Overwintering Ecology and Ecophysiology of *Neocalanus plumchrus*

by

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We accept this dissertation as conforming
to the required standard

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ABSTRACT

Neocalanus plumchrus is the most common copepod in the Northeast Pacific, and as such plays in important role in the ecosystems of that area. The bulk of *N. plumchrus*' annual life cycle is spent in a dormant overwintering state, and little is known of its ecology, behaviour, or physiology during that period. The goal of this thesis is to describe the physiological changes that occur during the overwintering period, and explain how they interact with the physical environment to produce observed life history patterns.

Lipid stores in *N. plumchrus* were primarily wax esters, and were in highest abundance in overwintering stage 5 copepodids. Consumption of wax ester stores began approximately two months prior to moulting *in situ*. Rates of lipid use in the *in situ* population and a number of laboratory incubations ranged from 0.3 - 1% d^{-1}, with 22 - 60% of total wax ester reserves used prior to moulting, presumably to fuel gonadogenesis. Concurrent measurements of protein content and glutamate dehydrogenase activity (an enzyme involved in protein catabolism) did not show any significant protein use during overwintering. Incubation experiments suggest that *N. plumchrus* has some concept of the time of year (i.e. an endogenous clock), but the use of external cues cannot be ruled out.

It is often assumed that the abundant lipids found in calanoid copepods play some role in buoyancy regulation. However, lipids are generally more compressible, and more thermally expansive than seawater, which means that neutral buoyancy will be inherently unstable. A simple model of mass density shows that (i) individuals will only be able to stay at depth if they are able to diagnose where they are neutrally buoyant, and (ii) the buoyancy properties

of an individual are extremely sensitive to its chemical composition.

In the Strait of Georgia, depth-specific measurements of abundance showed a shift towards deeper depth distributions over the course of the overwintering period. Model results suggest that lipid use could be responsible for those changes, though deep water renewal events that occur regularly in the Strait of Georgia in winter may also have been partially responsible.

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For my parents

CHAPTER 1
Introduction

1.1 General Introduction

Free-living marine organisms must deal with an environment that fluctuates seasonally, in physical conditions (e.g., temperature, light), as well as in biological factors such as food availability, predation risk, the presence and abundance of conspecifics/competitors, and the presence of potential mates. If an environment becomes unsuitable, the organisms inhabiting it can only persist if they migrate to a better habitat, change the habitat that they are in, or await the return of more favourable conditions. Dormancy is one mechanism commonly used by many plants and animals to survive unfavourable conditions, particularly at high latitudes. A period of dormancy is common in copepods (Arthropoda: crustacea), particularly among the large calanoid copepods that usually dominate high-latitude oceanic ecosystems, where a dormant stage is usually present during times when food is scarce (i.e. winter). Although there has been a resurgence in marine zooplankton research in recent years (e.g. GLOBEC, TASC), overwintering ecology remains largely a "black box" to zooplankton ecologists. Copepods of the genus *Neocalanus* dominate zooplankton communities in the subarctic North Pacific (both numerically and in terms of biomass) and undergo a prolonged diapause stage, which makes them an ideal model to study overwintering. The overall goal of this thesis is to fill in some of the gaps in the present knowledge of the physiological ecology of calanoid copepods, and to further explore how changes in physiology and biochemical composition can shape life history patterns.

1.2 Dormancy in the copepoda

Many free-living copepods (members of the orders Harpacticoida, Cyclopoida, and Calanoida) exhibit a period of dormancy at some stage in their life cycle (Dahms, 1995). Dormancy is not known to occur among the 7 orders that are exclusively parasitic (Williams-Howze, 1997). The life history stage that undergoes dormancy varies widely both within and among groups; eggs, larvae (i.e. nauplii), juveniles (i.e. copepodids) and adults are all known

to exhibit dormancy (Dahms, 1995; Alekseev and Starobogatov, 1996; Williams-Howze, 1997). Within individual species, dormancy is usually restricted to a smaller number of stages (the most common situation involving a single stage). For instance, many freshwater and marine calanoid copepods of the superfamily Centropagoidea produce only dormant eggs, while many freshwater cyclopoids and marine calanoids enter into a dormant phase during a specific copepodid or adult stage (Williams-Howze, 1997).

There are several terms used to describe dormancy in copepods (e.g. resting phase, hibernation, diapause, quiescence, torpor), and they are often used interchangeably or without strict operational definitions. As a result, there have also been numerous classification schemes proposed to define each of these terms (e.g. Elgmork and Nilssen, 1978; Alekseev and Starobogatov, 1996; Hirche, 1996), each of which has been adapted from schemes originally designed to describe dormancy in insects (e.g. Mansingh, 1971). In particular, two terms have received wide use in the copepod dormancy literature, diapause and quiescence, and it is therefore important to properly define these terms. Diapause is "a *period of retardation or suspension of development*" while quiescence is "*motionless, inactive, at rest*" (Brown, 1993). Following Dahms (1995), diapause may be further defined as: arrested growth or development, triggered by environmental (e.g. photoperiod, temperature) or endogenous factors (e.g. a "biological clock"; neurosecretions, i.e. hormones; lipids; metabolites). In this sense, diapause is programmed and obligatory (i.e. it is only induced or broken by the abovementioned endogenous or external factors) and, ultimately, genetically determined. Quiescence may be further defined as: arrested development or growth undertaken in direct response to some limiting factor, induced by the condition of an individual and the conditions of its immediate environment. In other words, while diapause is an evolutionary adaptation for the avoidance of reasonably predictable variations in the environment that is "programmed" into the organism, quiescence is an *ad hoc* reaction to a deterioration in current environmental conditions.

1.3 Physical and biological setting in the northeast Pacific

Large scale circulation in the subarctic Pacific is driven by the westerly winds. The strong western boundary current, the Kuroshio, flows northward and turns to the east near Japan, becoming the Kuroshio extension, and finally the North Pacific Current (Thomson, 1981). Along the eastern margin of the north Pacific, the North Pacific Current bifurcates into the (southerly) California and (northerly) Alaska currents (LeBlond, 1996). The Alaska current defines the eastern margin of the Alaska Gyre, a cyclonic circulation centered in the Gulf of Alaska (Cummins and Mysak, 1988). Abundant freshwater runoff from North America creates a coastal buoyancy current that eventually joins the Gyre, contributing to the relative freshness of surface waters, as well as the maintenance of a strong permanent halocline (Royer, 1981a, 1981b). Seasonal and long-term characteristics of the water column in the Subarctic Pacific have been reviewed by Whitney and Freeland (1999) for Ocean Station Papa (50°N 145°W), that lies approximately on the eastern margin of the Alaska Gyre. A permanent halocline is present over the entire year, at ~100 - 150 m. Above the halocline, temperature and salinity vary seasonally from ~13 to 5 °C and 32.65 and 32.5 psu, respectively. Below the thermocline, conditions are less variable, with temperature decreasing from ca. 5 to 4 °C and salinity increasing from 33.8 to 34.25 between 200 to 800 m.

The mesozooplankton of high latitude oceanic ecosystems are generally dominated by large calanoid copepods both in terms of abundance and biomass (Conover, 1988; Atkinson, 1998). In the central northeast Pacific (i.e. Alaska Gyre), the near-surface mesozooplankton community varies seasonally, with large calanoid copepods (*Neocalanus plumchrus, N. cristatus, N. flemingeri* and *Eucalanus bungii*) dominating in the summer (May-August). During the rest of the year, smaller calanoids and cyclopoids (e.g. *Calanus*, *Pseudocalanus*, *Metridia*, *Microcalanus, Oithona*) make up most of the biomass in the surface waters while the large calanoid copepods overwinter at depth (Mackas and Tsuda, 1999).

The arrival of the large, seasonally migrant copepods in the surface waters of the northeast

subarctic Pacific is believed to be closely linked to seasonal patterns in primary productivity. The oceanic subarctic Pacific is one of three high nitrate, low chlorophyll (HNLC) regions in the world ocean, and standing stocks of primary producers are generally low (< 10 μg C l^{-1}) over much of the year (Miller *et al.*, 1984; Boyd and Harrison, 1999). Primary productivity in the subarctic Pacific does vary seasonally, however, driven by changes in insolation and nutrient supply (Iron: Maldonado *et al.*, 1999), and is generally dominated by small phytoplankton (<3μm) with only occasional blooms of large diatoms (Boyd and Harrison, 1999). It is currently held that copepods in the Alaska gyre are not major grazers of the phytoplankton community (Dagg, 1993a). Instead, it has been observed that these copepods feed on sinking organic matter (Dagg, 1993b) or on the heterotrophic microplankton (small flagellates and ciliates) that do graze directly on phytoplankton (Gifford, 1993; Landry *et al.*, 1993). Thus, it is the production of heterotrophic microplankton during summer that is utilized by copepods in the surface waters (as well as sporadic blooms of diatoms, that are in the size range that can be handled by copepods: Boyd *et al.*, 1999), and it is during that time that much of the growth of copepods occurs.

Neocalanus plumchrus overwinters as copepodid stage 5 in the subarctic Northeast Pacific (copepodid and nauplii stages will hereafter be referred to as "C" or "N" and their stage number in roman numerals, e.g. copepodid stage 5 = CV). Moulting to adulthood, mating, and spawning all occur at depths between 500 and 2000 m (Miller *et al.*, 1984). Spawning females do not feed, and rely on stored energy reserves (primarily lipid, in the form of wax ester) for the production of eggs (Fulton, 1973). Eggs and nauplii are positively buoyant, and contain droplets of lipid, that are used in development during ascent to the surface (Fulton, 1973). Development of the nauplii in the open ocean is undescribed, but *N. plumchrus* in the Strait of Georgia progress rapidly from hatching to NIII, the stage where exogenous feeding first occurs (Pandyan, 1971; Gardner, 1972). The young copepods usually arrive at the surface as either final stage nauplii (N6) or as CI (Mackas *et al.*, 1998). Development progresses through CI to CV in the surface waters above the seasonal thermocline (generally in the top 50m), fueled by exogenous feeding on microzooplankton and phytoplankton

(Goldblatt *et al.*, 1999). The CV copepodids return to depth in mid-summer (June to July) to overwinter.

1.4 Physical and biological setting in the Strait of Georgia

The Strait of Georgia lies between the Coast mountains of mainland British Columbia and Vancouver Island, and is approximately 222 km long by 28 km wide. Depth in the Strait averages about 155 m overall, with the deepest waters (ca. 400 m) occurring in the central Strait, to the south of Texada Island (Thomson, 1981). Hydrography in the Strait is dominated by the outflow from the Fraser River in the south, and the Strait may thus be considered an estuarine system (LeBlond, 1983). At the mouth of the Fraser, a strong salt wedge is present, and its position is dependent on sea level changes caused by both tidal oscillations and variations in the magnitude of freshwater discharge (Tully and Dodimead, 1957; LeBlond, 1983). An extensive estuarine plume extends from the river mouth, resulting in a surface layer of low salinity water (S<20). During the spring freshet (which peaks from about May to July), the layer of low salinity water can overly much of the Strait (Lucas, 1929). A thermocline is also present from spring to autumn. Waters in the northern Strait are usually well-mixed in winter, when freshwater outflows are at the seasonal minimum and the plume is confined to the southern Strait (Waldichuk, 1957; Thomson, 1981). Surface salinities in the northern Strait approach 30 psu when freshwater inputs are minimal (Thomson, 1981). Temperatures in the surface layer (above 50 m) vary from 5 - 6°C in winter to >20°C in summer (LeBlond, 1983). Subsurface waters (below 50 m) remain fairly uniform, with temperature varying annually between 8 to 10 °C and salinity between 30.5 and 31 (Thomson, 1981). Northward flow out of the strait is restricted by several shallow straits. To the south, outflow is mostly through the large Haro and Rosario straits, in addition to a number of shallower passages through the Gulf and San Juan Islands. Flow to both the north and south is of a two-layer estuarine type, with freshwater outflow at the surface and deep water inflow at depth (Thomson, 1976; Thomson, 1981). Deep water in the Strait is renewed regularly, both by the sinking of cool, relatively fresh water in winter (Waldichuck, 1957), and return flows of deep, salty water when Fraser River outflows are at or near their

maximum (LeBlond, 1983; LeBlond *et al.*, 1993).

The mesozooplankton community in the Strait of Georgia is somewhat different from that of the oceanic northeast Pacific. Large calanoid copepods are represented by *Neocalanus plumchrus*, and small numbers of *E. bungii* (Black, 1984; Fulton, 1973). *Neocalanus flemingeri* and *N. cristatus* are either absent or rare (Miller and Clemons, 1988). Like the open Northeast Pacific, mesozooplankton biomass in the Strait is dominated by *N. plumchrus*, although other species (particularly *Calanus marshallae*, *C. pacificus* and *Pseudocalanus minutus*) may dominate to a lesser degree at other times (Harrison *et al.*, 1983). Numerous other species of small copepods also inhabit the surface waters throughout much of the year (e.g. *Acartia* spp., *Centropages* sp., *Chiridus gracialis*, *Metridia* spp., *Microcalanus pusillus*, *Paracalanus parvus*, *Pseudocalanus* spp.). However, work to date has remained mostly qualitative and the specific life history patterns of those species are not well known (McMurrich, 1916; Wailes, 1929; Légaré, 1957; Gardner, 1977; Harrison *et al.*, 1983). The use of older technical data reports to explore zooplankton community dynamics in the Strait is further complicated by changing species nomenclature. Harrison *et al.* (1983) present a list of known synonymies for some of the more common copepod species in the Strait.

The timing of the life history of *Neocalanus plumchrus* in the Strait differs from its conspecifics in the open ocean (Fulton, 1973). Overwintering of the Strait of Georgia population is more synchronous, and the time spent developing in the surface waters is compressed (~2 months versus ~5 months). It has been suggested that these differences result from differences in the feeding environment between the two areas (Miller *et al.*, 1984). Whereas the open northeast Pacific is dominated by the microbial loop, the pelagic ecosystem in the Strait is usually dominated by a "classical" planktonic food web, with a strong diatom bloom in the spring, followed by a smaller bloom in autumn (Harrison *et al.*, 1983). Concentrations of phytoplankton in the Strait are considerable in spring, on order of 10^2 to 10^3 $\mu g \cdot C\ L^{-1}$ (Stockner *et al.*, 1979), one or two orders of magnitude greater than

concentrations of phytoplankton and microzooplankton in the open ocean.

