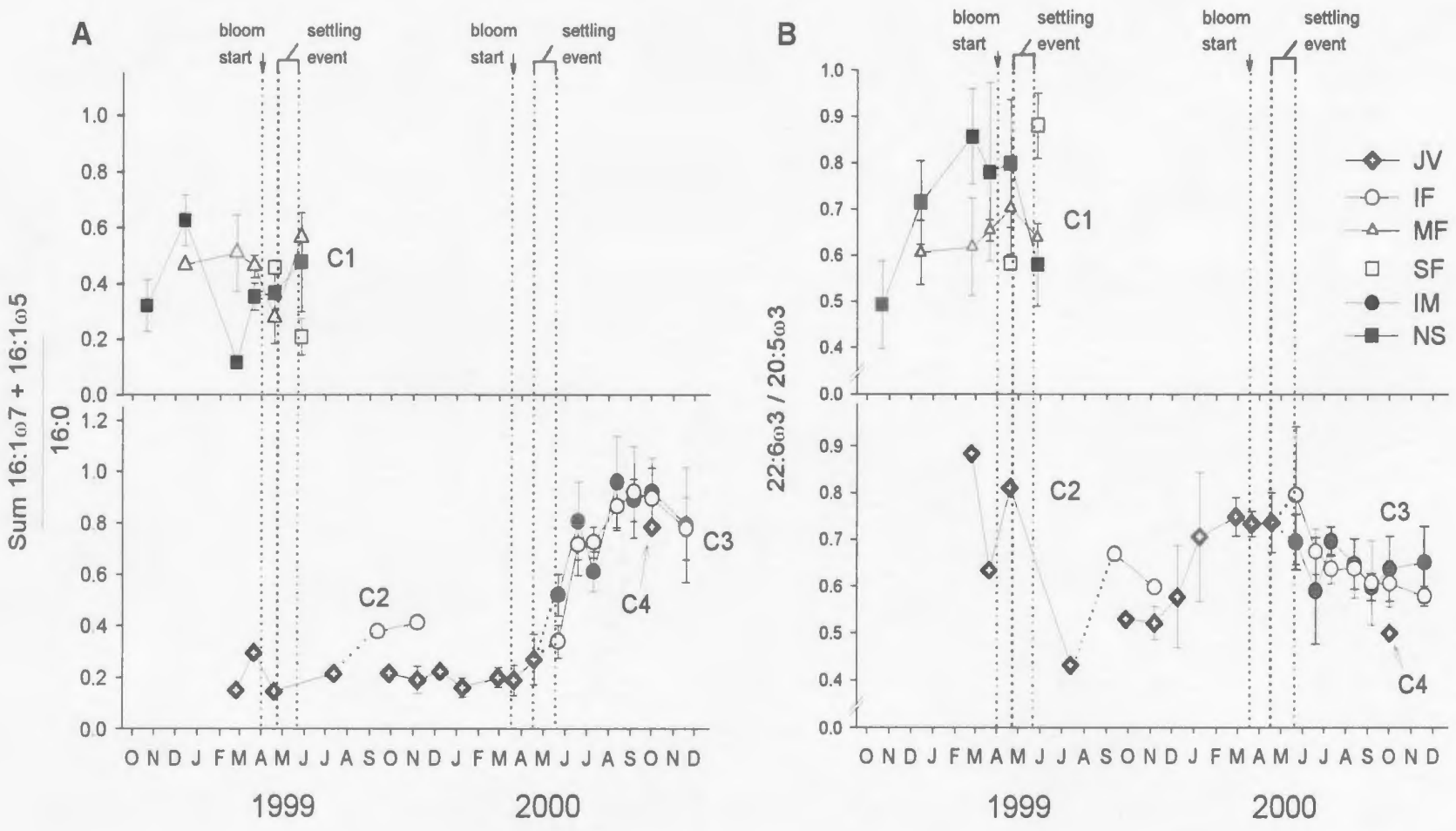

Fig. 6.6 *Acanthostepheia malmgreni*. Changes in (A) the (16:1ω7 + 16:1ω5)/16:0 ratio (diatom marker) and (B) the 22:6ω3/20:5ω3 ratio (dinoflagellate marker) in cohorts 1 - 4 *(additional details as in Fig. 6.5)*

