POPULATION DYNAMICS AND LIFE HISTORY CHARACTERISTICS OF BOREAL APPENDICULARIAN SPECIES IN CONCEPTION BAY, NEWFOUNDLAND, CANADA

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by

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Abstract

The primary goal of this study was to investigate the population dynamics and life history traits of boreal appendicularian species in Conception Bay, Newfoundland. Specific questions were; (1) What are the optimum environmental conditions for appendicularian species? (2) What are their generation time, and growth and secondary production rates? (3) How do length-at-age, age-at-maturity and fecundity vary under seasonally fluctuating environmental conditions and how do these demographic parameters relate to population growth rate? In order to study life history and demographic traits of naturally occurring populations of appendicularians, it was necessary to develop an *in situ* method of age determination.

The temporal and spatial niche of appendicularian species is defined primarily by temperature and salinity in which *Oikopleura vanhoeffeni* is a cryophilic, stenothermal and stenohaline species, *Fritillaria borealis typica* is eurythermal and euryhaline and *Oikopleura labradoriensis* is mesothermal and mesohaline. Throughout the year, more than 70 % of the individuals of each species were present above 100 m, indicating that a majority of them experienced seasonal variation in abiotic and biotic factors.

The presence of lipofuscin in the brain tissue, growth rings on the statolith and statolith diameter were explored as age indicators for appendicularians. The results indicated that statolith diameter was a feasible and reliable age indicator. A laboratory study of statolith diameter and somatic growth in *O. vanhoeffeni* showed that variability in statolith diameter-at-age was substantially lower than that in trunk length-at-age, and that variability in statolith-at-age remained constant with age whereas variability in trunk length-at-age increased with age, suggesting that statolith diameter should be a better *in situ* indicator of age than body size. Using statolith diameter as a proxy for age, trunk length-at-age of *O. vanhoeffeni* and *O. labradoriensis* in Conception Bay varied seasonally depending on food type and concentration, indicating that conventional decomposition of cohorts from trunk length frequency distributions would lead to inaccurate estimation of age structure and thus population growth rates.

Based on age structures represented by statolith diameter, the generation time of *O. vanhoeffeni* was one year and that of *O. labradoriensis* was between 8 months and one year. Somatic growth in both species was exponential, suggesting the absence of food-limitation and that there was no apparent energetic trade-off between growth and reproduction. Somatic growth rate of both species (mass basis) ranged from 0.007 to 0.043 d^{-1}. House production rate far exceeded somatic production in terms of carbon. The annual sum of somatic and house production of both oikopleurid species was equivalent to 49 - 95 % of mesozooplankton production and 3.8 - 7.3 % of primary production in Conception Bay.

Age-at-maturity of *O. vanhoeffeni* and *O. labradoriensis* increased over winter and spring as temperature decreased, while size-at-maturity and potential fecundity increased to a maximum during the spring diatom bloom. The population growth rate of *O. vanhoeffeni* peaked in spring as age-at-maturity and fecundity increased whereas the population growth rate of *O. labradoriensis* peaked in the fall when age-at-maturity and fecundity decreased. Thus, *O. vanhoeffeni* has a life history strategy based upon maximization of clutch size and *O. labradoriensis* has a life history based upon maximization of population turnover rate. Thus, the appendicularian tunicates appear to have multiple adaptations promoting niche separation including temporal segregation, different optimum temperature and salinity and differing life history adaptations, particularly involving variability in age and size-at-maturity and in individual fecundity.

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General Introduction

Understanding the determinants of population dynamics is pivotal in population ecology. A long history of investigation has revealed the complex nature of population dynamics. The geometric nature of population growth was described by Malthus in 1798, while density-dependent population growth was addressed in a logistic model by Verhulst (1838). Population dynamics in relation to demographic traits was described mathematically in the Euler-Lotka equation, $\sum l_x m_x e^{-rx} = 1$, where l_x is age-specific survival, m_x is age-specific fecundity and r is the Malthusian parameter of intrinsic rate of population growth (Euler 1760, Lotka 1925). Laughlin (1965) and May (1976) showed how population growth rate depends upon the number of offspring that survive to reproduce during each generation, and Lewontin (1965) and Caswell and Hastings (1980) described how population growth rate could be increased by decreasing generation time or increasing the number of surviving offspring. The development of a matrix representation of life history tables in the analysis of population dynamics based on demographic traits (Leslie 1945) enabled forecasting the pattern of population growth (Caswell 1989).

Current studies in population dynamics show how both external and internal factors affect demographic traits and consequently population growth rates. External factors can be abiotic variables such as temperature, condition of substrates, toxicity and climate (Johnson 2000, Krebs 2002, Sibly and Hone 2002, Pena et al. 2005) and biotic variables such as food supply, predation, competition and parasitism (Krebs 2002,Sibly and Hone 2002). Internal factors which exist within the populations can be population density, genetic variation, physiological plasticity and life history strategies (Levinton 1982, Sinclair 1989, Stearns 1992, Sibly and Hone 2002). However, external and internal factors may not independently influence demographic traits but may act together through various feedback mechanisms. For example, the effects of many external environmental factors can be population density dependent (Krebs 1995).

Determining the effect of external and internal factors on demographic traits and population growth rates of marine organisms in the field can be difficult for a variety of reasons. For example, most plankton populations are open to immigration and emigration, making it difficult to examine the time trajectory of a single cohort in a particular population. In addition, demographic studies of field populations require *in situ* methods for age determination and survivorship, conventionally involving mark-recapture techniques that are generally not feasible for zooplankton. For these reasons, studies of population dynamics in relation to demographic traits in the marine environment have been limited to organisms such as fish, mollusks, and mammals in which tagging and age determination are possible (Myers et al. 1997, Brazeiro and Defeo 1999, Pistorius et al. 1999, Hutchings 2005). The few studies of population dynamics in this context for invertebrates such as copepods and cladocerans have been conducted in the laboratory (Hann 1985, Lürling and van Donk 1997, Colin and Dam 2004, Mangas-Ramírez et al. 2004) where demographic traits can be more easily observed.

The main goal of this study was to further our knowledge about the population dynamics of appendicularians. Appendicularians are pelagic tunicates that are common and abundant in all oceans. Most species are found in the euphotic zone, however, new species have also been described from mesopelagic and bathypelagic depths (Fenaux et al. 1998, Hopcroft 2005). Appendicularians are semelparous and protandric hermaphrodites, except for a single species, *Oikopleura dioica*, which is dioecious (Fenaux 1998). The appendicularian life cycle can be divided into four distinct stages. After external fertilization and the hatching of eggs, metamorphosis occurs upon completion of organogenesis by a 180-degree rotation of the tail. Most somatic cells reach their definitive number early in development, and further growth is achieved by an increase in the volume of somatic cells accompanied by a high degree of endopolyploidy. Development is direct, and there are no separate instars or epidermal molts. When sexual maturity is reached, sperms are released via a spermiduct first and shortly after the oocytes are released by rupture of the gonad and body wall, resulting in the death of the animal (Fenaux 1976, Fenaux and Gorsky 1983, Galt and Fenaux 1990). Because the

time periods in the spawning of sperms and eggs overlap, self-fertilization is possible in *Oikopleura vanhoeffeni* (Chapter 2), but the degree of self-fertilization in nature is not known. It has been noted that some appendicularian species form mating aggregations, thereby increasing the probability of fertilization (Alldredge 1982).

Appendicularians play several important roles in marine ecosystems. Using their complex filtering structure, called the 'house', they are able to feed on a wide size range of particles ranging from DOM (dissolved organic matter) to large diatoms (Deibel 1986, Flood et al. 1992, Urban-Rich et al. 2006). Because appendicularians are prey for many invertebrates and fish (refs. in Purcell et al. 2005), they efficiently transfer energy within food webs by short-circuiting intermediate trophic links (Gorsky and Fenaux 1998). In favorable conditions appendicularians can grow and multiply quickly, forming dense blooms that consume up to 50 - 66% of the standing crop of phytoplankton daily (Alldredge 1981, Deibel 1988, Maar et al. 2004). The sinking of appendicularian fecal pellets and discarded houses transports a substantial portion of primary production to the benthos (Silver and Alldredge 1981, Taguchi 1982, Bauerfeind et al. 1997, Maar et al. 2004, Alldredge 2005, Dagg and Brown 2005, Robison et al. 2005).

Temporal and spatial variation in the abundance and biomass of some appendicularian species have been studied in relation to temperature, salinity, food availability and predator abundance (Fenaux 1963, Shiga 1985, Acuña and Anadón 1992, Acuña 1994, Nakamura et al. 1997, Tomita et al. 2003, Båmstedt et al. 2005, Lopéz-Urrutia et al. 2005, Hoover et al. 2006). However, there is no field information regarding how environmental variation influences demographic traits and life history traits, both of which contribute to population growth. A major problem in determining demographic and life history traits of appendicularians is the lack of an *in situ* method for determination of age.

The age of organisms can be estimated in various ways. Absolute age can be determined from chronologically accumulated markings (rings and striations) on calcified structures such as bones (Marmontel et al. 1996, Snover and Hohn 2004), statoliths (Lipinski 1986, Chatzinikolaou and Richardson 2007), otoliths (Campana and Neilson

1985) and shells (MacDonald and Thomas 1980). Relative age has been estimated using the concentration of lipofuscin, a fluorescent, pigment composed of lipid containing residues of lysosomal digestion that accumulate in postmitotic cells with age (Ettershank 1984). Other metrics of relative age include, telomere length, which shortens with age (Haussmann and Vleck 2002), and otolith size, which generally increases with age (McDougall 2004). None of these methods have been tested for age estimation of appendicularians.

The primary goal of this study was to investigate the population dynamics of boreal appendicularian species in Conception Bay, Newfoundland. Three species, *Oikopleura vanhoeffeni*, *Oikopleura labradoriensis* and *Fritillaria borealis typica* are found in Conception Bay. All three are transported to Newfoundland via the Labrador Current from the Arctic Ocean (Kramp 1942, Udvardy 1954, Grainger 1965). Conception Bay lies within the cold temperate Northwest Atlantic biogeographic province immediately below the Arctic realm (Spalding et al. 2007). This distribution provides the unusual opportunity to investigate the population dynamics of Arctic appendicularian species from a land-based laboratory at Memorial University.

In Chapter 1 I described the temporal and vertical distribution of the three appendicularian species described above in relation to seasonal variation in physical and biological factors (i.e., temperature, salinity and chlorophyll *a* concentration) over one year. The optimal range of temperature and salinity for each species was defined as the physical niche. The vertical distribution of appendicularian species was essential information for the study of life history and population dynamics in the subsequent chapters of the thesis, because it is important to know whether the appendicularians are exposed to seasonal variation in temperature and food concentration that occurs primarily within the upper 100 m of Conception Bay. I also presented a new morphological key in Chapter 1 which is used to identify small juveniles of *O. vanhoeffeni* and *O. labradoriensis*.

In Chapter 2 several methods for age determination of *O. vanhoeffeni* were explored, including lipofuscin in brain tissue, incremental rings on the statolith, and

statolith size (i.e., diameter). Laboratory and field studies indicated that statolith diameter is a robust age indicator for appendicularians while body size varies considerably with age and thus is not a reliable age indicator.

Using statolith diameter as an age proxy, several important questions regarding life history characters and population dynamics of two sympatric oikopleurid species, *O. vanhoeffeni* and *O. labradoriensis*, were answered in Chapter 3. Based on cohort analysis of age composition derived from statolith diameter, generation times, patterns of somatic growth and growth rates were determined. Somatic and house production rates were estimated and secondary production also determined. Finally, I determined how the population growth rates of these two oikopleurid species are related to variation in the demographic traits size-at-maturity, age-at-maturity and fecundity, under seasonally fluctuating temperature and food availability.

Chapter 1

Temporal and vertical distributions of appendicularians in Conception Bay, Newfoundland

1.1 Introduction

Appendicularians are pelagic tunicates found in all oceans and are one of the most abundant zooplankton in marine ecosystems. The success of appendicularians is attributed to their ability to quickly grow and increase in abundance when conditions become favorable (King 1982, Hopcroft and Roff 1995). These suspension feeders use a secreted mucous house containing a complex set of filters to consume a wide size range of particles from colloidal organic matter through pico- and nanoplankton to large diatoms (Alldredge 1977, Deibel and Turner 1985, Deibel 1986, Flood et al. 1992, Urban-Rich et al. 2006). They are preyed upon by numerous species of invertebrates, and larval and juvenile fish (Purcell et al. 2005). Thus, appendicularians bypass the microbial loop by directly transfering very small particulate organic matter to higher trophic levels (Azam et al. 1983, Gorsky and Fenaux 1998), creating a short circuit of energy within the food web. Appendicularians are also an important member of the 'biological pump', in that they transport organic matter from the euphotic zone to deeper layers by producing a large number of fecal pellets and by discarding mucous houses containing trapped organic matter (Taguchi 1982, Sato et al. 2003, Alldredge 2005, Dagg and Brown 2005).

In order to assess the ecological impact and understand the life history of appendicularians, it is important to know how environmental factors affect their temporal and spatial distribution. Previous studies of relationships between the temporal and vertical distributions of appendicularians and environmental variables have been conducted primarily in European and Japanese coastal waters on temperate and subtropical species (Fenaux 1968, Shiga 1985, Acuña and Anadón 1992, Acuña 1994, Tomita et al. 2003, Lopéz-Urrutia et al. 2005). Additional studies from other regions are necessary to gain a global perspective on the distribution of appendicularians in relation to environmental variation.

The focus of the present study is to determine the temporal and vertical distribution of cold-water appendicularian species in Conception Bay, Newfoundland. Three species, *Oikopleura vanhoeffeni* (max. trunk length, ca. 5 mm), *Oikopleura labradoriensis* (max TL, ca. 2 mm) and *Fritillaria borealis typica* (max TL, ca. 0.9 mm) occur in Conception Bay (Fig. 1.1). The distribution of *O. vanhoeffeni* extends from the Arctic Ocean to the boreal North Pacific and North Atlantic oceans (Lohmann 1895, 1896, Frost et al. 1933, Galt 1970; Shiga 1993) and that of *O. labradoriensis* extends from the Arctic Ocean to the temperate North Pacific and North Atlantic oceans (Lohmann 1896, Udvardy 1954, Fenaux 1963, Shiga 1976, 1985). *F. borealis typica* lives in both hemispheres, and is distributed from the Arctic and Antarctic oceans to the temperate zone of the Atlantic and Pacific oceans (Lohmann and Büchmann 1926, Grainger 1965, Wyatt 1973, Buchanan and Browne 1981, Tomita et al. 2003). Although the feeding ecology and bioenergetics of these species have been investigated (Deibel 1988, Lopéz-Urrutia et al. 2003) their life histories and population biology are essentially unknown. There have been few studies that address temporal variation in the abundance of these three species (Frost et al. 1933, Udvardy 1954, Davis 1982, Mahoney and Buggeln 1983) and only one study reporting their vertical distribution in Newfoundland waters (Deibel 1988). Information on the abundance of appendicularians in Newfoundland waters cannot be used to address their population biology on annual time scales because the published literature is either focused on a particular season or is generic, i.e. *O. vanhoeffeni* and *O. labradoriensis* are lumped into the Oikopleuridae because of difficulties in determining the species identity of small juveniles (Davis 1982). As the present results demonstrate, lumping these two taxa will obscure marked species-specific differences in life history characteristics and resultant population dynamics.

The objectives of this study were to describe the temporal and vertical distribution of appendicularians in relation to environmental variation in Conception Bay, Newfoundland, over a one year period, to determine the degree to which these sympatric species demonstrate niche separation, and to compare their environmental niches to those published for the same species from other boreal and temperate waters. Given that

routine differentiation between *O. vanhoeffeni* and *O. labradoriensis* of all life history stages was crucial to this study, I also report on a new morphological character that is useful in the identification of juvenile specimens.

1.2. Materials and Methods

1.2.1. *Study site*

Conception Bay, located on the northeast coast of Newfoundland, is approximately 70 km long and 32 km wide at its mouth, with a maximum depth of about 300 m (Fig. 1.2). At the mouth of the bay there is a sill at about 150 m that restricts entry of deep water. In some years, pack ice advected by the Labrador Current and pushed ashore by onshore winds covers the bay from mid-March to late April, but the formation of local ice is rare. Freshwater runoff is relatively unimportant compared to the influence of seasonal ice-melt upstream of the bay, which largely determines surface salinity variability (deYoung and Sanderson 1995). The residence time of water above 150 m ranges from 30-42 d (deYoung and Sanderson 1995).

1.2.2. *Sample collection*

Appendicularians were collected during daytime from July 3, 2002 to June 25, 2003 at a site in Conception Bay, Newfoundland, with a bottom depth of ca. 235 m (47°32.2'N; 53°07.9'W, Fig. 1.2). Samples were collected in 0-30, 30-100, and 100-225 m depth strata with an opening-and-closing Tucker Trawl, with a mouth area of 0.2 m^2, a total area of 1.5 m^2 (open-area to mouth-area ratio = 4.6) and a mesh size of 110 μm. The mesh size was selected to quantitatively collect newly-hatched appendicularians, which have a trunk length of ca. 150 μm (*O. vanhoeffeni*, laboratory observations). The depth strata were chosen to bracket the depths above, within, and below the seasonal thermocline and halocline. The speed of oblique towing was 0.12 m sec^{-1} and the volume of water filtered was determined with a TSK™ flowmeter mounted on the net. Upon retrieval of the net, samples were immediately fixed in Bouin's solution. Sampling was conducted monthly except during winter, when harsh weather conditions precluded

sampling. A CTD cast was made before each tow using a Seabird SBE 25-01 equipped with a Seatech fluorometer to measure temperature, salinity and *in situ* relative fluorescence. Relative fluorescence units (RFU) were converted to chlorophyll *a* concentrations (μg chl *a* L^{-1}) using the equation Chl *a* = (0.398 x RFU) + 0.281 (r^2 = 0.73, n = 244) which was developed using historical data from Conception Bay (Cold Ocean Productivity Experiment I, unpublished).

1.2.3. *Sample analysis*

Appendicularian abundance was determined from subsamples produced using a Motoda zooplankton sample splitter (Motoda 1959). The animals were viewed and counted under a Zeiss stereomicroscope at 40 x and 60 x magnifications. The number of *O. vanhoeffeni*, *O. labradoriensis* and *F. borealis* counted depended upon their seasonal abundance, ranging from 1-517, 3-464, and 116-132 $subsample^{-1}$, respectively (n = 27 subsamples for each species). The number of each species counted in each subsample resulted in median 95% confidence intervals (i.e. analytical error) of 18 %, 17 % and 16 % of the count for each species, respectively (Alden et al. 1982).

Taxonomic differentiation between *O. vanhoeffeni* and *O. labradoriensis* was possible in large specimens using conventional morphological characters, including the patterns of inclusion bodies in the house rudiment and subchordal cells in the tail. The inclusion bodies of *O. vanhoeffeni* were bean-shaped and randomly distributed over the surface of the house rudiment whereas the inclusion bodies of *O. labradoriensis* were rod-shaped and arranged in a single, folded line on each side of the house rudiment (Lohmann & Büchmann 1926, Büchmann 1969, Galt and Flood 1998). Inclusion bodies were stained with Coomassie Blue R to enhance their visibility. Many clustered subchordal cells of *O. vanhoeffeni* were aligned along the right side of the tail (viewed dorsally), covering at least half the length of the tail, while the few subchordal cells of *O. labradoriensis* were aligned in a row on the right side of the tail, covering only 1/3 to 1/4 of the length of the tail (Büchmann 1969, Shiga 1976, Mahoney 1981, Shiga 1993). However, inclusion bodies and subchordal cells could not be recognized in individuals

without house rudiments and with damaged tails. Moreover, these characteristics were difficult to recognize in small juveniles. In order to overcome this problem I examined their anatomy carefully, and discovered that the position of the endostyle on the oikoplast epithelium is diagnostic for these two species (Fig. 1.3). Viewing the trunk mid-ventrally, the endostyle of *O. vanhoeffeni* extended posteriorly (i.e. towards the anus) and reached the end of the oikoplast epithelium (Fig. 1.3a, c) whereas the endostyle of *O. labradoriensis* was located in the center of the oikoplast epithelium (Fig. 1.3b, d). This species-specific position of the endostyle relative to the oikoplast epithelium was the most reliable and easy to use taxonomic character in this study, particularly for juvenile life stages.

1.2.4. *Data analysis*

Relationships between water column abundance and environmental variables over the annual study period were determined using Spearman rank correlation. Parametric analysis was not suitable because the abundance data were not normally distributed even after transformation. Vertical distribution at each sampling time point was expressed as the percentage of abundance in each depth layer to the total abundance through the entire water column. Pearson correlation was used to determine the relationship between the percent of total abundance of each species in the surface layer (0-30 m) and environmental variables, after arcsine-transformation of the percent abundance.

1.3. Results

1.3.1. *Hydrography*

Near-surface temperature ranged from -1.5 to 16.6 °C over the annual cycle, whereas the temperature below 150 m remained constantly below 0 °C (Fig. 1.4a). The water column was isothermal in April (< 0 °C), but there was early indication of surface heating in May. The thermocline intensified over the summer as near-surface temperature reached a maximum in August and September before decreasing over the winter. Salinity ranged from 31.1 to 33.2 psu and generally increased with depth (Fig.

1.4b). Salinity of the upper 75 m showed a distinct seasonal cycle, decreasing from a maximum in April and May to a minimum in October, followed by a gradual increase through December. This warmer and less salty surface water was mixed downward by winter storms and deep convection between November and December (Fig. 1.4a, b). The concentration of chlorophyll *a* ranged from 0.6–3.5 $\mu g\ l^{-1}$, with evidence of a spring bloom in April and May and a minor, deep chlorophyll maximum in July and August at the base of the thermocline (Fig. 1.4c). These environmental data were averaged within the three depth strata from which the appendicularians were collected (Fig. 1.5). Most of the seasonal variation in mean temperature, salinity and chlorophyll *a* concentration occurred above 100 m, with greater variation in the 0-30 m than in the 30-100 m stratum (Fig. 1.5).

1.3.2. *Total abundance of appendicularians over the entire water column*

Vertically-integrated water column abundance of the three appendicularian species displayed a non-overlapping cycle of seasonal dominance (Fig. 1.6). The abundance of *O. vanhoeffeni* increased from April to May and peaked in June at 114,135 ind. m^{-2}, followed by an increase in the abundance of *F. borealis* from June to a maximum of 107,431 ind.m^{-2} in August, similar to the maximum abundance of *O. vanhoeffeni* (Fig. 1.6). *O. labradoriensis* peaked in October at 61,937 ind.m^{-2}. *O. vanhoeffeni* showed the highest seasonal variability (annual coefficient of variation, CV = 206%) and *F. borealis* had the least variability (CV = 96%) while *O. labradoriensis* showed intermediate variability (CV = 166%). Maximum total abundance of appendicularians (i.e. sum of the three species) occurred in June after the end of spring bloom (166,772 ind. m^{-2}), as a result of the peak abundance of *O. vanhoeffeni* and an increase in the abundance of *F. borealis*.

1.3.3. *Temporal and vertical distribution of appendicularians in relation to environmental variables*

The abundance of *O. vanhoeffeni* remained low (< 27 m^{-3}) from July 2002 to April 2003 and increased by 2 orders of magnitude in all three depth layers during May and June, reaching a peak abundance of 1124 m^{-3} in the surface layer (Fig. 1.7a). Variation in abundance over the year in all three depth layers was positively correlated with chlorophyll *a* concentration (Table 1.1). The vertical distribution displayed two distinct temporal modes annually (Fig. 1.7a). Abundance in the surface layer decreased below abundance in the middle and deep layers during summer (June to September) but increased above the middle and deep layers from autumn to spring (October to May) (Fig. 1.8a). Less than 1 % of the population inhabited the surface layer during summer as temperature increased, while the majority of the population remained in the middle and deep layers where temperature was lower (Fig. 1.8a). A large proportion of *O. vanhoeffeni* population remained in the surface layer during spring when temperature reached its annual minimum. Correlation analysis indicated that the percent of total abundance in the surface layer was inversely related to temperature (Table 1.2). The annual mean (±SD) proportion of the *O. vanhoeffeni* population occurring above 100 m depth was 71 $\pm$ 21 %.

The abundance of *F. borealis* in the surface layer increased from May to July, reaching a peak of 526 m^{-3} (Fig. 1.7b). Abundance then generally decreased until the following April. The temporal pattern of abundance in the middle and deep layers was similar to that in the surface layer, reaching maxima of 639 m^{-3} and 439 m^{-3}, respectively, during August. Throughout the year, the abundance of *F. borealis* in all depth layers was positively correlated with temperature and negatively correlated with salinity (Table 1.1). Vertical distribution of abundance (Fig. 1.7b) and relative abundance (Fig. 1.8b) indicates that the majority of animals occurred below the surface layer in August, April and May, but within the surface layer in July and October - January. This pattern of variation in the surface was not correlated with the environmental variables observed in

this study (Table 1.2). The annual mean (±SD) proportion of *F. borealis* in the upper 100 m was 80 ± 18 %.

Seasonal variation in the abundance of *O. labradoriensis* was strongest in the surface and middle depth layers (Fig. 1.7c). Between September and October there was a 1000-fold increase in abundance in the upper layer and a 100-fold increase in the middle layer, followed by a gradual decrease until spring (Fig. 1.7c). The abundance over all depths throughout the year was negatively correlated with salinity (Table 1.1). The vertical distribution of *O. labradoriensis* displayed two distinct temporal modes, one in which abundance was highest in the surface layer from October to April and one in which abundance was lowest in the surface layer from May to September (Fig. 1.7c). In fact, no animals were collected from the surface layer from May to August. The relative abundance of *O. labradoriensis* indicated that animals remained below the surface layer from May to September but were found in the surface layer from October to April (Fig. 1.8c). The seasonal pattern in relative abundance in the surface layer was negatively correlated with salinity, indicating a preference for the lowest salinities (Table 1.2). Similar to the other two species, the annual mean (±SD) proportion of *O. labradoriensis* in the upper 100 m was 75 ± 21 %.

1.3.4. *Appendicularian niche defined by temperature and salinity*

The above temporal and spatial differences in abundance of these three species of appendicularians result in clear differences in t-s preferenda, particularly between the oikopleurid congeners (Fig. 1.9). The optimum t-s range of *O. vanhoeffeni* defined as the range where greater than mean abundance occurred was narrow, from -1.3 to 4.6 °C and 32.3 - 32.7 psu (Fig. 1.9a), indicating a stenothermal (cryophilic) and stenohaline species. *F. borealis* displayed characteristics of a eurythermal and euryhaline species, with a wide optimum t-s range of -1.3 to 12.4 °C and 31.1 - 33.0 psu (Fig. 1.9b). *O. labradoriensis* was a mesothermal and mesohaline species, with a t-s range intermediate between those of *O. vanhoeffeni* and *F. borealis*, from 1.8 - 9.9 °C and 31.1 - 32.1 psu (Fig. 1.9c). The niche separation of *O. vanhoeffeni* and *O. labradoriensis* was best defined by salinity, as

the optimum range of the two species did not overlap and was sharply and clearly defined for each (Fig. 1.9a, c).

1.4. Discussion

Taxonomic differentiation between *O. vanhoeffeni* and *O. labradoriensis* required the use of three morphological characters. In large specimens, differences in the patterns of inclusion bodies on the house rudiments, patterns of subchordal cells on the tails, and the position of endostyle relative to that of the ventral epithelium were all easily determined. However, inclusion bodies and subchordal cells often could not be used as good taxonomic characters because they were not clearly visible in small juveniles, possibly as a result of ontogenetic variation in their rate of development. In addition, frequent absence of house rudiments and tissue damage in tails often precluded differentiation of species based on these conventionally used characters. The new morphological character found in this study, the position of the endostyle relative to the ventral epithelial tissue, proved to be diagnostic for all body sizes of the two species in all samples collected. The use of this morphological difference has not been reported before, and further observation of specimens from other boreal and Arctic regions is therefore required to document within and between species variability in this character.

The seasonal pattern and range of environmental characteristics in Conception Bay observed in this study are similar to those observed from 1996 to 2000 (Stead and Thompson 2003, Richoux et al. 2004). Previous measures of temperature, salinity and chlorophyll *a* concentration range from -1.5 to 15.9 °C, 32.0 to 34.0 psu and 0.14 to 5.3 $\mu g\ l^{-1}$, respectively. A distinct thermocline occurs in the summer whereas temperature below 150 m remains < 0 °C throughout the entire year. However, the persistent halocline present during summer and autumn in the upper 75 m is not described in previous studies. The annual minimum of surface salinity in October is thought to be a lagged signal of spring ice meltwater from Baffin Bay and Hudson Strait that is advected into Conception Bay via the Labrador Current (Myers et al. 1990, deYoung and Sanderson 1995). In general, the spring phytoplankton bloom begins in March and peaks

in May, when temperatures are still below zero (Deibel et al. 1992, Stead and Thompson 2003, Richoux et al. 2004 and data in 2002 in Chapter 2 of this thesis). Timing of the spring bloom is associated with a decrease in wind velocity that coincides with sufficient light intensity and inorganic nutrients (Deibel et al. 1992, Stead and Thompson 2003). The occurrence of a smaller, secondary phytoplankton bloom in summer (July-August) has been reported in previous years (Stead and Thomspon 2003, Richoux et al. 2004), however, no explanation for the mechanism behind the secondary bloom has been provided. In general, seasonal variability of the above conditions indicates that Conception Bay is a cold boreal system with Arctic influence.

Total abundance of appendicularians in Conception Bay peaked following the spring phytoplankton bloom, which is consistent with previous observations for Norwegian fjords and the Cantabrian Sea (Båmstedt et al. 2005, Lopéz-Urrutia et al. 2005). Thus, it seems that the total abundance of appendicularians is generally related to the phytoplankton biomass. The population response of appendicularians in Conception Bay was lagged by at least a month behind the peak of the spring phytoplankton bloom, which occurred in May when the temperature of the entire water column was 0 to -1 °C. This time lag is much longer than is typical in tropical waters, where appendicularian populations may respond to food pulses within days (Hoover et al. 2006). This latitudinal difference in the time lag between a pulse in food and a population response is likely a result of temperature effects on the rate of appendicularian development. For example, *O. vanhoeffeni* hatches 2 days after fertilization at 0 °C (laboratory observations, Appendix 1), whereas the tropical and temperate species *Oikopleura dioica* hatches 3 hours after fertilization at 22 °C (Fenaux 1976).

Temporal variation in the abundance of temperate and tropical appendicularian species is correlated with temperature and salinity. Fenaux (1961, 1963) documented species succession of appendicularians throughout the year in Mediterranean waters and attributed the seasonal pattern to differing temperature optima. Shiga (1985) showed that the seasonal occurrence of appendicularian species in Volcano Bay, Japan, coincided with intrusion of different water masses that could be defined by differences in

temperature and salinity. Lopéz-Urrutia et al. (2005) concluded that the seasonal occurrence of appendicularian species in four European coastal environments is regulated primarily by temperature and secondarily by salinity. My results also indicate significant relationships between physical factors and abundance of appendicularian species in Conception Bay. *F. borealis* increased during summer at depths where temperature was high and salinity was low and *O. labradoriensis* increased during autumn at depths where salinity was lowest. However, *O. vanhoeffeni* was most abundant during spring at depths where chlorophyll *a* concentration was highest, suggesting that physical parameters alone do not adequately explain the temporal distribution of appendicularian species.

Vertical distribution of oikopleurid species in Conception Bay was significantly correlated with temperature and salinity. Several studies from temperate regions have suggested that appendicularian species are vertically distributed according to temperature optima. In Volcano Bay, Japan, during summer, warm water species are distributed in the upper water column and cryophilic species are confined to the deeper, colder layer (Shiga 1985). In the Cantabrian Sea, cryophilic species are found in the colder, deeper layer during summer (Acuña 1994). Similarly in this study, the cryophilic *O. vanhoeffeni* was most abundant in deeper, colder layers during summer. However, in Conception Bay the vertical distribution of *O. labradoriensis* appeared to be strongly regulated by salinity, particularly during the annual salinity minimum in late summer and early fall. The importance of salinity as a determinant of the vertical distribution of appendicularians has also been reported in the brackish Bornholm Basin of the Baltic Sea, where tunicates were confined below the halocline (Schulz and Hirche 2007).

A few studies have shown that the vertical distribution of appendicularian species is homogeneous when the water column is vertically mixed during winter (Fenaux 1968, Shiga 1985). This pattern was not observed in Conception Bay, in that each species displayed different depth distributions in April and May when the water column was isothermal and almost isohaline. During this time, *O. vanhoeffeni* was more concentrated in the surface layer whereas *F. borealis* and *O. labradoriensis* were distributed at greater depths. The explanation for this pattern is not clear, but it is certain that the vertical

distribution of appendicularian species in Conception Bay is not a function of seasonal mixing and stratification of the water column.

In general, a large proportion (i.e. > 70 %) of the population of all three appendicularian species occurs within the upper 100 m over the year, perhaps as a result of higher concentrations of phytoplanktonic food above this depth. Tomita et al. (2003) observed a similar pattern in Toyama Bay, Japan, where most appendicularians were found in the upper 50 m throughout the year, where the chlorophyll *a* maximum consistently occurred and stated that vertical aggregation of appendicularians in the upper layer was due in part to higher food concentration.

Temperature-salinity diagrams clearly indicate species-specific spatial and temporal niches of appendicularians in Conception Bay (Fig. 1.9). The fact that *O. vanhoeffeni* is cryophilic and stenothermic, *O. labradoriensis* mesothermic and *F. borealis* eurythermic in Newfoundland coastal waters supports previous hypotheses about thermal niche of appendicularian species in southern Labrador and the Grand Banks (Frost et al. 1933, Udvardy 1954). These three species have similar relative thermal niches in temperate and boreal European coastal waters (Lopéz-Urrutia et al. 2005). In boreal Pacific waters, *F. borealis* displays a wider range of temperature and salinity preferenda than does *O. labradoriensis* (Shiga 1985), similar to our results from Newfoundland. Thus, the niche breadth of these three appendicularian species is becoming better defined on a global scale.

Niche separation of species in Conception Bay and around the world suggests that the distribution of appendicularians may be sensitive to climate forcing. Variation in surface temperature and salinity in Greenland, the Labrador Sea, and Newfoundland coastal waters is related to interdecadal cycles in the strength of the North Atlantic Oscillation as well as to global warming in general (Myers et al. 1988, Drinkwater 1995, Colbourne et al. 1997, Houghton and Visbeck 2002, Chylek and Lohmann 2005). Such changes in temperature and salinity in boreal and Arctic waters may affect the relative abundance of these three appendicularian species. With arctic temperature and rainfall both predicted to increase over the next century, the abundance of the stenothermic and

stenohaline *O. vanhoeffeni* may decrease, while that of *F. borealis* and *O. labradoriensis* may be expected to increase. In addition, recent surface warming is enhancing stratification and suppressing nutrient exchange through vertical mixing (Behrenfeld et al. 2006). Consequently, reduced availability of nutrients is leading to a decrease in the primary production and phytoplankton standing stocks in the ocean's upper layer (Behrenfeld et al. 2006). This trend may negatively affect the abundance of *O. vanhoeffeni*, in particular, because this species responds positively to an increase in phytoplankton biomass.