1.5 Rationale and objectives for this thesis

As a main consumer of phytoplankton, microplankton and detritus and an important prey species for higher trophic levels, *Neocalanus* represents a key link in the ecosystems of the north Pacific. Although it is no longer believed that grazing limits phytoplankton in HNLC regions (the "major grazer" hypothesis of Heinrich, 1962), the mechanism instead being iron limitation and grazing by microzooplankton (Miller *et al.*, 1991a; Boyd and Harrison, 1999), grazing is still important in the context of remineralization of nutrients (Hutchins and Bruland, 1994). In addition, *Neocalanus* grazing enhances material flux to depth by the packaging of phytoplankton and microzooplankton (*N. plumchrus* and *N. flemingeri*) or detritus (*N. cristatus* and *E. bungii*) into quick-sinking fecal pellets (Mackas *et al.*, 1993; Dagg, 1993a,b). Flux from the surface may also be reduced by sloppy feeding and disruption of sinking detritus (Dilling and Alldredge, 2000). *Neocalanus* is also an important prey item for higher trophic levels such as invertebrate predators, fish, birds and mammals (Mackas and Tsuda, 1999).

It has been observed recently that the life history timing of *Neocalanus plumchrus* can be quite plastic. The timing of maximal abundance in surface waters varies by weeks to months over the course of decades (Mackas *et al.*, 1998; Bornhold, 2000). Changes in life history timing could potentially alter the relationship among *Neocalanus* and both its predators and prey (e.g. "match-mismatch" scenarios : Cushing, 1975), which could alter ecosystem productivity in numerous ways. Thus, an improved understanding of the mechanisms involved in overwintering by these copepods is timely, and will be of use to those interested in modelling population dynamics in pelagic ecosystems as well as the energy fluxes within those ecosystems.

The objectives of this thesis are to answer the following questions:

1. What role does biochemical content play in maintenance of overwintering depth?

2. How does physiology change during overwintering in *N. plumchrus* in the Strait

of Georgia?

3. Where do *N. plumchrus* in the Strait of Georgia spend their time while overwintering?

1.6 Structure of the thesis

I have employed a variety of techniques to address the above questions, from models to field and lab studies, and this thesis has therefore been arranged around those three questions. Chapter 2 uses a simple model to project how the thermodynamics of the compression of lipids (that calanoid copepods have in abundance) affects their buoyancy properties, and that might be of use in maintaining (or changing) vertical position. The focus for that effort was a case study on *Calanus finmarchicus* in the Faroe-Shetland channel. Earlier model work was done with that population, and that focus was retained for comparative purposes and to reach a broader audience. The model is also applied to the *Neocalanus plumchrus* case in Chapter 5. A version of Chapter 2 (with Dr. John Dower as co-author) has been published in the journal Marine Ecology Progress Series (volume 263 pp. 93-99). Chapters 3 and 4 present the results of concurrent field and lab studies that describe the changes in physiology during the overwintering period, with particular reference to the termination of the overwintering state. Both chapters have been submitted to Marine Ecology Progress Series with Dr. Dower as a co-author. Some of the data used in Chapter 4 were collected in conjunction with Ms. Palmira Boutillier, who measured glutamate dehydrogenase (GDH) activity as part of her 4th year honours thesis, and Ms. Boutillier is therefore a co-author on the manuscript version of Chapter 4. The raw data collected by Ms. Boutillier were reanalysed by the author for that chapter. Chapter 5 presents the results from monthly field work in the Strait of Georgia involving depth-stratified net samples and measurements of plankton distributions with an optical particle counter.

CHAPTER 2

The role of lipids in the maintenance of neutral buoyancy by zooplankton

2.1 Introduction

Many planktonic organisms use lipids as an energy storage medium (Lee and Hirota, 1973; Childress and Nygaard, 1974; Sargent and Falk-Petersen, 1988). At atmospheric pressure, lipids are less dense than seawater; they often form a layer at the top of preserved zooplankton samples and have even been found to form surface slicks in the ocean (Lee and Williams, 1974). It is widely held that such lipids play a role in buoyancy control (Lewis, 1970, Sargent and Falk-Petersen, 1988). However, a plausible mechanism by which lipids may be used to regulate buoyancy has yet to be proposed.

Yayanos *et al.* (1978) measured the density of a lipid mixture (primarily wax esters) extracted from the calanoid copepod *Neocalanus plumchrus* and observed the mixture to be more compressible, and to have a much higher thermal expansion, than seawater. They suggested that because of those properties, a lipid-rich plankter that is positively buoyant at the surface will become less so as it moves deeper in the water column. They concluded that lipids may initially represent a "barrier" to downward vertical migration, in that copepods are more buoyant at the surface than at depth, and must therefore overcome buoyancy forces when moving from shallow to deeper depths.

Building on this work, Visser and Jónasdóttir (1999) fit a high order polynomial to the density measurements of Yayanos *et al.* (1978). Using that relationship, they produced a simple model for the density of a copepod in order to demonstrate how overwintering *Calanus finmarchicus* in the Faroe-Shetland channel can be positively buoyant at the surface, but neutrally buoyant at depth. Parameters for the Visser and Jónasdóttir model were calculated from field and laboratory measurements, as well as with the assumption that the copepods were neutrally buoyant at their depth of overwintering. They found that the vertical ascent rate attributable to buoyancy forces could be considerable (of order tens of

meters per day), particularly near the surface where the density difference between seawater and lipids is greatest.

The pressure and temperature dependence of the mass density of lipids clearly has the potential to affect the way that lipid-rich planktonic organisms relate to and perceive the pelagic environment. In this chapter, I propose that these properties may have a different relationship than that which has generally been assumed in the literature, and argue that the presence of large proportions of lipids requires some other buoyancy regulation mechanism(s) in the zooplankton.

2.2 Neutral buoyancy by lipids is not stable

Whether an animal floats or sinks depends on the density difference between it and the surrounding seawater. Thus, a neutrally buoyant animal must have the same aggregate density as the surrounding seawater. However, the greater compressibility of lipids than seawater means that any depth of neutral buoyancy will not be stable. In other words, below the depth of neutral buoyancy, lipid will become denser (as pressure increases), and thus the aggregate density of the animal will become greater as well. The converse is also true. Therefore, any displacement of the animal away from its depth of neutral buoyancy should result in it accelerating away from that depth. Thus, the presence of lipids is more than a barrier to downward migration, as suggested by Yayanos *et al.* (1978) or a means to promote upward migration as suggested by Visser and Jónasdóttir (1999); it actually represents an impediment to maintaining position in the water column. Moreover, as I will illustrate, the buoyancy properties of an animal are extremely sensitive to the relative composition of its biochemical constituents.

2.3 The role of lipids in buoyancy regulation: A copepod example

2.3.1 A simple model for mass density

Visser and Jónasdóttir (1999; VJ99 hereafter) divided their model copepod into three components. At its simplest, the mass of the model copepod can be expressed as the sum of

the masses of the components:

$$m_{copepod} = m_{Water} + m_{Lipid} + m_{Other} \quad (1)$$

If rearranged in terms of density (ρ) and volume (V; *i.e.* $m=\rho V$), this is equivalent to Eq. 3 of VJ99 (subscripts will be abbreviated hereafter). VJ99 further generalized their model with volume proportions (e.g. V_L/V_C). However, volume is not conservative with pressure (each component is compressible to some degree), while mass is, and so it is preferable to express the model in terms of mass proportions.

The density of the model copepod can also be expressed as:

$$\rho_c = \frac{m_c}{V_W + V_L + V_O} \quad (2)$$

This is conceptually identical to the VJ99 model, in that the copepod is divided into three components (*i.e.* $V_c=V_L+V_W+V_O$). Volume may be expressed in terms of mass and density (*e.g.* $V_L=m_L/\rho_L$), and mass proportions (*e.g.* $\delta_L=m_L/m_C$) may then be substituted into Eq.2 to yield:

$$\rho_c = \left(\frac{\delta_W}{\rho_W} + \frac{\delta_L}{\rho_L} + \frac{\delta_O}{\rho_O} \right)^{-1} \quad (3)$$

ρ_L can be modeled as a function of temperature and pressure (using the polynomial of VJ99, their Eq. 2). ρ_W can be determined as a function of pressure, temperature and salinity (assuming osmotic equilibrium between the animal and the seawater around it) using the UNESCO seawater equation of state (Millero *et al.*, 1980). ρ_o represents the "structural mass" of the copepod (*e.g.* protein, exoskeleton) and here will be held constant. VJ99 reported values for ρ_o of 1080 to 1240 kg m^{-3}. Although the structural components are not completely incompressible, they are considerably less so than lipids. Kharakoz (2000) cited a coefficient of compressibility (β) for protein on the order of $10\text{-}25\times10^{-6}$ Bar^{-1}. By comparison, β for copepod wax esters is of order 6.5×10^{-5} Bar^{-1} (calculated from Table 2 of

Yayanos *et al.* (1978), using an exponential fit to volume data at 5.1°C).

The changes in seawater and lipid densities with changes in pressure are generally quite small (of order 1% over 100 bar). However, since it is the *difference* between these densities that drives buoyancy forces, even very small changes in density can result in large changes in ascent or decent rates. Furthermore, only small changes in the relative proportion of the three model constituents (*i.e.* lipid, water and "other") are necessary to produce dramatic changes in the buoyancy properties of the model copepod. To demonstrate the sensitivity of the model to changes in the parameters, I have altered water and lipid contents while keeping ρ_o ("other") fixed, and calculated ascent rates given the density calculated for the model copepod (Fig. 1). In order to make the reformulated model comparable to that of VJ99, their volume proportions (α_o) were converted to mass proportions (δ_o; see appendix 1). VJ99 chose their parameters given lipid:dry weight ratios from 0.29 (copepods used in laboratory measurements of density and lipid content) to 0.59 (representative of overwintering *Calanus finmarchicus* in the Faroe-Shetland Channel). In order to assess a likely parameter space for the model, the range of calculated ascent rates given those upper and lower limits is indicated within the shaded areas of Figure 1.

In the examples presented here, a ~2% change in water (and lipid) content is sufficient to achieve neutral buoyancy over the entire 850 m water column (dashed zero contours of Fig. 1). However, most parameter combinations result in a model copepod that is always positively or negatively buoyant, regardless of pressure. The first scenario of VJ99 ("case 1"), overlaps the neutral buoyancy contour, while the second scenario ("case 2") does not. Neither scenario is consistent with neutral buoyancy over the range of the only published observations of water content for *Calanus finmarchicus* (~79-85%: Tande, 1982). Obviously, there are countless parameter combinations to chose from. However, the points I wish to make are (1) that the model is very sensitive to the choice of parameters, and (2) the buoyancy properties of the model copepod are extremely sensitive to its relative biochemical content.

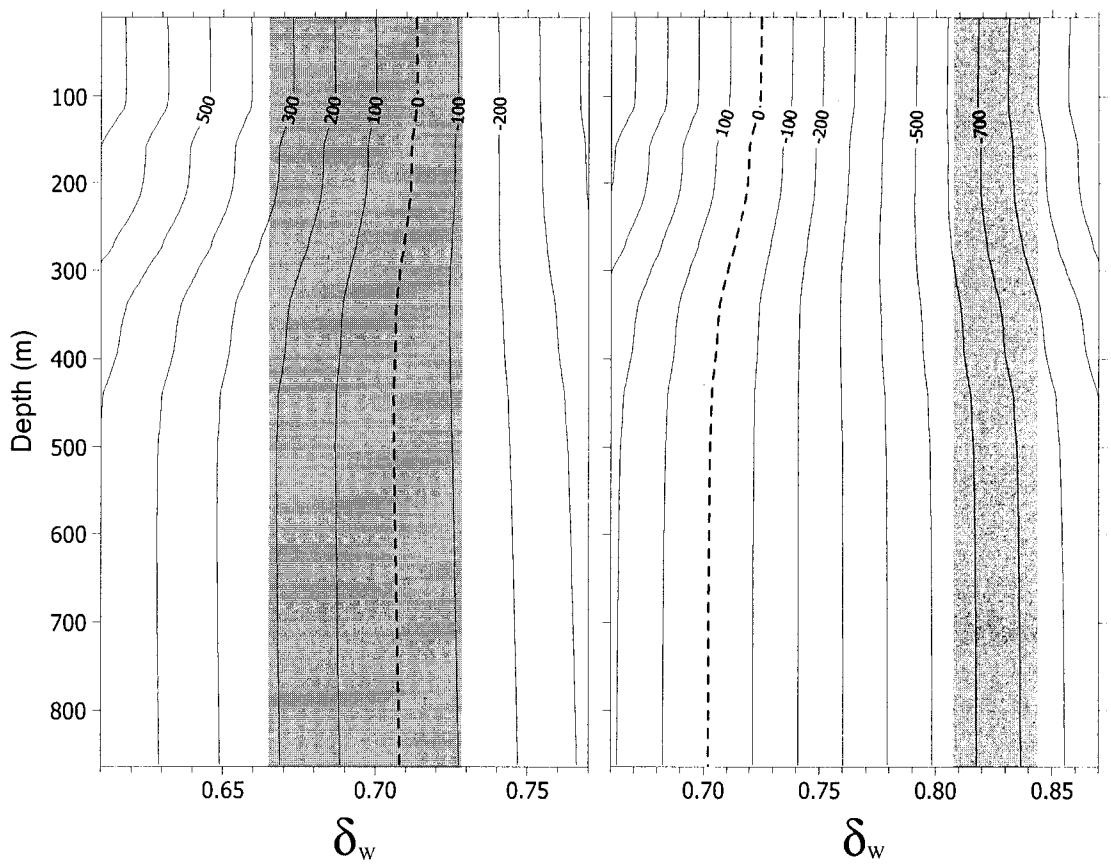

Figure 1. Contours of velocity attributable to buoyancy (meters per day, positive upwards) of a model copepod as a function of relative chemical composition (based on data for overwintering *C. finmarchicus* from the Faroe-Shetland Channel, March 1995). Rates were calculated with model results for ρ_C and $\rho_{seawater}$ and Stokes' law ($w=g\Delta\rho d^2/18\mu$, where w is ascent rate in m s^{-1}, g is acceleration due to gravity in m s^{-2}, $\Delta\rho$ is density difference in kg m^{-3}, d is effective diameter in m and μ is absolute viscosity in kg m^{-1} s^{-1}). Effective diameter (d) was 0.0013 m (Visser & Jónasdóttir 1999) and μ was calculated as a function of pressure, temperature and salinity with the equation of Matthäus (1972; values ranged from 1.48×10^{-3} to 1.83×10^{-3} kg m^{-3}). For these examples, δ_O has been held fixed, and δ_W and δ_L have been varied such that $\delta_W+\delta_L+\delta_O=1$ (*i.e.* lipid increases in direct proportion to a decrease in water, and vice-versa). Neutral buoyancy ($w=0$) is denoted by the dashed line. Parameter sets have been chosen to match those of Visser & Jónasdóttir (1999): Left panel: "case 1" ($\delta_O=0.12$ $\rho_O=1080$ kg m^{-3}), Right pane: "case 2" ($\delta_O=0.21$ $\rho_O=1240$ kg m^{-3}). See appendix 1 for details on conversion calculations . Grey boxes indicate "likely parameter space" for *C. finmarchicus* given lipid to dry weight ratios between 0.29 and 0.59.