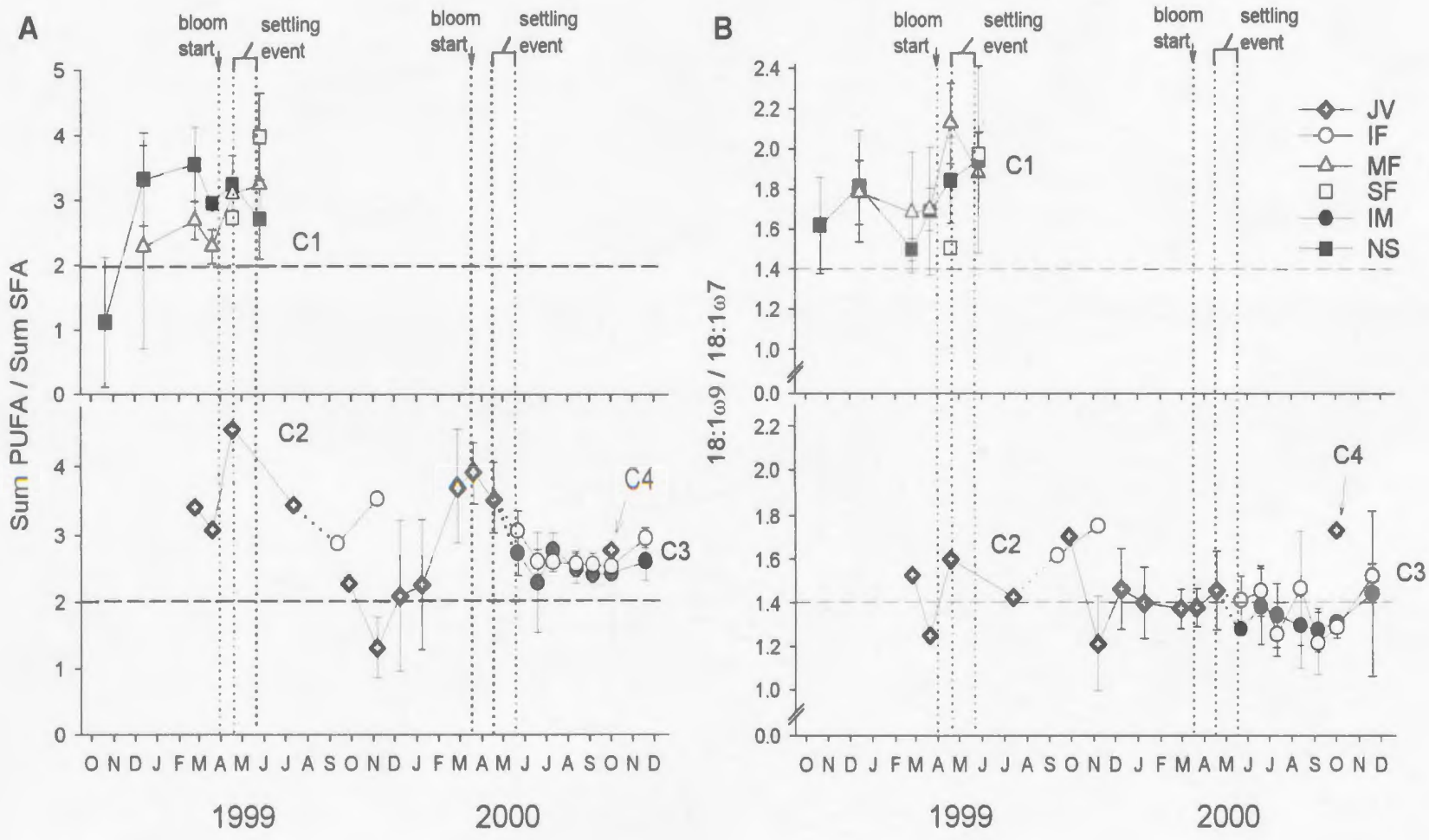

Fig. 6.7 *Acanthostepheia malmgreni*. Changes in (A) the sum PUFA/sum SFA ratio and (B) the 18:1ω9/18:1ω7 ratio (omnivory/carnivory markers) in cohorts 1 – 4. *Dashed horizontal lines* represent reference lines for comparisons between vertical panels *(additional details as in Fig. 6.5)*