Thus, temporal and vertical distributions of boreal appendicularian species are related to temperature, salinity, and chlorophyll concentration, indicating that these factors are important to the population dynamics of each species. Niche separation of appendicularian species in temperature-salinity space suggests that physiological tolerance to temperature and salinity levels may be important in predicting recruitment and survivorship of each species. In-depth understanding of interspecific variation in appendicularian abundance requires further study of how these physical and biological variables affect demographic parameters such as survival, reproduction and growth.

Table 1.1. Spearman rank correlation coefficients between abundances of appendicularian species (ind. m^{-3}) and environmental variables. N = 27, p-values are in parentheses. * $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$.

	Temperature	Salinity	Chlorophyll *a*
Oikopleura vanhoeffeni	-0.27	0.01	0.37*
	(0.18)	(0.95)	(0.06)
Fritillaria borealis	0.52***	-0.45**	0.01
	(0.005)	(0.02)	(0.95)
Oikopleura labradoriensis	0.12	-0.37*	0.30
	(0.57)	(0.06)	(0.13)

Table 1.2. Correlation coefficients between percent total abundances of appendicularian species in the surface layer (0-30 m) and environmental variables. N = 9, p-values are in parentheses. * $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$.

	Temperature	Salinity	Chlorophyll *a*
Oikopleura vanhoeffeni	-0.66**	-0.08	0.56
	(0.05)	(0.84)	(0.12)
Fritillaria borealis	0.18	-0.54	-0.50
	(0.64)	(0.13)	(0.17)
Oikopleura labradoriensis	-0.10	-0.66**	0.33
	(0.79)	(0.05)	(0.38)

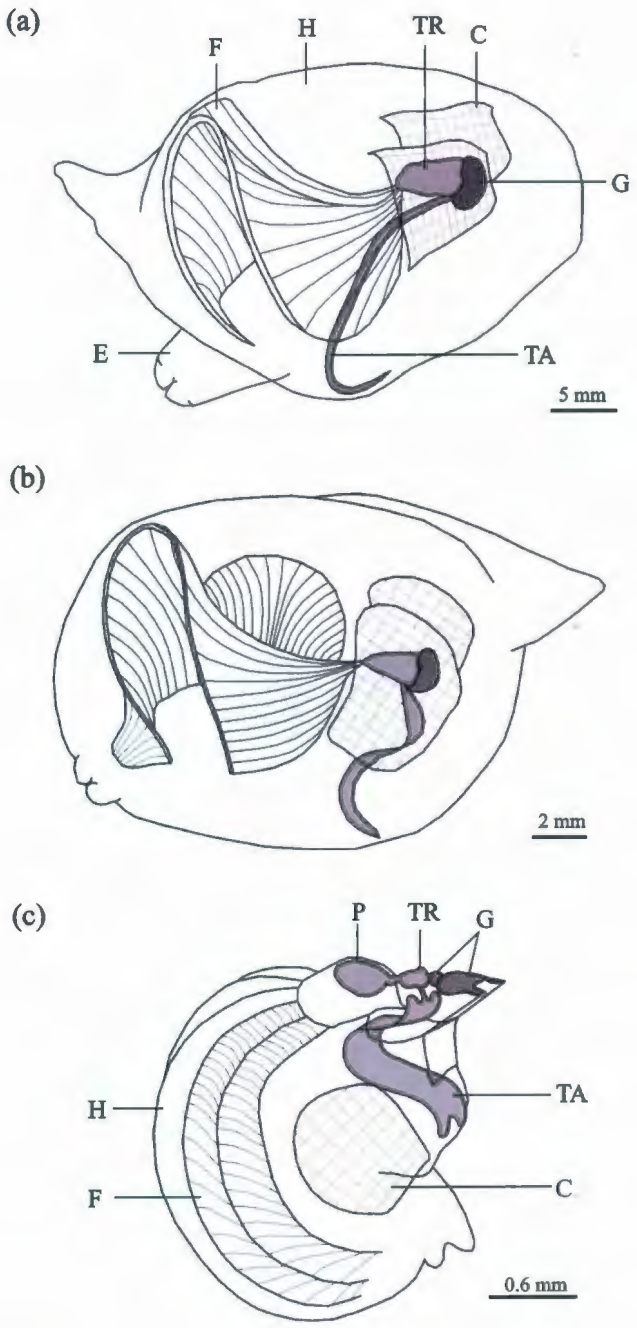


Fig. 1.1. Illustrations of three appendicularian species in Conception Bay. (A) *Oikopleura vanhoeffeni*, (B) *Oikopleura labradoriensis*, (C) *Fritillaria borealis*. Drawings are modified from photographs by P.R. Flood and figure by Flood (2003). C, coarse filter; E, exit spout; F, fine filter; G, gonad; H, house; P, pharynx; TA, tail; TR, trunk.

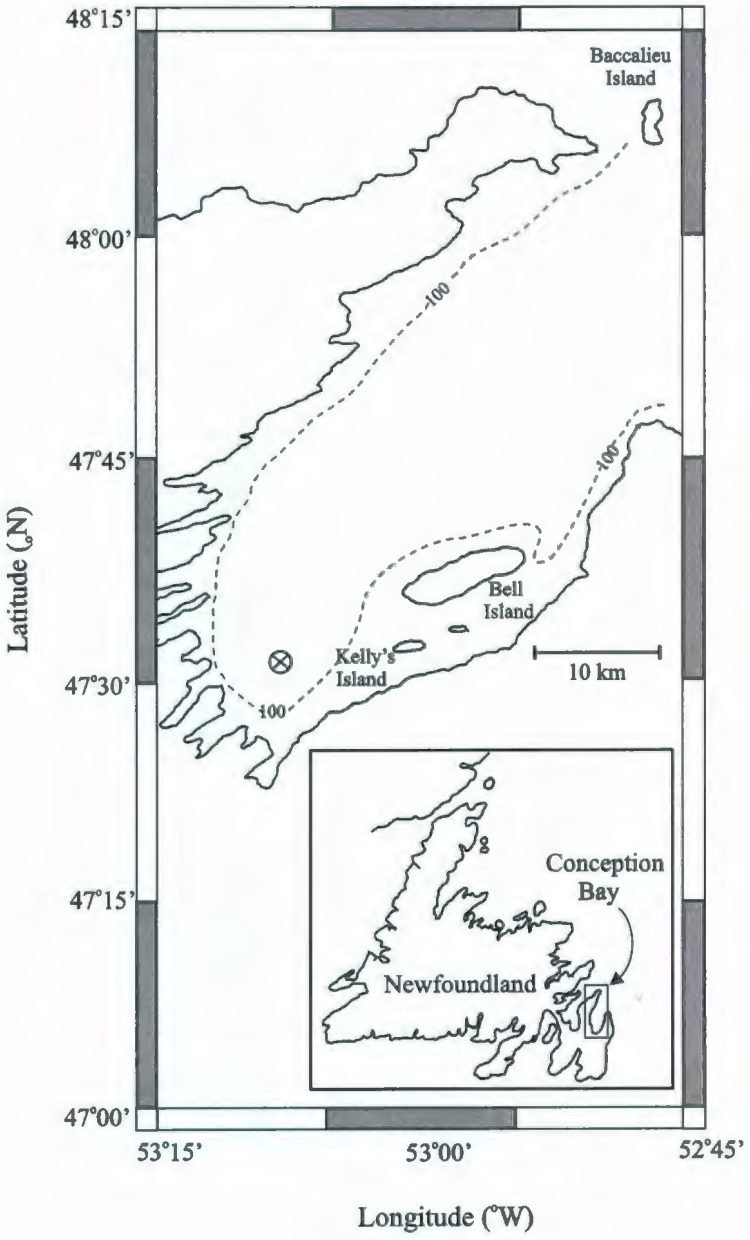


Fig. 1.2 Map of Conception Bay, Newfoundland. Sampling site is indicated as 'X.' The dotted line indicates the 100 m isobath.

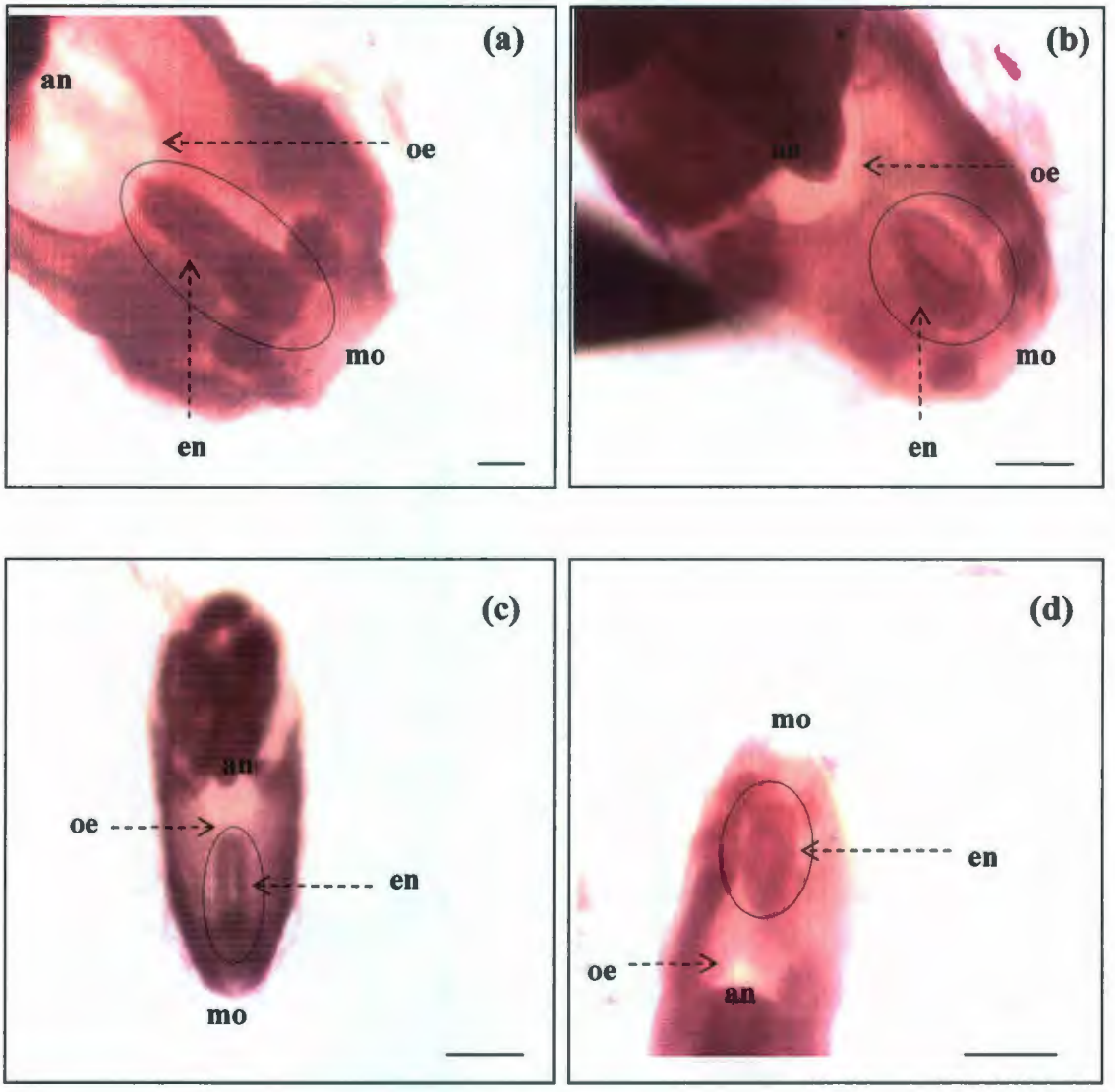


Fig.1.3. Morphology of the endostyles of *Oikopleura vanhoeffeni* (a, c) and *Oikopleura labradoriensis* (b, d) in ventral view.

Note that the endostyle of adult (a) and juvenile (c) *O. vanhoeffeni* extends posteriorly to the end of the oikoplastic epithelium, while that of adult (b) and juvenile (d) *O. labradoriensis* is located in center of the oikoplastic epithelium. 'an' = anus, 'en' = endostyle, 'oe' = oikoplastic epithelium, 'mo'= mouth. Scale bar = 100 μm.

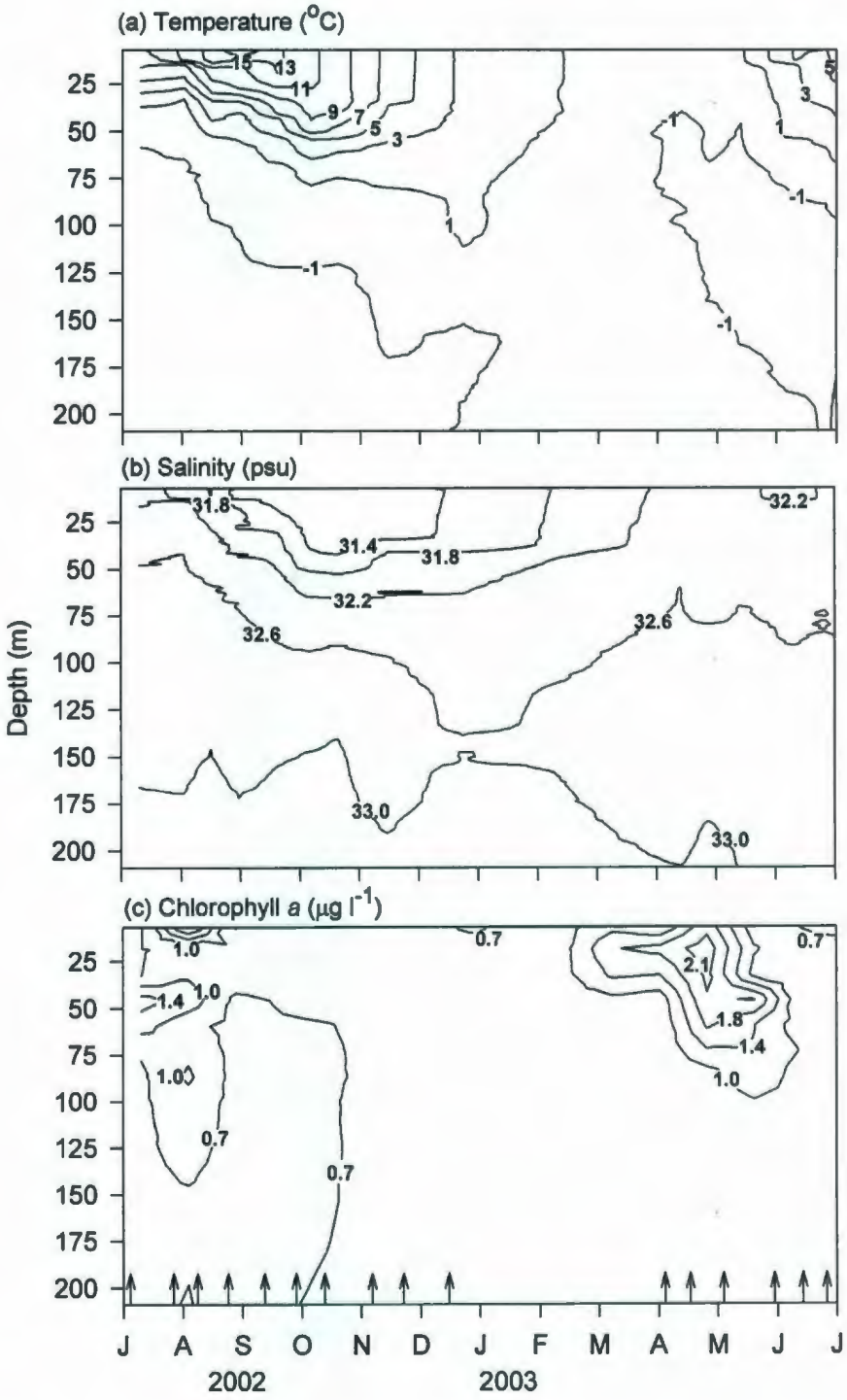


Fig. 1.4. Vertical profiles of (a) temperature, (b) salinity and (c) chlorophyll *a* concentration in Conception Bay from July 2002 to June 2003. Arrows indicate individual CTD casts.

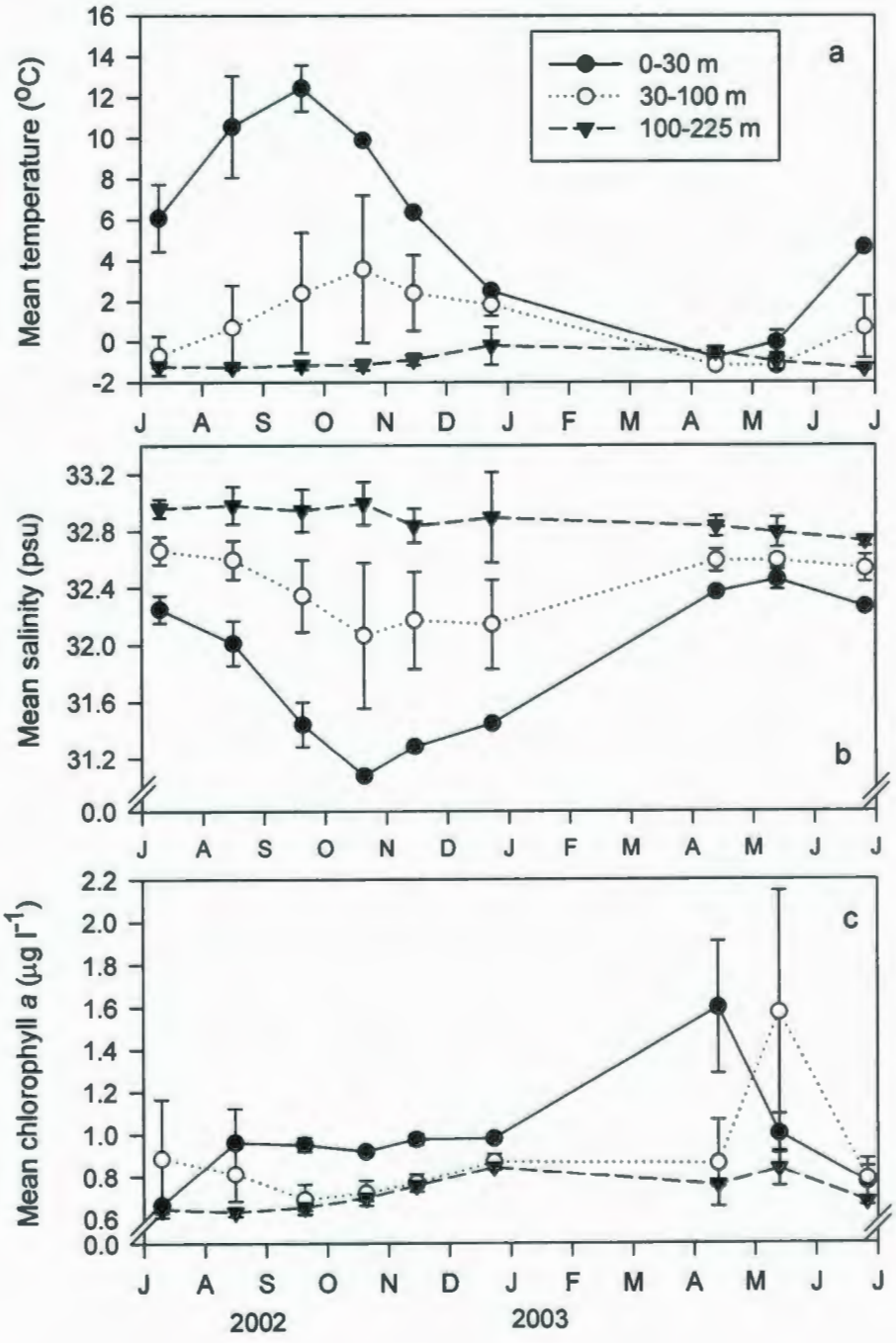


Fig. 1.5. Mean temperature (a), salinity (b), and chlorophyll *a* concentration (c) of Conception Bay over three depth strata from July 2002 to June 2003. Error bars represent standard deviation of the mean.

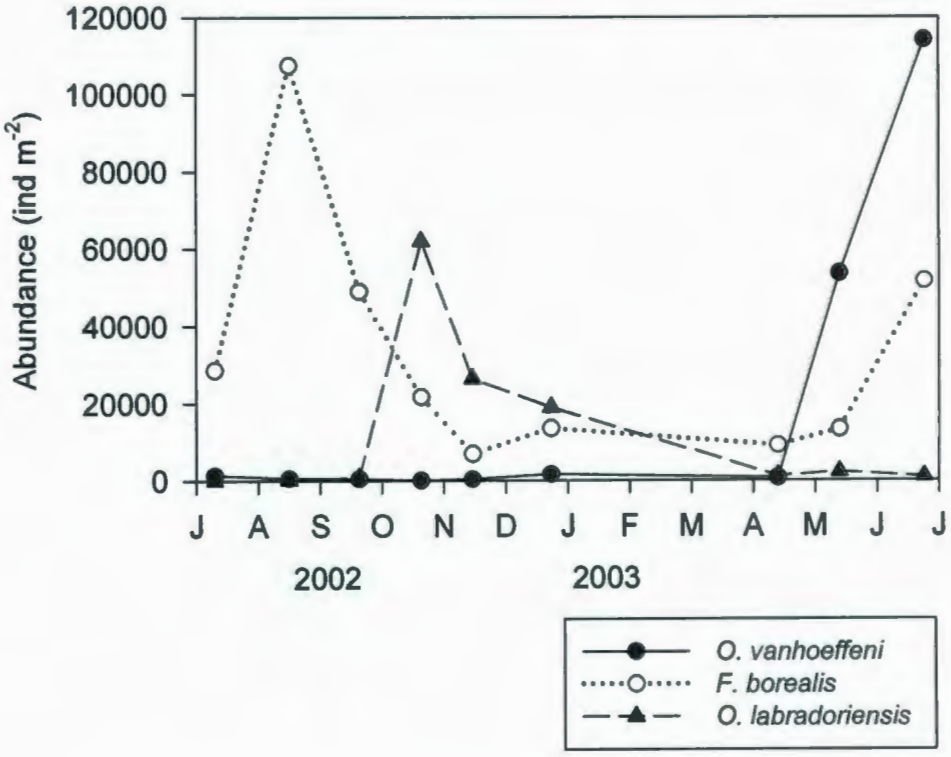


Fig. 1.6. Total abundance integrated over the water column of *Oikopleura vanhoeffeni*, *Fritillaria borealis,* and *Oikopleura labradoriensis* in Conception Bay, Newfoundland from July 2002 to June 2003.

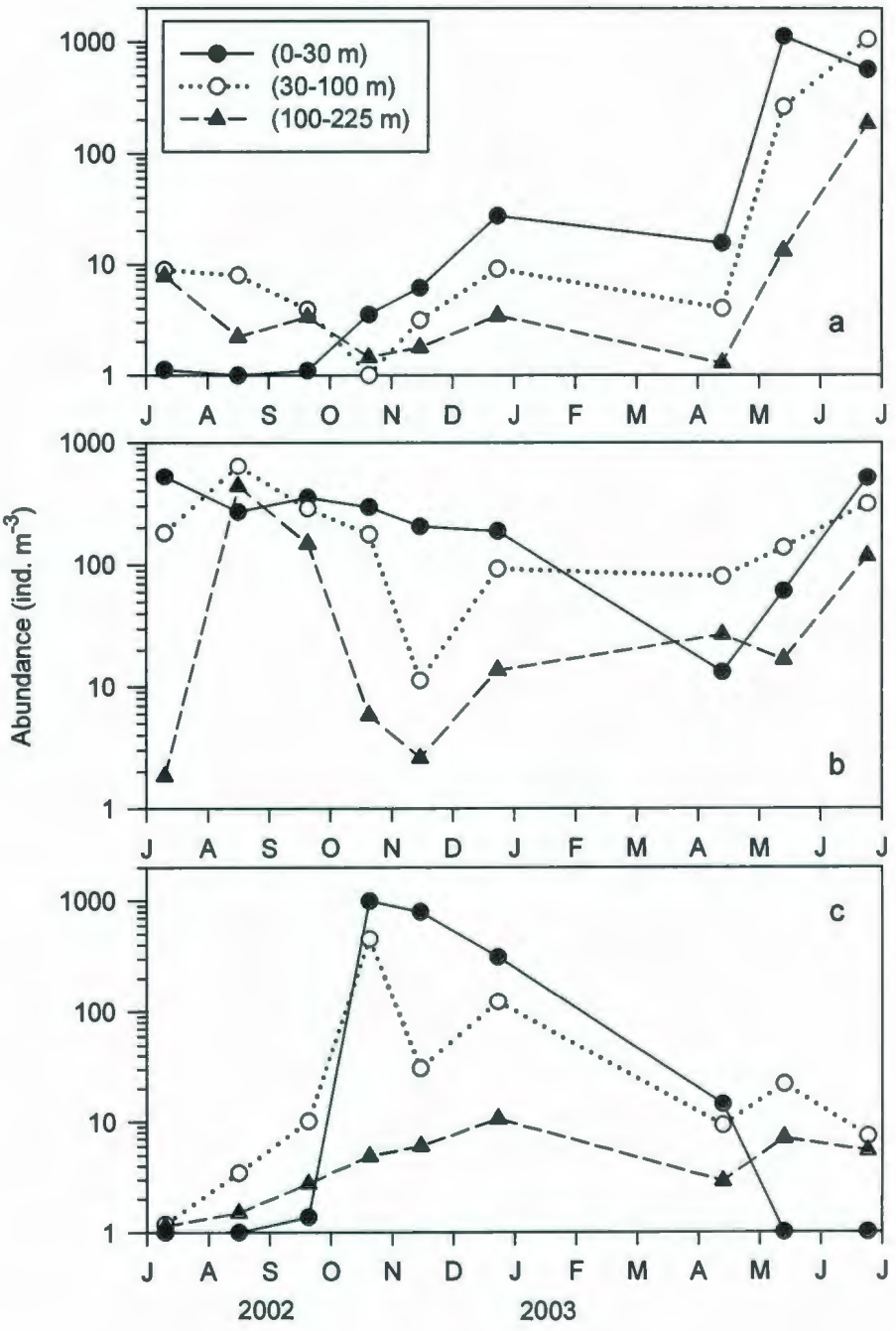


Fig. 1.7. Time series of volumetric abundance (m^3) in three depth strata from July 2002 to June 2003. (a) *Oikopleura vanhoeffeni*, (b) *Fritillaria borealis*, (c) *Oikopleura labradoriensis*.

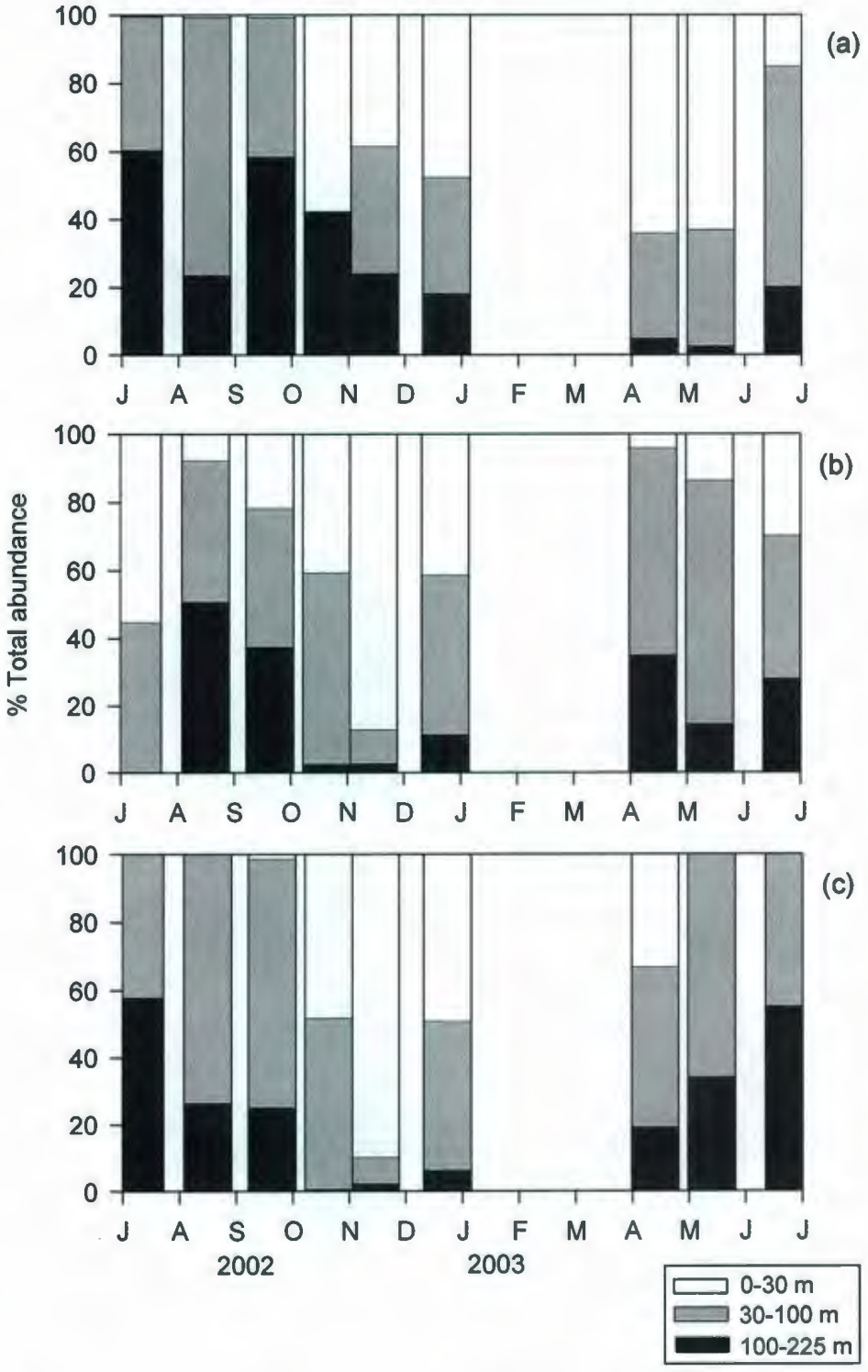


Fig. 1.8. Time series of relative abundance as a % total abundance of three appendicularian species in three depth strata of Conception Bay from July 2002 to June 2003.
(a) *Oikopleura vanhoeffeni*, (b) *Fritillaria borealis* and (c) *Oikopleura labradoriensis*.

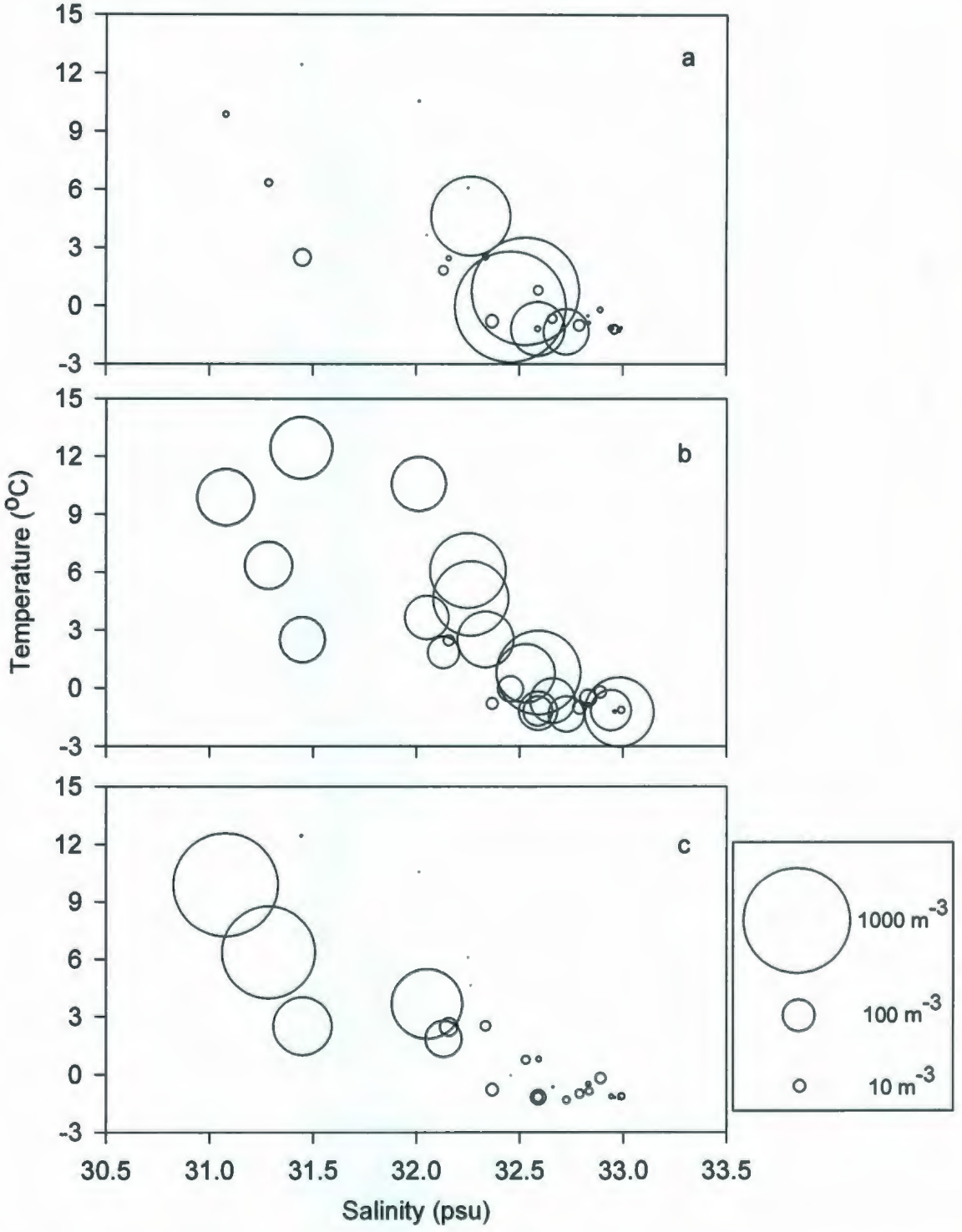


Fig. 1.9. Abundance of three appendicularian species in Conception Bay vs. temperature and salinity. (a) *Oikopleura vanhoeffeni*, (b) *Fritillaria borealis*, (c) *Oikopleura labradoriensis*.