2.3.2 Maintenance of overwintering depth

In the example of *Calanus finmarchicus* in the Faroe-Shetland channel, overwintering individuals are found primarily at depths below 600 meters, and appear to maintain their position at depth for periods of about 6 months (Heath, 1999). At those depths, even extremely small changes in the relative composition (parts per thousand) will result in very large changes in the buoyancy properties (100's of meters). However, *C. finmarchicus* overwintering at depth are generally quiescent, and have not been observed to swim (Hirche, 1996). How, then, are they are able to maintain position during the overwintering period?

If we take an average water content of 82% (Tande, 1982), and ρ_O=1260 kg m^{-3}, neutral buoyancy occurs in the Faroe-Shetland channel at approximately 690 m with δ_L=0.11 and δ_O=0.07 (Fig. 2). This corresponds to a lipid:dry weight ratio of 0.61, only slightly greater than the value of 0.59 given by VJ99 for lipid-rich *Calanus finmarchicus* from the Faroe-Shetland Channel. At depths near the point of neutral buoyancy, ascent rates are very low. The "ascent time", the time it takes a passive particle to reach 10 m or 860 m (i.e. the surface or the bottom), can be considerable. Positioning at or near the point of neutral buoyancy (e.g. between 635 and 777m, Fig. 2) does however allow the animal to remain at depth for an extended period (~six months). In other words, *if* an animal is able to find its depth of neutral buoyancy during the summer/autumn descent, one can expect it to maintain that position during the overwintering period, if changes in the biochemical constituents are ignored.

Metabolic rates in overwintering *Calanus finmarchicus* are quite low (Hirche, 1996; Ingvarsdóttir *et al.*, 1999), but some expenditure of biochemical contents is to be expected. Lipid reserves are consumed over the overwintering period, particularly during the latter portions when gonadogenesis begins (Hopkins *et al.*, 1984; Chapter 3). Protein contents may also decline (Orr, 1934; Kirkesæter, 1977 *cited in* Hirche, 1996; but see Chapter 4). In the case of our model copepod, it is assumed that its water component has the same density as seawater (as did VJ99). Consequently, the balance between the "other" component (which

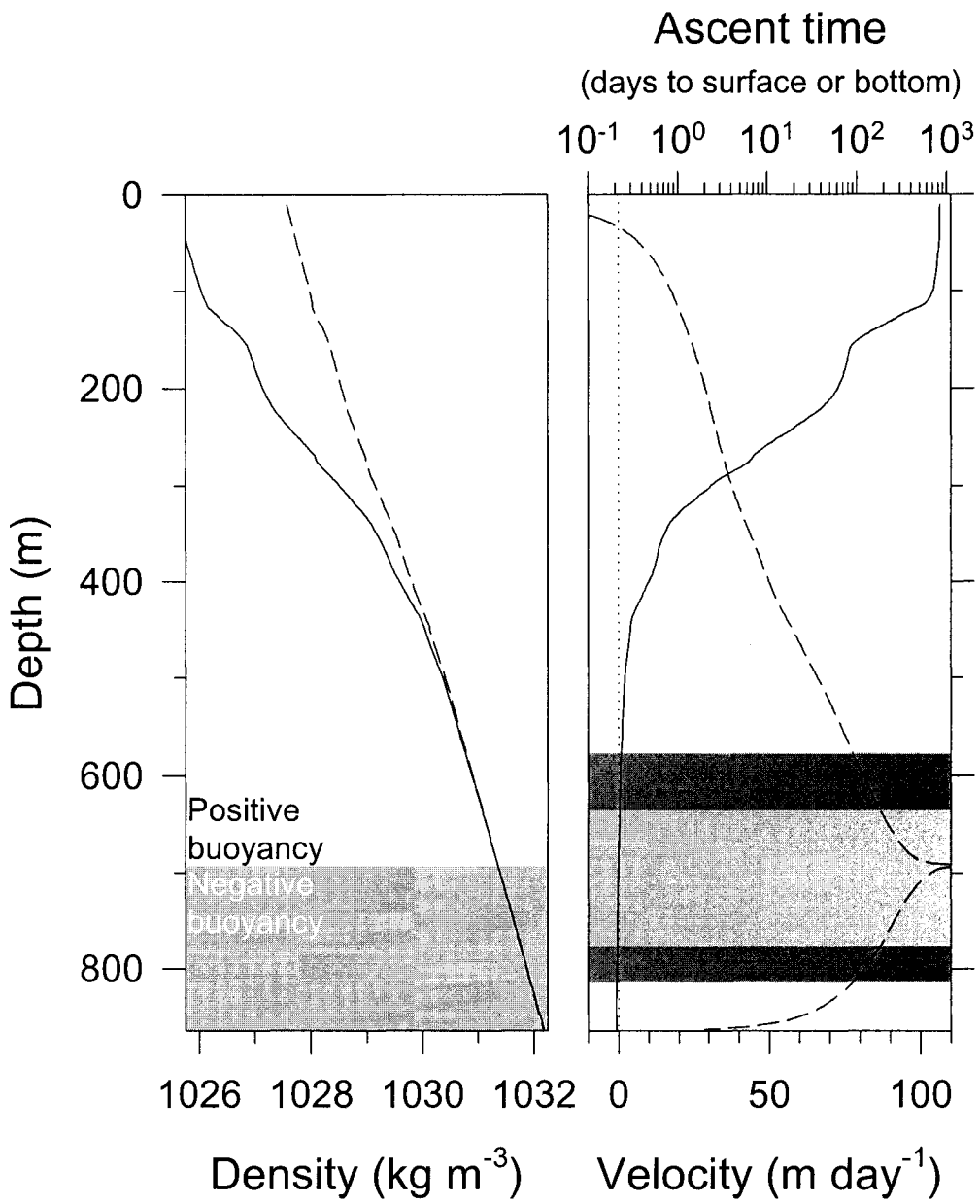

Figure 2. Left panel : In situ seawater density (dashed line), Faroe-Shetland Channel, March 1995 (same data as Visser & Jónasdóttir (1999), their figure 2) and calculated density for the model copepod (ρ_c: solid line), given δ_W=0.82, δ_L=0.11, δ_O=0.07 and ρ_o=1240 kg m^{-3}. See text for details. Right panel: Ascent velocity (solid line, positive upwards) given the calculated density difference, and calculated with Stokes' equation (see Fig. 1), and time to 10m (i.e. surface) or 860 m (i.e. bottom; dashed line). The dark grey area encompasses the depth range within which a passive particle will take 90 days to reach the surface or bottom, the light grey area encompasses the depth range within which a passive particle will take 180 days to reach the surface or bottom.

is denser than seawater), and the lipid component (which is less dense than seawater) determines its overall buoyancy. Therefore, a decrease in lipid composition will reduce the upward buoyancy force, tending to make a neutrally buoyant animal become negatively buoyant. Similarly, a decrease in the "other" component (e.g. protein) will tend to make a neutrally buoyant animal become positively buoyant. This balance between lipid and protein loss during the overwintering period may therefore partially balance each other in their effect on buoyancy. Currently, however, there is not sufficient information about loss rates of either component to realistically model this effect.

2.3.3 Termination of Overwintering

Termination of the overwintering state and moulting to adulthood by *Calanus finmarchicus* occurs at depth. Males appear first and move up slightly in the water column, to about 500m. Females appear shortly afterwards, and migrate to the surface, where spawning occurs (Heath, 1999). The maturation process consumes lipid reserves, and will presumably alter the buoyancy properties of an animal (a reduction in the proportion of lipids tending to make the animal more negatively buoyant).

The model shows us that ascent rates are extremely sensitive to relative biochemical content (Fig. 1). The ascent rates presented here, and by VJ99, should therefore be viewed with caution. Moreover, if the assumption of osmotic equilibrium between the copepods and seawater is violated (see below), ascent rates will also be affected. Nevertheless, the ascent rates calculated may be reasonable, if one is willing to accept certain assumptions. If we assume that the copepod begins from a state of *quasi neutral buoyancy*, as it moves actively towards the surface, its lipids will expand and upward buoyancy forces will increase, eventually resulting in positive ascent rates attributable to lipid expansion alone (Fig. 2). Above 500 meters, temperature begins to increase, and ascent rates also rise, driven by the large thermal expansivity of the lipids. The ascent rates calculated here are comparable to those of VJ99 (their Fig 4 and 5).

2.4 Implications

2.4.1 The case for a buoyancy control mechanism in copepods

For lipid-rich organisms like copepods and other zooplankton, any "depth of neutral buoyancy" is not a stable position. This will be true for any animal that contains a large proportion of any substance that is more compressible (and/or has a larger thermal expansivity) than seawater. Although the model shows that the depth of neutral buoyancy is not stable, it also shows that ascent/decent rates around some position of neutral buoyancy can be very small, permitting an animal to remain in the water column for long periods without adjustment (particularly in the case where temperature does not vary greatly with depth). However, the buoyancy properties of an individual are extremely sensitive to the relative biochemical composition (see above), and biochemical composition does change. In the example presented here, a change of only a few percent makes a large difference in the buoyancy properties of the animal (Fig. 1). It is not unreasonable to expect large changes in the buoyancy properties of individuals as they grow, mature, and reproduce. It is also not unreasonable to expect them to possess a buoyancy control mechanism of some sort to deal with those changes.

Buoyancy regulation has not been observed in the Copepoda beyond the supralittoral harpacticoid copepods of the genus *Tigriopus*, that maintain negative buoyancy by altering their osmotic balance (McAllen *et al.*, 1998). Ionic replacement, the selective transport of "heavier" ions (*e.g.* SO_4^{2-} and Mg^{2+}) and replacement with either "lighter" ions (*e.g.* Na^+, Cl^- and NH_4^+), or ions with a higher partial molal volume (*e.g.* trimethylamine, $Me_3NH_4^+$) has been observed in numerous taxa (Table 1). Among the invertebrates, ionic replacement is a very common buoyancy regulation mechanism, and has been observed in other crustaceans. In this way, an organism can remain iso-osmotic with the surrounding seawater, while selectively reducing or increasing its aggregate density. Ion replacement has not yet been investigated in the pelagic Copepoda, but if it does occur, then the assumption that $\rho_w=\rho_{seawater}$ used in the model is violated. Although it is currently unknown whether oceanic copepods are capable of buoyancy regulation, there is evidence that certain estuarine

Table 1. Review of identified buoyancy regulation mechanisms for aquatic organisms. The large literature on buoyancy in fish that possess swim bladders, and buoyancy in fish eggs is not reviewed exhaustively - rather, representative review articles are given.

Taxon	Buoyancy control mechanism	Reference
Vertebrates		
Fish (with swim bladders)	gas exchange	a,b (reviews)
Fish (without swim bladders; all nonmigrant bathypelagic species: *Bathylagus pacificus*, *B. milleri*, *Tactostoma macropus* and *Chauliodus macouni*)	buoyant glycosaminoglycan layers	c
Pleuragramma antarcticum	lipid sacs	d
Larval Fish (*Gadus morhua*)	Osmoregulation: unknown ion transport pathways	e
Fish eggs	Osmoregulation: free amino acids as	f (review)
Dogfish Shark (*Squalus acanthias*)	osmolytes	g
Whiskery shark (*Furgaleus ventralis*), Whaler shark (*Carcharhinus obscurus*) and Shovelnosed Ray (*Aptychotremata vincentiana*)	Accumlation of lipid (glyceryl ether) within the liver Sequestration of Urea and Trimethylamine oxide	h
Urochordates		
Phlebobranchid (*Corella willmeriana*)	Ion replacement: NH_4^+ sequestration	i
Salp (*Cyclosalpa pinnata*)	Ion replacement: SO_4^{2-} exclusion	j
Molluscs		
Nautilus and Cuttlefish	Gas filled chambers	k
Teuthoid squids	Ion replacement: NH_4^+ sequestration	l
Janthinid snails	Gas filled chambers	k
Heteropod (*Pterotrachea coronata*)	Ion replacement: SO_4^{2-} exclusion	m
Pteropod (*Cymbulia peroni*)	Ion replacement: SO_4^{2-} exclusion	m
Chaetognaths (*Sagitta* spp.)	Ion replacement: NH_4^+ sequestration, Na^+ exclusion	n
Crustaceans		
Harpacticoid copepod (*Tigriopus brevicornis*)	Osmoregulation of cell volumes with free amino acids and Na^+ transport	o
Deepwater Oplophorid Shrimp (*Notostomus gibbosus*)	Ion replacement: Na^+ exclusion, NH_4^+ and trimethylamine sequestration	p
Lobster (*Homarus vulgaris*; adult and larval forms)	Ion replacement: SO_4^{2-} and Mg^+ exclusion	q

Table 1 (cont.)