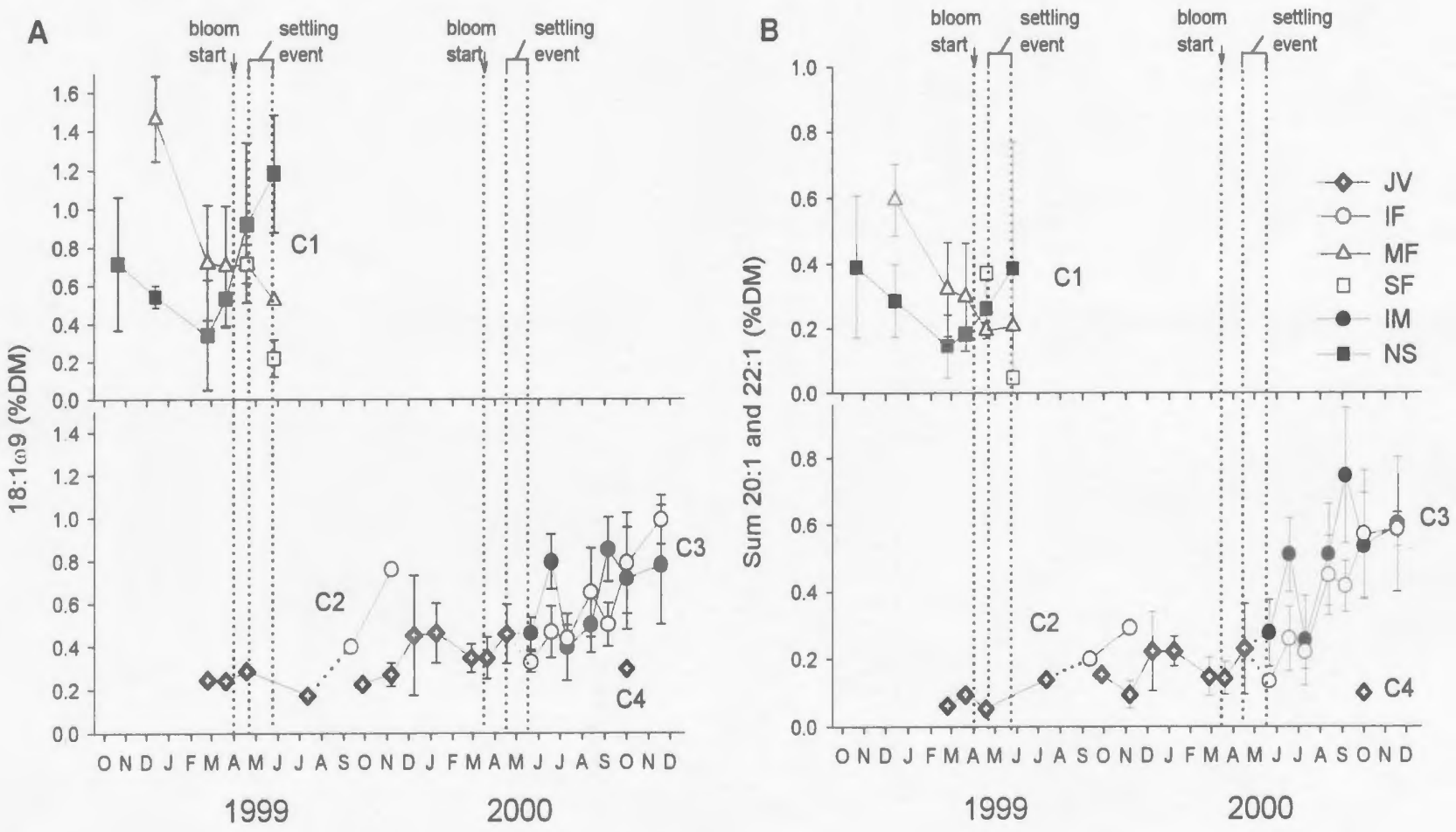

Fig. 6.8 *Acanthostepheia malmgreni*. Changes in (A) 18:1ω9 (% DM; carnivory marker) and (B) 20:1 + 22:1 fatty acids (% DM; copepod marker) in cohorts 1 - 4 *(additional details as in Fig. 6.5)*

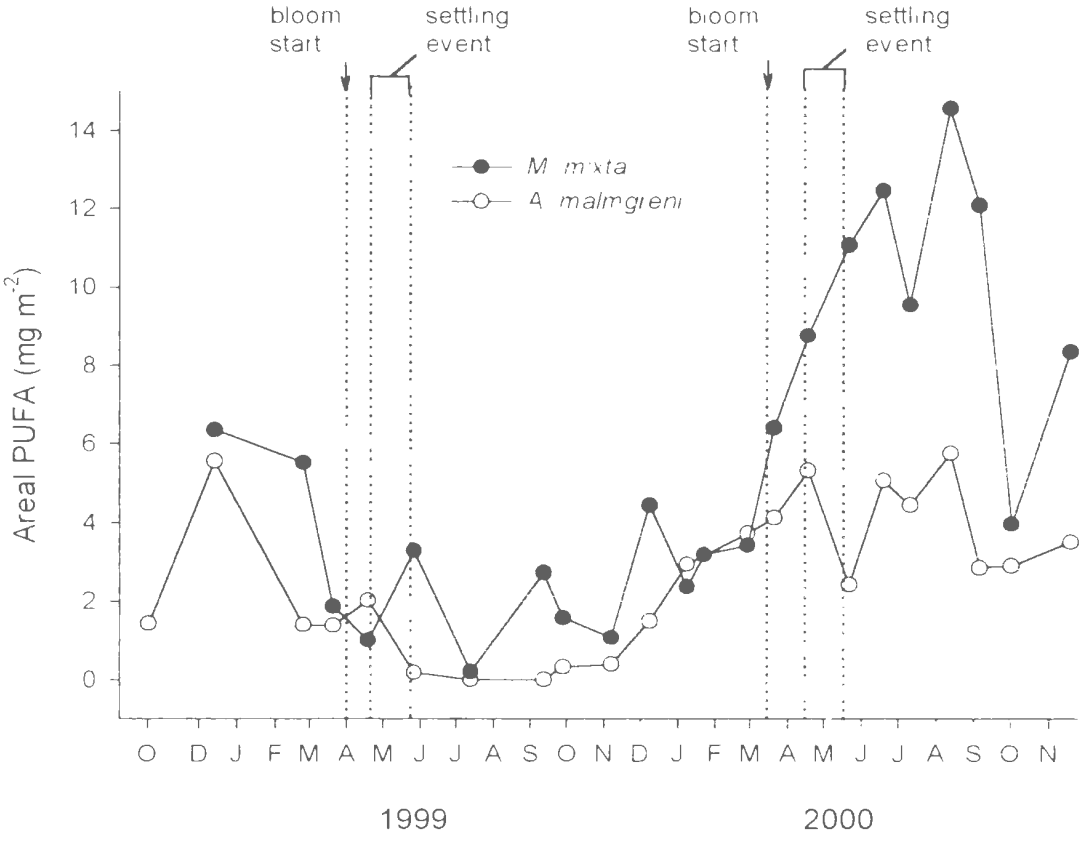

Fig. 6.9 *Mysis mixta*, *Acanthostepheia malmgreni*. Areal concentrations of polyunsaturated fatty acids (PUFA) within the hyperbenthos of Conception Bay. *Vertical dotted lines* represent bloom start and settling times