Chapter 2

Statolith diameter as an age indicator in the appendicularian tunicate *Oikopleura vanhoeffeni*

2.1. Introduction

Age determination is essential for the study of population dynamics, which requires demographic information regarding age-specific fecundity, age-specific mortality and generation time (Stearns 1992, Vandermeer and Goldberg 2003). For many marine invertebrates that lack discrete life-history stages and have continuous growth, age is conventionally estimated based on body size. However, body size can be a poor indicator of age because size-at-age may vary depending on the rate of growth, which can be regulated by temperature or food availability. For example, under severe food limitation, soft-bodied animals such as gelatinous zooplankton may experience degrowth as a result of using internal energy reserves and the digestion of somatic tissue to maintain metabolism (Hamner and Jensen 1974, Kremer 1976, Deason and Smayda 1982). For these reasons, various chemical and physical characters have been used for age estimation, including lipofuscin concentration in neural tissue, and age increments or dimensions of permanently calcified structures such as otoliths and statoliths.

Lipofuscin is a yellow-brown, autofluorescent material that accumulates over time in lysosomes of postmitotic cells such as neurons and cardiac myocytes in various vertebrates and invertebrates (reviews in Porta 1991, Yin 1996, Terman and Brunk 1998). This age pigment forms due to peroxidation of unsaturated lipids by oxygen-free radicals and polymerization of oxidized lipid compounds with protein residues (Donato 1981, Yin 1996, Terman and Brunk 2004). Lipofucinogenesis involves a series of chain reactions. Hydrogen peroxide, a byproduct of normal oxygen metabolism in the electron transport chain of mitochondria, partially diffuses through lysosomal membranes. Intra- and extracellular materials enter lysosomes through autophagy and hetrophagy and the materials are decomposed into molecules such as amino acids, fatty acids and simple sugars (Dice 2000). In the lysosome, degradation of iron-containing metalloprotein produces ferrous iron, which readily reacts with hydrogen peroxide resulting in formation

of extremely reactive oxygen derived hydroxyl radicals (Brunk et al. 1992). When the hydroxyl radicals attack unsaturated lipids, aldehydes are formed which react with amino groups within protein residues (Chio and Tappel 1969, Dillard and Tappel 1971). The end product of cross-linked aldehydes and protein residues is lipofuscin, which cannot be degraded by lysosomal hydrolases, nor exocytosed, and thus accumulates with age in lysosomes within post-mitotic cells (Brunk and Terman 2002, Terman and Brunk 2004).

Histochemical characteristics of lipofuscin include resistance to polar and non-polar solvent extraction, basophilia, osmiophilia, and stainability by periodic acid-Schiff and Sudan black B (Brunk and Ericsson 1972, Sohal 1981, Porta 1991). One of the most distinct characteristics of lipofuscin is yellow-orange to off-white autofluorescence produced when it is excited with blue or UV light (Porta 1991, Yin 1996, Bluhm 2001). The cumulative quantity of autofluorescence in post-mitotic cells has been used for determination of age in many organisms for which other methods of ageing are lacking or are in some respect unsatisfactory. Lipofuscin concentration has been particularly useful for ageing aquatic crustaceans that lack permanent calcified structures with age marks (Ettershank 1984, Sheehy and Wickins 1994, Ju et al. 1999, Bluhm and Brey 2001). Lipofuscin concentration has been determined in two ways. The first method involves analysis of pigment granules in histological sections using epifluorescent microscopy (O'Donovan and Tully 1996, Sheehy et al. 1996, Wahle et al. 1996, Belchier et al. 1998). The second method involves pigment extraction and measurement of bulk fluorescence by spectrophotometric analysis (Nicol 1987, Nicol et al. 1991, Ju et al. 1999). Using these methods, modal analysis of frequency distributions of lipofuscin concentration may produce additional age-classes not apparent in conventional size-frequency distributions for some organisms (Sheehy et al. 1998, Bluhm and Brey 2001). However, efficiency of extraction is variable, as Nicol (1987) found that pre-fixation in formaldehyde resulted in increased lipofuscin concentrations in copepods, euphausiids, and squid. Sheehy (1996) also noted that soluble autofluorescence intensity in the brain of freshwater crayfish did not represent lipofuscin alone but represented a mixture of unknown pigments and did

not match the concentration of lipofuscin determined by microscopy (Eldred and Katz 1989, Sheehy 1996).

Lipofuscin concentration may be affected by factors other than age. Tully et al. (2000) demonstrated that lipofuscin accumulation in the European lobster *Homarus gammarus* oscillated with the simulated seasonal temperature cycle and thus seemed to be affected by metabolic rate. Based on this result, the authors suggested that geographical and long-term temporal differences in temperature (such as degree days available for growth) need to be considered when converting physiological age indices, obtained from lipofuscin, to a chronological scale. Castro et al. (2002) reported that dietary antioxidants significantly reduce lipofuscin concentration in the shrimp *Panaeus japonicus* and that age estimation using lipofuscin may be biased when wild populations from diverse environments are compared. For all of the above reasons, the use and interpretation of lipofuscin concentration as an age proxy are difficult.

Daily and annual incremental rings that form in the otoliths, statoliths, and shells of fish, cephalopods, gastropods, and bivalves are commonly used for age and growth rate estimates (Sire and Bonnet 1984, Campana and Neilson 1985, Lipinski 1993, Sejr et al. 2002, Barroso et al. 2005). Incremental growth of such structures occurs through differential deposition of calcium carbonate on a proteinaceous matrix in the form of calcite or aragonite (Dunkelberger et al. 1980, Young 1992, Lipinski 1993), depending on environmental and physiological variables such as photoperiod, temperature, feeding, growth or an endogenous circadian rhythm (Simkiss 1974, Mugiya et al. 1981, Campana and Neilson 1985, Jackson 1994). Enumeration of age rings can be achieved using light or scanning electron microscopes, depending on the size of the structure, and often requires sectioning, polishing, and acid-etching in order to aid in clear visualization of surface ultrastructure.

Dimensions of calcified structures have been found to increase with age, particularly in fish. Use of otolith dimensions may provide a time and cost saving alternative to conventional counting of age rings for some organisms (McDougall 2004). Otolith length, width and weight are strongly related to age in several fish species

(Fujiwara and Hankin 1988, Secor and Dean 1989, Reznick et al. 1989, Kristoffersen and Klemetsen 1991, Newman et al. 1996, 2000, Newman 2002, McDougall 2004). Importantly, otolith size increases in fish at a constant rate even when food is limiting and somatic growth rate decreases (Templeman and Squires 1956, Mosegaard et al. 1988, Reznick et al. 1989, Secor and Dean 1989, Secor et al. 1989, Campana 1990, Pawson 1990, Cardinale et al. 2000). Finally, it has been shown in several fish species that the use of otolith weight-age relationships results in estimated age-frequency distributions that are not significantly different from those determined from otolith increment counts (Piling et al. 2003, McDougall 2004).

The objective of this study was to explore a method for age determination of appendicularian, *Oikopleura vanhoeffeni*, a pelagic tunicate commonly found in high concentrations in arctic and boreal waters (Udvardy 1954, Shiga 1993, Ashjian 1997), where it is often a dominant suspension feeder (Deibel 1988, Acuña et al. 2002). Appendicularians often contribute substantially to biogenic carbon fluxes in the form of discarded mucous-filter houses and fecal pellets (Bauerfeind et al. 1997, Alldredge 2005, Dagg and Brown 2005). Although the distribution and abundance of this species is known, population dynamics have not been studied. To better understand the population dynamics of this species, demographic parameters need to be determined, in which age determination is a crucial factor.

The development of method for age determination of appendicularians is in an early stage. One method has been developed using the number of endostyle cells as an index of age in appendicularian *Oikopleura dioica* (Troedsson et al. 2007). The hypothesis of this study is based on the developmental time model which uses the discrete cell cycle steps as a nondimensional biological clock for marine invertebrates (Aksnes et al. 2000a, b). In *O. dioica*, the number of endostyle cells increases with age, however the rate of increase in the cell number is dependent on temperature but independent from food concentration (Troedsson et al. 2007). Thus, age of *O. dioica* can be predicted from the number of endostyle cells and ambient temperature (Troedsson et al. 2007). The technique for enumeration of endostyle cells involes staining the

polyploid endostyle nuclei and counting the nuclei with a confocal microscope. Use of this method for analyzing the age structure of field populations has not been tested.

In this study, I explored the use of lipofuscin in brain tissue, incremental rings on the statolith, and statolith diameter for age determination of the appendicularian, *Oikopleura vanhoeffeni*. The results of this study suggest that statolith diameter can be used as an age indicator in appendicularians. Using statolith diameter, length-at-age of *O. vanhoeffeni* in Conception Bay, Newfoundland, was examined over two years in relation to variation in food availability, in order to test whether body size is a reliable indicator of age in field populations.

2.2. Materials and Methods

Sensory organs of *Oikopleura vanhoeffeni* are located in the anterior-dorsal region of the trunk and consist of the brain and a statocyte that contains the statolith (Fig. 2.1a, b).

2.2.1. *Lipofuscin analysis*

O. vanhoeffeni were collected from Logy Bay, Newfoundland, by SCUBA divers and preserved in 4 % buffered formaldehyde at the Ocean Sciences Centre (MUN) laboratory. Trunks of the animals were dehydrated in an ascending ethanol series, cleared in xylene, and embedded in paraffin. Embedded material was serially sectioned at 6-μm intervals. Sections were mounted on a glass slide, dewaxed with xylene, and covered with a non-fluorescent mounting medium (Gel Mount aqueous mounting medium, Sigma). For identification of lipofuscin, unstained sections of the brain were examined for characteristic yellow or off-white, granular autofluorescence under 450-490 nm (blue) excitation wavelength combined with > 515 nm emission wavelength and under 330-380 nm (UV) excitation combined with > 420 nm emission using a Nikon Eclipse E600 epifluorescence microscope.

2.2.2. *Statolith analysis*

Animals were preserved in 95 % ethanol, cleared in 1 % KOH, and then mounted in glycerol on glass slides to visualize the statolith. Statolith diameter was measured to the nearest 0.5 μm under a Zeiss Axiovert 35 microscope at 1000X magnification.

To view the statolith using transmission electron microscopy, *O. vanhoeffeni* were fixed for 1 hr in Karnovsky's fixative (5% glutaraldehyde, 4% paraformaldehyde, buffered with 0.2 M sodium cacodylate buffer, pH 7.4), followed by post-fixation for 30 min in 1 % osmium tetroxide buffered in 0.2 M sodium cacodylate, pH 7.4. The animals were serially dehydrated in an ethanol series followed by acetone and embedded in epoxy resin. Semi-thin (1 μm) sections were cut from the tip of the dorsal region of the mouth to the tip of the brain with glass knives and ultra-thin (0.5 μm) sections were cut through the entire brain region with a diamond knife (LKB Ultratome). Sections were placed on formvar coated grids, stained with uranyl acetate and lead citrate for examination with a Zeiss EM 109 transmission electron microscope.

For observation of the statolith using scanning electron microscopy, *O. vanhoeffeni* were preserved in 95 % ethanol and 2 % buffered glutaraldehyde. In order to view the entire statolith it was necessary to separate it from the statocyte. Manual dissection was difficult because of their minute size (ca. 8 to 18 μm), thus an indirect approach was taken by dissolving the brain and statocyte in sodium hypochlorite, leaving the statolith intact. Statoliths were rinsed with distilled water and stained with alizarin red to aid visualization. They were then collected by filtering onto cellulose acetate membrane filters, which were mounted on an SEM stub and sputter-coated with gold. The samples were viewed at 1500 to 4000 X power with a Hitachi 5570 scanning electron microscope.

2.2.3. *Growth of the statolith and trunk in the laboratory*

Fully mature *O. vanhoeffeni* were collected from Logy Bay, Newfoundland from April to the middle of June 2001. They contained an orange mass of sperm in the sperm sac and many oocytes packed in the ovary. Each individual was kept suspended in a

glass tank containing 20 L of seawater pre-filtered through 40 μm mesh. The tank was maintained at 0-1°C during the experiment, and was equipped with a clear, twisted plexiglass paddle mounted vertically, which was connected to a 12 V automobile windshield wiper motor (Volkswagen Motors AG, J.-L. Acuña, pers. comm.). A rheostat was connected to the motor to regulate the speed of rotation. This setup created a gentle, rotating current of water which kept the animals in suspension (Fenaux & Gorsky 1985, Fig. 2.2). Six mature individuals in the tanks spawned within one or two days of collection. The eggs hatched ca. two days after self-fertilization, and the new generation of animals inflated the first houses ca. six days after fertilization (Appendix 1). Animals within houses were moved to new seawater prefiltered through 40-μm mesh, but supplemented with 2×10^6 cells l^{-1} of *Isochrysis* sp. and 2×10^6 cells l^{-1} of *Thalassiosira pseudonana*. The animals were moved to new seawater with fresh food every two days with a wide-bore pipette. The size of the pipette used for transfer of the animals varied depending on the size of houses to prevent damage of the house which causes the animals to be trapped in the house. The animals outside the houses were not transferred because trunks and house rudiments were easily damaged during transfer, preventing the animals to successfully build new houses. When a large pipette was used, the bottom end of the pipette was shut tightly with a plastic cap attached with a handle extension after gently suctioning the animal in a pipette, to avoid spilling the content during the transfer. Individuals from six families were removed from the experiment randomly at intervals from 10 - 60 days after hatching and were preserved in 95 % ethanol. The number of individuals collected per family at each sample time ranged from 8 to 77 (Appendix 2). It was not possible to sample each family with a high and equal frequency because mortality was high and variable in each family throughout the rearing process. Trunk lengths, excluding the gonad, were measured to the nearest 25 μm under a Zeiss stereomicroscope at 40X magnification. After the measurements were made, animals were cleared in 1 % KOH solution and mounted in glycerol on a glass slide. Statolith diameter was measured as described above. In order to determine the growth pattern of statolith diameter and trunk length, linear or non-linear regressions were fit to the data.

2.2.4. *Field determination of body size as a function of statolith diameter (i.e. trunk length-at-statolith diameter, a proxy for length-at-age)*

Individuals of *O. vanhoeffeni* were collected from June 11, 2001 to June 25, 2003 at the same site in Conception Bay as described in Chapter 1. Vertical hauls were made from near the bottom (ca. 225 m) to the surface using a ring net with a mesh size of 110 μm. The speed of retrieval of the net was ca. 0.12 m sec^{-1}. Upon retrieval of the net, the entire sample was immediately preserved in 95 % ethanol. The frequency of sampling was bimonthly except during winter, when harsh weather conditions precluded sampling. On each sampling day, *in situ* relative fluorescence was measured with a Seabird SBE 25-01 CTD equipped with a SEATEC fluorometer. Relative fluorescence units (RFU) were converted to chlorophyll *a* concentration (μg Chl *a* l^{-1}) using the equation Chl *a* = 0.398 x RFU + 0.281 (r^2 = 0.72, n = 244), which was developed using historical data from Conception Bay (Cold Ocean Productivity Experiment, unpublished). Temperature and chlorophyll *a* data were bin-averaged at 1 m depth intervals before statistical analysis and plotting.

Animals were rinsed with 95 % ethanol before measuring trunk length, gonad maturity and statolith diameter. Animals from the field samples were not rinsed with water since doing so lowered the pH of the sample and completely dissolved the statoliths. Trunk lengths were measured to the nearest 25 μm under a Zeiss stereomicroscope at 40 X magnification, followed by clearing, mounting and the measurement of statolith diameter as described above. Trunk length and statolith diameter were measured from the individuals collected from a single tow sample at each time point of collection.

To observe the temporal variation in length-at-age, mean trunk lengths were calculated at 1-μm increments of statolith diameter in the individuals sampled from June 2001 to June 2003. Mean trunk lengths at statolith diameter from 9 to 16 μm were analyzed with polynomial regression ($Y = a_0 + a_1 x + a_2 x^2 + a_3 x^3 \ldots$). The order of best-fit polynomial function to each data set was determined by fitting the sequential orders of polynomials until the sums of squares for error were explained significantly (Christensen

1996). Relationships between mean trunk length at each statolith diameter (hereafter referred to as 'length-at-age') and temperature and concentration of chlorophyll *a* were explored with a linear regression model. Mean temperature and chlorophyll *a* in the upper 100 m of the water column were used in this model, because > 70% of the animals were located within this depth stratum (Chapter 1). Trunk lengths were log-transformed when residuals were not homogeneous and normally distributed.

2.3. Results

2.3.1. *Lipofuscin*

Yellow or off-white fluorescent lipofuscin granules were not detected in the brain sections of *O. vanhoeffeni* under blue and UV excitation light (Fig. 2.3), indicating either the lack of production of lipofuscin or the production of very low levels. Therefore, lipofuscin concentration could not be used as an age proxy in this study.

2.3.2. *Statolith*

Viewed under transmitted light, the statolith revealed four conspicuous rings (Fig. 2.4a), however, daily rings could not be resolved under transmitted light. Thus, I explored higher magnification approaches, but found that statoliths needed to be either sectioned or polished and etched to view ultrastructural details under TEM and SEM. Unfortunately, sections of the statolith could not be obtained for TEM analysis. The statoliths may have popped out from the sections because of their hard consistency or the brain region was not sectioned at an appropriate angle to include the statolith. Controlling the target angle of sectioning was difficult because the statoliths were so small in size. Under SEM, statoliths appeared to be composed of discrete layers, which may have been deposited at a fixed time interval (Fig. 2.4b). Statoliths need to be polished to the core and etched before viewing under SEM in order to reveal the presence of age-related deposition. This could not be done because of handling difficulties associated with their minute size. For all of the above reasons, visualization of the statolith using EM was not a feasible approach for age determination of appendicularians.

2.3.3. *Relationships between statolith diameter, trunk length and age in laboratory-reared individuals*

Statolith diameter of *O. vanhoeffeni* increased linearly for over 60 days after hatching in the laboratory (Fig. 2.5a), suggesting that statolith diameter is linearly related to age. This relationship does not appear to be dependent on the genetic origin of individuals because the data in Fig. 2.5a come from different parents. Most importantly, the coefficient of variation of mean statolith diameter-at-age did not increase with an increase in age (Fig. 2.5b), meaning that statolith diameter is a robust age indicator for individuals of all ages.

Trunk length of the laboratory population increased linearly over time (Fig. 2.6a). However, the coefficient of variation of mean trunk length-at-age showed an increasing trend with an increase in age (Fig. 2.6b), suggesting that trunk length is a less precise indicator of the age of older individuals than of younger individuals. In addition, the overall coefficient of variation for mean statolith diameter (6-11 %) was significantly less than that for mean trunk length (8-27 %) (t-test, $t(20) = -7.08$, $p < 0.001$). Thus, statolith diameter is a more precise indicator of age for individuals of all ages than is body size, even in laboratory conditions where temperature and food concentration were controlled.

2.3.4. *Temperature and chlorophyll* a *in Conception Bay*

In Conception Bay, temperature fluctuated seasonally in the upper 10 m with an increase to a maximum of 15.4-16.6 °C in late August and a decrease to a minimum of -1.0 to -0.8 °C in late March to early April (Fig. 2.7a). A thermocline developed within the upper 60 m from June to October and retreated as winter mixing occurred to a depth of 100 to 150 m. Temperature below 150 m remained < 0 °C throughout the study. Seasonal variation in chlorophyll *a* concentration occurred mostly within the upper 100 m (Fig. 2.7b). The spring bloom began in March and peaked in May with a maximum chlorophyll *a* concentration of 5.8 $\mu g\ l^{-1}$ in 2002 and 3.5 $\mu g\ l^{-1}$ in 2003. A minor fall bloom occurred in August 2001 (2.4 $\mu g\ l^{-1}$) and a weaker bloom occurred in late July

2002 (1.7 μg l^{-1}). The occurrence of the fall bloom was more variable from year to year compared to the major spring bloom (Stead and Thompson 2003, Richoux et al. 2004). Minimum concentrations of chlorophyll *a* were found in July 2001 (0.89 μg l^{-1}) and in October 2002 (0.98 μg l^{-1}).

2.3.5. *Relationship between trunk length and statolith diameter in field populations*

Using statolith diameter as an age proxy, seasonal variation in length-at-age was apparent, particularly in older age groups represented by statolith diameters from 14-16 μm (Fig. 2.8), in which trunk length was < 1000 μm from summer to early winter (July to December) but > 1000 μm in late winter and spring (February to June). Maximum trunk length in this age group was greatest during spring (April and May), reaching a maximum size > 2000 μm.

To examine this seasonal variation in length-at-age in detail, mean trunk length was calculated at 1-μm increments of statolith diameter. Mean trunk lengths at statolith diameters from 9-16 μm were examined because these age groups were present year round. Polynomial regression analysis of best-fit orders on the temporal progression of mean trunk length over the year 2001 to 2003 revealed no clear seasonal variation in the mean trunk length at statolith diameters from 9 to 11 μm but a distinct seasonal variation at the statolith diameters from 12 to 15 μm with a maximum length-at-age in spring and a minimum in summer (Fig. 2.9, Table 2.1). This seasonal trend in the older individuals repeated in two consecutive years.

Temporal variability in trunk length-at-age, expressed as the coefficient of variation of mean trunk length at each statolith diameter (CV = standard deviation expressed as the percentage of grand mean trunk length over 2 years), fluctuated from 8-10 % at statolith diameters from 9-12 μm, but increased linearly up to 31 % at statolith diameters from 13-16 μm (Fig. 2.10), suggesting that temporal variability in length-at-age increased with age.

2.3.6. *Relationships between length-at-age and temperature and chlorophyll* a *in field populations*

Trunk length at statolith diameters from 9 - 13 μm did not vary significantly with temperature, whereas mean trunk length at statolith diameters from 14 - 16 μm increased significantly as temperature decreased (Fig. 2.11, Table 2.2). There was no relationship between mean trunk length at statolith diameters from 9-11 μm and the concentration of chlorophyll *a*, but mean trunk length at statolith diameters from 12-16 μm increased with an increase in chlorophyll *a* (Fig. 2.12, Table 2.3).

2.4. Discussion

Age determination of appendicularians was explored using four methods: body size (mean trunk length), lipofuscin concentration in brain tissue, age rings in statolith, and size of statolith. Lipofuscin was not detected in the brain sections of *O. vanhoeffeni*. Analytical procedures did not differ from those used in other studies for aquatic organisms. The wavelength range for excitation of lipofuscin (450-490 nm, blue) used in this study included the wavelength used in most other studies of lipofuscin in other organisms such as crustaceans and gastropods (O'Donovan and Tully 1996, Sheehy et al. 1998, Bluhm and Brey 2001). UV light, under which lipofuscin fluoresces in many organisms (Porta 1991, Terman and Brunk 1998), did not produce a positive result in the brain sections of *O. vanhoeffeni*. It is unlikely that the negative result is caused by some discrepancy in the histological procedure because the procedure itself was simple without requiring staining. In addition, yellow or off-white fluorescent granules were not detected in the live brain tissue of *O. vanhoeffeni* under UV excitation (personal observation). Thus, present results suggest absence or an undetectable level of lipofuscin in *O. vanhoeffeni*. This negative result is unusual because lipofuscin is present throughout the animal kingdom and one of the universal features of the pigment is its autofluorescence (Porta and Hartroft 1969, Sohal 1981, Bluhm et al. 2002). However, occurrence of resolvable fluorescent lipofuscin granules is taxonomically widespread, but not universal. For example, Sheehy (1990) and Bluhm (2001) reported that lipofuscin

was either absent or inconspicuous in several crustacean species. So far, there are no studies that explain the variation in lipofuscin occurrence in these species.

Unfortunately, age rings on the statolith in the appendicularian could not be resolved because there is no protocol to handle the miniscule statoliths, which are about 10 times smaller than otoliths and statoliths in larvae of fish, squid, and gastropods. Thus, this option for determination of absolute age is left open for future studies. However, this study did suggest the possibility of using statolith size as a proxy for age in appendicularians. Statolith diameter was strongly related to age in *O. vanhoeffeni*, as has also been found in fish, squids and gastropods, in which otolith and statolith dimensions are strongly related to age (Morris and Aldrich 1985, Fujiwara and Hankin 1988, Secor and Dean 1989, Reznick et al. 1989, Kristoffersen and Klemetsen 1991, Newman et al. 1996, 2000, McDougall 2004, Chatzinikolaou and Richardson 2007).

Increase in statolith diameter over time was monotonic with constant variance under controlled temperature and food concentrations in the laboratory. This result was independent of the genetic origin of the individuals. In contrast, the variance of mean body size was not stable over time but increased as the individuals aged, even under fixed conditions of temperature and food concentration in the laboratory, suggesting that increasing variability in body size with age is an inherent character of *O. vanhoeffeni*. Furthermore, the overall variance of statolith diameter was less than that of body size, suggesting that statolith diameter is the more precise indicator of age over all life history stages. However, additional laboratory studies are required to examine the dependence of statolith diameter on age at various food concentrations and temperatures.

Trunk length-at-age of *O. vanhoeffeni* in Conception Bay varied over two years. The temporal variability in trunk length-at-age was particularly high in older groups represented by statolith diameters from 14 - 16 μm (Fig. 2.8). In these age groups, mean length-at-age doubled and tripled from fall to spring (Fig. 2.9), with length-at-age at statolith diameters of 14, 15 and 16 μm ranging from 364 to 707 μm, from 488 to 939 μm and from 438 to 1695 μm, respectively. Furthermore, temporal variability in length-at-age increased with age (Fig. 2.10). This high and inconsistent variability in length-at-age

suggest that body size is not a reliable age indicator in field populations. Therefore, using the conventional method of cohort separation based on modal progression analysis of length frequency data may lead to inaccurate estimation of age distribution.

Polynomial regression analysis of the temporal progression of trunk length-at-age revealed a distinct seasonal pattern in the trunk length-at-age in the older individuals (12-15 μm statolith diameter) but an absence of seasonal pattern in the younger individuals (9-11 μm statolith diameter), suggesting age-specific variation in growth. The increase to a maximum length-at-age in the older and larger individuals during spring may be associated with the ability of larger individuals to ingest large diatoms during the spring bloom in Conception Bay (Deibel and Turner 1985, Urban et al. 1992). Absence of increase in the length-at-age in the younger and smaller individuals during spring indicates that growth of younger individuals may not have been affected by the spring bloom because of their inability to ingest large diatoms. Thus, variation in the age-specific growth of appendicularians may be related to the availability of food types that are ingestible. Food limitation in the growth of appendicularians *in situ* has only been documented in one study where *Oikopleura dioica* showed a rapid increase in body size after a picoplankton bloom in summer (Nakamura et al. 1997). The present study, however, is the first to suggest that intraspecific variation in age-specific growth of appendicularians may result from *in situ* food concentration.

Appendicularians are thought to be suspension feeders that are adapted to efficient ingestion of small particles (Flood et al. 1992, Deibel and Lee 1992, Acuña et al. 1996, Fernández et al. 2004) with their somatic and population growth rates relying on the availability of pico- and nanoplankton (Nakamura et al. 1997, Nakamura 1998, Hoover 2006). However, this conclusion is based on studies of small species in temperate and tropical regions, and may not apply to large, cold water species such as *O. vanhoeffeni*. Growth in older and larger *O. vanhoeffeni* in Conception Bay is enhanced during the spring diatom bloom, and previous studies have shown that large *O. vanhoeffeni* in Conception Bay and in the Northeast Water Polynya (NE Greenland) are capable of ingesting large diatoms (Deibel and Turner 1985, Acuña et al. 1999).

Increased length-at-age during spring may lead to increased egg production and population growth rates (see Chapter 3).

In conclusion, this study demonstrated for the first time that statolith diameter is a robust indicator of age for the appendicularian *O. vanhoeffeni*. I found that; (1) length-at-age of field populations exhibits seasonal variation, indicating temporal variation in growth; (2) variation in length-at-age is related to the availability of ingestible particles in the field; (3) due to unstable variability in length-at-age both in controlled laboratory conditions and in field populations, body size is not a reliable indicator of age. Thus age structure in nature may be defined more accurately from the distribution of statolith diameter rather than the distribution of body size, a finding that has important implications for the study of the population dynamics of appendicularians.

Table 2.1. *Oikopleura vanhoeffeni*. Analysis of variance for the best-fit polynomial models describing the temporal trend in the mean trunk length at 1 μm increments of statolith diameter. See Fig. 2.9.

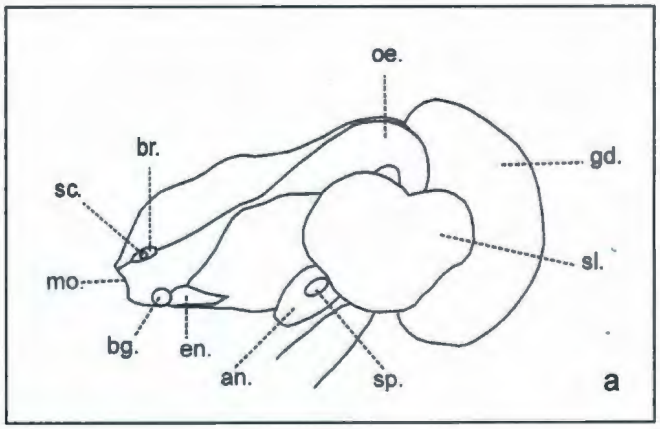
Statolith diameter	Best-fit polynomial order	Source	df	SS	MS	F	p
9 μm	2	Regression	2	2256	1128	10.48	0.001
		Error	15	1614	108		
		Total	17	3870			
10 μm	4	Regression	4	8191	2048	6.09	0.004
		Error	16	5382	336		
		Total	20	13573			
11 μm	2	Regression	2	5956	2978	6.73	0.006
		Error	21	9296	443		
		Total	23	15252			
12 μm	5	Regression	5	12930	2586	4.16	0.012
		Error	17	10570	622		
		Total	22	23500			
13 μm	5	Regression	5	43046	8609	7.81	0.001
		Error	17	18734	1102		
		Total	22	61780			
14 μm	5	Regression	5	132427	26485	9.85	< 0.001
		Error	16	43003	2688		
		Total	21	175429			
15 μm	5	Regression	5	401326	80265	12.07	< 0.001
		Error	15	99775	6652		
		Total	20	501101			
16 μm	None significant						

Table 2.2. *Oikpleura vanhoeffeni*. Regression analysis of the relationship between mean trunk length-at-statolith diameter and temperature. The independent variable is temperature and the dependent variable is trunk length (log-transformed).
* $p < 0.05$, ** $p < 0.01$. See Fig. 2.11.

Statolith diameter	n	r^2	Coefficients		p
			Constant	Temperature	
9 μm	19	0.009	2.325	0.002	0.702
10 μm	21	< 0.001	2.394	0.0004	0.948
11 μm	22	0.002	2.465	-0.001	0.834
12 μm	21	0.107	2.546	-0.008	0.148
13 μm	22	0.093	2.642	-0.009	0.167
14 μm	22	0.223	2.737	-0.019	0.026*
15 μm	21	0.396	2.877	-0.033	0.002**
16 μm	15	0.545	3.033	-0.070	0.002**

Table 2.3. *Oikopleura vanhoeffeni*. Regression analysis of the relationship between mean trunk length-at-statolith diameter and the concentration of chlorophyll *a*. The independent variable is concentration of chlorophyll *a* and the dependent variable is trunk length (log-transformed). * $p < 0.05$, ** $p < 0.01$. See Fig. 2.12.

Statolith diameter	n	r^2	Coefficients		p
			Constant	Chlorophyll *a*	
9 µm	19	0.001	2.324	0.003	0.902
10 µm	21	0.017	2.378	0.016	0.573
11 µm	22	0.049	2.439	0.024	0.325
12 µm	21	0.194	2.484	0.050	0.046*
13 µm	22	0.355	2.536	0.091	0.003**
14 µm	22	0.414	2.571	0.137	0.001**
15 µm	21	0.344	2.666	0.164	0.005**
16 µm	15	0.301	2.740	0.219	0.034*



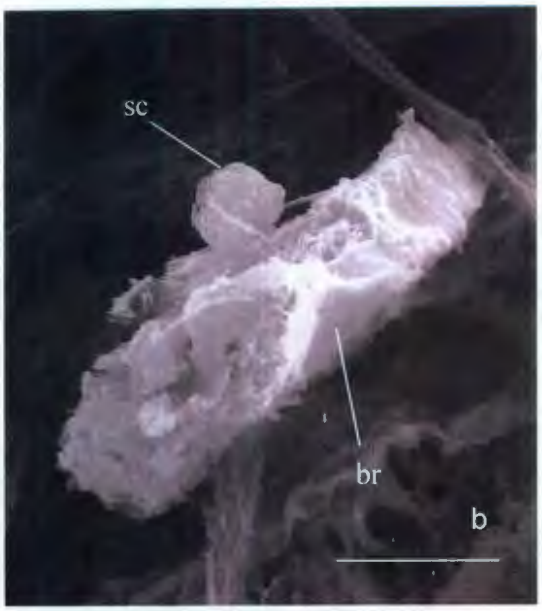


Fig. 2.1. *Oikopleura vanhoeffeni*. (a) General morphology of trunk. 'br' brain, 'sc' statocyte, 'mo' mouth, 'bg' buccal gland, 'en' endostyle, 'an' anus, 'sp' spiracle, 'sl' stomach lobe, 'gd' gonad, 'oe' oesophagus. (b) SEM view of brain and statocyte. Scale bar = 15 μm.

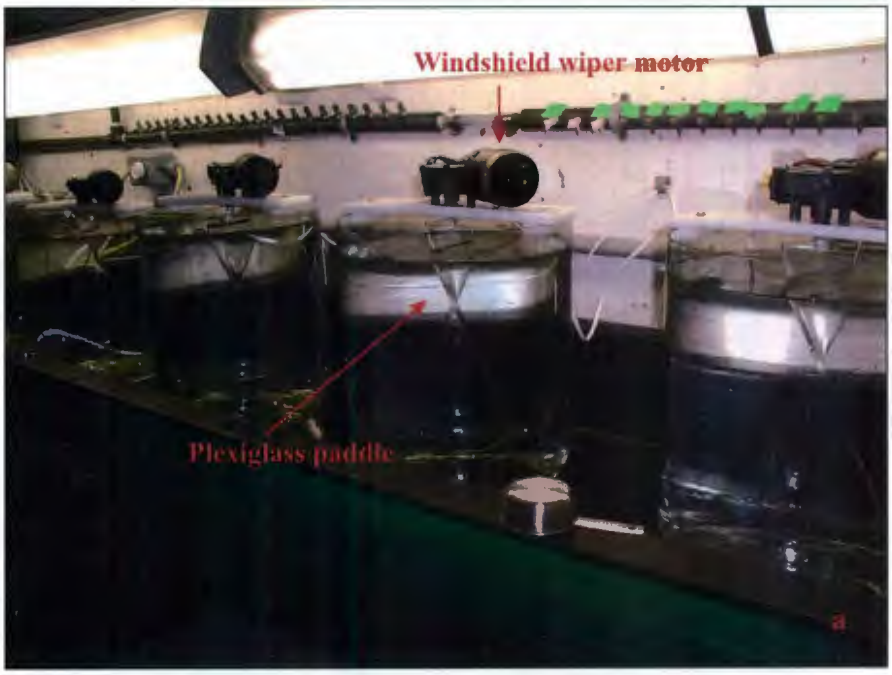


Fig. 2.2. Diagram of larvacean culture set-ups. (a) Glass tanks holding 20 l of seawater with a twisted plexi glass paddle driven by a windshield wiper motor. Gentle rotation of water kept the animals suspended. Tanks were kept in a cold room at a temperature 0-1°C. (b) Rheostat control board for controlling the speed of rotation of plexi glass paddles.