Cnidarians		
Siphonophorea (Physonectae, Calycophorae and Semaeostomeae)	Ion replacement: SO_4^{2-} exclusion	j
Siphonophorea(Physonectae and Cystonectae)	Gas filled inclusions	r (review)
Schyphozoan (*Chrysaora quinquecirrha*)	Ion replacement: SO_4^{2-} exclusion	s
Hydrozoan (*Obelia* spp.)	Ion replacement: SO_4^{2-} and Mg^{+} exclusion	q
Ctenophores		
Beroe cucumis and *Cestum veneris*	Ion replacement: SO_4^{2-} exclusion	j
Pleurobrachia pileus	Ion replacement: SO_4^{2-} and Mg^{+} exclusion	q
Echinoderm larvae (*Asterias bipinnaria*)	Ion replacement: SO_4^{2-} and Mg^{+} exclusion	q
Dinoflagellate (*Pyrocystis noctiluca*)	Ion replacement: SO_4^{2-} exclusion, NH_4^{+} sequestration	t

key to references: a, Cocker 1978; b, Denton 1961; c,Yancey et al. 1989; d, Devries & Eastman 1978; e, Sclafani et al. 2000; f, Craik & Harvey 1987; g, Malins & Baron 1970; h, Withers et al. 1994; i, Lambert and Lambert 1978; j, Bidigare & Biggs 1980; k, Denton, 1964; l, Voight 1994; m, Denton & Shaw 1961; n, Bone et al. 1987; o, McAllen et al. 1998; p, Sanders & Childress 1988; q, Newton & Potts 1993; r, Mackie et al. 1987; s, Wright & Purcell 1997; t, Kahn & Swift 1978.

copepods (e.g. *Acartia*, *Temora*, *Eurytemora* and *Centropages* spp.) are capable of osmoregulation (Gaudy *et al.*, 2000; Lance, 1963; Roddie *et al.*, 1984; Bayley, 1969 respectively). If oceanic calanoids are able to exploit this pathway in order to alter water or ionic content, they should also be able to regulate their apparent buoyancy.

2.4.2 Effects in surface waters

Although the present example deals with overwintering calanoid copepods, it is important to note that both the steepest gradient of *in situ* seawater density, and the greatest density contrast between the model copepod and seawater occurs near the surface. This is driven by temperature, which has a larger effect on lipid mass density (though in terms of our example of overwintering copepods this is not relevant). Many copepods (as well as other zooplankton) undergo active diel vertical migrations of tens to hundreds of meters (reviewed by Forward, 1988; Pearre, 2003). Presumably, they expend a significant deal of energy to do so, swimming being energetically costly to most zooplankton (Klyashtorin, 1978; Torres *et al.*, 1982; Mauchline, 1998 and references therein). The strongest temperature and density gradients are almost always found in surface waters (Pickard and Emery, 1990), and so it is possible that even slight alterations to buoyancy (to aid ascend or descent) could represent considerable energetic savings to a vertically migrating animal.

For instance, the drag force (F_D, N) on a particle moving at intermediate Reynolds numbers can be calculated with Rayleigh's formula:

$$F_D = C_D(\tfrac{1}{2}\rho_W U^2 A) \quad (4)$$

Where C_D is the drag coefficient ($C_D=24[1+0.15Re^{0.687}]/Re$ for $100>Re>1$: Clift *et al.*, 1978), U is velocity (m s^{-1}) and A is the cross sectional area (m^2). The buoyancy force (F_B) is:

$$F_B = mg\left(\frac{\rho_W}{\rho_C} - 1\right) \quad (5)$$

where m is mass (kg) and g is acceleration due to gravity (m s^{-2}; Glickman, 2002). With the

parameters in the example given here for *Calanus finmarchicus* (Fig. 2), assuming a wet weight of 1000 μg (Tande, 1982) and a swimming speed of 1 cm s^{-1}, $F_D \approx (10^0)(10^3)(10^{-4})(10^{-6}) \approx 10^{-7}$N, and $F_B \approx (10^{-3})(10^1)(10^{-3}) \approx 10^{-5}$N at the surface. At the depth of neutral buoyancy, F_D is essentially unchanged, while F_B=0.

Clearly, buoyancy forces can be much greater than drag forces, though the latter will be size and velocity dependent. However, since the buoyancy force increases as depth decreases (*i.e.* the animal accelerates), any organism exploiting this mechanism to move upwards must possess a way to rapidly change mass density in order to stop its ascent when nearing the surface. Similarly, any animal moving downwards from quasi-neutral buoyancy at the surface will experience progressively less buoyancy force as it descends and will need to change its mass density in order to stop sinking once the desired depth has been reached.

Given the large thermal expansion of lipids, it is possible for true neutral buoyancy to occur in areas where temperature increases with depth. An increase in temperature decreases lipid density, so that an animal moving deeper will experience an increase in the upward buoyancy forces of its lipids. Conversely, a move upwards would cause an increase in lipid density, and a decrease in upward buoyancy forces. Increases in temperature with depth do occur in neritic zones (e.g. Gulf of Maine) and large estuaries (e.g. Gulf of St. Lawrence) and could be exploited in those areas by plankton to achieve neutral buoyancy. Again, whether an individual can passively achieve neutral buoyancy, or be positively or negatively buoyant over the entire water column will depend on its relative chemical composition.

2.4.3 Measurements of mass density and acoustic backscatter

Since lipids are more compressible than seawater, measurements of mass density made at atmospheric pressure (Knutsen *et al.*, 2001 and references therein) can be expected to be underestimates of *in situ* values. Because lipid constituents will expand following collection at depth, mass density measured at the surface will be less than mass density under pressure. Similarly, mass density measurements will be affected by the temperature at which they are

made, and the greater thermal expansivity of lipids means that it is much more important that measurements be made at the *in situ* temperature from which the animals were collected. Measurement of mass density at *in situ* temperature and pressure certainly add to the already considerable methodological challenges of measuring mass density (reviewed by Knutsen *et al.*, 2001), but is not insurmountable.

The differential compressibility of lipids also has practical implications. Measurements of acoustic backscatter are commonly used to estimate zooplankton biomass. Acoustic backscatter, however, is extremely sensitive to the density contrast between the ensonified particles and the fluid they are suspended in (Greenlaw, 1979; Køgeler *et al.*, 1987). The effect is strongly size- and frequency- dependent, but Knutsen *et al.* (2001) report a 1% change in density causing a 2 dB change in backscatter. There have been measurements of the density contrast of several types of zooplankton, particularly euphausiids and copepods (see Knutsen *et al.*, 2001), but all have been made at atmospheric pressure. That density contrast changes with depth and temperature (and can perhaps even change sign) is a potentially confounding factor for *in situ* measurements of acoustic backscatter.

2.5 Conclusions

The high compressibility of lipids makes any position of neutral buoyancy unstable. Although I have used a copepod example, this principle holds true for any organism containing a large proportion of any substance more compressible than seawater. It follows, then, that a lipid-rich organism attempting to maintain neutral buoyancy will have to actively maintain that position in some way. The model also shows that the density difference (and resulting buoyancy force) is highly sensitive to changes in the relative chemical composition of the organism (with some assumptions, outlined above). Again, this will apply to any organism (copepod or otherwise) that does not actively maintain its buoyancy in some way. In fact, since ascent or descent rates at low to intermediate Reynolds numbers scale with the square of size, larger organisms (*e.g.* decapods, cnidarians and fish) should ascend or descend at much greater rates than those presented here. This mechanism for moving up and

down in the water column could result in significant energetic savings for animals undergoing large vertical migrations on a regular basis (*e.g.* Euphausiids, Mychtophids).

CHAPTER 3

Ecophysiology of overwintering in the copepod *Neocalanus plumchrus*: Lipid contents and stage composition

3.1 Introduction

Mid- to high- latitude pelagic ecosystems typically display strong seasonality in primary production, and the large calanoid copepods that dominate the mesozooplankton in those ecosystems have a similarly seasonal life history (e.g. Conover, 1988). Growth of these copepods is generally confined to the fairly narrow period when primary production (and biomass) is high, with the remainder of the year spent in a dormant overwintering state, usually at considerable depth (Hirche, 1996).

Dormancy can be defined as a "state of suppressed development" and may be the result of quiescence or true diapause (Dahms, 1995). Strictly speaking, diapause is a reduction in development triggered by environmental stimuli, and is ultimately under physiological (i.e. endocrine) control, while quiescence is an *ad hoc* reaction to a local environmental requirement (e.g. food, temperature: Danks, 1987). Diapause is therefore obligatory, while quiescence is generally facultative. In insects, diapause is also persistent: diapause is not terminated until the proper token stimuli are received and the required physiological processes have occurred. In other words, true diapause is not terminated spontaneously, even if conditions become favourable (Tauber *et al.*, 1986). However, all activity need not necessarily be curtailed during true diapause. Growth, mobility and feeding have all been observed in certain diapausing insects (Hodek, 2002). The neurosecretions observed by Carlisle and Pitman (1961) in *Calanus finmarchicus,* and the low levels of ecdysteroids in *Calanus pacificus* observed by Johnson (2003), are consistent with an endocrine mediated diapause stage in calanoid copepods.

The nature of dormancy in calanoid copepods is poorly described. The proximate cues to initiate dormancy are not known (Miller *et al.*, 1991b; Hirche, 1996), but following the insect

literature (e.g. Tauber *et al.*, 1986), temperature, food availability and photoperiod are usually invoked. Similarly, the cues triggering the termination of dormancy have yet to be identified. Because water temperatures do not usually vary much at the depths where most calanoid overwinter (usually hundreds of meters), it has been suggested that changes in photoperiod may be used as a cue to prompt termination (Miller *et al.*, 1991b). However, the photoperiod signal at depths >500 m is very small. In addition, a modelling study by Hind *et al.* (2000) suggests that changes in photoperiod cannot explain the timing of emergence observed in geographically separated populations of *Calanus finmarchicus*. Instead, they found that normal development processes, operating at reduced (but temperature controlled) rates, best explained observed patterns in timing. Other researchers have observed decreases in dry weight and lipid contents during overwintering (Gatten *et al.*, 1979; Tande, 1982; Hopkins *et al.*, 1984), while some have found no significant reductions until dormancy is completed and moulting begins (Ohman *et al.*, 1989; Evanson *et al.*, 2000).

Prior work with *Calanus finmarchicus* and *Neocalanus plumchrus* suggests that overwintering dormancy is a fragile state (Gardner, 1972; Grigg and Bardwell, 1982; Hirche, 1983, 1989; Miller and Grigg, 1991). Gardner (1972) incubated *N. plumchrus* collected from the Strait of Georgia (October 1971) in the dark and at "field photoperiod", and observed that moulting began after 19 days. Grigg and Bardwell (1982) incubated individual *C. finmarchicus* in low, diffuse light and found that moulting occurred quickly post-capture. Moulting occurred after a lag of 7-10 days early on in the overwintering period (August - November), and occurred immediately after capture later on in the season (January onwards). Miller and Grigg (1991) similarly incubated groups of *C. finmarchicus* from the Gulf of Maine, but in various light regimes. Moulting was observed after a ~1 month lag in individuals kept in constant illumination, and moulting was less consistent in treatments with different photoperiods (and no evidence for a photoperiodic cue to terminate dormancy was found). Moulting was much more infrequent in dark treatments and with different photoperiods, usually with very little moulting occurring by the end of the experiments (which were terminated after ~1.5 months). Hirche (1983, 1989) kept individual *C.*

finmarchicus in the dark, and observed that moulting also occurred after about one month. There is some question whether or not the animals used by Hirche (1983) were indeed dormant, because (i) they were collected from the near surface (>100 m), and (ii) the animals had higher digestive enzyme activity than those found at greater depths.

In the oceanic subarctic Northeast Pacific, mesozooplankton biomass is dominated by species of the genus *Neocalanus* (e.g. Miller *et al.*, 1984). *Neocalanus* spp. (simply referred to as *Neocalanus*, hereafter) spend most of the year at depth in a dormant state. Arousal, moulting, and spawning all occur at depth as well. Naupliar and copepodid stages migrate to the surface, where growth and development occurs, followed by a downward migration by late stage copepodids (stage 4-6, depending on species and locale: Mackas and Tsuda, 1999). *Neocalanus* differs from other calanoid copepod genera in that all development and spawning is fueled by endogenous lipid reserves. *Neocalanus* thus represents an excellent model with which to study physiological changes during the termination of overwintering, because the potentially confounding factor of food requirements is absent.

Given the paucity of information on the physiological changes undergone during overwintering dormancy by calanoid copepods in general, and in *Neocalanus plumchrus* specifically, the objectives of this chapter were to quantify the changes in stage composition and lipid contents over the course of the life history of *N. plumchrus*. In order to accomplish this, I have used measurements from both the *in situ* population in the Strait of Georgia, and from laboratory incubations, which could be sampled at higher frequency. I chose the Strait of Georgia for the study site because it hosts a population of *N. plumchrus* that can (i) be sampled with relative ease throughout the year, and (ii) exhibit a highly synchronized life history (Fulton, 1973).

3.2 Methods

3.2.1 Collection

Animals were collected during approximately monthly excursions to a single site over the

deepest part (~400 m) of the Strait of Georgia (49°15' N 123°45' W) using a closing SCOR-type net (236 μm mesh) towed vertically at ~0.5 m s^{-1}. Four strata (0-100m 100-200m, 200-300m and 300-400m) were sampled. Upon retrieval, the net was rinsed down and the sample split with a Motoda splitter (Motoda, 1959), and half of the sample preserved in 3-4% formalin for subsequent enumeration. From the second half, approximately 5 lots of *Neocalanus plumchrus* (usually 4 individuals per lot) were picked under a stereomicroscope and placed into folded, precombusted 25 mm glass fibre filters, then frozen immediately in liquid nitrogen. Replicate samples were not collected between December 2001 and May 2002 (i.e. one lot of 4 individuals only). The time between collection and freezing was <15 minutes. Hydrographic properties at the sampling site were measured with a SeaBird model 19-plus or model 25 CTD.