6.4 Discussion

Fatty acid composition in *Mysis mixta* and *Acanthostepheia malmgreni* varied with life-history stage and season, although changes in *M. mixta* were generally more rapid and pronounced. The relatively few comprehensive studies on ontogenetic and seasonal changes in fatty acid composition of zooplankton in the field have focussed primarily on copepods (mainly *Calanus* spp.) or euphausiids (e.g. Kattner & Krause 1987, Tande & Henderson 1988, Kattner et al. 1994, Hagen et al. 2001, Stübing et al. 2003). In general, cold-ocean calanoids synthesize long-chain MUFA and utilise or maintain PUFA levels throughout development (Kattner & Krause 1987, Tande & Henderson 1988, Kattner et

al. 1994), and polar euphausiids show high variability in fatty acid composition depending on feeding modes and environmental conditions (Falk-Petersen et al. 2000). In polar and temperate regions, ontogenetic changes in fatty acid composition of zooplankton have been associated with changes in lipid biosynthesis, behaviour, diet, seasonal environmental productivity, or a combination of factors.

The ability and extent to which *M. mixta* and *A. malmgreni* assimilate and store organic material produced during and following the annual phytoplankton bloom was of particular interest in this study. In 1999 and 2000, the spring bloom in Conception Bay began in March and reached a maximum in late April to mid-May (chl *a* maximum was 2.22 μg l^{-1} in 1999 and 3.64 μg l^{-1} in 2000, Chapter 2). Settling of the material to the hyperbenthos occurred in May each year, and an additional secondary bloom appeared in July 1999 (Chapter 2). As is typical for Newfoundland fjords, the upper 50 m of Conception Bay is characterized by a recurring successional pattern of plankton each year. Generally, large diatoms dominate the plankton biomass early in the spring, whereas flagellates, bacteria and meso- and microzooplankton flourish in late summer and early autumn (Parrish et al. 2000, Tian et al. 2003). Consequently, hyperbenthic and benthic organisms experience a seasonal shift in food availability and quality. The dense diatomaceous material produced during the bloom is quickly and efficiently transferred to the seafloor, and high concentrations of essential fatty acids produced within the euphotic zone have been found in membrane and storage lipids of benthic organisms from Conception Bay (Parrish et al. 1996). Input of organic matter to the hyperbenthos decreases following the spring bloom, primarily because the increased dominance of the microbial food web augments the retention of organic carbon within the upper mixed layer (Tian et al. 2003). By August, many calanoid copepods that have spent the summer accumulating lipid stores within the euphotic zone descend through the water column to begin diapause (Davis 1982, Deibel et al. unpublished). The downward migration of these lipid-rich late-copepodite stages provides an added food source for deep-living organisms in autumn and winter. The lipid composition of stage V calanoids collected from Conception Bay during the winter, characterized by relatively low levels of PUFA and

high levels of monounsaturated 20:1ω9 and 22:1ω11 (Stevens 2003), is typical of diapausing *Calanus* spp. from other marine systems (Lee 1974).

Chlorophyll *a* and total fatty acid concentrations vary with environmental conditions and algal physiology, and chl *a* fluctuations are generally consistent with variations in total fatty acids (Reuss & Poulsen 2002). In addition, clear relationships exist between algal fatty acid composition and taxonomy, providing an opportunity for tracing trophic paths back to the primary producers within a food web. Phytoplankton produce essential PUFA that are necessary for proper membrane structure and function, and are therefore useful as biomarkers (Sargent & Falk-Petersen 1988). PUFA are particularly important in cold-water organisms because cell membranes must remain sufficiently fluid despite the tendency for increased rigidity with low temperatures (Hall et al. 2000). Altering fatty acid composition to effect changes in membrane fluidity is one of the principal methods that organisms use to adapt to changes in temperature (Gurr & Harwood 1991). As a result, variations in temperature can influence both total lipid and fatty acid composition in zooplankton on a seasonal basis. Because water below 80 m depth in Conception Bay does not fluctuate far from 0°C year-round (Chapter 2), temperature is not considered a highly influential variable in the present study.

Prior to the spring bloom in 1996, PUFA levels in plankton from the upper 80 m of Conception Bay were ~29% TFA (Parrish et al. unpublished). After the bloom started in March, PUFA levels increased up to 53% TFA and remained above 40% TFA until July (Parrish et al. unpublished). PUFA levels in sediment trap contents at 220 m increased from 21% TFA, prior to the bloom, up to 35% TFA after the bloom material had settled in April. PUFA in the deep sediment trap material returned to low levels by June 1996 (Parrish et al. unpublished). In Trinity Bay, a nearby fjord with similar physical and biological characteristics to those in Conception Bay, more detailed analyses of plankton fatty acids in the upper mixed layer during the 1996 spring bloom revealed maximum quantities of diatom fatty acids in May (Parrish et al. 2000). During such periods of high phytoplankton production, zooplankton typically contain increased proportions of PUFA, particularly ω3 fatty acids, in their neutral lipid stores (e.g. Sargent et al. 1985, 1987, Falk-Petersen 1987, Fraser et al. 1989b). Accumulation of phytoplanktonic ω3 PUFA in

lipid stores is particularly important in copepods because high concentrations of these membrane fatty acids are required for gonad formation, egg production, embryogenesis and early naupliar development (Fraser et al. 1989a).