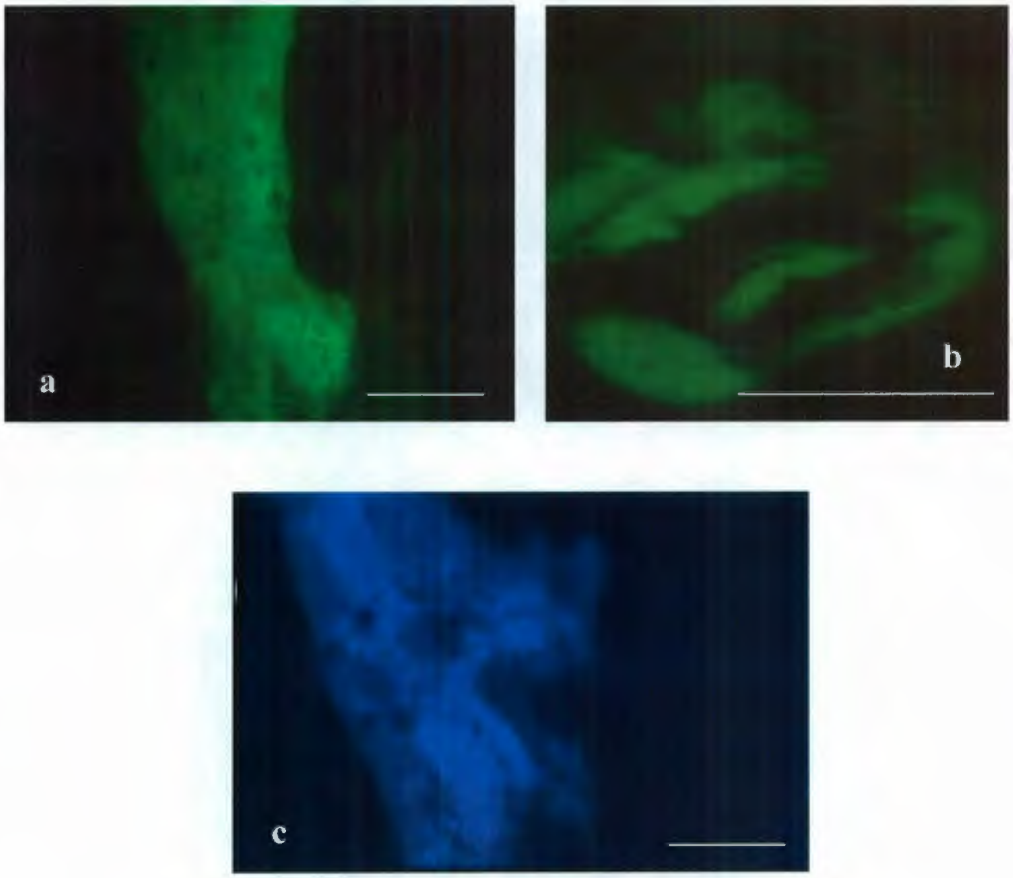


Figure 2.3. *Oikopleura vanhoeffeni*. (a) Longitudinal and (b) transverse sections of brain under blue excitation light showing absence of yellow or white, autofluorescent lipofuscin granules. (c) Longitudinal section of brain under UV excitation light showing absence of lipofuscin granules. Scale bars = 10 μm.

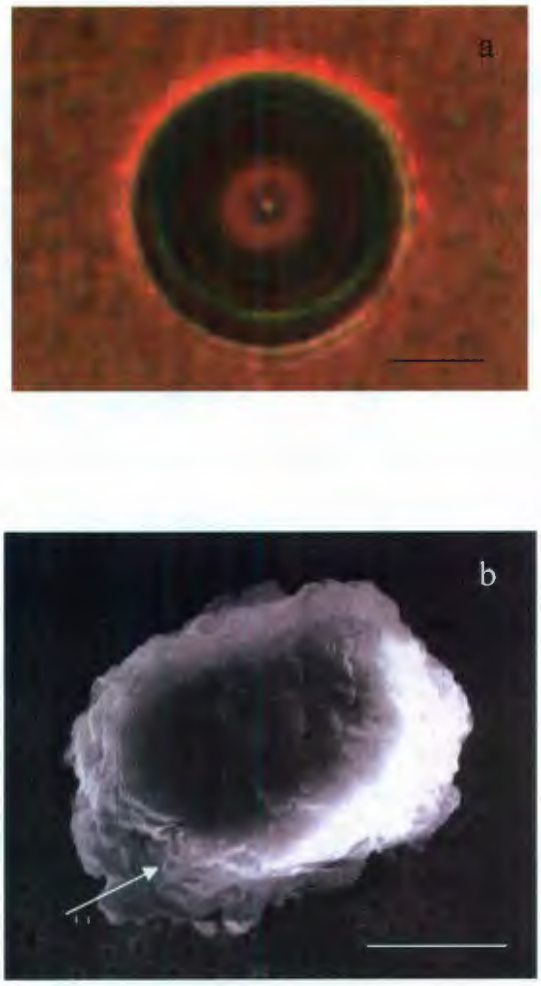


Fig. 2.4. *Oikopleura vanhoeffeni*. (a) Statolith under light microscope. (b) SEM view of statolith preserved in glutaraldehyde. Arrow is pointed where layers of accretion are shown. Scale bars = 5 μm.

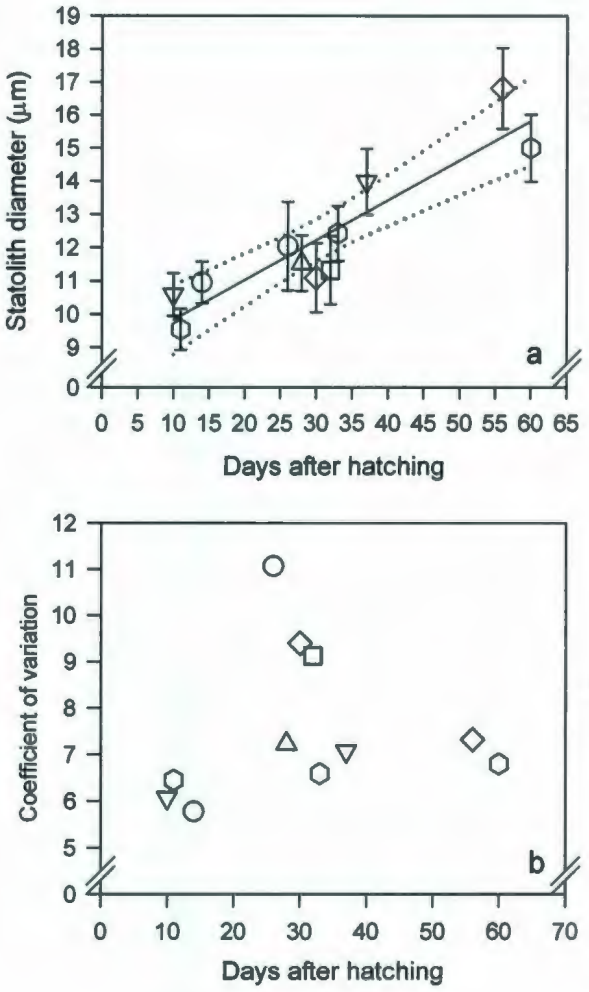


Fig. 2.5. *Oikopleura vanhoeffeni*. (a) Statolith diameter vs. time post hatching at 0-1°C in the laboratory. Data symbols represent offspring from different self-fertilized parents. The solid line shows the least squares linear regression and the dotted lines indicate 95% confidence intervals. $r^2 = 0.83$, $F_{(1,10)} = 44.3$, $p < 0.001$. (b) The coefficient of variation of mean statolith diameter vs. days after hatching. Pearson correlation, $r = 0.13$, $p = 0.36$).

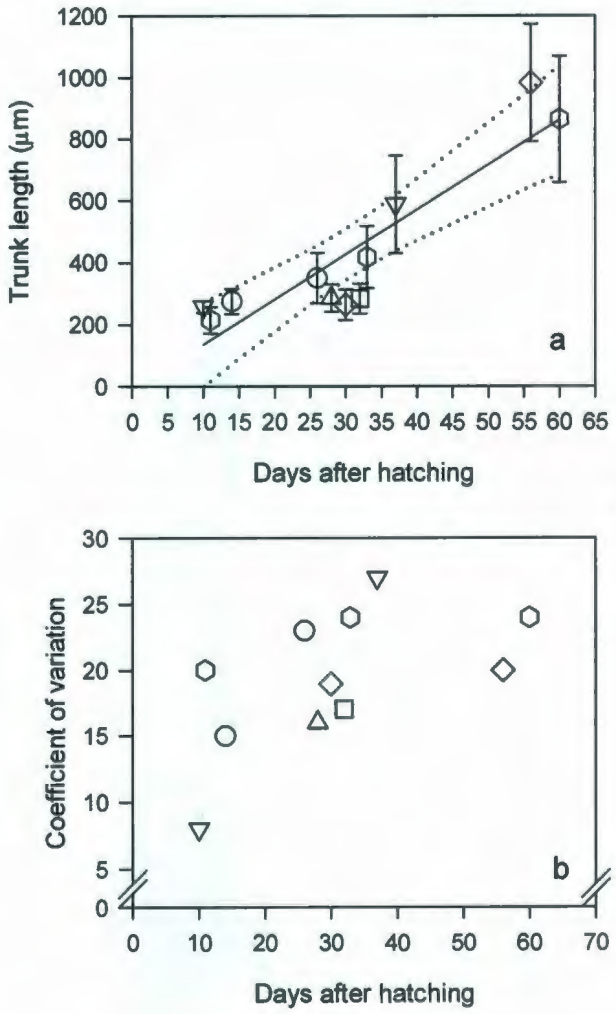


Fig. 2.6. *Oikopleura vanhoeffeni*. (a) Trunk length vs. time post hatching at 0-1°C in the laboratory. Data symbols represent offspring from different self-fertilized parents. The solid line shows the least squares linear regression and the dotted lines indicate 95% confidence intervals. $r^2 = 0.81$, $F_{(1,10)} = 38.2$, $p < 0.0001$. (b) The coefficient of variation of mean trunk length vs. days after hatching. Pearson correlation, $r = 0.57$, $p = 0.03$.

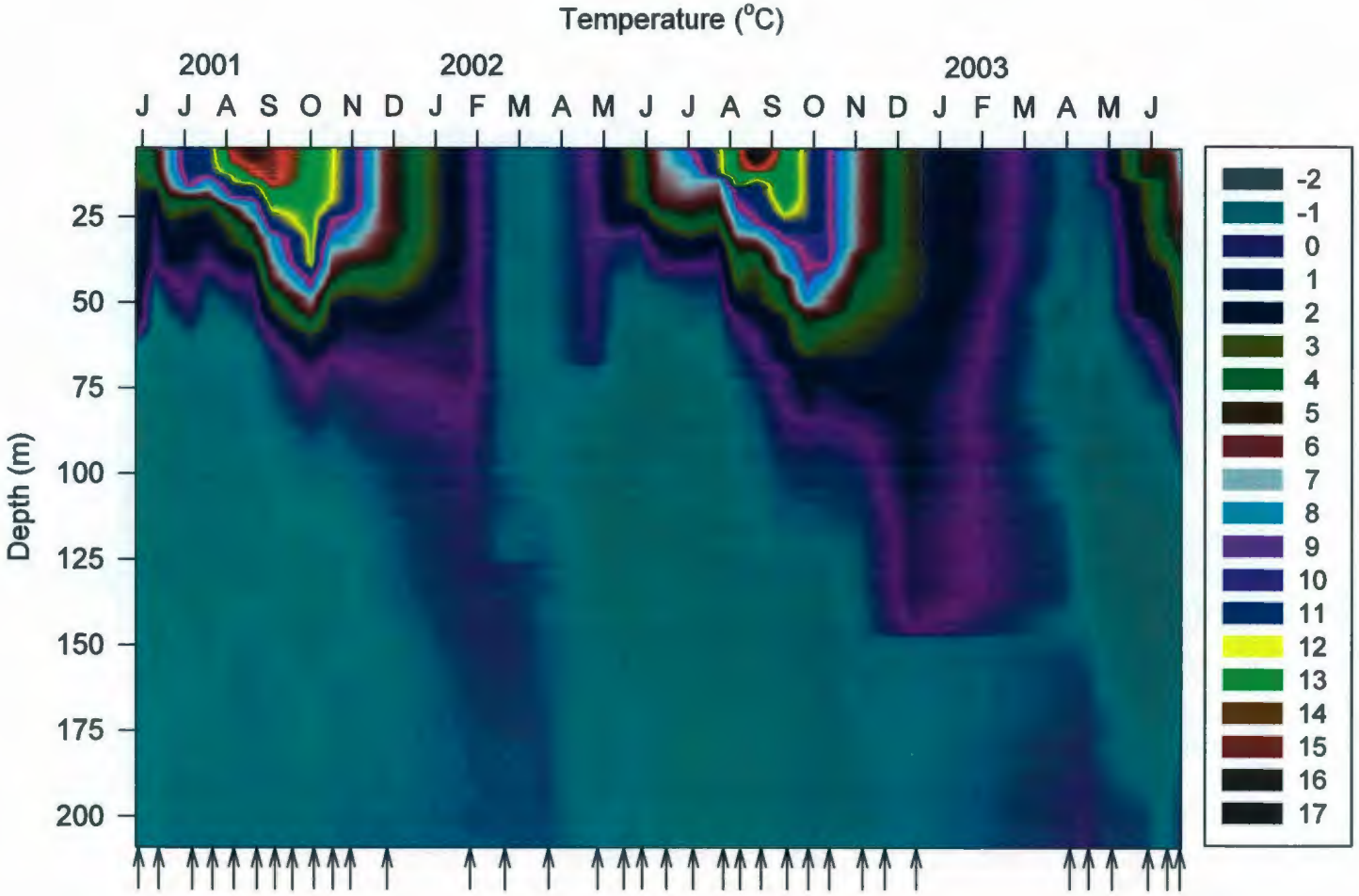


Figure 2.7 a. Vertical profile of temperature in Conception Bay from June 2001 to June 2003. Arrows on the bottom x-axis indicate when the CTD casts were taken.

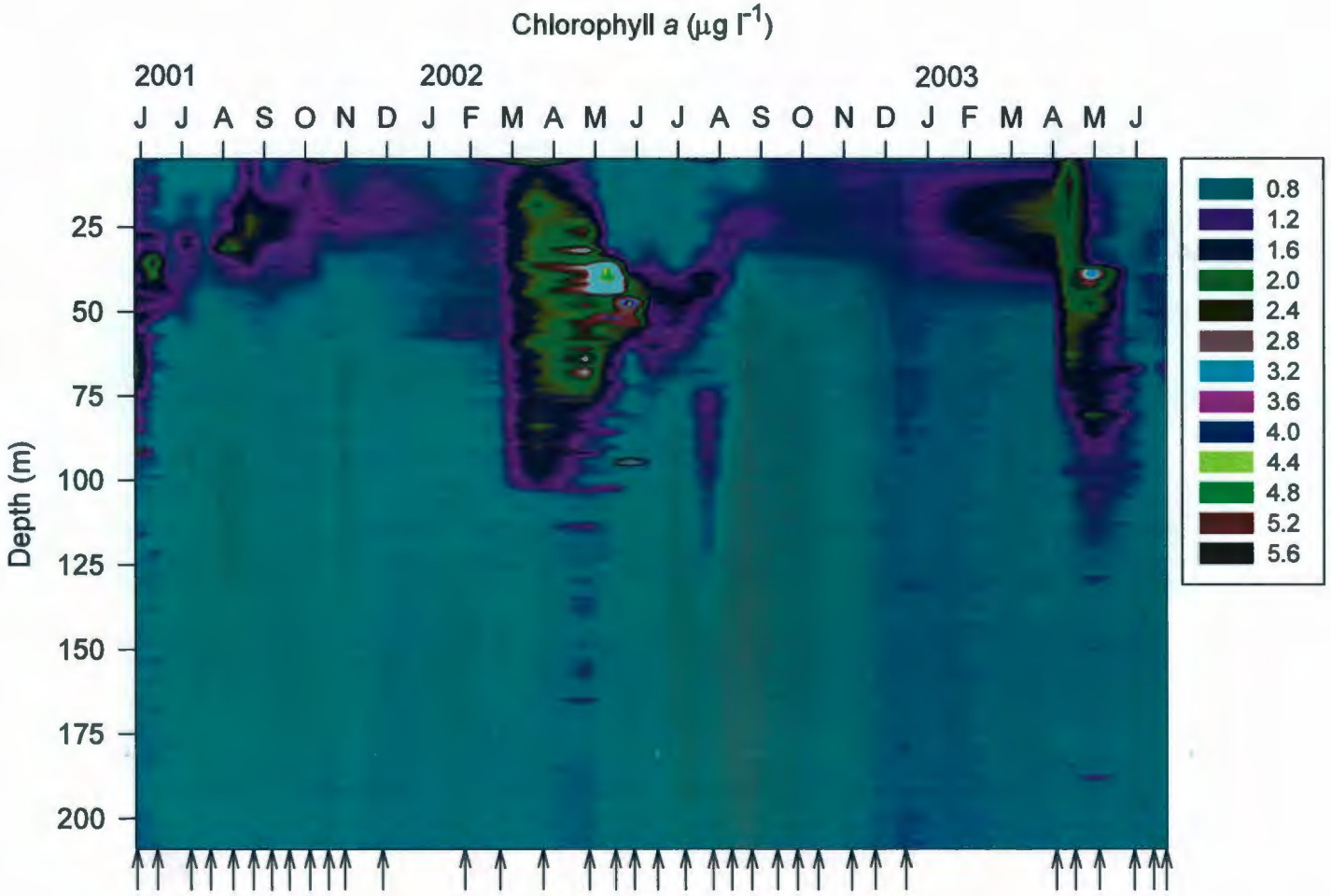


Figure 2.7 b. Vertical profile of chlorophyll *a* concentration in Conception Bay from June 2001 to June 2003. Arrows on the bottom x-axis indicate the time when CTD casts were taken.

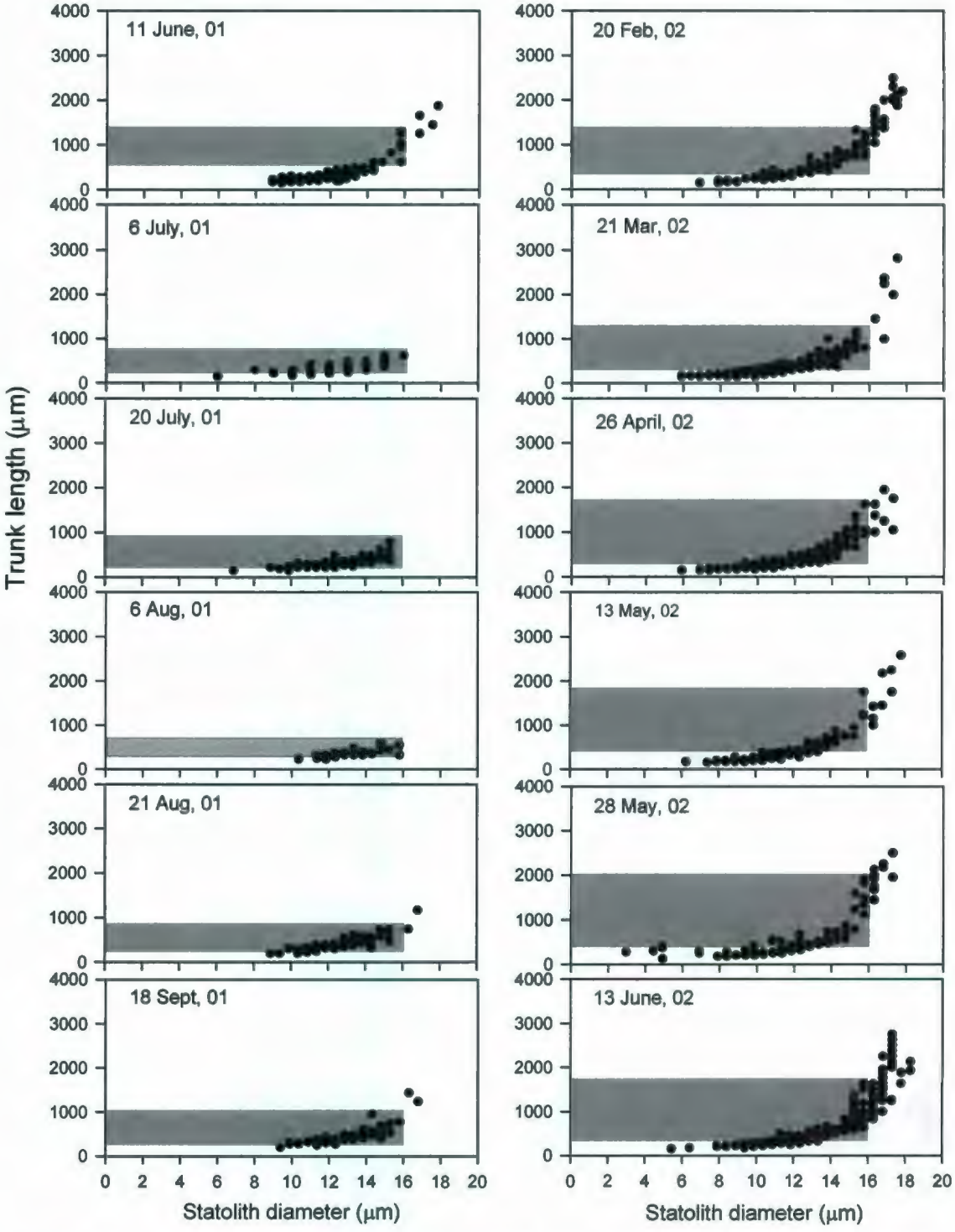


Fig. 2.8. *Oikopleura vanhoeffeni*. Trunk length vs. statolith diameter from June 2001 to June 2003. Grey area depicts trunk length at statolith diameters from 14 to 16 μm.

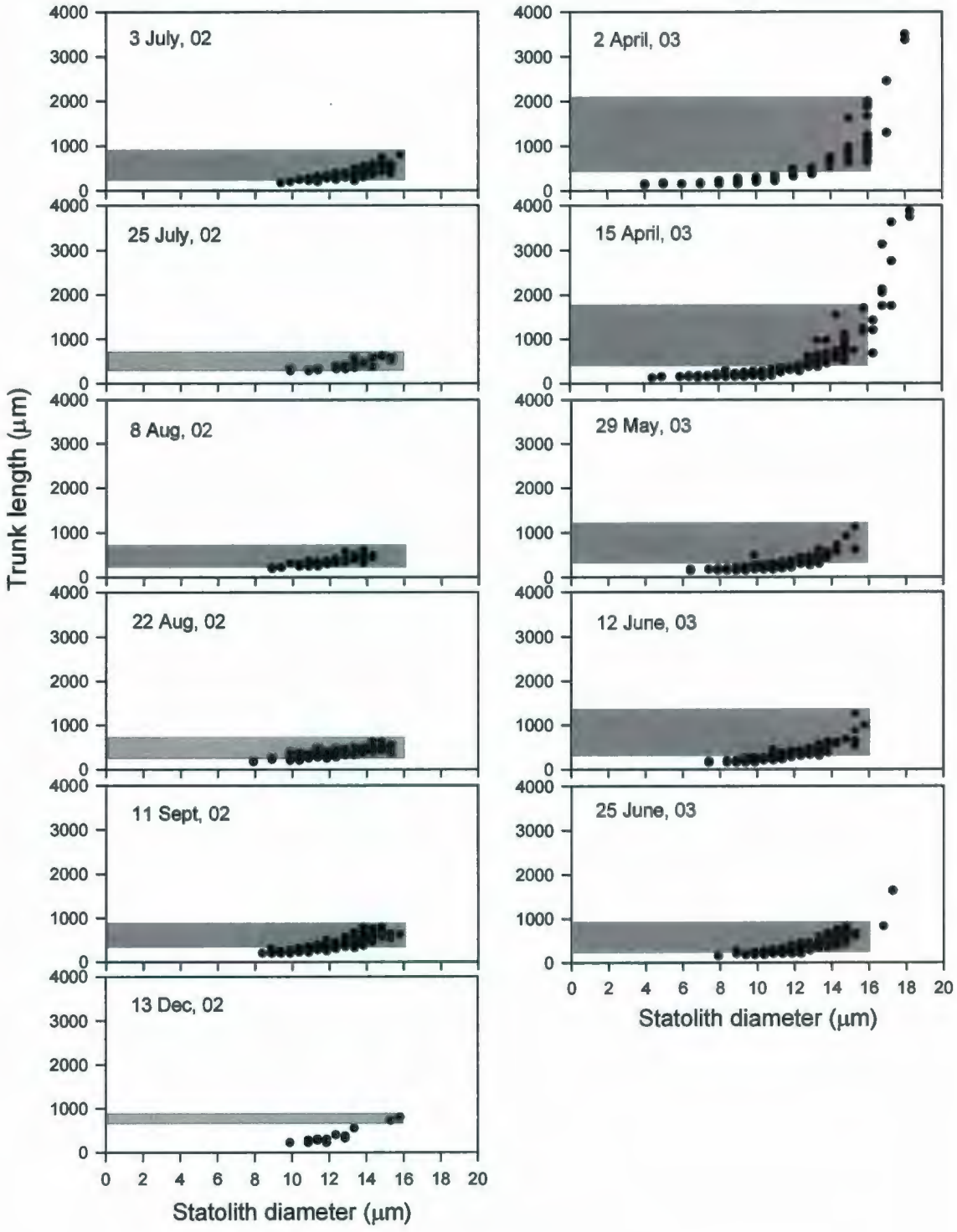


Fig. 2.8 continued.

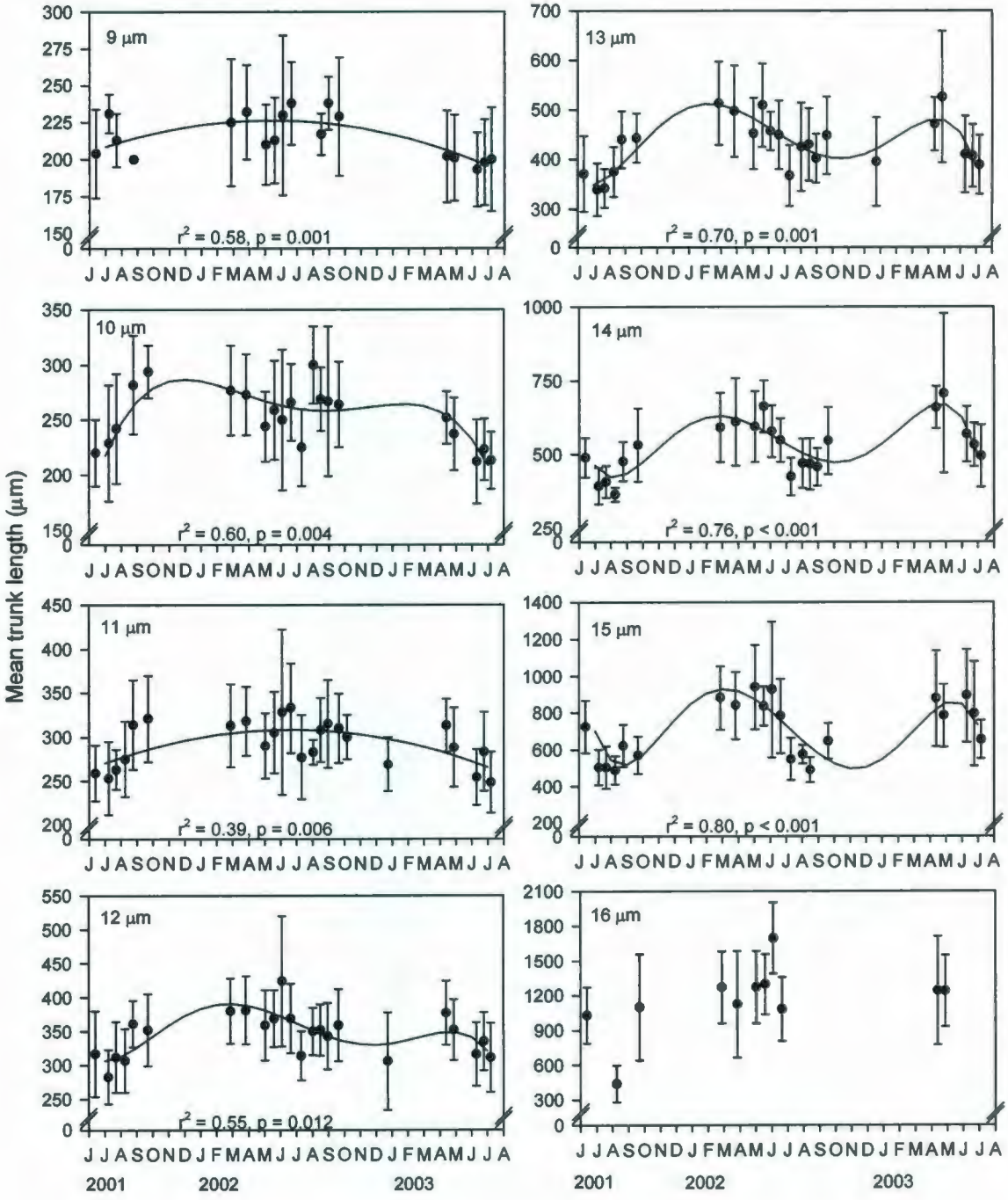


Fig. 2.9. *Oikopleura vanhoeffeni*. Mean trunk length-at-statolith diameter vs. time from June 2001 to June 2003. Lines represent polynomial regression of best-fit orders. No significant polynomial function was found for the mean trunk length-at-16 µm statolith diameter. See Table 2.1.

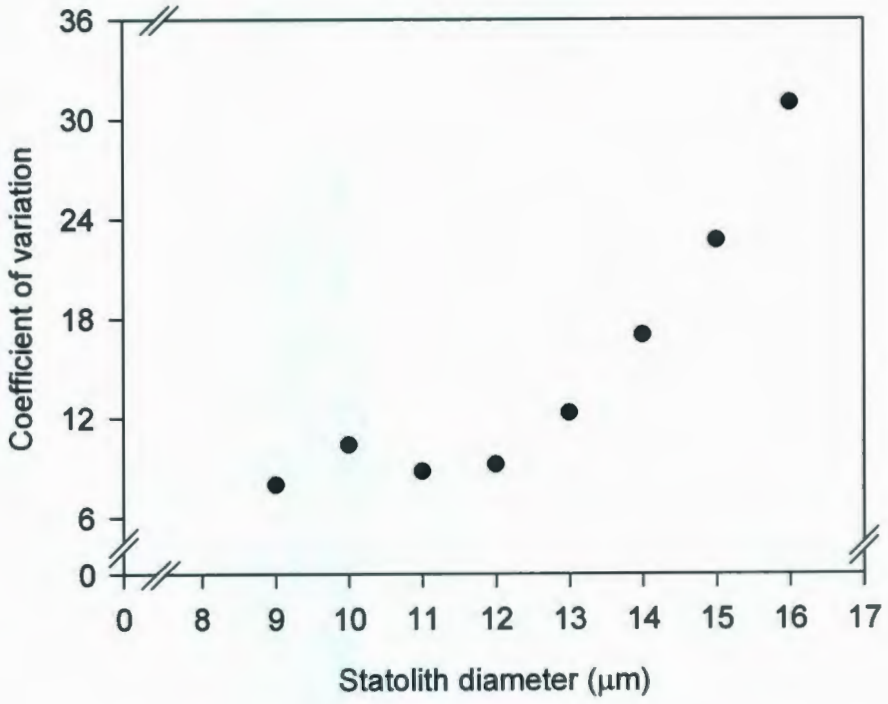


Fig. 2.10. *Oikopleura vanhoeffeni*. Coefficient of variation of mean trunk length at 1 μm statolith diameter intervals from June 2001 to June 2003.

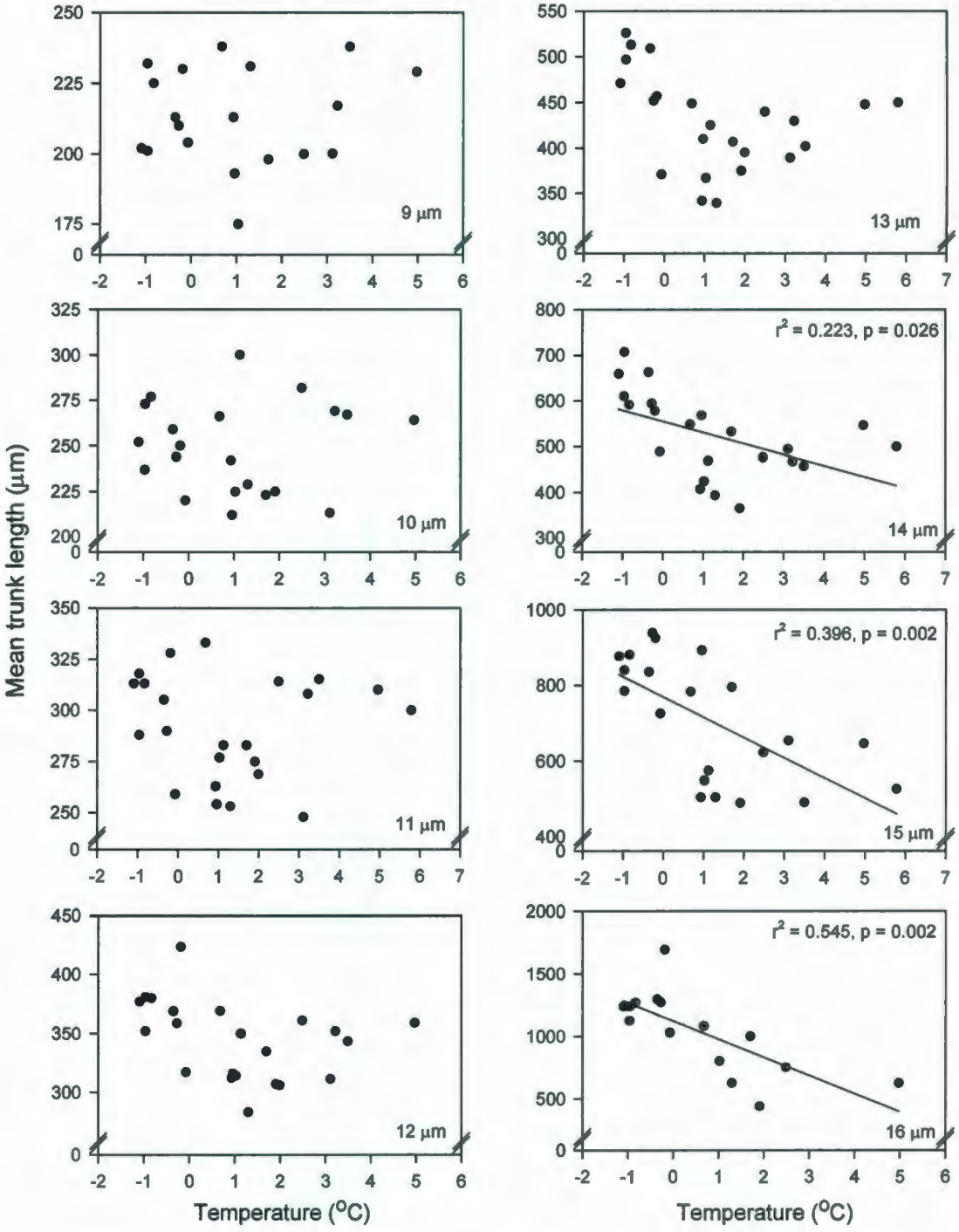


Fig. 2.11. *Oikopleura vanhoeffeni*. Mean trunk length-at-statolith diameter vs. temperature from June 2001 to June 2003. Temperature was averaged over the upper 100 m of the water column. Lines represent least squares linear regression.