In 2002, only CV copepodids were collected for lipid analysis, while in 2003 adult males and females were also collected. Females were split into three groups: "undeveloped" (gonad undeveloped, oviducts empty), "gravid" (gonad developed, oocytes present in oviducts), and "spent" (gonad and oviducts empty, or with <6 individual oocytes). The former two categories are analogous to stage 1 and stages 3 -7 of Runge (1987) respectively. Individuals in intermediate stages of development (e.g. oocytes present in ovaries, but not fully developed) were encountered infrequently, and included as "gravid".

Animals to be used for incubations were collected using the same SCOR net, with a closed codend attached, towed vertically from 400-200 m at ~0.25 m s^{-1}. Upon retrieval, the codend was immediately emptied (without rinsing the net) into a cooler filled with ~30 l of 300 m seawater. Groups of 25-30 individuals were removed from the cooler and transferred immediately into 2-2 litre glass jars filled with water from 300 m depth that had previously been filtered through a G/F75 filter (0.7μm nominal pore size). Jars were filled to the brim and sealed without any air bubbles, and transported back to the laboratory in the dark in seawater filled coolers with ice packs.

3.2.2 Incubations

Upon arrival in the laboratory, jars were placed in a dark, 9°C cold room. Preliminary incubations had experienced oxygen depletion (from bacterial respiration following moulting by the animals), but it was preferable to not disturb the animals during the incubation by changing the water. Therefore, each jar was initially spiked with 1 ml of broad spectrum antibiotic (Invitrogen PSN antibiotic mixture, cat #:15640055). The concentrations of antibiotics used were very low (penicillin and streptomycin: 2.5 $\mu g\ ml^{-1}$, neomycin: 5$\mu g\ ml^{-1}$), but sufficient to prevent microbial oxygen depletion during the course of the incubations.

At selected intervals, individuals were removed from a jar, the stage of each individual was noted, and groups of 1-4 individuals were frozen in liquid nitrogen (i.e. in the same manner as the field collected animals). Time points were spaced closely together at the beginning of the incubations (1-3 days), and spaced more widely (2-4 weeks) as the incubations progressed. The number of jars per incubation depended on the number of animals collected, and time constraints at sea, so time points were not the same among all incubations. Instead, they were spaced out to cover the individual incubation times as well as possible. Two jars were sacrificed at each time point, one for lipid measurements, and one for the measurement of enzyme activities and protein contents (Chapter 4). The observations of stage compositions from both jars will be discussed in this chapter. Mortality during the incubations was low initially (<8%), and generally occurred after moulting. At later time points (post spawning) mortality was high, of order 80%, involving large numbers of spent adults (i.e. with no visible lipid stores or musculature). Only live animals were retained for lipid analysis. Moulting failure was uncommon; out of all the incubations, only three individuals were found entangled in their exuvium.

3.2.3 Lipids

Each filter (with its group of 1 to 4 frozen animals) was placed in an extraction medium of 2:1 (v/v) chloroform:methanol, to which a known concentration of 3-hexadecanone had been

added as an internal standard. The extraction medium and sample were homogenized with a Branson model 250 ultrasonic homogenizer (2 minute duration, 75% duty cycle, power level 40 Watts). The filter was removed prior to homogenization, and temperature rise was reduced by suspending the sample in a dry ice-ethanol bath. The homogenate and filter were stored at -15˚C for 24 h after homogenization to complete the extraction. Following the extraction period, 0.9% NaCl solution was added to bring the extraction medium to 8:4:3 chloroform:methanol:water (v/v/v; Folch *et al*, 1957) and the sample centrifuged for 5 minutes at 1500 x g in a swinging basket centrifuge to allow phases to form. The filter was then moved up into the upper phase for 5 minutes to allow any chloroform to sink out. The sample was then recentrifuged for 5 minutes and the lower phase was pipetted out, dried under argon gas, and resuspended in hexane. The amount of hexane used was adjusted depending on the number of individuals in the lot, and varied between 75 (one individual) and 300 µl (four individuals).

Analysis of lipid classes was performed using an Iatroscan MK-5 TLC/FID analyzer. A 1 µl aliquot of the concentrated lipids in hexane was spotted onto an S-III chromarod, with a custom spotter similar to that of the mkII spotter of Read (1985) and 2 µl Hamilton syringe. Sample application by the syringe was controlled with a microprocessor-controlled electronic linear actuator and the chromarod was rotated at ~20 RPM by a second small motor during application. Separation of lipid classes was done with a modification of the double-development techniques of Ohman (1988) and Miller *et al* (1998). The chromarods were developed twice in 95:5 (v/v) hexane:diethyl ether for 30 minutes and once in 66:14:0.1 (v/v/v) hexane:diethyl ether:formic acid for 19 minutes. The chromarods were humidified over saturated $CaCl_2$ for 5 minutes before each development, and dried at 60˚C for 5 minutes after each development.

Recording of the chromatograms during FID scans and calculation of peak areas was done with a PeakSimple® chromatography data system (SRI Instruments). Analytical standards were tripalmitin (triacylglycerides: TAG), palmitic acid (free fatty acids: FFA), cholesterol

(sterols: ST) and DL-α-Phosphatidylcholine, dipalmitoyl (polar lipids: PL). Wax ester (WE) standards were purified from lots of field collected individuals (~300) and the method of Ohman (1997).

3.3 Results

3.3.1 Strait of Georgia

Temperature and salinity at depth changed only slightly during the overwintering period (Fig. 3). Relative to the time that the animals descended to depth (April-May), temperature and salinity increased by approximately 0.5°C and 0.5 ppt beginning in September 2003, as a consequence of deep water renewal processes that usually occur in late summer (LeBlond *et al.*, 1991; see also Chapter 5).

The overwintering stock of *Neocalanus plumchrus* was composed primarily of CV copepodids (one precocious male was captured in late June of 2003; Fig. 4), and was most abundant at depths below 200 m (see Chapter 5). Adults appeared in late December or early January. In 2001, only adult males were captured in December, while adult males and immature females were observed coincidently in January of 2003. Adults were distributed slightly shallower in the water column than were CVs, and were captured in the three strata between 100 m and the bottom. CV copepodids were only observed shallower than 200 m in March and April 2003, and presumably represented the 2003 generation.

Lipid contents (for all classes of lipids measured) were generally high in overwintering animals, and declined progressively towards the end of the overwintering period, although small increases in ST and FFA contents were apparent towards the end of the overwintering period (Fig. 4). WE content of CV copepodids declined by 26% between October 2002 and January 2003, from an average of 627±168 (±s.d.) to 461±73 μg. The rate of lipid utilization may be inferred by regression of WE content through time. Fitting an exponential model ($WE=\alpha e^{\beta t}$, where t is time in days) over that four month period corresponds to a lipid utilization rate (β) of 0.0028 μg μg^{-1} d^{-1} (r^2=0.20, p_{ANOVA}=0.006), or an overall decrease of

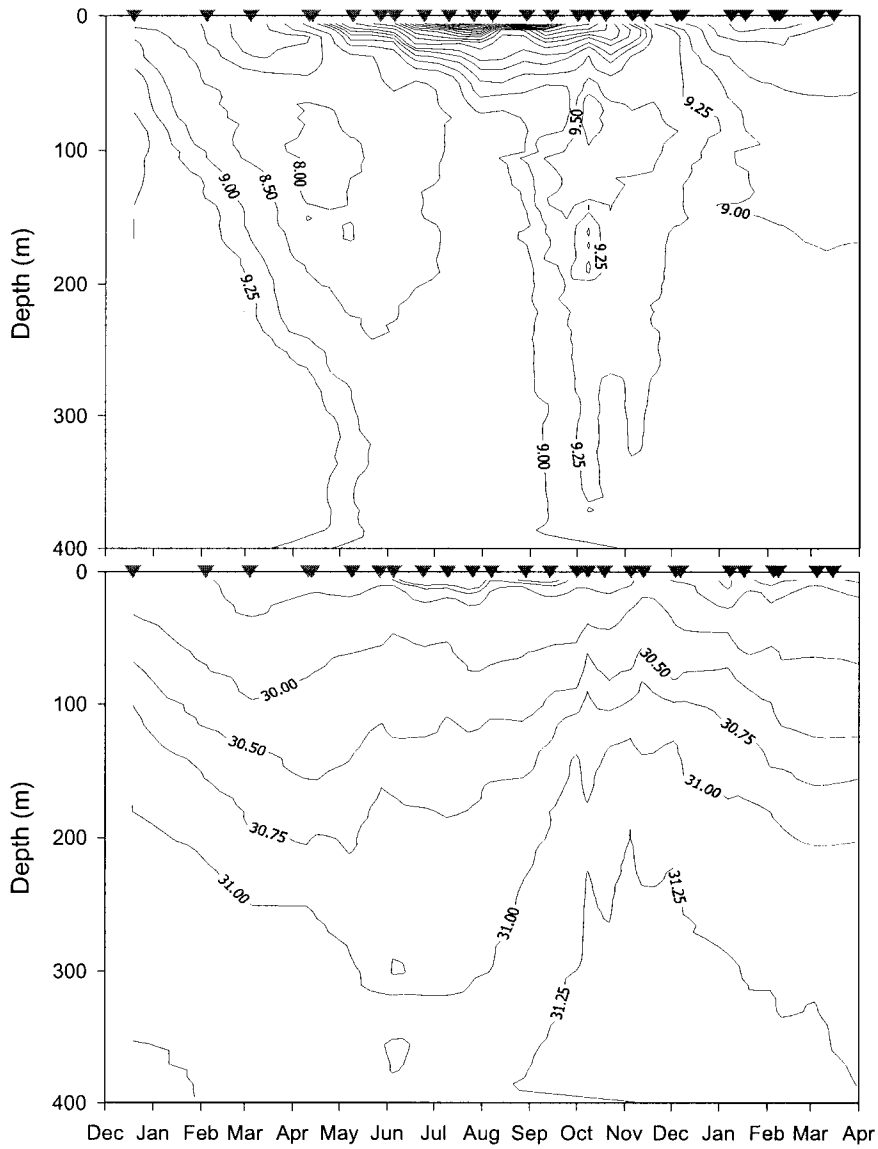

Figure 3. Seasonal variation in temperature (top panel) and salinity (bottom panel) in the Strait of Georgia (49°15' N 123°45' W) during the present study (December 1, 2002 - April 1, 2003). Isotherms and isohalines were fit by linear interpolation (5 m vertical spacing, 14 day temporal spacing) of 1 m binned CTD data. Inverted triangles along the top of the figure denote sampling dates. Data from the STRATOGEM project (www.stratogem.ubc.ca) was incorporated, along with CTD casts done during net sampling instances to improve temporal resolution.

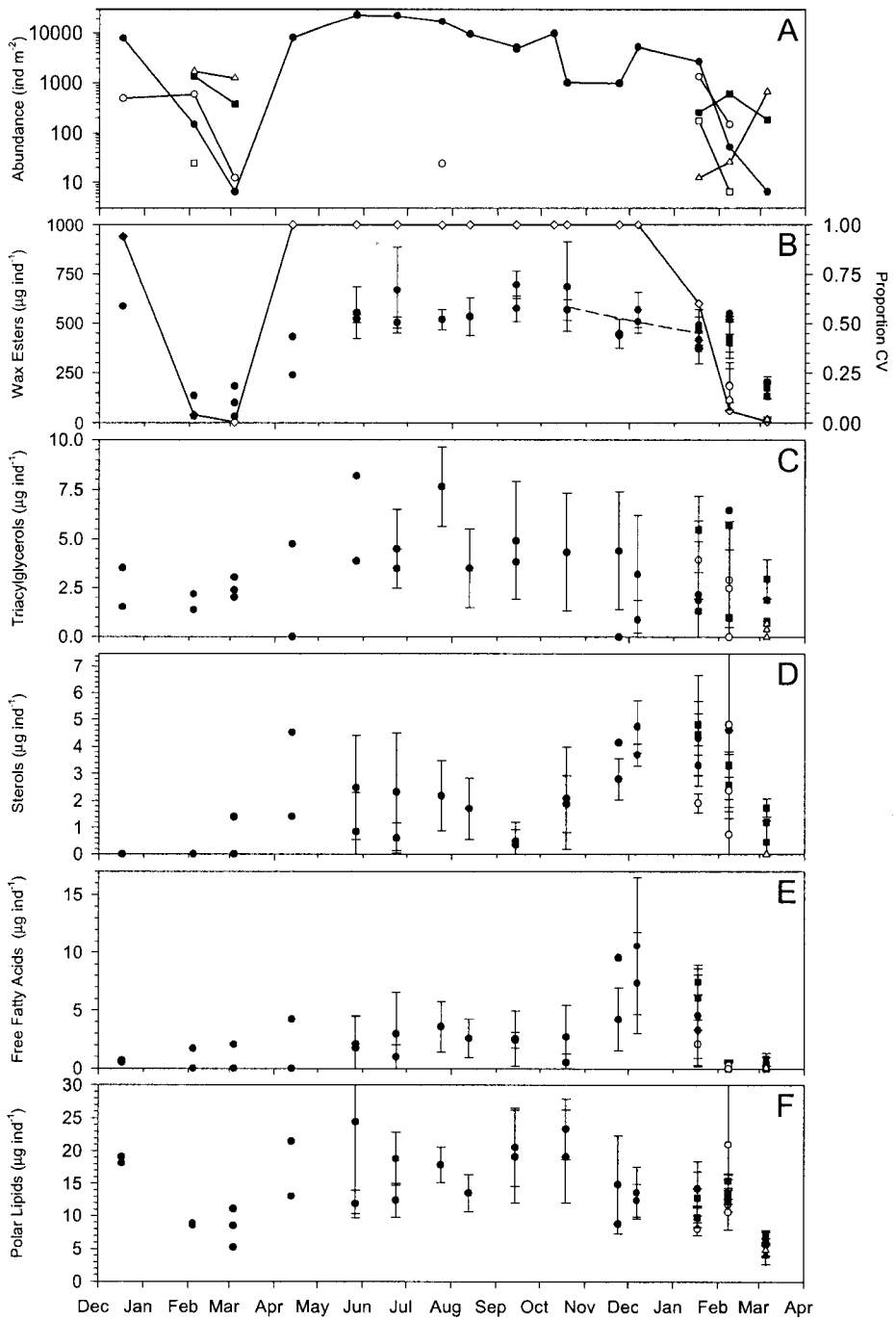

Figure 4. Seasonal changes in stage and lipid composition of *N. plumchrus* in the Strait of Georgia (49°15'N 123°44.9'W) from December 2001 to March 2003. Panel A: Abundance (ind m^{-2}) of stage V copepodids and adults, summed over all depths sampled. Panel B (right axis, open diamonds): proportion of adults. Panels B-F: average lipid composition for wax Esters, triacylglycerols, sterols, free fatty acids and polar lipids, respectively (mean±sd for replicate samples from the same depth stratum). Symbols denote different life history stages: ● = CV, ○ = ♂, □ = immature ♀, ■ = gravid ♀, △ = spent ♀.