Accumulation of all three fatty acid groups (PUFA, MUFA and SFA) increased in immature female *M. mixta* from Conception Bay after the spring bloom started in 1999 and 2000. Similar increases were also apparent in immature males from C2, although immature males from C3 showed decreased rates of PUFA and MUFA accumulation following the spring bloom. MUFA concentrations peaked in early-stage brooding female *M. mixta* from C2 (MUFA in C3 females appeared to level out once maturity was reached). In contrast, PUFA and SFA concentrations both leveled out as immature females became fully mature. Like TL and TAG (Chapter 4), either no change or significant decreases in PUFA, MUFA and SFA occurred in mature male *M. mixta* until death. All three fatty acid types were utilised by mature female *M. mixta* throughout brooding (with the exception of PUFA in C1 mature females), although MUFA were utilised at the highest rates. Other than the small cohort 2, PUFA, MUFA and SFA concentrations in *A. malmgreni* also increased with the development of sexual characteristics, although highest accumulation rates in *A. malmgreni* were far below those calculated from *M. mixta*. In addition, the disparities between pre- and post-bloom rates were not as pronounced in *A. malmgreni*, and marked increases did not begin until after the bloom material had settled in May. These differences in accumulation rates of fatty acids in the two species show that energy cycles in the *M. mixta* population are more tightly coupled to primary production in the upper water column. A connection also exists between water column production and *A. malmgreni* dynamics, but the nature of that relationship is less clear and probably trophically complex (i.e. several trophic levels involved). As in *M. mixta*, utilisation of MUFA and SFA occurred in mature female *A. malmgreni*, presumably to meet the energetic costs of brooding, although PUFA concentrations did not decrease significantly during the brooding period in *A. malmgreni* females. This may result from the females preferentially conserving PUFA, or from consumption and rapid catabolism of dietary-derived PUFA. The first alternative is more likely since PUFA levels remained constant even before the bloom had occurred.

Areal concentrations of PUFA were similar in *M. mixta* and *A. malmgreni* from December 1998 until March 2000, when concentrations in the *M. mixta* population increased markedly until they peaked in August. Maximum areal concentrations of PUFA in the *M. mixta* population represents 4.2% of the integrated input of seston PUFA to the hyperbenthos of Conception Bay (345 mg m^{-2}, measured from sediment trap material collected at 220 m during the spring bloom of 1996; Parrish et al. unpublished), whereas highest areal PUFA concentrations in *A. malmgreni* represent only 1.7% of integrated input. These disparate results are particularly interesting because areal concentrations of TL and TAG were remarkably similar in the two species, albeit with rapid accumulation periods occurring at slightly different times during a year (Chapters 4, 5). Lower areal concentrations of PUFA in the *A. malmgreni* population probably reflect decreased PUFA content in decomposed organic material that has settled to the hyperbenthos, whereas *M. mixta* has access to PUFA-rich material and zooplankton prey found higher in the water column.

High levels of algal fatty acids in *M. mixta* and *A. malmgreni* suggest that phytoplankton is an important dietary component for these hyperbenthic zooplankton. High concentrations of 16:1ω7 and 20:5ω3, with 16:1ω7 increasingly prevalent in older life-history stages, demonstrate the importance of diatoms as a source of nutrition in both species (Sargent & Falk-Petersen 1988). *M. mixta* contained consistently higher concentrations of 16:1ω7 and 20:5ω3 (% DM) than did *A. malmgreni*. The dinoflagellate marker 22:6ω3 was also a major fatty acid in both species. The polyunsaturated acid 18:4ω3, commonly regarded as an indicator for dinoflagellates, was prevalent only in *M. mixta* (occurred ~1% TFA in *A. malmgreni*). Dinoflagellates are an extremely diverse group of algae. As a result, 18:4ω3 and 22:6ω3 are not always reliable biomarkers for dinoflagellates, or even flagellates in general, and their prevalence in an ecosystem generally depends on the species composition and physiological status of the phytoplankton (Reuss & Poulsen 2002). Generally higher quantities of phytoplankton-derived fatty acids in *M. mixta* provide further evidence that this species has increased access to fresh algal material through its nightly migrations towards the euphotic zone, and/or possesses a superior ability to feed on a broad range of food items, including

phytoplankton, detritus and herbivorous zooplankton, than does *A. malmgreni*. *M. mixta* in the Baltic Sea is commonly known as an opportunistic omnivore with a high degree of versatility with regards to prey types (Viherluoto & Viitasalo 2001). In contrast, owing to its apparent confinement to the hyperbenthos and its prehensile feeding appendages, *A. malmgreni* likely derives the bulk of its essential PUFA indirectly through the capture of herbivorous and omnivorous prey, including copepods, within the hyperbenthos. Owing in part to high total fatty acid content in *M. mixta*, the copepod biomarkers 20:1ω9 and 22:1ω11 (Sargent & Falk-Petersen 1988) were present at higher concentrations (% DM) in the mysids than in the amphipods. Unlike PUFA concentrations, which stopped increasing shortly after the bloom had settled in May, 20:1 + 22:1 levels in *M. mixta* continued to increase in the autumn, possibly reflecting a dietary shift from phytoplankton to lipid-rich copepods and or to an ontogenetic shift in fatty acid utilisation related to the reproductive cycle. Although concentrations (% DM) were generally lower, post-bloom accumulation rates of absolute quantities of 20:1 and 22:1 fatty acids (collectively) by the amphipods were similar to accumulation rates of the major fatty acids found in this species, whereas accumulation rates of the major fatty acids in *M. mixta* far exceeded accumulation rates of 20:1 + 22:1. These between-species differences in relative accumulation rates suggest that copepods become increasingly important as a dietary component, as does phytoplankton, in maturing *A. malmgreni*. In addition, the lower concentrations of calanoid copepod markers in *A. malmgreni* probably reflect the relatively lower total fatty acid content and a diet composed of several zooplankton types that may include mysids and chaetognaths (Chapter 3).