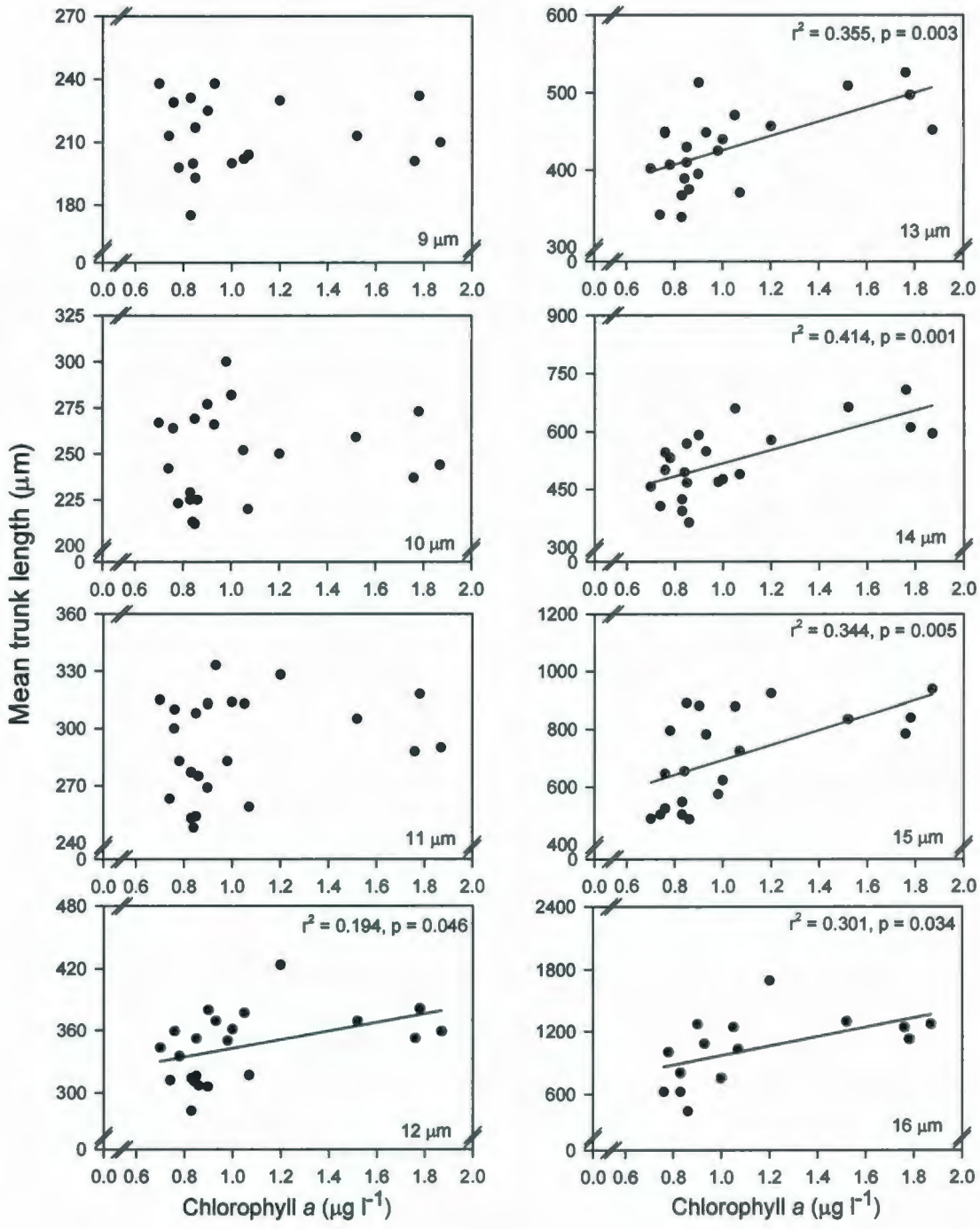


Fig. 2.12. *Oikopleura vanhoeffeni*. Mean trunk length-at-statolith diameter vs. the concentration of chlorophyll *a* from June 2001 to June 2003. Concentration of chlorophyll *a* (binaveraged at 1 m intervals) was averaged over the upper 100 m of the water column. Lines represent least squares linear regression.

Chapter 3

Life history and population dynamics of the cold water appendicularians *Oikopleura vanhoeffeni* and *O. labradoriensis* in Conception Bay, Newfoundland

3.1. Introduction

Appendicularians are important secondary producers in marine ecosystems. Although the biomass of these gelatinous zooplankters is generally lower than that of copepods, because of their high growth potential (Paffenhöfer 1976, Fenaux 1976, Hopcroft and Roff 1995, Nakamura et al. 1997, Hopcroft et al. 1998), production of appendicularians can be 30-100 % of copepod production in eutrophic environments (Hopcroft and Roff 1995, Nakamura et al. 1997, Hopcroft and Roff 1998). The grazing impact of appendicularians on phytoplankton is often significant, because they can remove up to 50 - 66 % of daily primary production (Alldredge 1981, Deibel 1988). In addition, appendicularians are important contributors to the vertical flux of organic matter by means of their production of fecal pellets (Taguchi 1982, Dagg and Brown 2005). Appendicularians continuously secrete mucus houses which they use to collect food particles. The daily production of houses may equal or exceed somatic production (Clarke & Roff 1990, Hopcroft & Roff 1998, Tomita et al. 1999). Also, discarded houses are used by various organisms as a source of food and as a surface habitat (Alldredge 1972, 1976, Ohtsuka and Kubo 1991, Ohtsuka et al. 1993, Steinberg et. al. 1994, Steinberg 1995, Mochioka and Iwamizu 1996). When houses are not consumed they may contribute to the vertical transport of organic matter to deeper layers (Silver & Alldredge 1981, Bauerfeind et al. 1997, Alldredge 2005). Using mucus filters, appendicularians ingest a large size range of food particles, from large diatoms to submicron particles (Deibel and Turner 1985, Urban et al. 1992, Acuña et al. 1996, Fernández et al. 2004) and colloidal and dissolved organic matter (Flood et al. 1992, Bedo et al. 1993). Because they are prey for other invertebrates such as chaetognaths, and larval and adult fish (Shelbourne 1962, Kimmerer 1984, Gadomski and Boehlert 1984, Keats et al. 1987, Purcell et al. 2005), appendicularians serve to short-circuit energy flow from the

microbial loop to higher trophic levels, thereby increasing the transfer efficiency of energy in marine food webs.

Life history, population dynamics, and production of appendicularians have been studied primarily in tropical and temperate regions, and little is known about species living in cold environments. Because there are no methods to accurately determine either the absolute or relative ages of appendicularians, *in situ* measurements of age-related life history characters that are important for understanding population dynamics, such as generation time, growth rate, age-specific fecundity and age-specific survival, remain a challenge. Generation times and growth rates of appendicularians have been estimated in the laboratory (Paffenhöfer 1976, Fenaux 1976, Gorsky 1980, Sato et al. 1999) and in mesocosms where artificial cohorts were created and food particle size and predation effects controlled (Nakamura et al. 1997, Hopcroft et al. 1998). Estimation of production has been based on field biomass data combined with growth rates determined in the laboratory at simulated natural temperatures (Uye and Ichino 1995, Tomita et al. 1999). In the present study, life history characters and production of two sympatric cold water appendicularians, *Oikopleura vanhoeffeni* and *Oikopleura labradoriensis*, were determined *in situ* in order to understand their population dynamics and their role as secondary producers in Conception Bay.

Age structure is an essential component of population dynamics. In aquatic organisms, age structure can be defined by separating cohorts based on various age-related characters. For fish and molluscs, age structure of populations can be determined from counting age rings on calcareous otoliths and shells (MacDonald and Thomas 1980, Lipinski 1986, Campana 2001), whereas in many other invertebrates age structure can be determined from frequency distributions of body size or developmental stages (Hygum et al. 2000, ref. therein) or from lipofusin concentration in post-mitotic cells (Sheehy et al. 1994, Bluhm and Brey 2001). I have shown that age rings in statolith and lipofuscin in brain tissue cannot be identified and therefore cannot be used to determine the age of *O. vanhoeffeni* (Chapter 2). However, I have also shown that statolith diameter is a more reliable age indicator than trunk length for two main reasons (Chapter 2); (1) variability

in statolith diameter-at-age is substantially less than that of trunk length-at-age (i.e., 7.5 % vs. 19.4%, respectively), and (2) variability in statolith diameter-at-age remains constant as statolith diameter increases but variability in trunk length-at-age increases as trunk length increases. Therefore, in the present chapter age structure was determined from frequency distributions of statolith diameter in order to estimate the generation time and growth rates of identifiable cohorts of *O. vanhoeffeni* and *O. labradoriensis* in Conception Bay. An assumption made in this study is that statolith diameter is also a reliable age indicator for *O. labradoriensis*.

Generation time and growth rate of appendicularians are influenced by temperature and food concentration. Faster growth and shorter generation time have been observed at high temperatures (Fenaux 1976, Troedsson et al. 2002, Touratier et al. 2003, Lopéz-Urrutia et al. 2003) and high food concentrations (Paffenhöfer 1976, Hopcroft et al. 1998, Touratier et al. 2003). Food limitation of growth depends on body size and varies ontogenetically. Hopcroft et al. (1998) found that the growth rate of larger genera in Jamaican waters is positively related to the concentration of picoplankton and nanoplankton, but that growth in smaller species is unrelated to food concentration. Lopéz-Urrutia et al. (2003) compiled information regarding feeding, metabolic and growth rates of tropical and temperate appendicularian species and applied the growth model of Huntley & Boyd (1984) to *Oikopleura dioica*. Their results suggest that *O. dioica* probably experiences food-limited growth during early developmental stages. In Conception Bay, temperature, food concentration, and food type vary seasonally in the upper 100 m of the water column (Stead and Thompson 2003, Richoux et al. 2004, Chapter 2), where most appendicularians are concentrated (Chapter 1). Thus, seasonal variation in generation time and growth may occur in oikopleurid species in Conception Bay.

Age and size-at-maturity are key demographic traits that influence population dynamics (Stearns and Koella 1986). Early maturation can lead to higher population growth rates because the probability of survival to maturity increases if the period spent as a juvenile is reduced (Cole 1954, Lewontin 1965). Delayed maturity can also lead to

higher population growth if further growth results in a larger size-at-maturity and higher fecundity (Tuljapurkar 1990, Stearns 1992). Age and size-at-maturity depend upon environmental conditions. In ectotherms, these traits are generally a function of temperature (Bergmann 1847, Shaw and Bercaw 1962, Atkinson 1994, Gillooly 2000); age- and size-at-maturity increase with a decrease in temperature. Food availability and predation pressure may also be important determinants of age- and size-at-maturity (Randall et al. 1986, Rasmussen and Giske 1994, Doksaeter and Vijverberg 2001, Shertzer and Ellner 2002, Law 1979, Abrams and Rowe 1996, Belk 1998, Chase 1999). A positive relationship exists between food availability and size-at-maturity (Rasmussen and Giske 1994, Doksaeter and Vijverberg 2001). Size-specific predation leads to variation in age- and size-at-maturity in which predation on small and juvenile individuals results in an increase in age and size-at-maturity (Reznick 1982, Belk 1995) whereas predation on larger and older individuals results in a decrease in age and size-at-maturity (Rodd and Reznick 1997, Hutching 2005).

In a laboratory study, Troedsson et al (2002) demonstrated that population growth rates of *Oikopleura dioica* can be increased either by a decrease in generation time or by an increase in fecundity. The highest intrinsic rate of natural increase was observed when generation time decreased and fecundity increased simultaneously under elevated temperature and food concentration. However, no field study has considered the effect of environmental variability on age- and size-at-maturity and consequential population growth rates of appendicularian species. The present study addresses the question whether age- and size-at-maturity and fecundity of appendicularians vary with temperature and food concentration in Conception Bay and how this potential variability affects population growth rates of appendicularians in nature.

Specific questions addressed in this chapter about life history and population dynamics of the boreal appendicularians *O. vanhoeffeni* and *O. labradoriensis* in Conception Bay are: (1) What is the generation time? (2) What is the growth rate? (3) What is the annual production? (4) How do age- and size-at-maturity vary under

seasonally fluctuating temperature and food concentration? (5) How does population growth rate change in relation to variation in age- and size-at-maturity?

3.2. Materials and Methods

3.2.1. *Sample collection*

Samples were collected from April 23, 2001 to June 25, 2003 at a site in Conception Bay described in Chapter 1. Vertical hauls from near the bottom (225 m) to the surface were made using a WP-2 net with a mesh size of 110 μm. The speed of retrieval was 0.13 m s^{-1} and the volume of water filtered was measured with a mechanical flowmeter (Model 2030 R6, General Oceanics, Inc.). Upon retrieval of the net, samples were immediately fixed in 95 % ethanol for statolith analysis and in 2 % Bouin's solution for histological analysis of the gonad. Triplicate samples were collected biweekly except during winter, when harsh weather conditions precluded sampling. An additional tow was made on each sampling day using a large ring net (1 m mouth diameter) with a 10 L cod end to collect mature individuals without damaging their gonads. From June 11, 2001 to June 25, 2003, a CTD cast (Seabird, SBE 25-01) was made before each tow to measure temperature and *in situ* relative fluorescence. Relative fluorescence units (RFU) were converted to chlorophyll *a* concentration (μg Chl *a* l^{-1}) using the equation Chl *a* = 0.398 x RFU + 0.281 (r^2 = 0.73, n = 244) which was developed using historical data from Conception Bay (Cold Ocean Productivity Experiment, unpublished). The temperature and chlorophyll *a* data were bin-averaged at 1 m depth intervals before contour plotting.

3.2.2. *Cohort analysis*

Appendicularians were sorted from the samples which had been preserved in 95 % ethanol. It was important to rinse the samples with 95 % ethanol instead of water before sorting appendicularians because addition of distilled water to the tow samples decreases the pH and dissolves the statolith. The cause of this chemical reaction is not clear. Trunk lengths of appendicularians were measured from the tip of the mouth to the posterior tip of the stomach, excluding gonads, to the nearest 25 μm under a Zeiss stereo

microscope at 40X magnification. After trunk length measurements, the individuals were cleared in 1% KOH and mounted in glycerol. The diameters of statoliths were measured to the nearest 0.5 μm using a Zeiss Axiovert 35 inverted microscope under transmitted light and bright field optics at 1000X magnification.

Cohorts of *O. vanhoeffeni* and *O. labradoriensis* were identified using the Bhattacharya Method (Bhattacharya 1967) on statolith diameter frequency distributions binned at 1 μm intervals. The FiSAT II software package (FAO-ICLARM Fish Stock Assessment Tools, Version 1.2.0) was used to separate the components of the normal distributions of statolith diameter from the total frequency distribution, starting on the left-hand side. Component normal distributions were removed iteratively until they could no longer be distinguished using the separation index $SI = \Delta L_k / \Delta \delta_k$, where ΔL_k is the difference between two successive means of component curves and $\Delta \delta_k$ is the difference between their estimated standard deviations (Sparre & Venema 1998). The separation of cohorts was statistically reliable when the SI value was above 2 (Hasselblad 1966, McNew and Summerfelt 1978, Clarke 1981).

3.2.3. *Generation time, growth rate and production*

Generation time of each cohort was estimated as the number of elapsed days between its appearance and disappearance. *O. vanhoeffeni* and *O. laboradoriensis* are semelparous and die immediately after spawning. For *O. vanhoeffeni*, the elapsed time from egg release until the juveniles reach sufficient size to be quantitatively collected in the plankton tow at 0-1 °C is approximately 7 d (laboratory observations), which may be the maximum underestimation of generation time in this study. Since information on embryonic development time and early stage growth rate of *O. labradoriensis* is not available, error in the estimation of generation time for this species is not known but is assumed to be ≤ 7 d.

Trunk lengths of individual *O. vanhoeffeni* and *O. labradoriensis* within each cohort were converted to carbon weight using the equations $C\ (\mu g) = 4.03\ TL(mm)^{3.45}$ (Deibel 1988) and $C\ (\mu g) = 7.43\ TL\ (mm)^{2.86}$ (Riehl 1992) respectively. Trunk lengths of

individuals fixed in 95 % ethanol were corrected for shrinkage by 18.0 $\pm$ 3.0 % (n = 14, Appendix 3) before conversion to weight.

To test if the somatic growth pattern of the cohorts was exponential, the relationship between ln-transformed weight and time for each cohort was examined for linearity. If this linear relationship was statistically significant, instantaneous growth rate was estimated by fitting the exponential growth function $W_t = W_0 e^{gt}$, where W_0 represents the initial mean weight, W_t the mean weight of each cohort at time 't' and 'g' the instantaneous growth rate (d^{-1}). If the relationship between ln-weight and time was not significantly linear, One-Way Anova and post-hoc analysis (Tukey test) were used to determine the time periods when significant growth occurred, and the instantaneous growth rate within these time periods was estimated using the equation $W_t = W_0 e^{gt}$. If the growth pattern of the cohort was linear, a linear regression equation was fitted to the untransformed data and the growth rate was determined from the slope. All statistical analyses were performed with SPSS 9.0.0 (SPSS Inc., Chicago, IL).

Daily somatic production was computed as Pg = g x B, where g (d^{-1}) represents the instantaneous growth rate and B (mg C m^{-2}) the biomass in carbon weight at each sampling day. Biomass was estimated by converting the trunk length frequency distribution into a carbon weight frequency distribution and multiplying the distribution by the mean abundance data from triplicate samples. Production of *O. vanhoeffeni* from 2001 to 2002 and 2002 to 2003 was calculated using the instantaneous growth rates of cohort 2 and cohort 3 respectively (see Results). For *O. labradoriensis*, instantaneous growth rates of cohort 1 and 2 were averaged for estimation of production in 2001/2002 and those of cohort 3 and 4 were averaged for estimation of production in 2002/2003. Daily production was integrated over each year to estimate annual production, assuming that each data point remained constant between the sampling intervals. Daily somatic production was integrated over each year using the midpoint rule:

$$Pg = \int_a^b f(x)\,dx \approx \sum_{k=1}^{n} [Y_k + (Y_{k+1}-Y_k)/2]\ (X_{k+1}-X_k)$$

where the integration of production over time series (a to b) was approximated by summing the rectangular area under the time series curve where the area of the rectangle is centered at the midpoint between two successive sample points, k and k+1. Y and X represent daily production in mg C m^{-2} and sampling time in days, respectively.

The daily house production (Pe) of *O. vanhoeffeni* ranged from < 1 to 6 houses d^{-1} with a mean of 1.6 ± 1.0 houses d^{-1} at temperatures from -1 to 6 °C (Riehl 1992). Given that the carbon content of a clean house is about 23 % of body carbon (Deibel 1986), *O. vanhoeffeni* produces approximately 37 % of its body weight in houses each day (Deibel 1988). The daily house production and carbon content of houses of *O. labradoriensis* are not known, but are assumed to be similar to those of *O. vanhoeffeni* in this study. Thus, the daily house production rates of *O. vanhoeffeni* and *O. labradoriensis* were estimated as 37 % of the somatic weight. Annual house production was integrated between each collection interval and summed over each year of the study using the midpoint rule.

3.2.4. *Age- and size-at-maturity and potential fecundity*

Mature individuals (with a well-developed gonad expanded and covering the area of the entire posterior trunk, Shiga 1976, Shiga 1993) were sorted from the samples preserved in ethanol or Bouin's solution. Mature individuals in ethanol were cleared in 1% KOH and the statolith diameter measured by light microscopy to the nearest 0.5 µm. Mean statolith diameter-at-maturity was calculated at each sampling time point whenever mature individuals were found and these mean values were considered as a proxy for age-at- maturity.

Mature individuals in Bouin's solution were sorted and their trunk lengths measured. Mean trunk length-at-maturity was calculated at each sampling time point whenever mature individuals were found. Replicate individuals at these mean trunk lengths (2-3 individuals) were removed from each sample, dehydrated in a graded ethanol/water series and cleared in xylene. They were then embedded in paraffin and serially sectioned at 6 µm intervals. Sections were stained with hematoxylin and eosin to help visualize the oocytes and ovary (thin section micrographs are shown in Appendices

4 and 5). The total area occupied by the oocytes and their mean diameter were measured at 4 X to 20 X magnifications using ImagePro software (Version 4.0). Potential fecundity was calculated as:

Potential fecundity = Total volume of oocytes / Mean volume of an oocyte;

Total volume of oocytes = volume of ovary x mean % of ovary area occupied by oocytes.

Volume of ovary = Σ area of ovary in every consecutive N^{th} section x N x 6 μm, where N = 1/10 of total thin sections taken. Mean % of ovary area occupied by oocytes was calculated from all thin sections of ovary observed. Mean volume of oocytes = $4/3\ \pi$ x (mean radius of oocyte)^3, and the mean volume of an oocyte was calculated from all the measured oocytes. The number of oocytes measured for *O. vanhoeffeni* ranged from 23 to 177 oocytes and for *O. labradoriensis* from 41 to 207 oocytes. Potential fecundity is likely an overestimate of actual fecundity, because not all oocytes reach maturity and those that fail to mature are eventually reabsorbed (Last 1972). True fecundity was not determined in this study because of the difficulty in obtaining individuals with fully mature but unruptured gonads using net tows. However, true fecundity could be approximately 50 % of potential fecundity if oogenesis of *Oikopleuria* species in this study is similar to that of *O. dioica*, in which nearly 50 % of oocytes are reabsorbed at the end of maturation (Last 1972).

3.2.5. *Population growth rate*

For determination of population growth rate, animals from the triplicate net tow samples were counted. Population growth rate was calculated using the equation, $r = (\ln N_{ti+1} - \ln N_{ti})/(t_{i+1} - t_i)$ (Odum 1971), where N represents mean abundance and 't' time in days at the i^{th} time point. In order to smooth the data to reduce stochastic variability a three-point moving average was applied to the abundance data prior to the calculation of population growth rate (Diggle 1990). Throughout the study, it was assumed that the individuals sampled in Conception Bay all belonged to a single population, because the actual spatial scale of the entire population of both species is not known. It has been shown for other marine species inhabiting the large bays of eastern Newfoundland that

single populations span the region and that population dynamics are largely synchronized over the entire eastern Newfoundland shelf (Leggett et al., 1984).

3.3. Results

3.3.1. *Temperature and chlorophyll* a *concentration*

Temperature fluctuated seasonally in the upper mixed layer with an increase to a maximum of 15.4-16.6 °C in late August and a decrease to a minimum of 1.0 to -0.8 °C in late March to early April (Fig. 2.1a, Chapter 2). A thermocline was present within the upper 60 m from June to December which eroded as winter mixing occurred to a depth of 100 to 150 m. The temperature below 150 m remained < 0 °C throughout the time series. Seasonal variation in chl *a* concentration occurred mostly within the upper 100 m (Fig. 2.1b, Chapter 2). The spring bloom began in March and peaked in May with a maximum chl *a* concentration of 5.8 μg L^{-1} in 2002 and 3.5 μg L^{-1} in 2003. A minor fall bloom occurred in August 2001 (2.4 μg L^{-1}) and a weaker bloom occurred in late July 2002 (1.7 μg L^{-1}). Occurrence of the fall bloom is more variable from year-to-year than is the spring bloom (Stead and Thompson 2003, Richoux et al. 2004). Minimum concentration of chl *a* was found in July 2001 (0.9 μg L^{-1}) and in October 2002 (1.0 μg L^{-1}). Given that most appendicularians live in the upper 100 m of the water column year around (Chapter 1), habitat temperature and chl *a* used for statistical analyses are represented by the mean values of 100, 1-m depth bins within the upper 100 m of the water column.

3.3.2. *Recruitment and generation time*

Four cohorts of *Oikopleura vanhoeffeni* were identified between June 2001 and June 2003 (Fig. 3.1). Based upon the first appearance of cohort 3 (2002) and cohort 4 (2003), it appears that the primary annual spawning event began between mid-December and mid-February. Because oikopleurid appendicularians are semelparous and die immediately after spawning, the final appearance of cohorts 1 (2001), 2 (2002) and 3 (2003) suggests that the annual spawning event ended between mid-April and mid-June. Further evidences of this spawning window are the emergence of individuals with

statolith diameter less than 8 μm and the annual increase in abundance in May-June (Fig. 3.2a,b) which took place just after the peak of the spring bloom in April (Fig. 3.2c). Some individuals in cohort 2 and 3 became sexually mature early, in the fall of their first year at a small statolith diameter (shaded ranges of statolith diameter-at- maturity in Fig. 3.2a). However, there is no evidence in the cohort analysis or in the time series of abundance that successful recruitment occurred in the fall. Based upon all of the evidence above, the generation time of *O. vanhoeffeni* was essentially one year.

Five cohorts of *Oikopleura labradoriensis* were identified between August 2001 and April 2003 (Fig. 3.3). Cohort 2 appeared in September 2001 while cohort 3 appeared in March 2002. Both cohorts originated from cohort 1 because cohort 1 alone contained mature individuals (shaded ranges of statolith diameter-at-maturity in Fig. 3.4a). Similar to reproductive phenology between the year 2001 and 2002, cohort 4 appeared in October 2002 and cohort 5 in April 2003; both cohorts originated from cohort 3. Strangely, there is no indication of maturation and production of progeny by cohort 2 and cohort 4. Thus, there were two spawning events in each year, one in the fall and one in the spring. Further evidence of these spawning windows is the emergence of individuals with statolith diameters less than 7 μm and an increase in abundance during fall and spring (Fig. 3.4a, b). Although individuals with statolith diameters less than 7 μm appeared on January 25, 2002, their frequency was too small for them to be captured as a new cohort. Based upon the time series of abundance (Fig. 3.4b), a major recruitment occurred during fall, when temperature reached its annual maximum (Fig. 3.4c). However, it is not clear when these cohorts ended because they were not present in samples between May and July, perhaps due to massive mortality or advection out of the bay (Fig. 3.4a). Assuming that the earliest time that the cohort could have ended was in April, the generation time of cohorts 2 and 4 was at least 8 months and that of cohort 3 was at least one year.

3.3.3. *Individual growth, population biomass and production*

Animals from cohort 2 of *O. vanhoeffeni* grew exponentially over an entire year (Fig. 3.5a, b) with an instantaneous rate of $0.017 d^{-1}$ (Table 3.1). However, animals from

cohort 3 did not grow exponentially (Fig. 3.5c), confirmed by a nonlinear relationship between ln-transformed weight and time (Fig. 3.5d). Cohort 3 animals did not grow significantly until April 2003 ($p < 0.001$), therefore growth rate until December 2002 was considered to be zero. The instantaneous growth rate of animals from cohort 3 estimated from December 2002 to April 2003 was 0.043 d^{-1} (Table 3.1).

Animals from cohorts 1, 3 and 4 of *O. labradoriensis* grew exponentially (Fig. 3.6), with instantaneous growth rates of 0.007-0.011 d^{-1} (Table 3.1). The growth of cohort 2 animals was best fit by a linear equation (Fig. 3.6) with an instantaneous growth rate of 0.011 d^{-1}, similar to the growth rates of animals from the other three cohorts (Table 3.1).

The population biomass of *O. vanhoeffeni* ranged from 0.01 to 483 mg C m^{-2} and increased during April and May with an annual mean and standard deviation of 66.5 $\pm$ 128 mg C m^{-2} from June 2001 to June 2002 and 25.0 $\pm$ 48.5 mg C m^{-2} from July 2002 to June 2003 (Fig. 3.7a, Table 3.2). The increase in population biomass during spring was due to the increase in abundance of large, mature individuals (Fig. 3.2b, Fig. 3.5). Daily somatic production of the *O. vanhoeffeni* population ranged from < 0.01 to 8.12 mg C m^{-2} with maximum production occurring during spring (Fig. 3.7a); annual production was 343 mg C m^{-2} y^{-1} in 2001/2 and 359 mg C m^{-2} y^{-1} in 2002/03 (Table 3.2). The annual $P_{somatic}/B$ ratio was 5.2 in 2001/2 and 14.3 in 2002/3 (Table 3.2). Daily house production of the *O. vanhoeffeni* population ranged from < 0.01 to 179 mg C m^{-2} with a prominent increase in spring (Fig. 3.7a). The annual house production rate was 8.4 g C m^{-2} y^{-1} in 2001/2 and 3.4 g C m^{-2} y^{-1} in 2002/3 (Table 3.2), giving a P_{total}/B ratio (where $P_{total} = P_{somatic} + P_{house}$) of 130 in 2001/2 and 150 in 2002/3.

The population biomass of *O. labradoriensis* ranged from 0.06 to 22.1 mg C m^{-2} and increased consistently during October/November and April with an annual mean of 6.8 $\pm$ 7.1 mg C m^{-2} from August 2001 to April 2002 and 8.5 $\pm$ 7.4 mg C m^{-2} from August 2002 to April 2003. These values are far less than those for *O. vanhoeffeni* (Fig. 3.7b, Table 3.2). Increase in population biomass during fall was the result of an increase in total abundance (Fig. 3.4b) while the large spring biomass was primarily a result of the

appearance of large animals (Fig. 3.6). Daily somatic production of the *O. labradoriensis* population ranged from < 0.01 to 0.24 mg C m^{-2}, increasing during fall and spring (Fig. 3.7b); annual production was 21.9 mg C m^{-2} y^{-1} in 2001/2 and 27.8 mg C m^{-2} y^{-1} in 2002/3 (Table 3.2). The annual $P_{somatic}/B$ ratios were similar in both years with a value of 3.2 in 2001/2 and 3.3 in 2002/3 (Table 3.2). Daily house production of the *O. labradoriensis* population ranged from 0.02 to 8.18 mg C m^{-2} with an increase during fall and spring. The annual house production rate was 0.8 g C m^{-2} y^{-1} in 2001/2 and 1.2 g C m^{-2} y^{-1} in 2002/3 (Table 3.2), resulting in a P_{total}/B ratio of 121 in 2001/2 and 144 in 2002/3.

3.3.4. *Relationship between life history characters and environmental variables*

The statolith diameter-at-maturity of *O. vanhoeffeni* and *O. labradoriensis*, which is a proxy for age-at-maturity, increased during winter and spring (Fig. 3.8) as temperature decreased and chlorophyll *a* concentration increased (Fig. 3.9). The linear, inverse relationship with temperature accounted for a greater proportion of the total variation in statolith diameter-at-maturity in both species (ca. 70 %) than did the non-linear, positive hyperbolic tangent relationship with chlorophyll *a* concentration (59 %) (Fig. 3.9).

Trunk length-at-maturity in both species increased during winter and spring as temperature decreased and chlorophyll *a* concentration increased (Fig. 3.10, Fig. 3.11). Trunk length-at-maturity ranged from 0.5 to 3.4 mm (680 %) in *O. vanhoeffeni* and from 0.5 to 1.3 mm (260 %) in *O. labradoriensis* over the entire sampling period. The range of trunk length-at-maturity was equal in both species (0.5-0.9 mm) from July to December but greater in *O. vanhoeffeni* (0.7-3.4 mm) than in *O. labradoriensis* (0.8-1.3 mm) from January to June. *O. vanhoeffeni* matured at a trunk length about two to three times greater than that of *O. labradoriensis* during spring (March-May). Stepwise multiple regression was used to examine whether temperature and chlorophyll *a* concentration were related to the seasonal variation in trunk length-at-maturity. Because the results from analysis of somatic growth (Results 3.3.3) suggest that both species grew

continuously, the older individuals that matured at low temperature during winter and spring were obviously larger than younger individuals that matured at higher temperature during summer and fall. The effect of chlorophyll *a* on trunk length-at-maturity may be less clear because a significant relationship between trunk length-at-maturity and chlorophyll *a* concentration could be a result of the covariation between temperature and chlorophyll *a* concentration. For these reasons, stepwise forward regression was used, where temperature was included first in the model and chlorophyll *a* concentration second. Temperature explained 57 % of the variation in trunk length-at-maturity of *O. vanhoeffeni* and chlorophyll *a* concentration explained an additional 10 % of the variation ($F(2, 23) = 21.0$, $p < 0.001$). Similarly, temperature explained 43 % of the variation in trunk length-at-maturity in *O. labradoriensis* and chlorophyll *a* concentration explained an additional 13 % of the variation ($F(2, 20) = 11.5$, $p < 0.005$).

Potential fecundity of both species also varied seasonally, increasing during winter and spring and decreasing during summer and fall (Fig. 3.10). *O. vanhoeffeni* showed a greater annual range of potential fecundity (79-4976 oocytes ind^{-1}) than did *O. labradoriensis* (90-803 oocytes ind^{-1}). Potential fecundity of *O. vanhoeffeni* was negatively correlated with temperature ($r = -0.88$, $p = 0.02$, $n = 9$) and positively correlated with chlorophyll *a* concentration ($r = 0.63$, $p = 0.07$, $n = 9$) at 10 % significance level. Potential fecundity of *O. labradoriensis* was also negatively correlated with temperature ($r = -0.59$, $p = 0.04$, $n = 12$) and positively correlated with chlorophyll *a* concentration ($r = 0.91$, $p < 0.001$, $n = 12$). Because potential fecundity of both species was a function of trunk length-at-maturity (Fig. 3.12), seasonal variation in potential fecundity is most likely related to variation in temperature and chlorophyll *a* concentration, as it is for trunk length-at-maturity.