22%. Following moulting, adults rapidly used up their remaining lipid contents over the next 1-2 months. There was little indication of any conversion of WE to TAG, TAG usually only made up a small proportion of total lipids of order 1 - 2 % of WE (Fig. 5).

3.3.2 Incubations

Post-capture behaviour of overwintering *N. plumchrus* was similar to that observed in *Calanus finmarchicus* by Hirche (1983). Immediately following capture, most individuals were quiescent, many exhibiting the "overwintering posture" described by Hirche (1983) with first antennae folded ventrally. They were also positively buoyant, and did not respond to handling. Several hours (~3-6) later, however, most individuals were actively swimming, and initiated escape responses if handled. Several days to one week post-capture, most individuals were observed to be neutrally buoyant and did not swim (except for occasional hops), but still exhibited escape responses when handled.

Moulting from CV to adult occurred from 1-2 months post capture (Table 2, Fig. 6). Moulting did not occur at all during the first incubation (based on one observation at 104 days), and there were still CVs present in the second incubation when it was terminated. Moulting was most prolonged during the third incubation. Otherwise, in those incubations initiated during the early part of the *in situ* overwintering period (August to October) adults appeared at about the same time over the course of the incubations (after 56-58 days), and moulting occurred over approximately the same time span (92-98 days). Moulting occurred sooner, and over a shorter time span, in early December, and occurred very quickly in January. There was very little consistent change in TAG, ST and PL over the course of the incubations. This was probably a result of lower overall sample sizes in the incubations, (a consequence of dividing the small number of individuals in each jar into stage and developmental categories), and the very small amounts of those lipid classes present in a given individual. I will therefore confine my analysis of lipids from the incubations to measurements of WE which, as the predominant lipid class (~60% of dry weight), are most quantitative. Measurements of WE in the CVs are also the most robust, because they were

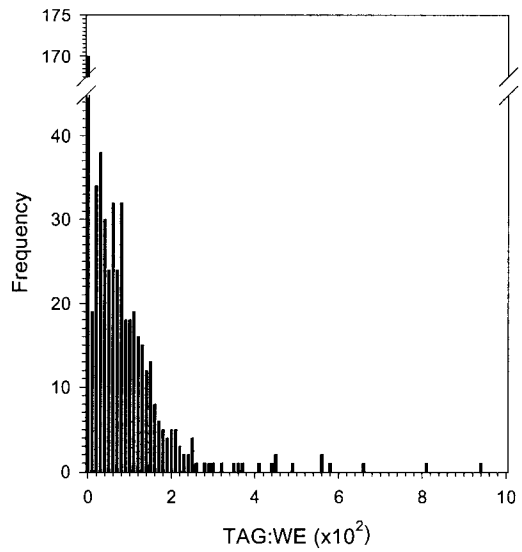

Figure 5. Frequency histogram of TAG:WE (x10^2), all samples combined (incubations and field samples).

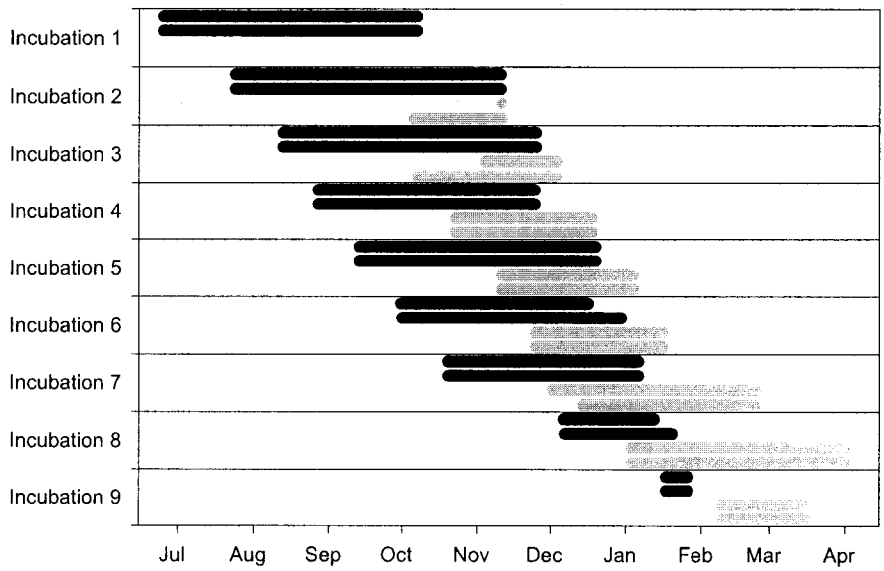

Figure 6. Summary of stage compositions in the incubation experiments. Black bars denote CVs and grey bars denote adults. Left side of the bars is the start time of the incubations (for CVs), or the time point when adults were first observed in the incubations. Right side of bar is the last time point that CVs were observed, or the last time point of the experiment (for adults). Two jars were taken down at each time point, and the top bar (for each stage) is the "lipid" jar and the bottom bar is the "enzyme" jar.

they were the only stage present early on in the incubations, and thus provided good replication (usually 5 replicates of 4 individuals).

The higher frequency measurements of WE content at the beginning of the incubations indicate that there was some variability among incubation jars. Appreciable declines in WE content in CVs were apparent after 2-4 weeks (Fig. 7). The rate of lipid use (inferred from an exponential model, see above) increased in later incubations, and varied from approximately 0.5% to 1 % d^{-1} (Table 2). Overall, lipid content declined more quickly in the incubations than in the field samples, and a greater proportion of lipid was used, (of order 50% as opposed to 22% in the field samples). As observed in the *in situ N. plumchrus* population, lipid contents declined more quickly in adults.

3.4 Discussion

3.4.1 Lipids

Overwintering calanoid copepods are usually characterized by low metabolic rates over much of the overwintering period. However, our observations are consistent with an increase in metabolic activity well in advance of spawning. In Balsfjorden, northern Norway, biochemical constituents (as evidenced by a change in dry weight) have been observed to decline in *C. finmarchicus* in advance of the appearance of adults (Tande, 1982; Hopkins *et al.*, 1984). Maturation of gonads in phenotypic males and females occurs at the same time (Tande and Hopkins, 1981). A similar pattern in lipid content has also been observed in *C. finmarchicus* in the English Channel (Gatten *et al.*, 1979).

Work with *Neocalanus tonsus* in the Southern Pacific, and *Neocalanus plumchrus* from the same station sampled in this study suggests that metabolic reserves are not usually mobilized until very near the time of moulting to adulthood (Ohman *et al.*, 1989; Evanson *et al.*, 2000). The results presented here tell a different story - lipid reserves were mobilized, and considerable proportions (~22% of WE) of those lipids were used, well in advance of actual moulting. The increase in FFA prior to moulting is consistent with lipid use, since wax

Table 2. Results of incubation experiments. Third, fourth and fifth columns are the last day that CV were present, the day when adults were first observed, and the day when only adults were observed, respectively. Observations from the enzyme jars are included, see chapter 4 for results of enzyme measurements. Regression parameters are for fits of $WE=\alpha e^{-\beta t}$ (where t=time in days) for CV only, see fig. 1. Lines were fit to unaveraged data. Proportion WE used is the amount of WE used over the time when CV were present, assuming an exponential decrease (i.e. $1-[\alpha e^{(\text{days CV present})}/\alpha e^{(0)}]$).

Incubation	Start Date	Measurement jar	CV present (d)	Adults first observed (d)	100% adults (d)	α: WE at t=0 (μg)	β: WE use (μg μg^{-1} d^{-1})	n	r^2	p_{ANOVA}	Proportion WE used
1	06/25/2002	lipid	104	-	-	643	0.005	42	0.33	<0.0001	0.41
		enzyme	104	-	-						
2	07/25/2002	lipid	109	73	-	650	0.0081	38	0.54	<0.0001	0.59
		enzyme	109	109	-						
3	08/13/2002	lipid	104	56	113	548	0.0088	33	0.40	<0.0001	0.60
		enzyme	104	84	113						
4	08/28/2002	lipid	89	56	98	583	0.0051	35	0.48	<0.0001	0.36
		enzyme	89	56	106						
5	09/14/2002	lipid	97	58	97	595	0.0079	41	0.47	<0.0001	0.48
		enzyme	97	58	112						
6	10/01/2002	lipid	90	55	97	631	0.0056	27	0.40	0.0005	0.35
		enzyme	77	55	91						
7	10/20/2002	lipid	78	56	92	643	0.0076	35	0.37	0.0001	0.45
		enzyme	78	43	92						
8	12/07/2002	lipid	44	28	51	957	0.0106	29	0.36	0.0042	0.37
		enzyme	37	28	44						
9	01/18/2003	lipid	9	23	23	-	-	-	-	-	-
		enzyme	9	23	23						

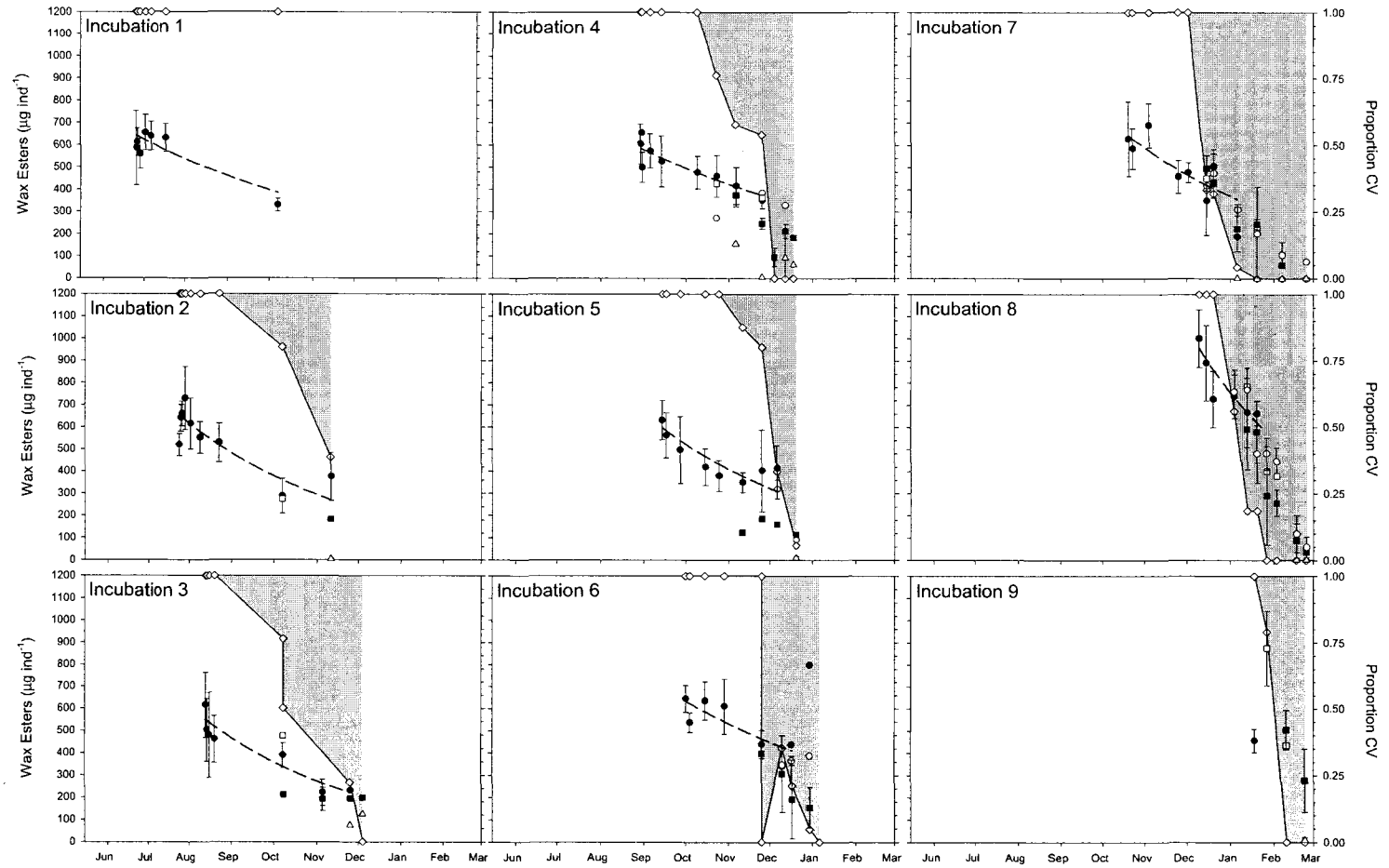

Figure 7. Time course of WE content in incubations. Open triangles indicate proportion of CV (right axis), other symbols as in fig. 4. Dashed lines indicate nonlinear regression of WE content with time (CV only), see table 2 for

esters must be hydrolyzed into their component fatty acid and alcohol prior to being catabolized (Gurr and Harwood, 1991). These differences may be because the former study (Ohman *et al.*, 1989) had poor temporal resolution (a ~2.5 month gap) just prior to moulting, and the gravimetric measurements of total lipid used in the latter study (Evanson *et al.*, 2000) are sensitive to removal of non-lipid materials (Ohman, 1997). The observations presented here confirm that *Neocalanus plumchrus* uses stored lipid well in advance of moulting to adulthood (2-3 months).