Examination of additional marker ratios further demonstrates the association of both hyperbenthic species with production by diatoms and dinoflagellates in the euphotic region of Conception Bay. The ratio of 16:1ω7/16:0, interpreted to describe the proportion of diatoms in the diet (Claustre et al. 1989), shows clear increases in *M. mixta* and *A. malmgreni* after the start of the spring bloom each year. The increases in the 16:1ω7/16:0 ratio correspond to rapid decreases in the marker ratio 22:6ω3/20:5ω3 in both populations. The 22:6ω3/20:5ω3 ratio is used to indicate the predominance of dinoflagellates over diatoms in environments like Conception Bay and Trinity Bay,

Newfoundland, where these two phytoplankton taxa are the major producers of 22:6ω3 and 20:5ω3, respectively (Parrish et al. 2000). Although they varied seasonally, both marker ratios did not exceed 1.0 in *A. malmgreni*, whereas ratios in *M. mixta* exceeded 1.5 at certain times of the year. This disparity supports the hypothesis that a stronger link exists between upper-mixed-layer dynamics and hyperbenthic *M. mixta*, relative to *A. malmgreni*. The marker ratio of C16 to C18 fatty acids, also used to deduce the prevalence of diatoms or dinoflagellates in the diet, exhibited a clear pattern in *A. malmgreni*, similar to changes in 16:1:16:0. C18 fatty acids are present in high concentrations in a variety of flagellates, and an increase in C18 fatty acids is frequently observed when dinoflagellates become dominant (Kattner et al. 1983; Claustre et al. 1989). Surprisingly, there was no discernible pattern of C16:C18 in *M. mixta*, perhaps indicating that ontogenetic processes are more influential than dietary factors in this instance.

The monounsaturate 18:1ω9, often associated with animal prey (Sargent & Falk-Petersen 1988) or detritus (Scott et al. 2002), was the second most important fatty acid in both species. Changes in 18:1ω9 resembled variations in the copepod marker 20:1 + 22:1, with increased accumulation occurring after the spring bloom had started (*M. mixta*) or settled (*A. malmgreni*). Maximum concentrations of 18:1ω9 occurred in mature female *M. mixta* during autumn, suggesting that in addition to copepod prey, the mysids had access to other prey types at that time. The ratio of 18:1ω9/18:1ω7 can reflect different feeding behaviours and trophic levels. Graeve et al. (1997) found increasing ratios from benthic suspension-feeders consuming freshly settled material (lowest ratio 0.1) to benthic predatory decapods and scavenging amphipods (highest ratio 3.6), therefore high ratio values are generally associated with higher trophic levels. The key premise of the 18:1ω9/18:1ω7 ratio is that 18:1ω9 is derived from animal prey, whereas 18:1ω7 is formed *in vivo* by chain elongation of 16:1ω7 produced by diatoms (Graeve et al. 1997, Falk-Petersen et al. 2000). Significant regressions of 18:1ω7 on 16:1ω7 for both Conception Bay species [(μg individual^{-1}); $p < 0.0001$, $r^2 > 0.8$, $n = 174$ (*M. mixta*), $n = 119$ (*A. malmgreni*)] confirmed that the isomers were closely correlated, and some interesting patterns in the 18:1 isomer ratio were apparent. In *M. mixta*, the recurring peaks of

18:1ω9/18:1ω7 in mid-winter each year may indicate the reliance of this species on a primarily non-phytoplankton diet prior to the bloom. In turn, the rapid decrease in the ratio with the onset of the bloom could represent the transition period to a phytoplankton-based diet. The less variable 18:1ω9 18:1ω7 ratios in *A. malmgreni* reflect a more uniform diet year-round, and less direct reliance on spring bloom production. Although high ratios have traditionally been associated with an increased degree of carnivory, the overall higher ratios in *M. mixta* may simply reflect a broader diet composition and different lipid biosynthesis capabilities compared with *A. malmgreni*. Ratios of 18:1ω9 to 18:1ω7 also increase in starved zooplankton (Auel et al. 2002), but it is clear from the lipid storage cycles in field-collected *M. mixta* and *A. malmgreni* that there is ample food available year-round (Chapters 4, 5).

Saturated fatty acids, although important components of lipids, are not particularly valuable as trophic markers because they are readily synthesized by most organisms and they occur at various concentrations in all algal groups (Napolitano 1999). On the other hand, high proportions of PUFA relative to SFA have been used to indicate increased carnivory in antarctic krill after they were fed an animal diet for 16 days (Cripps & Atkinson 2000). The highest ratio measured in the carnivorous krill was 5, whereas krill fed with diatoms exhibited low ratios of ~1 (Cripps & Atkinson 2000). This ratio has not been a successful indicator of carnivory in all trophic studies, possibly due to differences in relative PL content among zooplankton species and/or to regional differences in feeding strategies in zooplankton and phytoplankton taxonomy and fatty acid composition (Auel et al. 2002, Stevens 2003). Feeding strategies, PL content (Chapters 4, 5), and consequently PUFA/SFA ratio variations, differ between *M. mixta* and *A. malmgreni*. Ratios in *A. malmgreni* peaked at around 5 during or prior to the phytoplankton bloom and then decreased to ~2.5 shortly thereafter. In contrast, PUFA/SFA ratios in immature male *M. mixta* peaked at ~2 during mid-winter, and in immature females at ~2 during the spring bloom. The ratio of PUFA to SFA in *M. mixta* remained relatively level until autumn, after which it gradually decreased. The differences between male and female *M. mixta* may be due to differences in dietary preference, although such differences would presumably have been reflected in the other trophic markers. The sustained peak in *M.*