3.3.5. *Population growth rate in relation to life history characters*

O. vanhoeffeni recruitment occurred primarily in spring, with maximum population growth rates of 0.08 d^{-1} at the end of April 2001 and 0.18 d^{-1} in May 2002. *O. vanhoeffeni* suffered high mortality during summer with negative growth rates of -0.15

d^{-1} in August of both years (Fig. 3.13a). There was little or no evidence of overwintering mortality. Population growth rate increased as statolith diameter-at-maturity (i.e. age-at-maturity) increased (Fig. 3.13b) and as potential fecundity increased (Fig. 3.13c). *O. labradoriensis* recruitment occurred primarily in the fall, with maximum population growth rates of 0.16 d^{-1} at the beginning of October 2001 and 0.15 d^{-1} at the end of September 2002. *O. labradoriensis* experienced net mortality during summer with negative population growth rates of -0.05 to -0.10 d^{-1} from May to August of all years (Fig. 3.14a). Unlike *O. vanhoeffeni*, the population growth rate of *O. labradoriensis* increased as age-at-maturity decreased (Fig. 3.14b) and was unrelated to potential fecundity (Fig. 3.14c). Thus, population growth of *O. vanhoeffeni* was associated with increasing clutch size whereas that of *O. labradoriensis* was associated with increasing spawning frequency.

3.4. Discussion

The boreal appendicularians *O. vanhoeffeni* and *O. labradorienis* in Conception Bay experience subzero temperatures for about six months of the year and their generation times range from eight months to a year. These generation times are similar to previous estimates of an annual life span for *O. vanhoeffeni* in the Foxe Basin, Canadian Arctic archipelago (Grainger 1959) and for *O. labradoriensis* in the fjords of east Greenland (Ussing 1938), based on the observation of seasonal variation in the abundance and appearance of small juveniles. Generation times of appendicularians living in cold environments are much greater than those of species that live in temperate and tropical regions, which range from 1-27 d at 7-29 °C and vary inversely with temperature (Lopéz-Urrutia et al. 2003 and references therein).

Thus, the generation time of appendicularians on a global scale shows a negative relationship with temperature from -1 to 29 °C, as is generally the case in poikilotherms (Shaw and Bercaw 1962, Gillooly 2000). One clearly cannot apply individual and population growth and production rates from tropical and temperate species to cold ocean species when constructing simulation models of arctic and boreal energy flow.

The cohort analysis of *O. labradoriensis* showed that a portion of cohort 1 reproduced in the fall and in the spring of 2001 and 2002 and the repeated event occurred when cohort 3 reproduced in the fall and in the spring of 2002 and 2003. These two separate major spawning events in each year may have originated from two reproductively isolated populations since Conception Bay is not an enclosed region but is open to advection from the Arctic via the Labrador Current (Myers et al. 1990, deYoung and Sanderson 1995). Alternatively, if the assumption made in this study that appendicularians in Conception Bay all belonged to a single population is true, then the two separate major spawning events of *O. labradoriensis* suggest a polymorphism which is a presence of multiple phenotypes in a population. The variation in reproductive timing may have evolved in *O. labradoriensis* thereby spreading the risk of survival in a temporally heterogeneous environment. Such strategy is described as bet hedging (Slatkin 1974, Siaiah and Perrin 1990, Wilber and Rudolf 2006) or spreading of risk in time (den Boer 1968).

Individual growth of the cohorts of the two species was generally exponential, except for the discontinued growth of cohort 2 of *O. vanhoeffeni*. This continuous growth demonstrates that individuals obtained sufficient energy to maintain growth throughout the year, including over winter. The omnivorous and efficient feeding behaviour of these species may explain continuous growth given that appendicularians are able to feed on a wide range of food material that includes dissolved organic matter to bacteria, flagellates and large diatoms (Deibel and Turner 1985, Flood et al. 1992, Bedo et al. 1993, Acuña et al. 1996, Fernández et al. 2004). Appendicularians in Conception Bay are able to ingest most of the seasonally variable prey species, ranging from a large proportion of bacteria and flagellates in summer and fall, supplemented by diatoms in spring (Urban et al. 1992). Regardless of prey type, the food concentration was adequate to support continuous growth in most cases.

Continuous, exponential somatic growth during sexual development in both species indicates that individuals obtained sufficient energy to maintain growth and to fuel reproductive maturation, and that energy was not diverted from somatic growth

during development. However, the field samples collected in this study did not include animals that were in the final stages of gametogenesis, presumably because fully developed, fragile gonads ruptured during net collection. At this final stage in the maturation of gametes, secretion of house rudiments ceases and autolysis of oikplastic and digestive cells occurs as the autolysed energy is apparently re-invested in gonad development (Gorsky 1980, Fenaux and Gorsky 1983, personal observation).

O. vanhoeffeni grew faster than *O. labradoriensis* averaged over an entire generation (Table 3.2, *O. vanhoeffeni* 0.017-0.043 d^{-1}, *O. labradoriensis* 0.007-0.011 d^{-1}). Animals from cohort 2 of *O. vanhoeffeni* during 2001/2 attained up to six times the mean weight of animals from cohort 2 of *O. labradoriensis*, and animals from cohort 3 of *O. vanhoeffeni* during 2002/3 attained up to nine times the mean weight of animals from cohort 3 of *O. labradoriensis* (Fig. 3.5, Fig. 3.6). The interspecific variation in growth occurred primarily during spring and was most obvious in trunk length-at-maturity of the two species. Both species matured at the same range of trunk length during summer and fall (Fig. 3.10) at an age of about 6 to 9 months (Fig. 3.2, Fig. 3.4). However, *O. vanhoeffeni* matured at a trunk length about two to three times that of *O. labradoriensis* during spring, when they were about 10 to 12 months old. These results suggest that *O. vanhoeffeni* grew faster than *O. labradoriensis* during spring and that growth of *O. vanhoeffeni* was more affected by the spring diatom bloom than was that of *O. labradoriensis*, implying that food quality is an important determinant of interspecific variation in growth of appendicularians.

The weight-specific growth rates of appendicularians in Conception Bay are lower than those of other species from temperate and tropical regions (Table 3.2) and also lower than the range of 0.26 to 3.31 d^{-1} obtained from laboratory and field measurements at temperatures from 7 to 29 °C (Lopéz-Urrutia et al. 2003). The somatic production and P/B ratio of appendicularians in Conception Bay are also lower than values for species in temperate and tropical regions (Table 3.2), in part because growth rate is lower and biomass is higher in Conception Bay than in warm-water systems. This pattern is a typical example of the low turnover rate of energy in cold water ecosystems that results

from lower growth rate, higher biomass, and longer generation time (Waters 1977, Brey and Gerdes 1998). However, because of the high biomass, annual population house production rate is high and comparable to warmer systems, even though the daily house production rate of individual *O. vanhoeffeni* (i.e. 37 % of body weight, Deibel 1988) is lower than that of warm water species (40 to 300 % of body weight, Clarke and Roff 1990, Hopcroft and Roff 1998, Tomita et al. 1999, Sato et al. 2001). Population house production rates far exceed somatic production rates in *O. vanhoeffeni* whereas population house production by warm-water species is similar to somatic production (Table 3.2). Thus, it is essential to include house production in estimates of total carbon production by appendicularians, especially in cold water systems.

Assuming a mean annual primary production for Conception Bay of 131 $\pm$ 5 g C m^{-2} yr^{-1} (Tian et al. 2003), the total annual carbon production of *O. vanhoeffeni* and *O. labradoriensis* (somatic + house production) was 7.3 % of primary production in 2001/2 and 3.8 % in 2002/3. These are remarkably high figures, considering that the transfer efficiency between primary and secondary producers in marine ecosystems is approximately 10 to 13 % (Pauly and Christensen 1995, Ware 2000). Based on an estimate of mean mesozooplankton production (primarily copepods) of 10.1 g C m^{-2} yr^{-1} in Conception Bay (Tian et al. 2003), production of oikopleurids represented 95 % of mesozooplankton production in 2001/2 and 49 % in 2002/3. This range is similar to estimates from studies in other locations. Appendicularian production in Kingston Harbour, Jamaica, is at least 50 % of copepod production (Hopcroft and Roff 1998) and may exceed copepod production (Hopcroft and Roff 1995). Furthermore, temperate epipelagic appendicularians can represent an average of 10 % and up to 40 % of total mesozooplankton production in productive environments (López-Urrutia et al. 2003).

The ranges of statolith diameter of *O. vanhoeffeni* and *O. labradoriensis,* whose generation times ranged up to one year, were similar throughout the year (*O. vanhoeffeni,* 9-17 μm, Fig. 3.2a, *O. labradoriensis,* 8-17 μm, Fig. 3.4a). However, the ranges of body size of these species differed greatly (*O. vanhoeffeni* 0.6-110 μg C, Fig. 3.5, *O. labradoriensis,* 0.5-13 μg C, Fig. 3.6). These results suggest that statolith diameter is a

general age indicator in oikopleurid species but that body size does not relate to age in a direct and simple way. The fact that rates of increase in statolith diameter are similar in both species also suggests that growth of this organ may be decoupled from physiological processes.

Statolith diameter-at-maturity in both species was maximal during spring and minimal during late summer and fall. This seasonal variation showed a strong, linear inverse relationship with temperature for both species, accounting for ca. 70% of the total variation. This pattern indicates slower development during winter and more rapid development during summer, in accordance with the general paradigm of the relationship between developmental rate of poikilotherms and temperature (Allen 1976, Vidal 1980, Gillooly 2000). The weaker, positive, non-linear relationship between chlorophyll *a* concentration and statolith diameter-at-maturity is most likely indirect, as a result of the non-linear, inverse relationship between temperature and chlorophyll *a* concentration.

Stepwise regression analysis showed that trunk length- at-maturity in *O. vanhoeffeni* and *O. labradoriensis* in Conception Bay was negatively correlated with temperature and positively correlated with chlorophyll *a* concentration. Only one other field study has described the relationship between environmental variables and trunk length-at-maturity of an appendicularian. Trunk length-at-maturity of *Oikopleura dioica* in the Inland Sea of Japan varies seasonally and is inversely related with temperature (Uye and Ichino 1995). However, laboratory studies have not confirmed this relationship. For example, trunk length-at-maturity of *O. dioica* in the laboratory remained the same at 15 °C and 25 °C (Fenaux 1976) and did not change at different food concentrations (Troedsson et al. 2002). This lack of variability in trunk length-at-maturity in the laboratory may be a result of low genetic variation or to a container effect. However, this question requires further laboratory research.

Both species experience negative population growth from June to August. This period of high mortality may result from intense competition for food during summer when the plankton food web is most active. The abundance of zooplankton in Conception Bay e.g. the copepods *Calanus finmarchicus*, *Pseudocalanus* spp. and

Oithona similis, and the appendicularian *Fritillaria borealis,* increases between June and September (Davis 1982). Predation may also contribute to high mortality in oikopleurids during summer. Recruitment of the chaetognath *Parasagitta elegans*, a major predator of appendicularians (Feigenbaum 1982, Alvarez-Cadena 1992, Purcell et al. 2005), also occurs in the upper water column of Conception Bay during July and August (Davis 1982, Choe and Deibel 2000). Furthermore, the larvae of many fish species increase in Conception Bay and in other Newfoundland coastal regions in July and August (Frank and Leggett 1981, Davis 1982, Frank and Leggett 1983, Laprise and Pepin 1995). The causes of negative population growth of cold ocean appendicularians in late summer require further research, but it is noteworthy that population growth rates are lower in late summer than in mid-winter.

In Conception Bay, *O. vanhoeffeni* recruits primarily at the coldest time of the year, when the water column is well mixed and isothermal, with spawning ending following the spring phytoplankton bloom, when phytodetritus is sinking and the thermocline is beginning to form in the upper 20 m of the water column. During this time, age-at-maturity, size-at-maturity and fecundity are at a maximum. Thus, *O. vanhoeffeni* has a life history strategy that maximizes clutch size. In contrast, *O. labradoriensis* recruits primarily in the fall, when the upper mixed layer is warmest and when age-at-maturity, size-at-maturity and fecundity are at a minimum. Thus, *O. labradoriensis* has a life history strategy that maximizes spawning stock size and high population turnover rates. It appears that a trade-off between the timing of reproduction (adult survival) and number of offspring (juvenile survival) occurred in both species but in opposite directions. Life history theory predicts that optimum age-at-maturity can be determined by variation in age-specific mortality (Gadgil and Bossert 1970, Charnov and Schaffer 1973, Michod 1979). High juvenile mortality but low adult mortality is predicted to select for delayed maturation (Gadgil and Bossert 1970, Schaffer 1974, Lonsdale 1981, Belk 1998, Barata et al. 2001) and high adult mortality is predicted to select for early maturation (Schaffer 1974, Hernaman and Munday 2005, Depczynsky and Bellwood 2006). If this theory holds true for oikopleurids, adult mortality should be

lower in *O. vanhoeffeni* than in *O. labradoriensis*. Further examination of age-specific mortality in relation to selective pressures such as predation, food availability, and suitability of the physical environment is crucial for an understanding of interspecific variation in life history strategies of appendicularians.

Table. 3.1. *Oikopleura vanhoeffeni* and *Oikopleura labradoriensis*

Parameters for growth equations. The exponential growth equation, $W_t = W_0 e^{gt}$, was fitted to all cohorts except cohort 2 of *O. labradoriensis*, for which linear equation, $W_t = gt + W_0$, was fitted. n = number of time points in which the growh rate was determined. Error estimates are standard errors.

Species	Cohort #	$W_0 \pm$ SE	$g \pm$ SE	r^2	p	n
Oikopleura vanhoeffeni	2	0.191 ± 0.047	0.017 ± 0.001	0.96	< 0.001	12
	3	0.494 ± 0.044	0.043 ± 0.001	0.99	0.014	3
Oikopleura labradoriensis	1	0.743 ± 0.132	0.010 ± 0.001	0.86	< 0.001	12
	2	0.628 ± 0.301	0.011 ± 0.003	0.74	0.003	9
	3	0.299 ± 0.085	0.008 ± 0.001	0.82	< 0.001	12
	4	0.557 ± 0.105	0.007 ± 0.002	0.73	0.007	8

Table 3.2. Mean biomass (B), instantaneous growth rate (g), annual somatic production (Pg), annual house production (Pe), and Pg/B ratio of appendicularian species.

	Location	T (^{o}C)	B (mg C m^{-2})	g (d^{-1})	Pg (g C m^{-2} yr^{-1})	Pe (g C m^{-2} yr^{-1})	Pg/B	Reference
O. dioica	Seto Inland Sea	8.9-28.2	12	0.26-3.0	7.15	-	596	Uye and Ichino (1995)
Appendicularians	Off Lime Cay	27-29	5.5	1.56	1.9-4.6	1.2-2.4	346-836	Clarke and Roff (1990)
Appendicularians	Kingston Harbour	27-30	15.5	2.03-2.49	14	7.1-14.3	903	Hopcroft and Roff (1998)
O. longicauda	Toyama Bay	11.1-23.5	25.6	0.592	4.5	11.3	176	Tomita et al. (1999)
O. vanhoeffeni	Conception Bay	-1- 6 *	25-66	0.017-0.043	0.343-0.359	3.4-8.4	5.2-14.3	This study
O. labradoriensis	Conception Bay	-1- 6 *	6.8-8.5	0.007-0.011	0.022-0.028	0.8-1.2	3.2-3.3	This study

* Mean temperature was taken above 100 m where most of the oikopleurids were found. Surface temperature ranged from -1 to 17^{o}C.

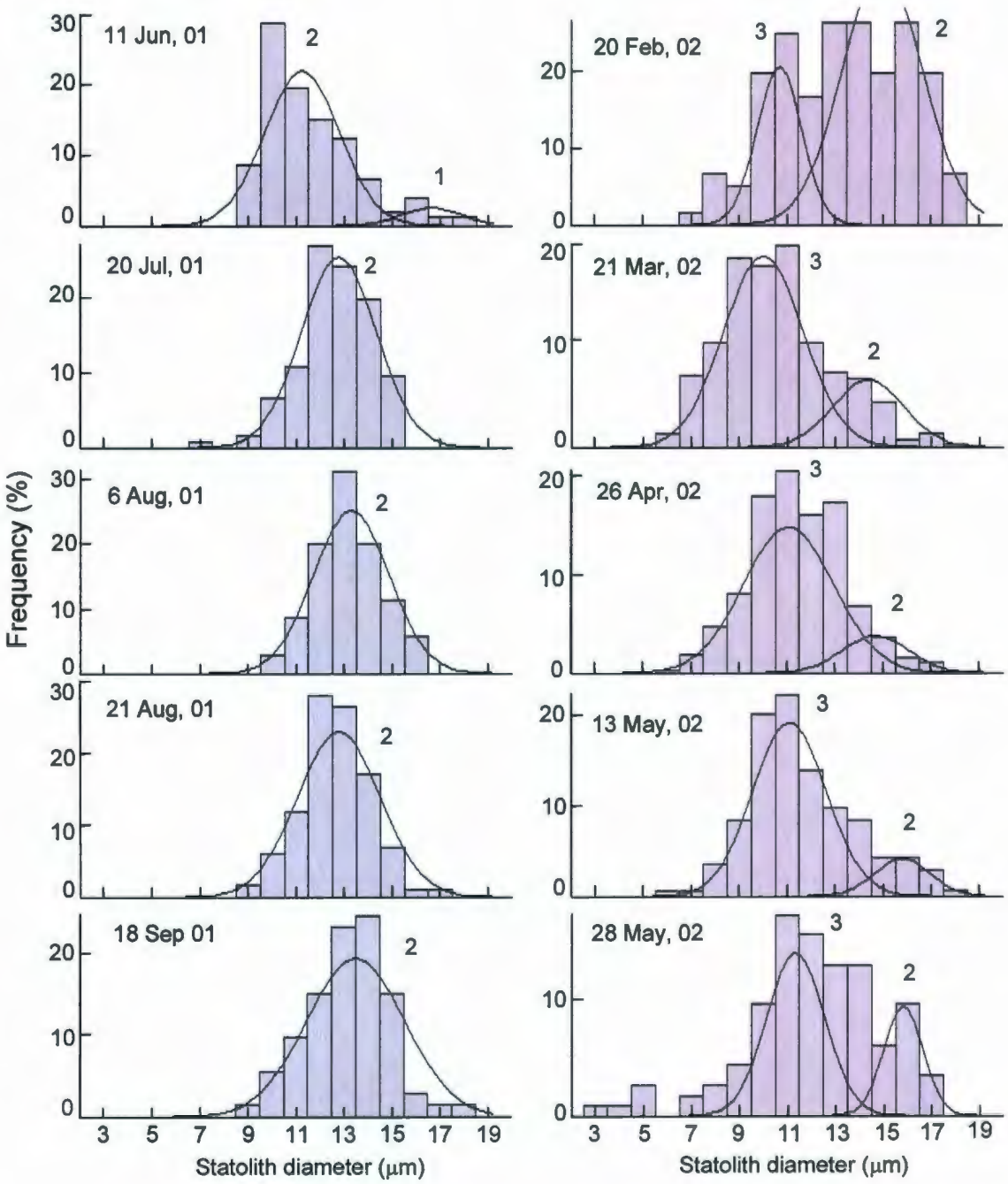


Fig. 3.1. *Oikopleura vanhoeffeni*
Frequency distributions of statolith diameter from samples collected between 11 June 2001 and 25 June 2003. Individual cohorts were defined as normally distributed components of the sample distributions (see Methods).

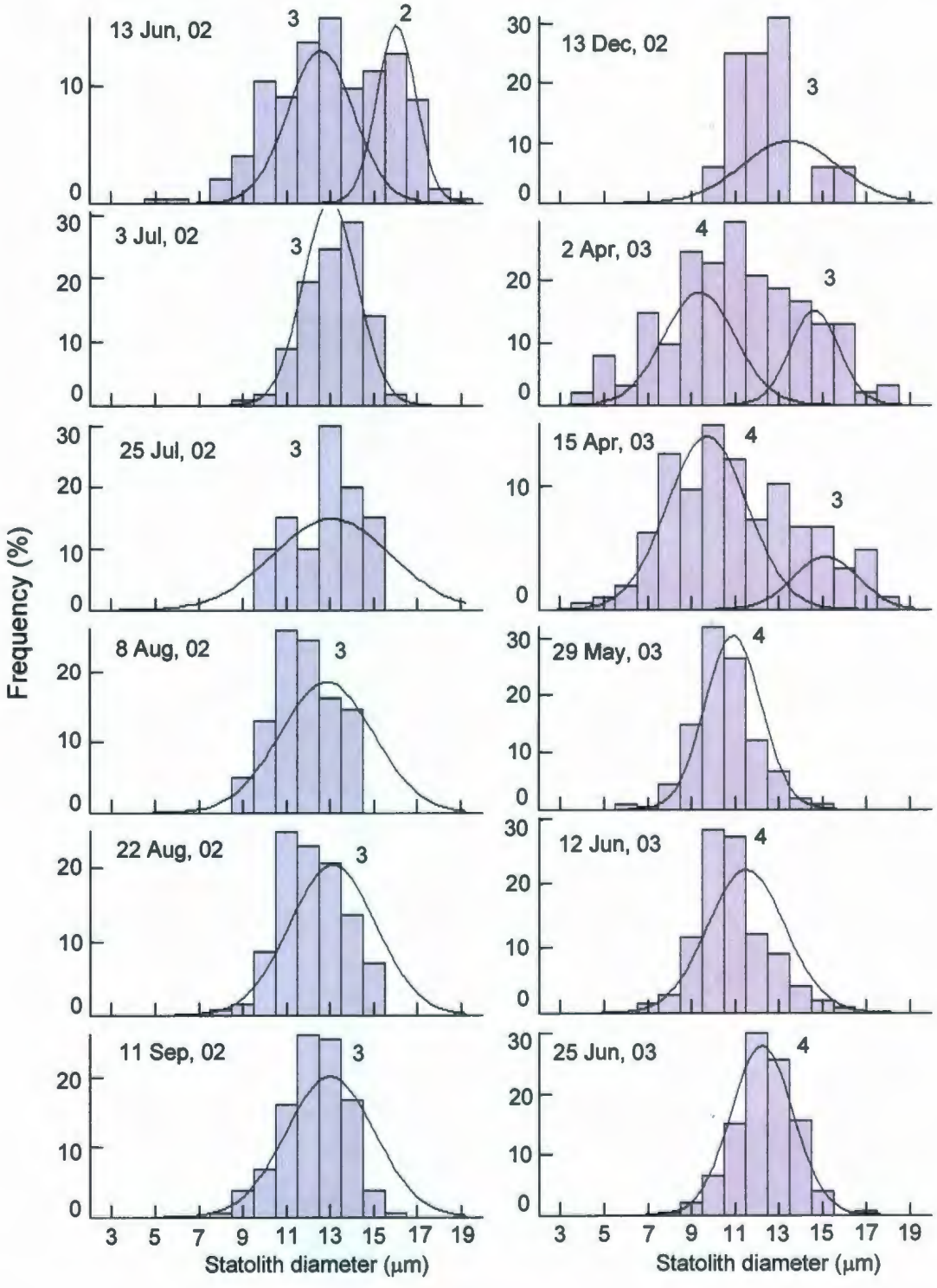


Fig. 3.1. Cont'd. *Oikopleura vanhoeffeni*

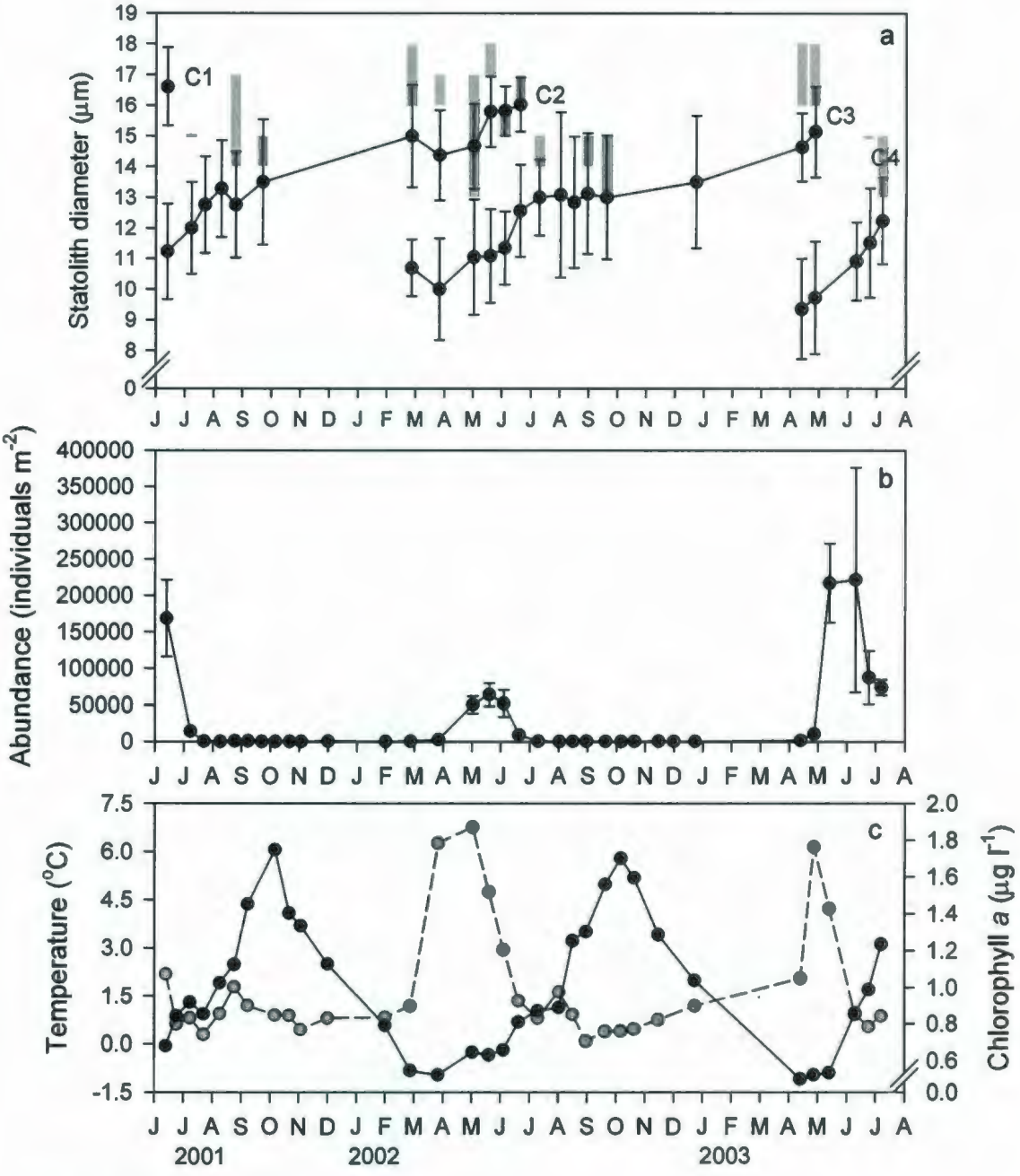


Fig. 3.2. *Oikopleura vanhoeffeni.*
(a) Time series of statolith diameter for cohorts 2, 3, and 4. Cohorts were defined as in Fig. 3.1. Shaded areas represent the range of statolith diameter at maturity. (b) Time series of mean areal abundance. (c) Time series of mean temperature (solid line) and concentration of chl *a* (dashed line) over the upper 100 m water column. Temperature and Chl *a* measurements were bin-averaged at 1 m depth intervals before computation of the vertically integrated mean.

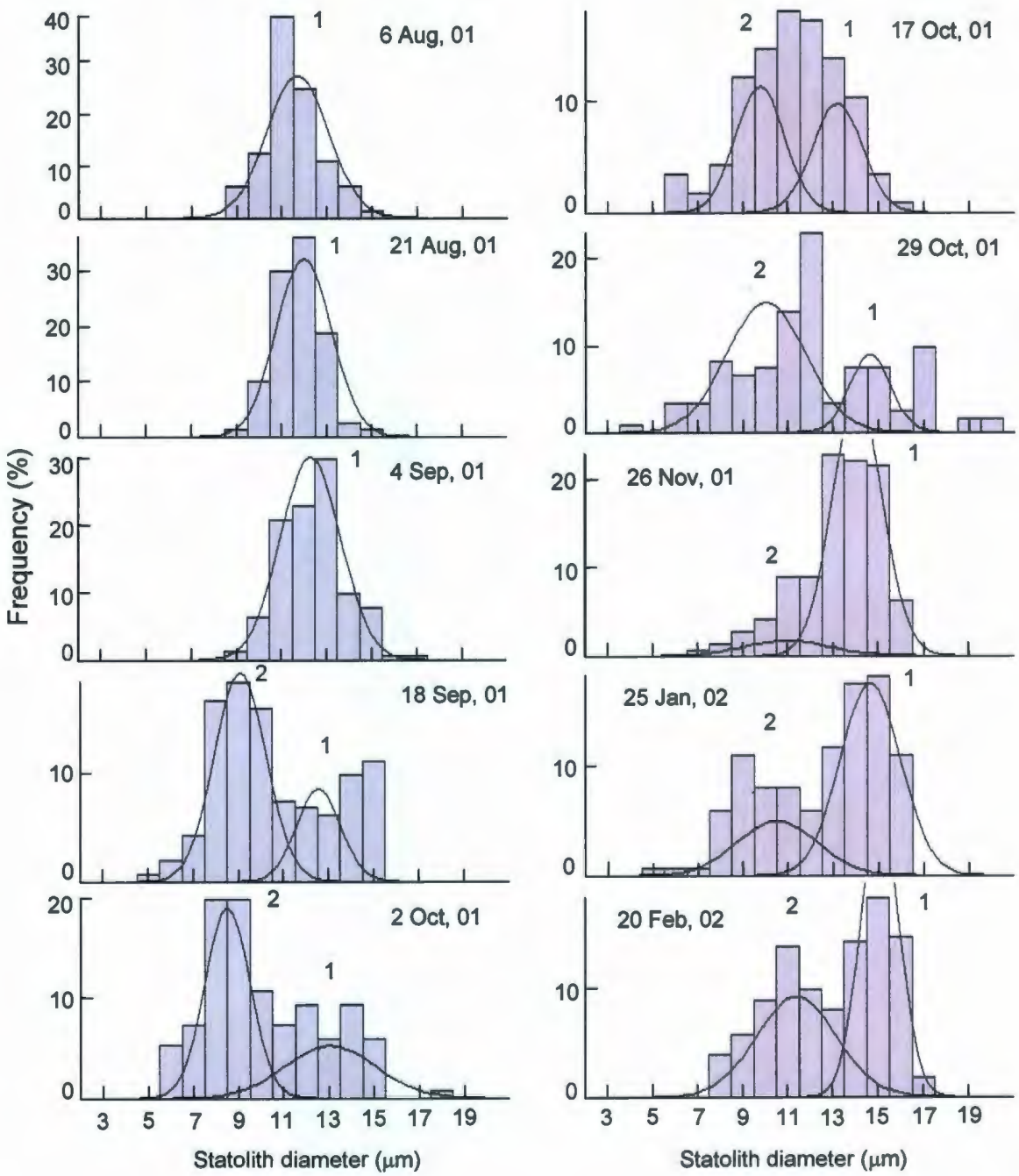


Fig. 3.3. *Oikopleura labradoriensis*
Frequency distributions of statolith diameter from samples collected from 6 August 2001 to 15 April 2003. Individual cohorts were defined as normally distributed components of the sample distributions (see Methods).