In a demographic modelling study, Hind *et al.* (1999) suggested that normal development processes, operating at reduced, temperature controlled rates could explain observed patterns in emergence for *Calanus finmarchicus* in the open North Atlantic. The results presented here (and those of Tande, 1982 and Gatten *et al.*, 1979) suggest that metabolic rates are low during much of the overwintering period (May to October, in this study), but do increase in advance of moulting. In many areas of the open ocean, there is no seasonal signal in temperature at depth. For instance, in Balsfjorden, deepwater renewal does not occur until April (and temperature and salinity are invariant prior to then), while moulting to adulthood begins in late January (Eilertsen *et al.*, 1981; Tande, 1982). Likewise, during the present study, there was only a small increase (0.5˚C) in temperature at depth (Fig. 3) during the overwintering phase. The model of Hind *et al.* (1999) may therefore be an oversimplification, since it is clear that there are physiological (and perhaps behavioural) changes over the course of the overwintering period

The rate of WE use by CVs from the *in situ* population was the lowest observed, and the total amount of WE used was smaller as well (22% as opposed to 35-60%). There is some circulation at depth in the Strait of Georgia (LeBlond *et al.*, 1991), and so it is possible that different groups of individuals were sampled at different times (i.e. individuals with different nutritional histories from different parts of the Strait). Whether or not the population in the Strait receives "new" individuals through Juan de Fuca Strait is uncertain (Harrison *et al.*, 1994; discussed in detail in Chapter 5). The considerable variability in lipid contents may

thus have masked changes in the *in situ* population. Nevertheless, a not insignificant amount of lipid (between one quarter and one half of WE reserves) was used well in advance of moulting.

Low levels of TAG were observed in both the *in situ* time series and the incubations, and TAG made up only a small proportion of the total lipids. TAG is generally considered a "metabolically active" lipid, and appears to be derived primarily from feeding on phytoplankton (Håkanson, 1984). Given that TAG concentrations were very low, and displayed very little seasonality (beyond a decrease in adults), it would appear that remobilization of TAG from WE does not occur during development in *Neocalanus plumchrus*. The decline in TAG observed prior to spawning in *N. tonsus* by Ohman *et al.* (1989; though the temporal resolution is suboptimal, see above), is also consistent with this observation. There is very little evidence for transformation of WE to TAG in calanoid copepods. In short term laboratory incubations, starved *Calanus pacificus* used all TAG within ~3 days (Håkanson, 1984). Miller *et al.* (1998) found elevated levels of TAG in *C. finmarchicus* from the Gulf of Maine in February and March, but the depths from where they were collected and whether or not they were feeding was not reported (and feeding has been observed during winter months in that location: Durbin *et al.*, 1997). Jónasdóttir (1999) observed a small increase in TAG content in deep (>400 m) *C. finmarchicus* in the Faroe-Shetland Channel between January and February. However, there is no guarantee the animals they sampled were from the same population, with the same nutritional history (There is a large amount of transport in that area: Backhaus *et al.*, 1994), and the average values given are statistically indistinguishable (mean±sd of 3.3±3.5 and 5.4±2.2 µg TAG ind^{-1} with n=9 and n=4 gives mean and 95% confidence intervals of 3.3±2.3 and 5.4±2.2 for January and February respectively; Jónasdóttir, 1999, her Fig. 5). The low, fairly constant TAG levels observed here may therefore reflect TAG that is bound up in structural components or physiological mechanisms, as suggested by Miller *et al.* (1998).

Sterols are a constituent of cell membranes and are precursors for numerous compounds used

in regulating endocrine function (Svoboda and Thompson, 1985). Arthropods are incapable of *de novo* sterol synthesis, and all sterols must therefore come from dietary sources (Grieneisen, 1994). The sterol biochemistry of copepods is poorly described, but the dominant forms are known to be cholesterol and the cholesterol precursor desmosterol (O'Hara *et al.*, 1978; Ballantine and Roberts, 1980), from which other sterol compounds are presumably derived. Since *N. plumchrus* does not feed from overwintering CV stages onwards, the increase in sterol concentrations observed in the *in situ* population most likely results from transformations of endogenous sterol reserves to other sterol containing compounds. FID response is dependent on the amount of ionizable carbon within a given lipid class (Sipos and Ackman, 1978). This suggests that the apparent increase in sterol concentration would have been caused by an increase in the amount of carbon within sterol compounds, rather than an increase in sterols *per se*. Moulting steroids (e.g. ecdysone: $C_{27}H_{44}0_6$) have the same 27 carbon arrangement as cholesterol ($C_{27}H_{46}0$), and would presumably have the same FID response, and other hormones in the crustacea appear to be derived from compounds other than sterols (Quackenbush, 1986). Small amounts of C_{28} and C_{29} sterols have been observed in calanoid copepods (Ballentine and Roberts, 1980), but their function is not known.

The polar lipids (as measured by TLC/FID) include phospholipids (structural components of membranes), glycolipids (functional components of membranes such as receptors), pigments, and non-lipid residues (Parrish, 1987). In zooplankton, polar lipids appear to be primarily phospholipids (Parrish, 1998), with smaller amounts of plant-derived glycolipids and pigments (Parrish *et al.*, 2000). The production of gonads would, of course, require phospholipids for the construction of membranes, and given that *Neocalanus plumchrus* does not eat during gonadogenesis, any phospholipids used would need to come from internal reserves. The drop in polar lipid content observed in females (Fig. 4) would then have been caused by the losses due to spawning. The phospholipid content of males was also low (Fig 4), implying that phospholipids may also be used as a source of energy, given the smaller gonad of males.

3.4.2 Termination of dormancy

It appears that dormancy in overwintering calanoid copepods is relatively plastic. Although overwintering dormancy is usually characterized by low physical and physiological activity, (see Hirche, 1996), the dormant state is interrupted if the animals are disturbed. In this study, animals that were initially quiescent when captured did not return to the dormant state. Rather, they appeared to switch towards development of gonads (as evidenced by a decrease in lipid), and moulting to adulthood. There was, however, a lag of several weeks before moulting occurred, and moulting tended to occur more quickly as it became closer and closer to the "natural" moulting period of individuals *in situ* (Fig. 6).

The CVs in at least the first six incubations (prior to November) were in a dormant state when collected, assuming one accepts that the lack of significant lipid use in the *in situ* population indicates low metabolic activity (enzyme assays in part corroborate this: see Chapter 4). In those incubations, moulting was less likely to occur (i.e. CVs were present longer) and adults took longer to appear (never appearing in the first incubation, though the temporal resolution is poor). In the latter four incubations (collected when lipid contents were already declining *in situ*) there was a progression towards earlier, and more synchronized, moulting (Fig.6, Table 2). This is consistent with observations by Fulton (1973), who observed that *Neocalanus plumchrus* collected in September had not moulted and still contained significant lipid in December. Such a change in behaviour in the absence of any external environmental cues suggests that *N. plumchrus* possesses an endogenous clock that modulates physiology over longer time scales, but that development becomes imperative once it has been switched on. Similarly, Conover (1965) found that when kept in the laboratory without any environmental stimuli, *C. hyperboreus* moulted sychronously with *in situ* populations almost one year after collection.

During "classical" diapause (*sensu* Danks, 1987), dormancy is maintained until some stimulus is received that prompts the organism to terminate dormancy. The suggestion of an endogenous rhythm is consistent with a physiologically controlled diapause state, and

endogenous rhythms have been identified in other invertebrates during diapause (Blake, 1959; Olive and Garwood, 1983; reviewed in insects by Hodek, 2002). Results from the incubations suggest that, during the process of being collected, the animals receive enough stimulation to prompt them to terminate dormancy and continue their development. Although I made every effort to capture the animals as gently as possible, a certain amount of disturbance (and a short term increase in light and temperature) was unavoidable. I cannot conclude from these data which stimulus (or combination of stimuli) prompts dormancy termination in *Neocalanus plumchrus*.

Why do *Neocalanus plumchrus* exhibit a highly synchronized life history, but do not exhibit dormancy in laboratory incubations? Although the dormant state appears to be fragile, it does not appear to terminate spontaneously in the *in situ* population. Results from the incubations suggest that the animals do have some knowledge of the time of year, and delay moulting when collected earlier in the year. One obvious cue that was missing from the incubations is pressure. If *N. plumchrus* has a pressure preference used to maintain position in the water column, but is unable to achieve that preferendum, this may trigger a "decision" to break overwintering and continue development. *Calanus finmarchicus* changes its behaviour in response to small changes in pressure encountered during diel migrations (Rice, 1962), and a mechanism for baroreception has recently been observed in a decapod crustacean (Fraser *et al.*, 2001). The pressure physiology of copepods is essentially unknown, and merits further study.

Dormancy in *Neocalanus plumchrus* appears to be a rather fragile state. The conditions experienced by overwintering zooplankton are considerably more dynamic than those experienced by terrestrial insects. Overwintering copepods must maintain their vertical position in the water column, where they are presumably displaced by tidal and residual currents. The Strait of Georgia is a large estuarine system, and deep water renewal occurs regularly in late summer, when gravity flows over the sill to the south end of the Strait inject warm, salty water to depth (LeBlond *et al.*, 1991; Fig 3). Deepwater renewal events may

therefore result in vertical displacements of overwintering *N. plumchrus*, and will alter *in situ* seawater density, which can affect the buoyancy properties of the animals (see Chapter 2). A "hardwired" dormancy state would be disadvantageous in such an environment, and it seems more likely that the overwintering animals fine tune their position occasionally, and then return to dormancy once they experience the proper conditions. Some degree of activity during the dormant state is not contrary to a true diapause state, as activity has also been noted in several insect species during diapause (Hodek, 2002). In an environment as dynamic as the marine pelagial realm some activity may be adaptive.

Copepods are significant grazers of oceanic primary production (e.g. Calbet, 2001), and prey to higher trophic levels (e.g. Cushing, 1975), and a thorough knowledge of the factors mediating their life history is of value. The use of field studies to elucidate physiological mechanisms clearly has drawbacks - sampling at a high enough frequency is difficult and expensive, and there is no guarantee that the same population will be present (thus increasing the apparent variance). Similarly, simulation of overwintering conditions *in vitro* presents considerable challenges, though they are not insurmountable (e.g. see Torres *et al.*, 1982). Development of techniques that allow the stimulation and maintenance of copepod dormancy in the laboratory will be required to properly describe physiological changes during the life history.

CHAPTER 4

Ecophysiology of overwintering in the copepod *Neocalanus plumchrus*: Enzyme activity and protein content

4.1 Introduction

Overwintering calanoid copepods generally exhibit low metabolic rates, presumably as a strategy to conserve metabolic and structural reserves to hedge against the highly seasonal primary production characteristic of mid- to high-latitude marine ecosystems (e.g. Hirche, 1996). In the Northeast Pacific, the dominant mesozooplankton (numerically and in terms of biomass) are copepods of the genus *Neocalanus*, particularly *Neocalanus plumchrus* (Miller *et al.*, 1984). *N. plumchrus* is an interzonal migrant (Vinogradov and Arashkevich, 1969), that spends a large proportion of its life history at depth in a dormant state (Fulton, 1973). It is particularly important for *N. plumchrus* to conserve its metabolic reserves, because it does not resume feeding once it emerges from overwintering. Thus, development from CV to adult, moulting and spawning are all fueled by endogenous energy reserves.

Respiration rates observed in overwintering copepods are usually low (Ingvarsdóttir *et al.*, 1999), presumably due to a reduction in lipid metabolism in order to preserve lipid reserves for use in gonadogenesis and reproduction (Conover, 1988; Hirche, 1996). Observations of lipid contents usually indicate no significant decreases, or a decrease at very low rates, during overwintering (see Chapter 3). It has also been suggested that overwintering copepods may use protein as an energy source (Hirche, 1996; Evanson *et al.*, 2000). Evidence for protein use during overwintering includes observed declines in total protein content (Orr, 1934; Kirkesæter, 1977, *cited in* Hirche, 1996); declines in nitrogen content (Tande, 1982; Ohman *et al.*, 1989); low but measurable rates of ammonium excretion (Conover and Corner, 1968; Cowen, 1982); and low O:N ratios (Conover and Corner, 1968; Kosobokova, 1990).

Measuring metabolic rates in zooplankton is a nontrivial process. *In vitro* incubations can be used to observe changes in respiratory or excretory products, but require large numbers

of individuals and are particularly sensitive to crowding and behavioural artefacts. To avoid these issues, enzymatic measurements of metabolic activity have often been used because they provide rapid estimates of the physiological state of small number of individuals, and avoid incubation artefacts (reviewed by Mayzaud, 1986; Ikeda *et al.,* 2000).

Oxygen consumption during respiration occurs inside mitochondria, via a series of linked enzymes known as the electron transport system (ETS). Zooplankton respiration rates have been routinely estimated by assaying the activity of the electron transport chain, whereby the amount of reduction of INT (2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride) in a cell-free homogenate is measured, and assumed to be proportional to the rate of respiration. The most widely used method has been that of Owens and King (1975), in which INT reduction is measured following the addition of substrates (NADH and NADH) in unlimited concentration. Citing wide variability in published values of the ratio of measured respiration rates to ETS activity (R:ETS), Båmstedt (2000) has recently suggested that ETS activity is not controlled by the amount of respiratory enzymes (of which the Owens and King method is a proxy). Instead, it was suggested that the ETS is substrate limited, and that substrates should not be added. Rather, cell free homogenates should be incubated in the presence of INT, and allowed to react to completion (so that the reduction of INT is an indication of how much substrate was reduced).

Calanoid copepods are primarily ammonotelic, whereby ammonium production results from the catabolism of proteins and nucleic acids (Corner and Cowey, 1968). In order to be catabolized, protein is broken down into amino acids by various proteases, then transaminated with α-ketoglutarate to produce α-keto acids and glutamate. Glutamate is then oxidized by glutamate dehydrogenase (GDH) into ammonium, NADH, and a proton (Bidigare and King, 1981). GDH activity may therefore be assayed by observing changes in the production of NADH in cell-free homogenates to which substrate has been added. Comparisons of GDH activity to ammonium production have shown good correlations in some studies (Bidigare and King, 1981; Ikeda *et al.,* 2000 and references therein). However,

Berges *et al.* (1993) found that GDH activity was related to body size but not to growth or feeding condition in *Artemia salina*, and Hernández-León and Torres (1997) found considerable variability in GDH:ammonium production ratios in mixed zooplankton.