mixta is also difficult to interpret, as one would expect a carnivory index to decrease rapidly with the onset of the bloom, as observed in the 18:1ω9 to 18:1ω7 ratio in *M. mixta* and the PUFA/SFA ratio in *A. malmgreni*. A high proportion of PUFA can also indicate starvation (and preferential conservation of PUFA over other fatty acids), but this is not the case here because absolute TAG and PUFA content increase during the periods of increased PUFA:SFA ratios in both *M. mixta* and *A. malmgreni*. Future studies on hyperbenthic zooplankton in this region will help to determine the correct applications and interpretations of these trophic markers.

6.5 Summary

Analyses of fatty acid composition and specific marker ratios show that sexual maturation in one-year-old *M. mixta* and *A. malmgreni* is fueled by phytoplankton produced in the upper water column during and following the spring phytoplankton bloom. The succession of the plankton from diatoms to dinoflagellates is reflected in the fatty acid composition and in specific marker ratios, particularly in *M. mixta*. After the bloom is exhausted, zooplankton become increasingly important components of the diet. Depletion of fatty acids occurs only in mature post-spawned males and females, therefore overwintering is not accompanied by the food shortages typical of other cold-ocean regions (e.g. Antarctica, Hagen et al. 2001). The diel vertical migrations into the water column and the broad range of prey items available to *M. mixta* confer upon this species increased opportunities to assimilate and store the fatty acids necessary for successful growth and reproduction. Fatty acid composition and accumulation rates in *A. malmgreni* indicate that this species is restricted to a different and lower quality diet than is *M. mixta*. Nevertheless, there exists a clear seasonal trophic link between the hyperbenthic amphipod population and production in the water column. Even if *A. malmgreni* incorporates fatty acids from its food without modification, it is likely that the lipid composition of any settling material used as a food source is altered considerably as a result of catabolic processes and intermediate trophic pathways (Santos et al. 1994), particularly during non-bloom periods when settling rates are slow. In addition, different patterns in areal concentrations of PUFA in the sympatric populations indicate different

requirements for or availability of PUFA, even though maximum areal concentrations of total lipid and triacylglycerol are nearly equivalent in *M. mixta* and *A. malmgrem* from Conception Bay (Chapters 4, 5).

This study represents the first comprehensive documentation of the seasonal and ontogenetic changes in the fatty acid composition of *M. mixta* and *A. malmgremi* living in any environment. While the majority of zooplankton fatty acid studies have focussed on trophic links within food webs, the ontogenetic changes in fatty acid composition of plankton have been largely overlooked. Nearly every marker fatty acid and ratio examined in *M. mixta* and *A. malmgrem* varied in some manner during development. These results reiterate the need for increased emphasis on the life-history component, in addition to the environmental component, in trophic studies that utilise fatty acid biomarkers. Comprehensive and multi-tiered studies are needed to understand the underlying cause for the variability of fatty acid composition in an ecological context.

6.6 References

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Chapter 7. Conclusion

7.1 Summary of thesis

This research documents the first comprehensive data on the population and energy-storage characteristics of *Mysis mixta* and *Acanthostepheia malmgreni* from Conception Bay, Newfoundland. Conception Bay is influenced by the year-round input of cold water from the Labrador Current; therefore, many features exhibited by *M. mixta* and *A. malmgreni* are somewhat typical of zooplankton species inhabiting polar oceanic regions that are characterized by low, stable temperatures and marked seasonal patterns in photoperiod and primary production (e.g. Clarke 1983, Ward 1984). Such features include slow and seasonally variable growth rates, seasonal breeding and lipid accumulation, and a lengthy brooding period. Fortunately, boreal systems like Conception Bay are considerably more accessible than polar and deep-sea regions, and they provide opportunities for long-term and comprehensive studies (rather than snapshot, one-tiered studies) on zooplankton species and their roles in benthic-pelagic coupling.

Despite taxonomic and biological differences, there were remarkable similarities in the life cycles and population dynamics of the sympatric species *M. mixta* and *A. malmgreni*: both lived for 2–5 years from spawning to death, and juveniles were released during the spring bloom sedimentation event following a 5-month brooding period. Life cycles in both species showed close coupling with annual phytoplankton flux from the euphotic zone, although *M. mixta* exhibited behavioural adaptations (e.g. diel vertical migrations and a broad range of prey types) that allowed it to take immediate advantage of spring bloom production. Enhanced plasticity in the life cycle, growth, motility, diet and lipid storage in *M. mixta*, compared with *A. malmgreni*, suggests that the mysid population is better able to adapt to inter-annual and seasonal changes in the energetics of the Conception Bay ecosystem. Consistent annual recruitment, density, growth, biomass, and secondary production confirm that *M. mixta* is a highly flexible and adaptive species.