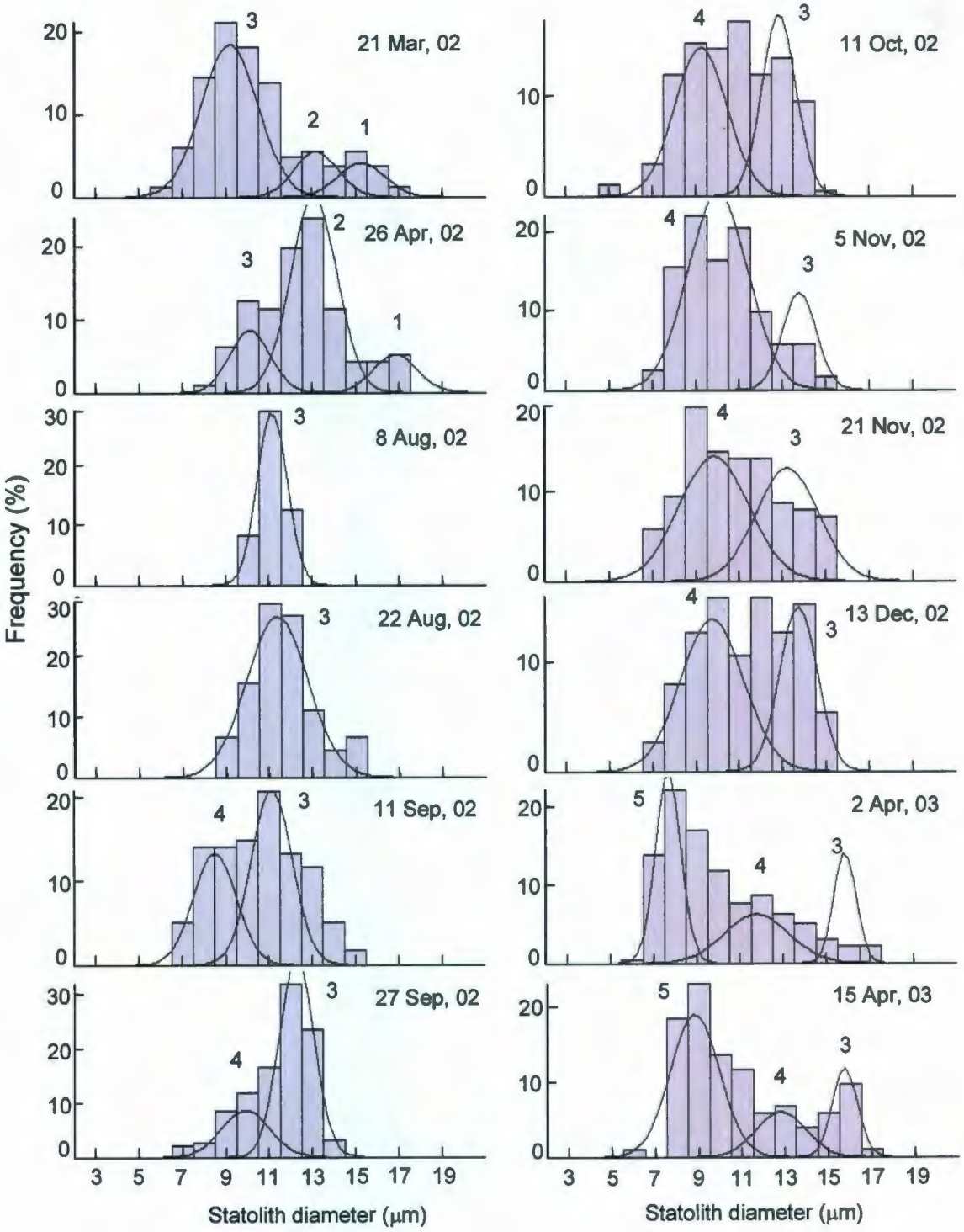


Fig. 3.3. Cont'd. *Oikopleura labradoriensis*

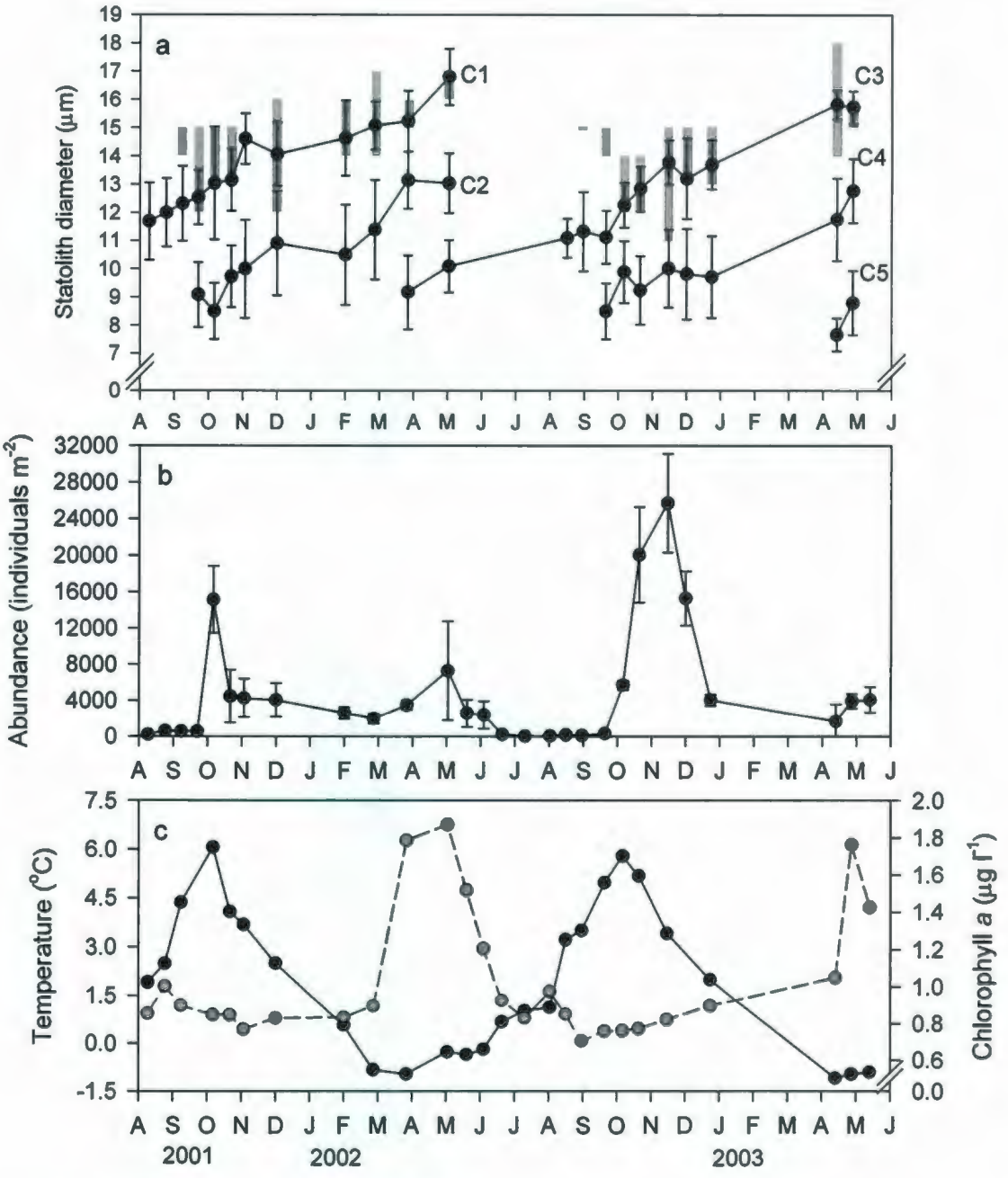


Fig. 3.4. *Oikopleura labradoriensis*
(a) Time series of statolith diameter for cohorts 1 to 4. Cohorts were defined as in Fig. 3.3. Shaded areas represent the range of statolith diameter at maturity. (b) Time series of mean areal abundance. (c) Time series of mean temperature (solid line) and Chl *a* concentration (dashed line) over the upper 100 m of the water column. Temperature and Chl *a* measurements were bin-averaged at 1 m depth intervals before computation of the vertically integrated mean.

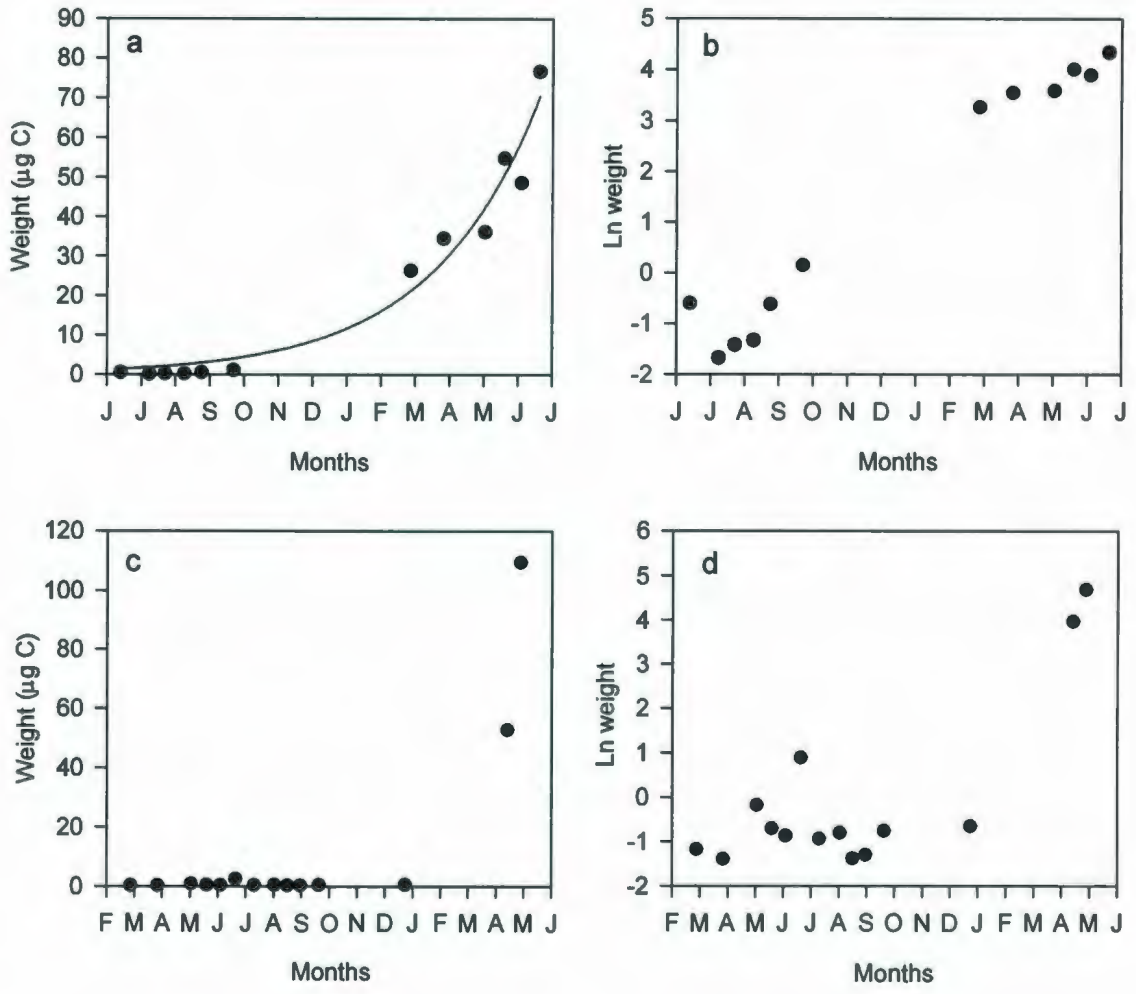


Fig. 3.5. *Oikopleura vanhoeffeni*.
(a) Weight of animals in cohort 2 vs. time. (b) ln-transformed weight of cohort 2 vs. time.
(c) Weight of animals in cohort 3 vs. time. (d) ln-transformed weight of cohort 3 vs. time.

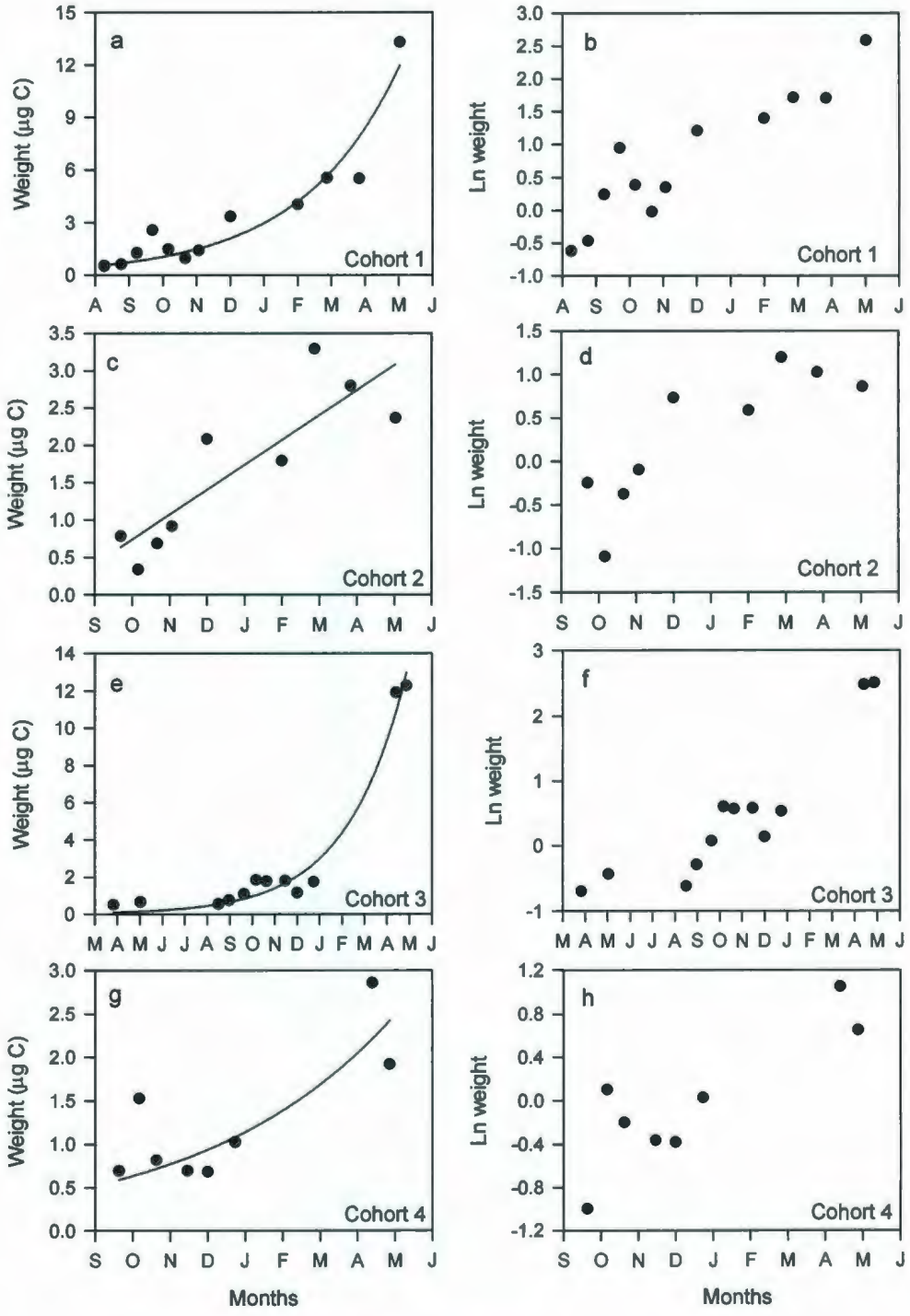


Fig. 3.6. *Oikopleura labradoriensis*.
a, c, e, g. Weight of animals in cohorts 1, 2, 3 and 4 vs. time.
b, d, f, h. ln-transformed weight of cohorts 1, 2, 3, and 4 vs. time.

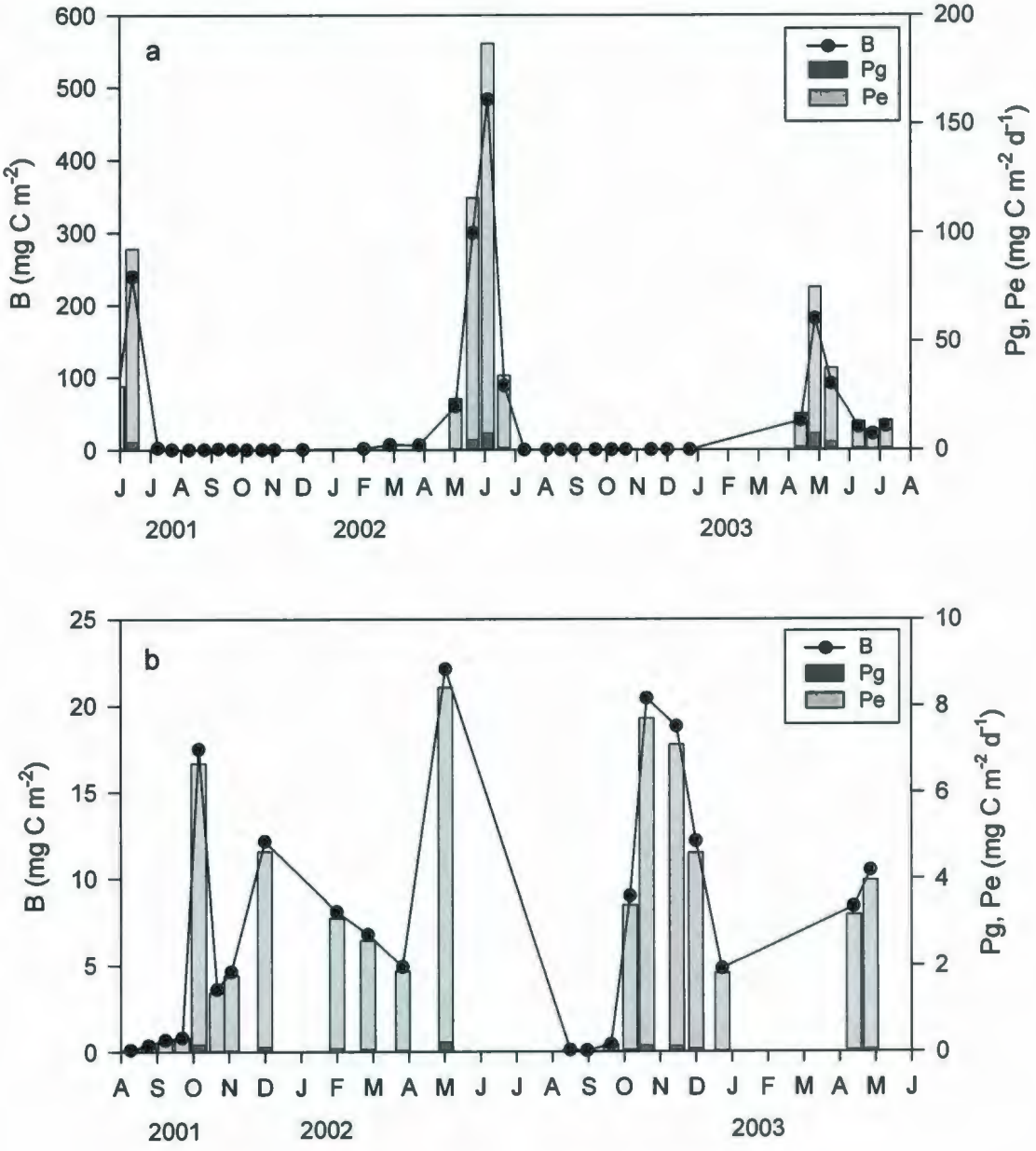


Fig. 3.7. *Oikopleura vanhoeffeni* and *Oikopleura labradoriensis*
(a) Biomass (B), somatic production (Pg) and house production (Pe) of *O. vanhoeffeni*. (b) Biomass, somatic production and house production of *O. labradoriensis*.

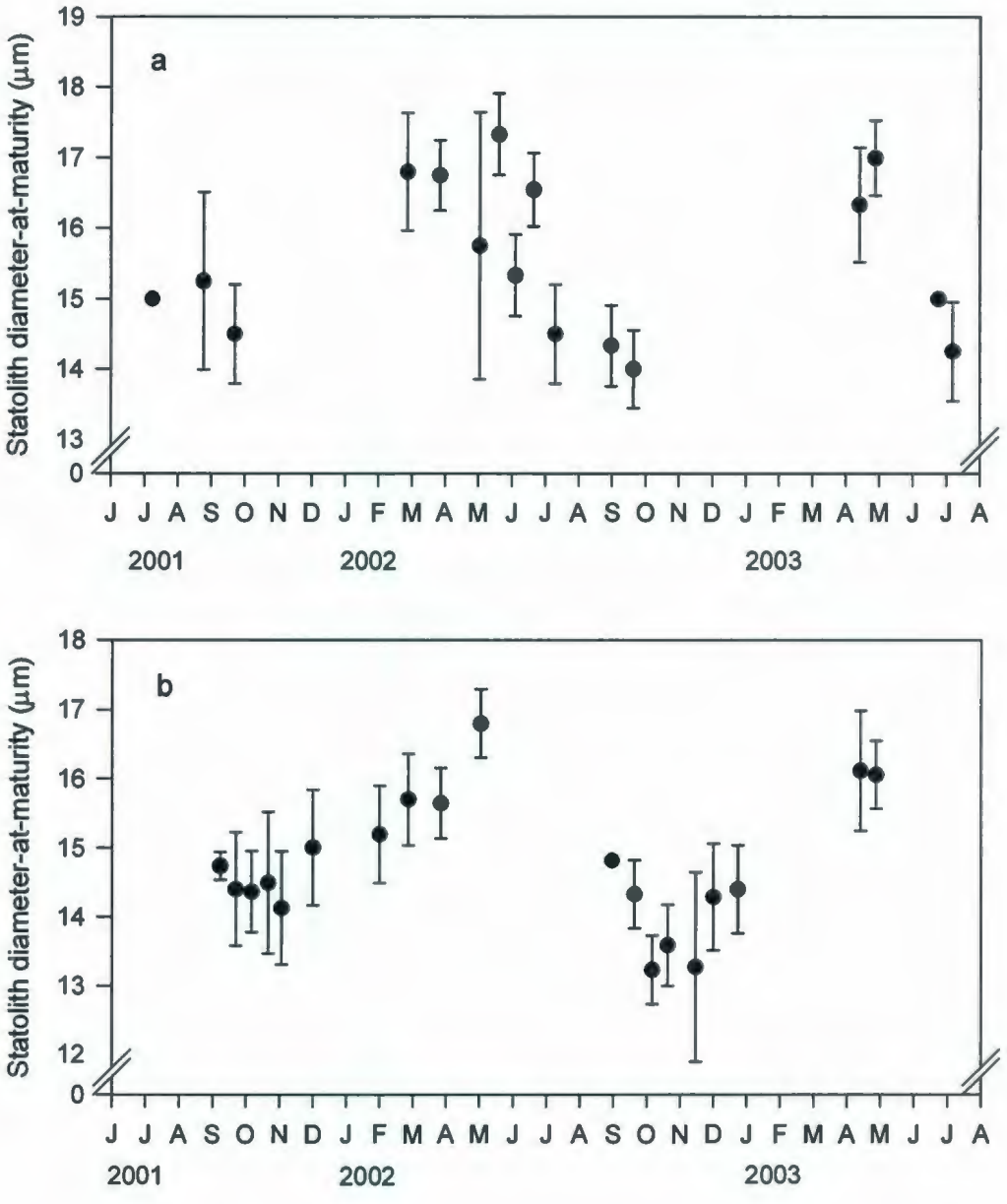


Fig. 3.8. *Oikopleura vanhoeffeni* and *Oikopleura labradoriensis* Time series of statolith diameter-at-maturity for (a) *O. vanhoeffeni* and (b) *O. labradoriensis*.

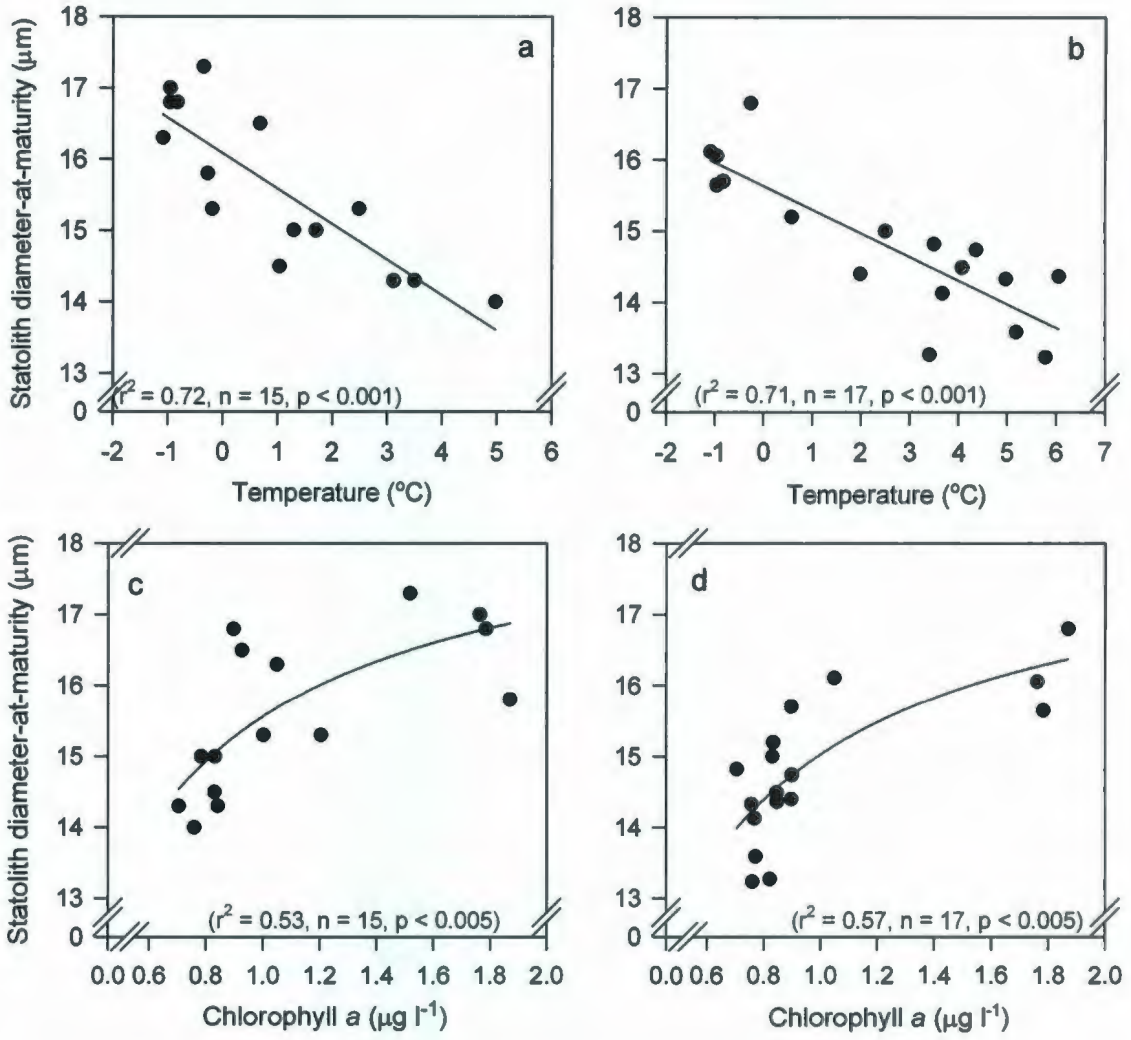


Fig. 3.9. *Oikopleura vanhoeffeni* and *Oikopleura labradoriensis*
Statolith diameter-at-maturity vs. temperature for (a) *O. vanhoeffeni* and (b) *O. labradoriensis*. Statolith diameter-at-maturity vs. concentration of chlorophyll *a* for (c) *O. vanhoeffeni* and (d) *O. labradoriensis*. A linear function was fitted to the relationship between statolith diameter-at-maturity and temperature and a rectangular hyperbola function, $y = ax/(b+x)$ was fitted to the relationship between statolith diameter-at-maturity and concentration of chlorophyll *a*.

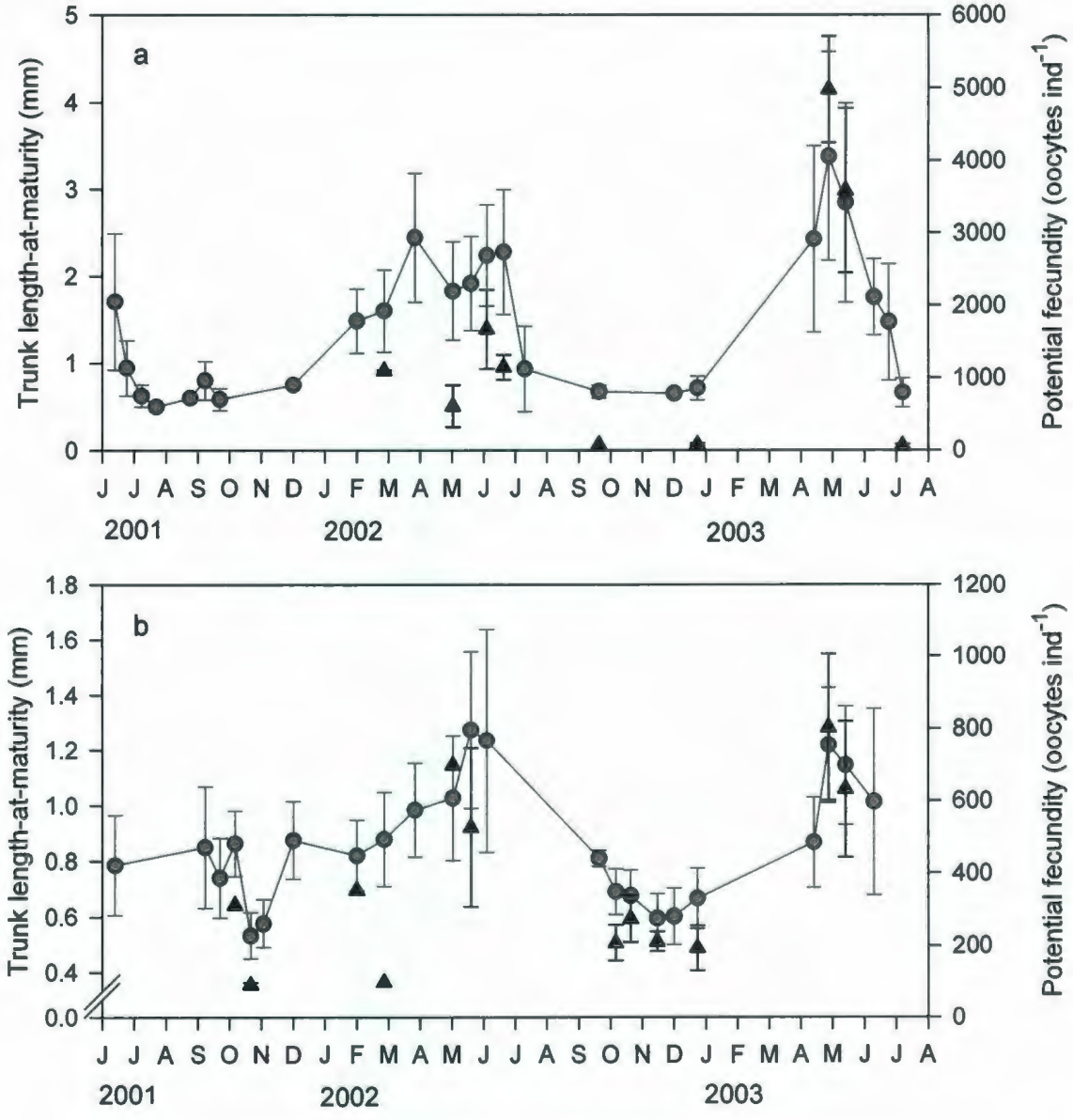


Fig. 3.10. *Oikopleura vanhoeffeni* and *Oikopleura labradoriensis* Time series of trunk length-at-maturity (circle) and potential fecundity (triangle) for (a) *O. vanhoeffeni* and (b) *O. labradoriensis*. The best estimate of actual fecundity is potential fecundity x 0.5 (see Methods).

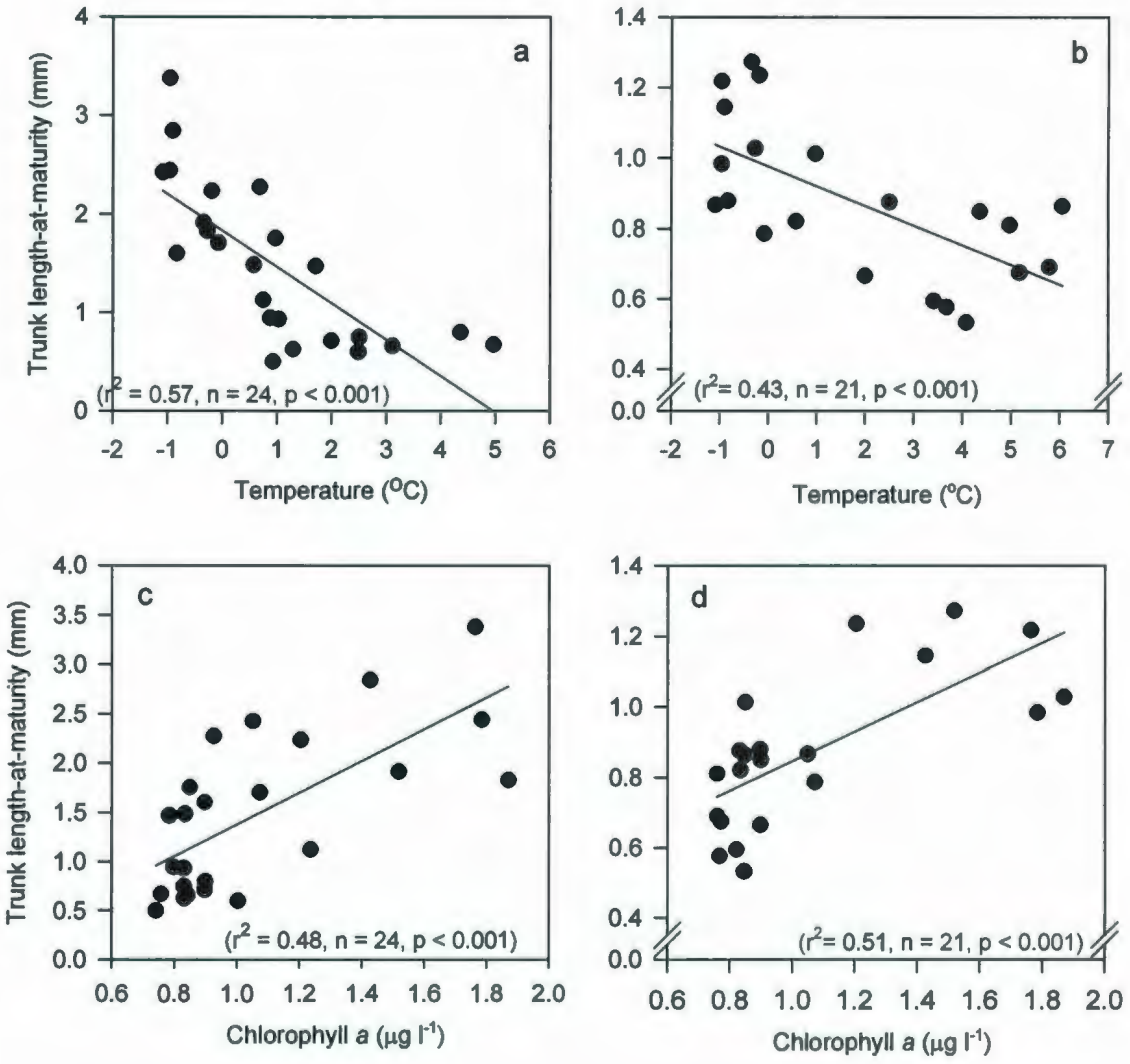


Fig. 3.11. *Oikopleura vanhoeffeni* and *Oikopleura labradoriensis* Trunk length-at-maturity vs. temperature for (a) *O. vanhoeffeni* and (b) *O. labradoriensis*. Trunk length-at-maturity vs. concentration of chlorophyll *a* for (c) *O. vanhoeffeni* and (d) *O. labradoriensis*. The solid lines represent the best fit least squares linear regression equations.