M. mixta and *A. malmgreni* exhibited seasonal changes in energy storage and utilisation as a result of complex interactions among food availability, environmental conditions, growth and reproduction. Both hyperbenthic species accumulate significant

quantities of lipids and are likely to be important trophic links in Conception Bay. *M. mixta*, in particular, plays a significant role in the coupling of the benthic and pelagic regions owing to its rapid and pronounced build-up of lipid reserves each year when the spring phytoplankton bloom is initiated. It appears that *A. malmgreni* is unable to utilise the bloom material until it settles to the benthos. In turn, the strong seasonality in reproduction and neutral lipid accumulation in both species demonstrates the overall importance of the annual spring bloom to the success of each hyperbenthic population. As expected, phospholipid concentration increased slowly with growth of the organisms, but it remained relatively stable with season.

Unlike polar copepods that accumulate large stores of wax esters to survive and reproduce during long periods of food shortage, *M. mixta* and *A. malmgreni* accumulate stores in the form of triacylglycerols to fuel reproduction and growth. The preferential storage of triacylglycerol rather than wax ester corresponds with omnivory and year-round feeding by *M. mixta* and *A. malmgreni*. Several studies have shown that wax esters are more abundant in herbivorous zooplankton, whereas omnivorous and carnivorous zooplankton tend to have lower amounts of wax esters and higher amounts of triacylglycerols (e.g. Lee & Hirota 1973, Sargent & Falk-Petersen 1981, Sargent et al. 1981, Clarke 1983, Graeve et al. 1994, Kattner et al. 1994). Analyses of laboratory-starved amphipods also indicate that *A. malmgreni* is not able to over-winter and successfully reproduce the following year without an adequate supply of food.

Food quality, and thus growth, also depends upon essential components including unsaturated fatty acids (Anderson & Pond 2000). Fatty acid analyses provided additional information regarding the trophic interactions within the Conception Bay ecosystem, particularly in reference to the seasonal phytoplankton dynamics. Increased accumulation rates of PUFA by *M. mixta* and *A. malmgreni* in the spring indicated that the source of these essential nutrients was the phytodetritus originating from autotrophs during the bloom. Both species appeared to fuel sexual development using material recently acquired from phytoplankton. Changes in fatty acids reflected the sequence of plankton taxa during and following the development of the spring bloom, starting with diatoms and dinoflagellates and ending with copepods. Fatty acid composition and accumulation rates

in *A. malmgreni* further confirm that this species is subject to a lower quality diet than is *M. mixta*.

One key theme throughout this research is the quantification of entire hyperbenthic populations to determine their relative ecological importance within Conception Bay. Calculations of annual biomass, density, secondary production and areal concentrations of total lipid, triacylglycerol and polyunsaturated fatty acids serve as important means to quantify the organic material consumed, and potentially recycled, by *M. mixta* and *A. malmgreni*. Although population characteristics of *A. malmgreni* varied significantly between years, density, secondary production and areal concentrations of total lipids and triacylglycerols were similar in *A. malmgreni* and *M. mixta*. Estimates of biomass and areal concentrations of polyunsaturated fatty acids were more disparate in the two species. On the whole, the data suggest that *M. mixta* and *A. malmgreni* are important energetic components in Conception Bay, regardless of physiological and behavioural differences, and this research provides a reliable foundation for further studies within this cold-ocean system.

7.2 Future studies

In the majority of cases, plankton, benthos and hyperbenthos are not independent of each other and should not be studied as discrete units. Broad-based approaches to study benthic-pelagic coupling are needed to assess the degree of connections in any aquatic ecosystem. One of the primary obstacles faced by investigators attempting to model benthic-pelagic coupling is the inability to predict export of material produced in the euphotic zone (Wassmann, 1998). Before export can be predicted on a global basis, investigators must first collect and then pool data that encompasses primary production and vertical flux over the entire range of marine ecosystems. At this time, generalizations regarding benthic-pelagic coupling are difficult to make and often lead to misconceptions.

The role of zooplankton species in linking benthic and pelagic regions requires further investigation, particularly with respect to highly motile and omnivorous species such as *M. mixta*. Basic information on feeding selectivity and rates, vertical migration patterns and overall energy budgets of keystone species are needed to provide a reliable

description of trophic coupling. Some ecosystems have been well-characterized, but between-studies comparisons are difficult because researchers report data using a variety of methods and units; for example, lipid content has been reported as a proportion of dry mass, ash-free dry mass, lipid-free dry mass and wet weight. Comparisons are further precluded because different zooplankton species contain varying amounts of refractory material such as chitin. Increased uniformity of collection methods and measurement units would facilitate comparisons among species and regions.

Furthermore, continued research involving tracers is needed to determine the paths of organic and inorganic compounds throughout food webs. Biomarker studies have successfully identified trophic links between pelagic and benthic organisms. Species composition of plankton, organic carbon accumulation rates, C/N ratios, stable isotopes, and chemical tracers are used in addition to lipid biomarkers to distinguish sources of organic matter. Comprehensive studies utilising one or more of these biomarker approaches may help to further elucidate the complex trophic dynamics in poorly understood marine food webs.

Finally, the quantification of entire populations with respect to each other and to the availability of resources provides great promise for studies of aquatic ecosystems in all regions of the world. Once the key populations are identified and then quantified in terms of variables such as annual secondary production and areal lipid concentrations, researchers can determine how resources are distributed to the different components within a food web and how these dynamics change as a result of environmental or anthropic effects.

7.3 References

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