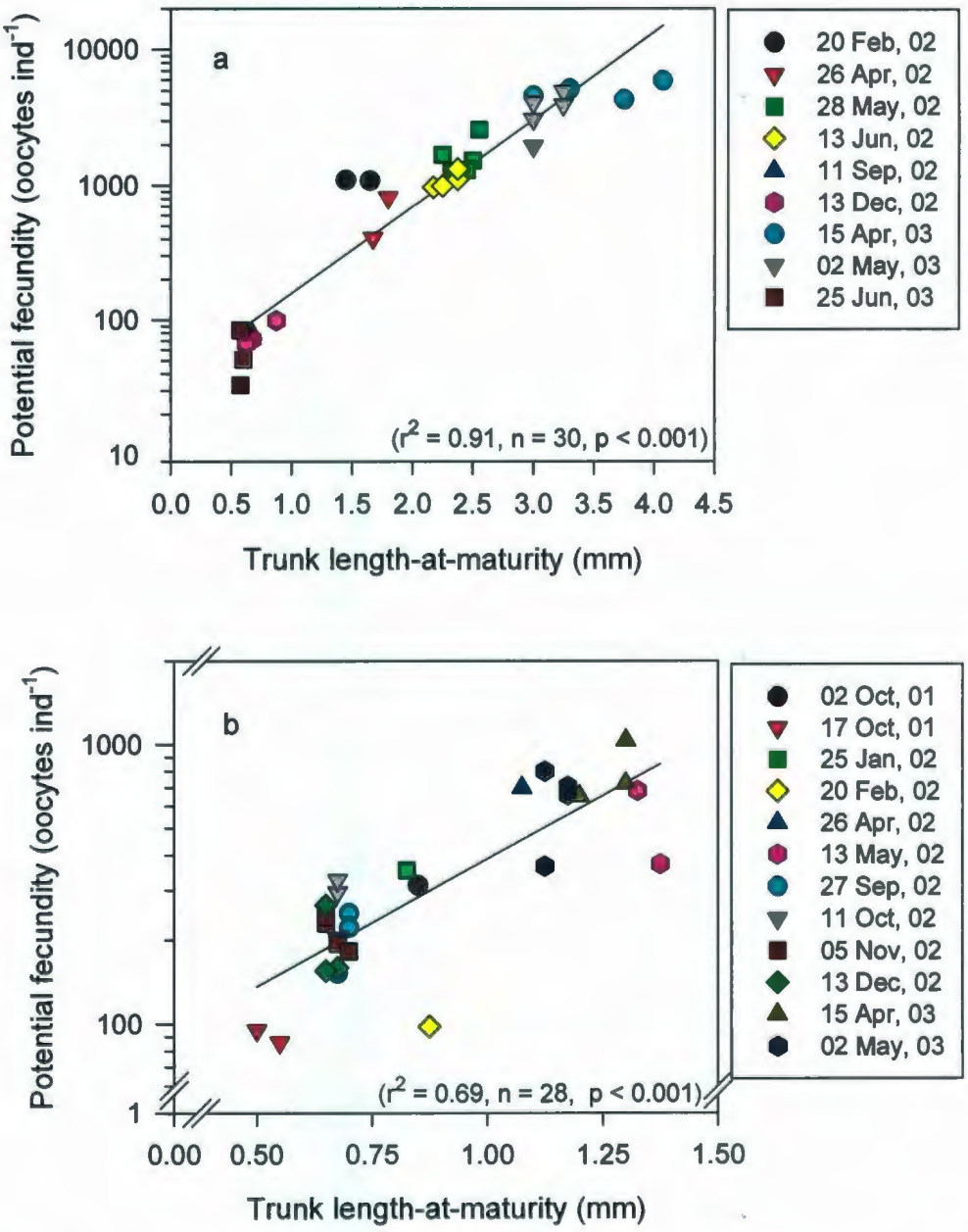


Fig. 3.12. *Oikopleura vanhoeffeni* and *Oikopleura labradoriensis* Trunk length-at-maturity vs. potential fecundity for (a) *O. vanhoeffeni* and (b) *O. labradoriensis*. The best estimate of actual fecundity is potential fecundity x 0.5 (see Methods).

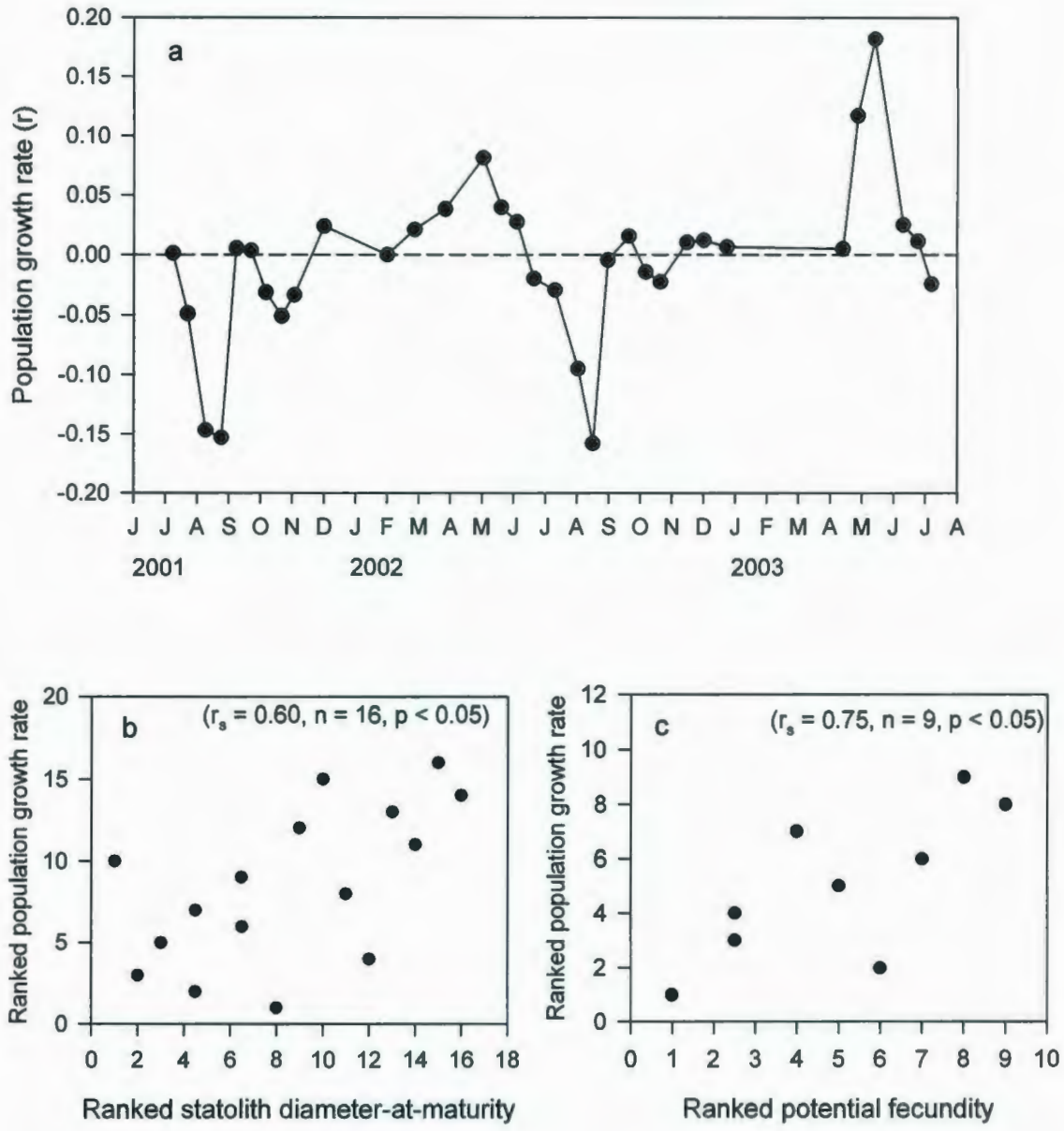


Fig. 3.13. *Oikopleura vanhoeffeni*
(a) Time series of population growth rate. (b) Spearman correlation by ranks between population growth rate and statolith diameter-at-maturity. (c) Spearman correlation by ranks between population growth rate and potential fecundity.

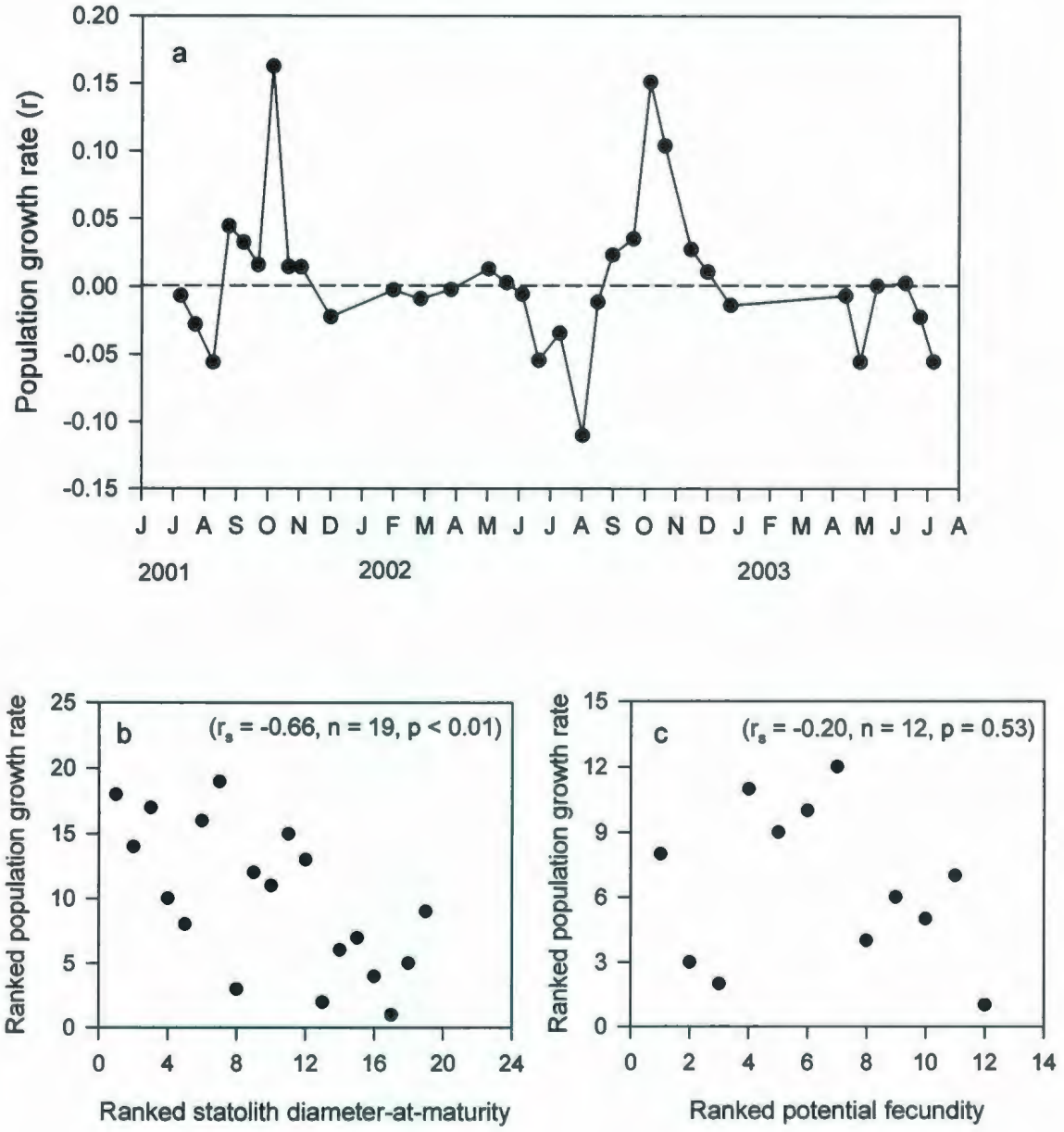


Fig. 3.14. *Oikopleura labradoriensis*
(a) Time series of population growth rate. (b) Spearman correlation by ranks between population growth rate and statolith diameter-at-maturity. (c) Spearman correlation by ranks between population growth rate and potential fecundity.

General Discussion

The main purpose of this thesis was to examine the population dynamics of boreal appendicularians in Conception Bay, Newfoundland in relation to variation in life history characters under fluctuating environmental conditions. The study required general understanding of temporal and vertical distribution of the species and a method that allows accurate assessment of age structure of appendicularian populations. The following discussion summarizes the main finding of this study and the questions raised.

Temporal and vertical distribution of appendicularian species in Conception Bay is defined by temperature and salinity. The clear niche separation in temperature-salinity space suggests that the level of physiological tolerance to temperature and salinity is important in predicting survivorship of each species. Embedded within this strong physical niche is evidence of a secondary biotic factor, food quality. The seasonal succession in appendicularian species in order of increasing body size, from *Fritillaria borealis*, the smallest species in summer, through the intermediate-sized *Oikopleura labradoriensis* in the fall to the largest *Oikopleura vanhoeffeni* in the spring, corresponds with the seasonal succession of prey species size, from picoplankton in summer through nanoplankton in fall to large diatoms in spring (Deibel and Turner 1985, Powell et al. 1987, Urban et al. 1992, Prasad and Haedrich 1993, Putland 2000). Evaluating the significance of food quality in the seasonal succession of appendicularian species requires further studies on the diet and relative nutritional contribution of assimilated food types to reproduction and growth. For these suspension feeders, in which size of ingestible particles is limited by the size of mesh on the house which, in turn, varies with body size (Flood and Deibel 1998), availability of prey species in a particular size range will likely influence population dynamics.

Evidence from my laboratory and field studies showed that statolith diameter is a robust and precise age indicator. In the laboratory, variability in statolith diameter-at-age remained low and constant as the individuals aged, whereas variability in trunk length-at-age was generally higher and increased with age. Using statolith diameter as an age

indicator for Conception Bay, NL field populations, trunk length-at-age in *O. vanhoeffeni* displayed clear seasonal variation, demonstrating that body size is not a reliable age indicator in field populations and that conventional cohort separation using modal progression analysis of length-frequency data may lead to inaccurate estimation of age distributions. In light of the fact that this is the first attempt to use statolith size as an age indicator for appendicularians, additional studies are necessary to test the general validity of this approach, particularly under various conditions of temperature and food quantity and quality.

Throughout the two years of this study, trunk length-at-age of *O. vanhoeffeni* varied seasonally and as a function of the different age groups. The most noticeable pattern was that length-at-age in the older and larger individuals increased in response to the spring diatom bloom while length-at-age of the younger individuals did not change, suggesting that older individuals with larger body size were able to feed on large diatoms and grow faster during spring. These results indicate that the intraspecific variation in growth was related to availability of ingestible particles. More importantly, the results imply that trunk length-at-age can be used as an index of growth status in appendicularians. This conclusion needs to be tested in the laboratory, where the absolute age of individuals as a function of variation in food concentration and size of food particles is known. In the field, future observations of length-at-age can be accompanied by an independent growth index, such as RNA content or RNA/DNA ratio (Sutcliff 1970, Båmstedt and Skjoldal 1980, Ikeda 1989, Acharya et al. 2004).

O. vanhoeffeni and *O. labradoriensis* grew exponentially throughout the year, indicating the absence of food limitation. Temperatures below zero during winter and spring did not have a negative affect on growth, suggesting that appendicularians may achieve temperature compensation in some of their physiological processes. In general, temperature compensation in physiological rates may involve two different mechanisms: basal metabolism of organisms at low temperatures may decrease, resulting in an increase in growth efficiency (Clarke 1987, 1991), or physiological rates are conserved in the presence of temperature variation (Hochachka and Somero 1984). The latter mechanism

has been observed in marine invertebrates and fishes in which both growth and metabolic compensation can be accomplished at lower temperatures (Nicieza and Metcalfe 1997, Vetter 1998, Hurst et al. 2005, Pörtner 2006). Thus, in order to understand how appendicularians maintain growth during winter, future studies will need to examine physiological energetics in relation to seasonal variation in temperature, which has never been done in appendicularians.

Production of appendicularian species was large in proportion to primary production or total copepod production. Thus, appendicularians represent an important part of the energy cycle in boreal marine ecosystem. The estimated population house production rates of *O. vanhoeffeni* and *O. labradoriensis* were far greater than were somatic production rates (Chapter 3, Table 3.2), in that house production represented 89 to 98 % of total somatic + house production. The annual house production of both species was approximately 3.5 to 7.0 % of primary production, indicating that a substantial portion of primary production may be exported from the euphotic zone to the benthos via sinking of appendicularian houses. In addition, the estimation of house production did not include adhered food particles and fecal pellets. Discarded houses can trap as much as 30 % of filtered particles (Gorsky 1980) and the concentration of particulate organic matter in the house is 10 times higher than that in the ambient water (Taguchi 1982). However, care should be taken in the interpretation of these data since the degradation rate of appendicularian houses during sinking is not known and sinking houses represent an important food source and substrate for microbial populations (Alldredge and Youngbluth 1985, Caron et al. 1986, Davoll and Silver 1986, Davoll and Youngbluth 1990), copepods (Ohtsuka and Kubo 1991, Ohtsuka et al. 1996) and eel larvae (Mochioka and Iwamizu 1996). No studies have yet shown that discarded appendicularian houses are a food source for benthic organisms, but this question is compelling.

Oikopleurid species in Conception Bay display different patterns of population growth. Population growth of *O. vanhoeffeni* is associated with an increase in size-at-maturity and fecundity and with a delay in age-at-maturity, whereas population growth of

O. labradoriensis is associated with a decrease in age-at-maturity. An important question is to determine the selection pressure that shapes variation in the life history pattern of these appendicularian species. Life history theory predicts that optimum age-at-maturity can be determined by variation in age-specific mortality (Gadgil and Bossert 1970, Charnov and Schaffer 1973, Michod 1979) in which external variables such as physical conditions, food availability, and predation pressure play important roles (Reznick et al. 2002). My study suggests that the lowest temperatures in winter and spring provide optimal physical conditions for the recruitment and survival of *O. vanhoeffeni* and that the spring diatom bloom results in an increase in growth rates of larger, older individuals, leading to increases in size-at-maturity and fecundity. Furthermore, the lowest salinities and highest temperatures in fall provide optimal physical conditions for *O. labradoriensis*, and the fall bloom may lead to enhanced rates of maturation and reproduction at an early age. Hypotheses regarding the causes of negative population growth in both species during summer e.g. intense resource competition and predation, remain to be tested. Thus, it will be important to study the age-specific mortality of appendicularians in order to gain better understanding of population dynamics through demographic analysis.

In conclusion, the temporal niche separation of co-occurring appendicularian species in a seasonally variable environment is defined by different optimal ranges of temperature and salinity. Within the optimal physical settings, each species displays different modes of population growth in which age-at-maturity and fecundity play significant roles. Seasonal variation in age-at-maturity, size-at-maturity and fecundity are closely related to temperature and food availability, suggesting a tight coupling between environmental change and population dynamics. The study also suggests that the effect of climate change on temperature and phytoplankton productivity and community structure (Richardson and Schoeman 2004, Hays et al. 2005, Hare et al. 2007) may influence the life history and demographic traits that regulate the population dynamics and secondary production of appendicularian species.

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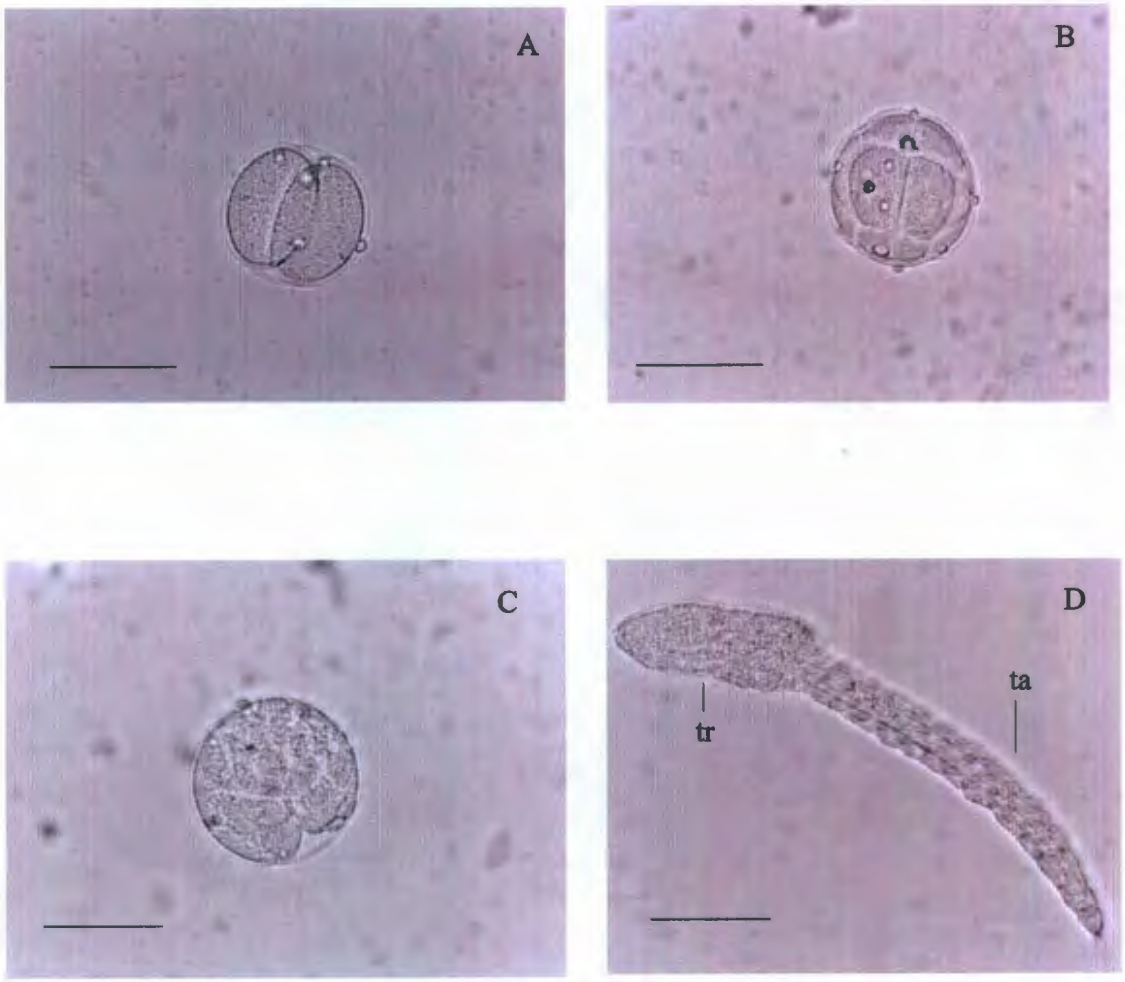
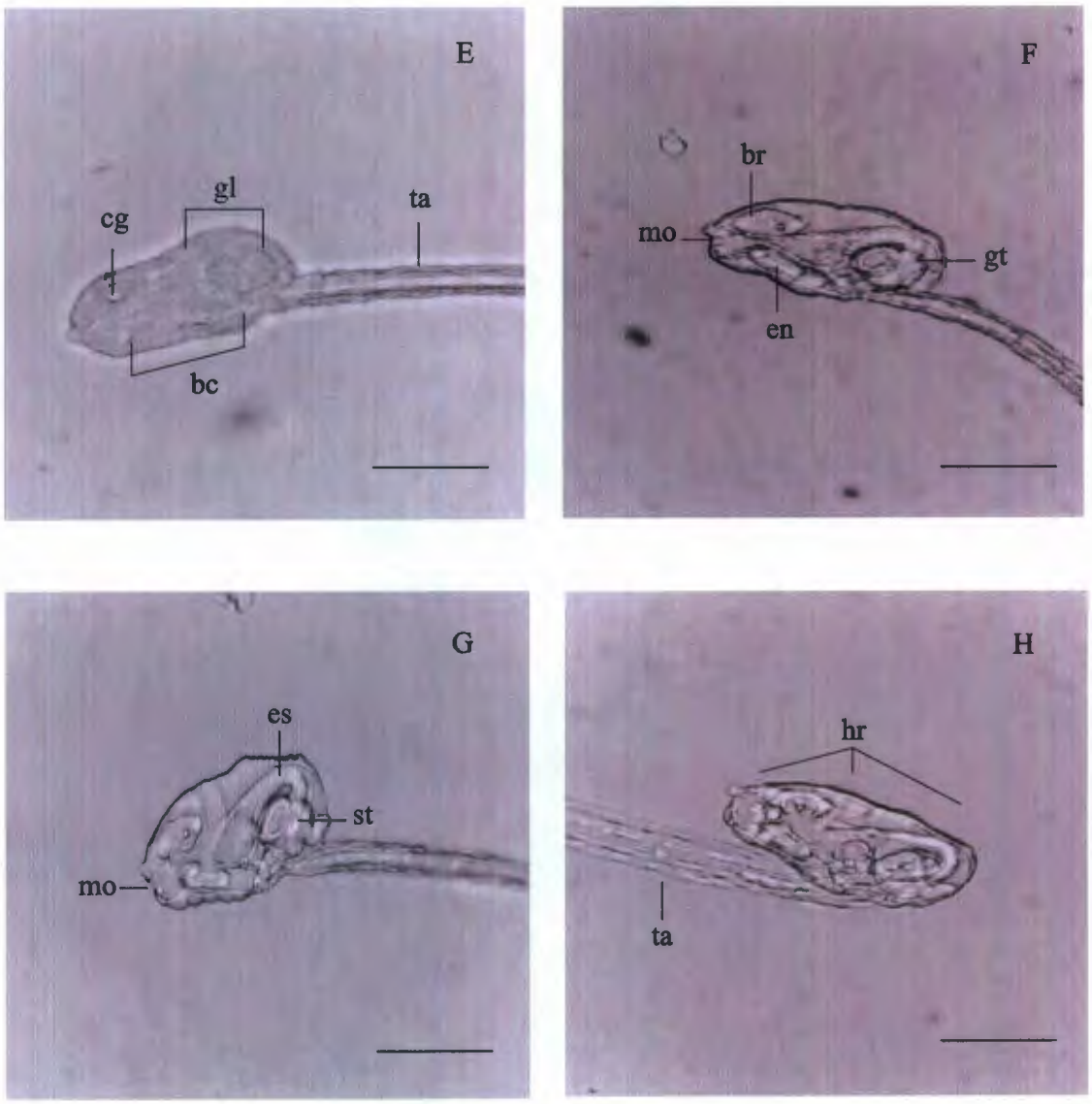


Appendix 1. Early developmental stages of *Oikopleura vanhoeffeni* from fertilization to metamorphosis at 0-1 °C. Scale bars = 100 μm.

A. Day 1 First cleavage of egg.
B. Day 1 Egg division continues.
C. Day 2 Embryo.
D. Day 3 Hatching. Labels 'tr' and 'ta' denote trunk and tail, respectively.



Appendix 1. Cont'd. Development of *Oikopleura vanhoeffeni* from fertilization until metamorphosis at 0-1 °C. Scale bars = 100 μm.

E. Day 4 Beginning of organogenesis. Cerebral ganglion (cg) develops. Gut lumen (gl) and body cavity (bc) appear.

F. Day 5 Mouth (mo) begins to appear. Brain (br), gut (gt) and endostyle (en) are well-developed.

G. Day 6 Organogenesis is completed. Mouth is open. Stomach (st) and esophagus (es) are clearly visible.

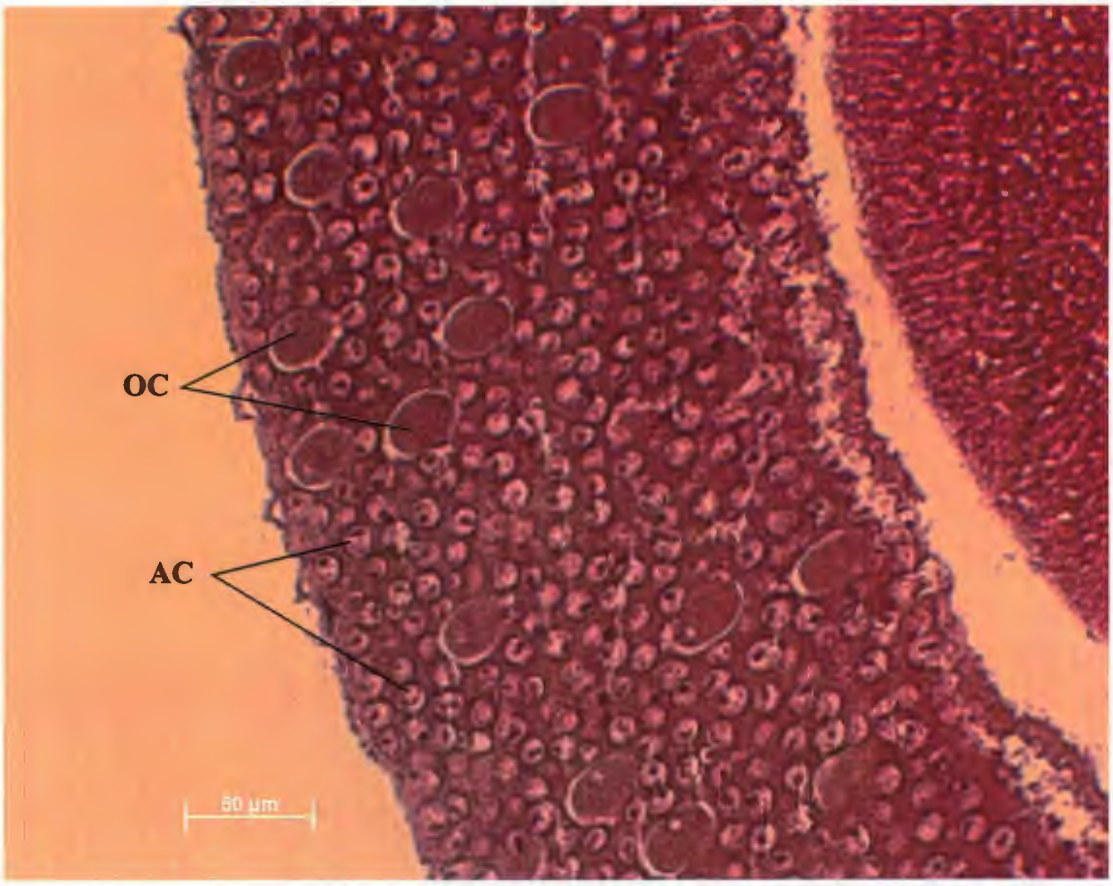
H. Day 7 Tail is shifted antero-ventrally. House rudiment (hr) is present. First house is built and feeding is initiated.

Appendix 2. *Oikopleura vanhoeffeni* . Number of animals sampled for measurement of statolith diameter and trunk length during 60 days post hatching. Family represents a group of offsprings from a single, hermaphroditic parent.

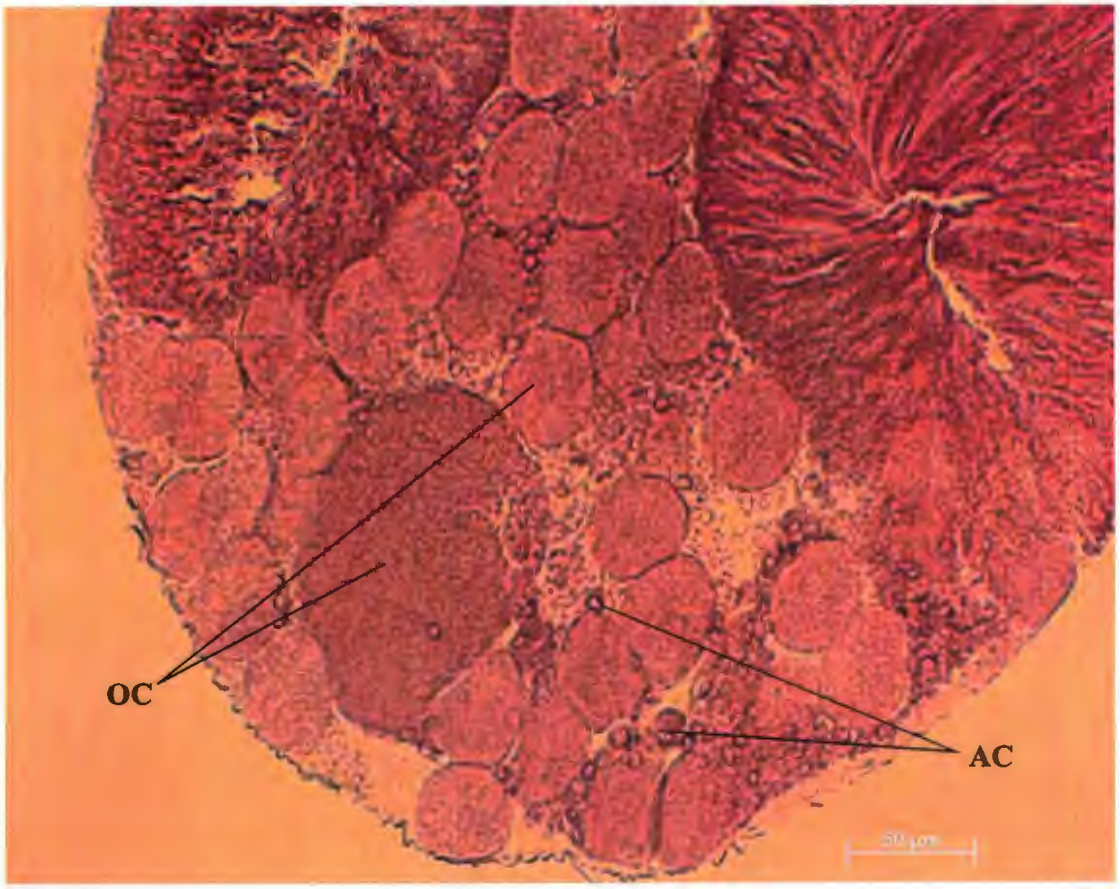
Family	Days post hatching	Number of measured individuals
1	11	27
	33	45
	60	16
2	10	17
	37	77
3	30	8
	56	18
4	28	9
5	32	17
6	14	58
	26	30

Appendix 3. Shrinkage of *Oikopleura vanhoeffeni* after fixation in 95 % ethanol and 2 % bouin's solution

Fixative	Trunk length (μm) before fixing	Trunk length (μm) after fixing	%Shrinkage	Mean	Standard deviation
ETOH	3870	3300	14.7		
ETOH	4050	3300	18.5		
ETOH	3780	3200	15.3		
ETOH	4050	3300	18.5		
ETOH	4800	3875	19.3		
ETOH	4700	3625	22.9		
ETOH	3100	2450	21.0		
ETOH	3300	2750	16.7		
ETOH	3900	3375	13.5		
ETOH	3700	3000	18.9		
ETOH	4300	3750	12.8		
ETOH	6500	5000	23.1		
ETOH	5300	4300	18.9		
ETOH	2900	2375	18.1	18.0	3.0
Bouin's	4320	3500	19.0		
Bouin's	4860	4000	17.7		
Bouin's	3600	2900	19.4		
Bouin's	4050	3300	18.5		
Bouin's	4140	3100	25.1		
Bouin's	4500	4000	11.1		
Bouin's	4230	3100	26.7		
Bouin's	3870	3600	7.0		
Bouin's	3600	2800	22.2		
Bouin's	3780	3400	10.1		
Bouin's	4800	4250	11.5		
Bouin's	3200	2625	18.0		
Bouin's	4200	3325	20.8		
Bouin's	5200	4250	18.3		
Bouin's	4300	3375	21.5		
Bouin's	3900	3050	21.8		
Bouin's	3500	2750	21.4		
Bouin's	4000	3250	18.8	18.3	5.1



Appendix 4. *Oikopleura vanhoeffeni*. A section of maturing ovary stained with hematoxylin and eosin. OC, oocyte; AC, accessory cell.



Appendix 5. *Oikopleura labradoriensis*. A section of maturing ovary stained with hematoxylin and eosin. OC, oocyte; AC, accessory cell.