In Chapter 3, I documented changes in the lipid composition during time series measurements of natural and simulated populations of *Neocalanus plumchrus* from the Strait of Georgia. In this chapter, I present the results of concurrent measurements of enzyme activity and protein composition, the goal being to demonstrate how the physiological state of *N. plumchrus* changes over its life history, particularly during the termination of the overwintering state.

4.2 Methods

4.2.1 Collection and incubations

Collection, handling and incubations of animals followed the procedures detailed in chapter 2. From the field samples, aliquots of approximately 20 animals were removed from the second half of the sample and filtered onto a small square of nitex mesh that was then folded, placed in a cryovial, and frozen in liquid nitrogen. Part way through the study it was found that it was not possible to stage adults accurately upon thawing (such samples are designated as "mixed"). Thereafter, beginning in March 2002, groups of 10-20 individuals were sorted by stage and frozen separately on combusted glass fibre filters. Terminated incubations were enumerated for stage composition, and also frozen *en masse* on a nitex square in liquid nitrogen.

To determine protein content and enzyme activity, the methods of Bidigare and King (1981), Smith *et al.* (1985) and Båmstedt (2000) were modified in order to make multiple measurements from the same homogenate. For each sample, the nitex square or filter was thawed, and ≤20 individuals placed in 1 ml of homogenization medium (0.1 M Tris buffer with 2 ml l^{-1} Triton X-100 made up to pH 8.5 with HCl). The animals and homogenization medium were then sonicated with a Branson model 250 ultrasonic homogenizer (1 minute

duration, 70% duty cycle, power level 40 Watts). The sample was suspended in a dry ice-ethanol bath during homogenization in order to reduce temperature rise. The homogenized samples were centrifuged for 5 minutes at 1500 x g in a swinging basket centrifuge prior to removing aliquots for ETS assays and protein determinations. Samples were kept on ice prior to all assays in order to reduce losses of enzyme activity.

4.2.2 Protein content

Protein content was determined using the binchoninic acid assay of Smith *et al.* (1985). The method uses a single standard working reagent (SWR), that is 50 parts reagent "A" (10 g l^{-1} bicinchoninic acid, 20 g l^{-1} Na_2CO_3, 1.6 g l^{-1} sodium tartrate, 4 g l^{-1} NaOH and 9.5 g l^{-1} $NaHCO_3$, adjusted to pH 11.25 with 10M NaOH) to 1 part reagent "B" (40 g l^{-1} copper (II) sulphate pentahydrate). SWR is stable for at least one week, but a fresh batch of SWR was made up for each homogenization. Protein content was measured by mixing the homogenate with SWR 1:20 (v/v), incubating for 30 minutes at 40°C, and measuring absorbance against a homogenization buffer blank at 562 nm. Typically, 100 µl aliquots of homogenate were mixed with 2 ml of SWR, though in samples with a large number of individuals (that saturated the absorbance), smaller aliquots were removed and topped up to 100 µl with homogenization buffer. Standards for calibration curves were made up with bovine serum albumin, and absorbance was read on an LKB Ultrospec II spectrophotometer.

4.2.3 ETS activity

ETS activity was assayed using a modification of Båmstedt's (2000) technique. In this study, 400 µl of homogenate was mixed 1:1 by volume with a modified ETS reagent (0.02 M Na_2HPO_4, 0.15 mM $MgSO_4$, 3 mg ml^{-1} polyvinylpyrrolidone, and 2 mg ml^{-1} INT, adjusted to pH 8.5 with HCl). Note that all concentrations (except INT) are twice those given by Båmstedt (2000), and were diluted to the proper concentration upon adding the modified ETS reagent to the homogenate. It was not possible to dissolve INT into solution at 4 mg ml^{-1}, so 2 mg ml^{-1} was used. The concentration of INT was therefore half that used by Båmstedt (2000), but was still sufficient to produce a response. Following addition of the

modified ETS reagent to the homogenate, it was incubated at 40°C for 1 hour, quenched, fractionated with chloroform/methanol, diluted with methanol, and the absorbance read at 475 nm (against a homogenization buffer blank) on an LKB Ultrospec II spectrophotometer. The modified ETS reagent was standardized as per Båmstedt (2000) in order to create a calibration curve relating the amount of INT reduced to absorbance. The amount of methanol used to dilute the fractionated mixture was adjusted (between 1 and 3 ml) to place the absorbance reading within the range of the calibration curve.

4.2.4 GDH Activity

GDH activity was assayed using modifications of the techniques of Bidigare and King (1981) and Bidigare *et al.* (1982). Since our homogenates contained a smaller amount of material, we improved the response by using a larger volume of homogenate (350 µl), and adjusting the amount and concentration of reagents accordingly, in order to achieve the same final concentrations as Bidigare *et al.* (1982). The reaction mixture had a volume of 1.1 ml, and contained 2 mM ADP, 1.2 mM NAD^+, and 40 mM glutamate. All reagents were added separately (125, 125 and 500 µl respectively), and glutamate was always added last. Prior to running the assay, samples were centrifuged at 10000g for 10 minutes in a fixed angle rotor in a refrigerated centrifuge at 2°C in order to remove any suspended matter. GDH activity was estimated by measuring the increase in absorbance at 340 nm following the addition of glutamate, using a CARY 5 spectrophotometer fitted with a temperature controlled block operating at 9°C. All reagents were stored in unused cells of the block prior to the assay, to allow them to cool to 9°C as well. Absorbance was measured at 1 second intervals, and the difference in absorbance in the linear region of the curve (between 20 and 70 seconds) was used to calculate the rate of change of absorbance. Occasionally, local minima were observed in the early part of the absorbance trace, and were attributed to resuspended particulates from the centrifuge tube wall that were aspirated while pipetting the homogenate. In those instances, a shorter time interval (but still ending at 70 s) was used to avoid the minimum.

4.2.5 Calibrations

In order to relate the observed enzyme activities to actual respiration and ammonium excretion rates, a number of calibration incubations were carried out using individuals collected from the study station (49°15' N 123°45' W) in April 2003. A total of four incubations were set up in 300 ml stoppered DO bottles containing between 22 and 51 individuals in 0.7 μm filtered sea water, as well as initial (T_0) controls and filtered sea water only controls. T_0 controls were measured upon return to the lab (approximately 3 hours after the incubations were set up). Incubations and animal-free controls were measured following a 24 h incubation period. Oxygen concentrations were measured with a Hanna HI9142 Oxygen electrode, and ammonium concentrations were measured as per Koroleff (1983). Following measurements of oxygen concentration and removal of water for ammonium determinations, all individuals were removed from the bottles, frozen in liquid nitrogen, and ETS and GDH activities determined as described above. Respiration rate:ETS activity (mean±sd) from those incubations was 7.8±1.1 (μg O_2 mg protein^{-1} d^{-1}:μg O_2 used in assay mg protein^{-1}) and GDH activity:ammonium excretion was 15.3±4.3 (ΔA340nm:μmol NH_4^+ ind^{-1} d^{-1}).

In order to include the effect of temperature on metabolic rate, enzyme activities not measured at *in situ* temperatures are often corrected with the Arrhenius equation (Owens and King, 1975). For the purposes of this study, I have not corrected measured enzyme activities, because the focus is primarily on changes in physiology, not excretory or respiratory products. The Båmstedt (2000) method is independent of temperature (since all substrate is used), and the GDH assay was done at 9°C. The temperatures experienced by *Neocalanus plumchrus* during overwintering at depth (200-400 m) in the Strait of Georgia do not vary greatly (~9-9.5°C), and the incubations were held at a constant 9°C. I have used the measured R:ETS and GDH:NH_4^+ excretion ratios to convert measurements of activity to excretion or oxygen consumption rates (the currency used by other workers), but point out that rates calculated for individuals at the surface (where temperatures were higher) are likely underestimates of actual *in situ* rates.

4.3 Results

4.3.1 Strait of Georgia population

Protein content was highest in overwintering animals, and showed a progressive decline in adults in both mixed and stage-discrete samples. The lowest values were recorded in spent females (Fig. 8A). Surface-dwelling stage 5 copepodites (CV) had relatively low protein content (mean±sd 84.6±23.6 μg) in April 2002, but a relatively high (175.6±2.6 μg) protein content in April 2003. There were no significant depth-based differences in protein, GDH or ETS activity, so samples will not be distinguished by depth. There was no significant decline in protein contents during the 2002 overwintering period, and the slope of a linear regression of protein content vs day for CVs (May 2002 to January 2003) was not significantly different from zero (p_{ttest}=0.79).

ETS activity displayed a significant positive correlation with protein content (Pearson's product moment: r=0.74, p<0.001). GDH activity, however, was not correlated with protein content r=-0.01, p=0.91; Fig. 9). Therefore, ETS activity will be expressed in protein specific units, and GDH activity on a per-individual basis. The two enzyme assays used here measure very different aspects of the physiology of the animals. GDH "activity" is actually a measurement of the amount of NAD^+ that is reduced to NADH by GDH, in the presence of unlimited substrates (glutamate and ADP). Therefore, any GDH that is present will be operating at V_{max}, and the amount of NADH produced over a small time period will simply be a function of the amount of enzyme present. Thus, although the term GDH "activity", is used here, what has actually been measured is a proxy for the amount of enzyme present in the homogenate.

Båmstedt (2000) has suggested that measuring what is essentially the amount of enzyme present does not accurately reflect the amount of "activity" for a given enzyme system (in this case, ETS activity). Rather, it measures the "capacity" of that system at the point in time that it is measured. Båmstedt's (2000) method differs in that it allows the enzymes that are present to convert all available substrate into product. It is therefore a proxy for the amount

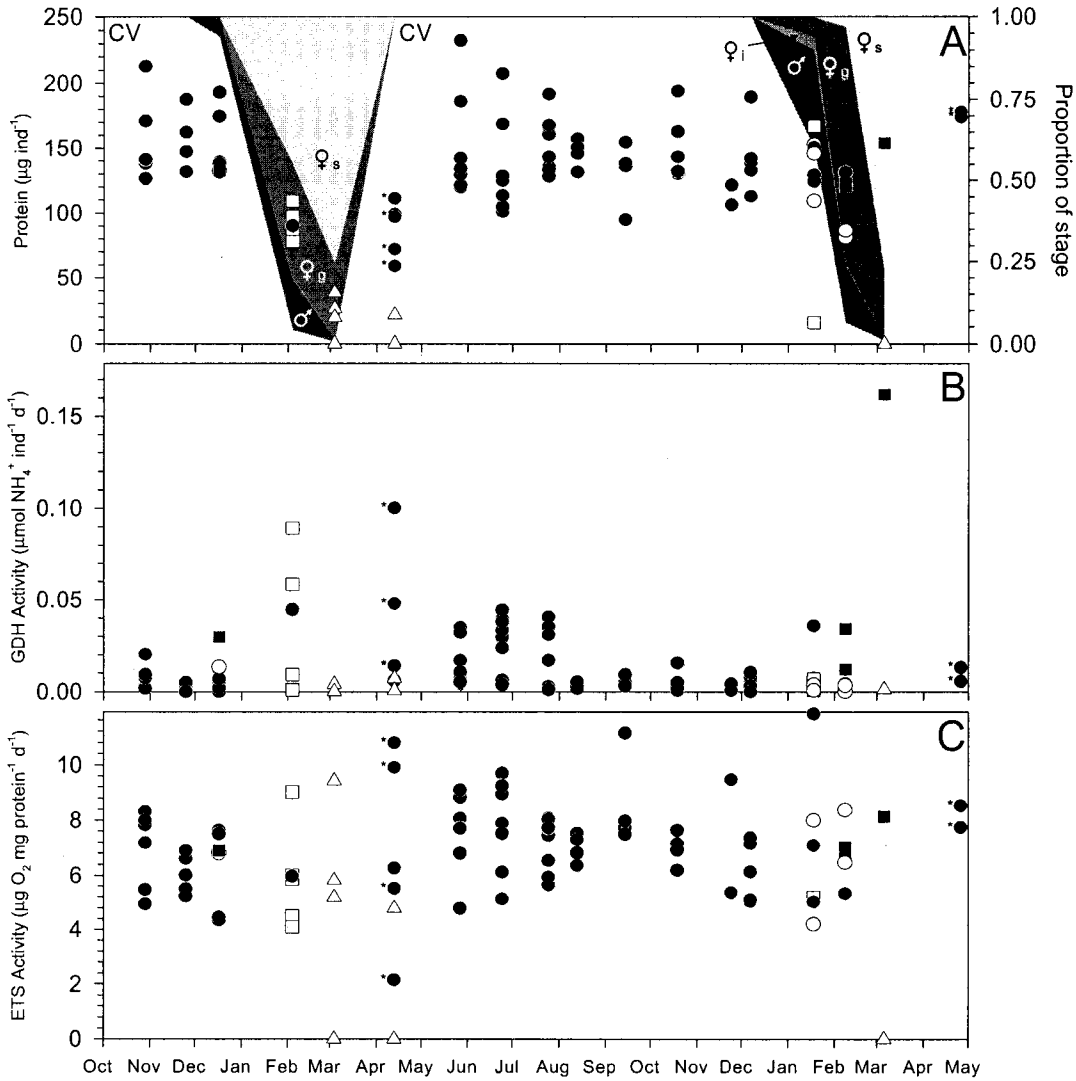

Figure 8. Seasonal variation in protein contents and enzyme activities in *N. plumchrus* from the Strait of Georgia, October 2001 - May 2003. Panel A: Protein content, shaded background and right axis are relative proportions of stage/reproductive state from depth-integrated counts ($♀_i$: immature females; $♀_g$: gravid females; $♀_s$: spent females). Panel B: GDH activity. Panel C: ETS activity. Symbols denote stage/reproductive state: ● = CV, ○ = ♂, ■ = gravid ♀, △ = spent ♀, and □ = mixed adults (not sorted by stage). Asterisks denote samples from surface stratum (0-100 m).

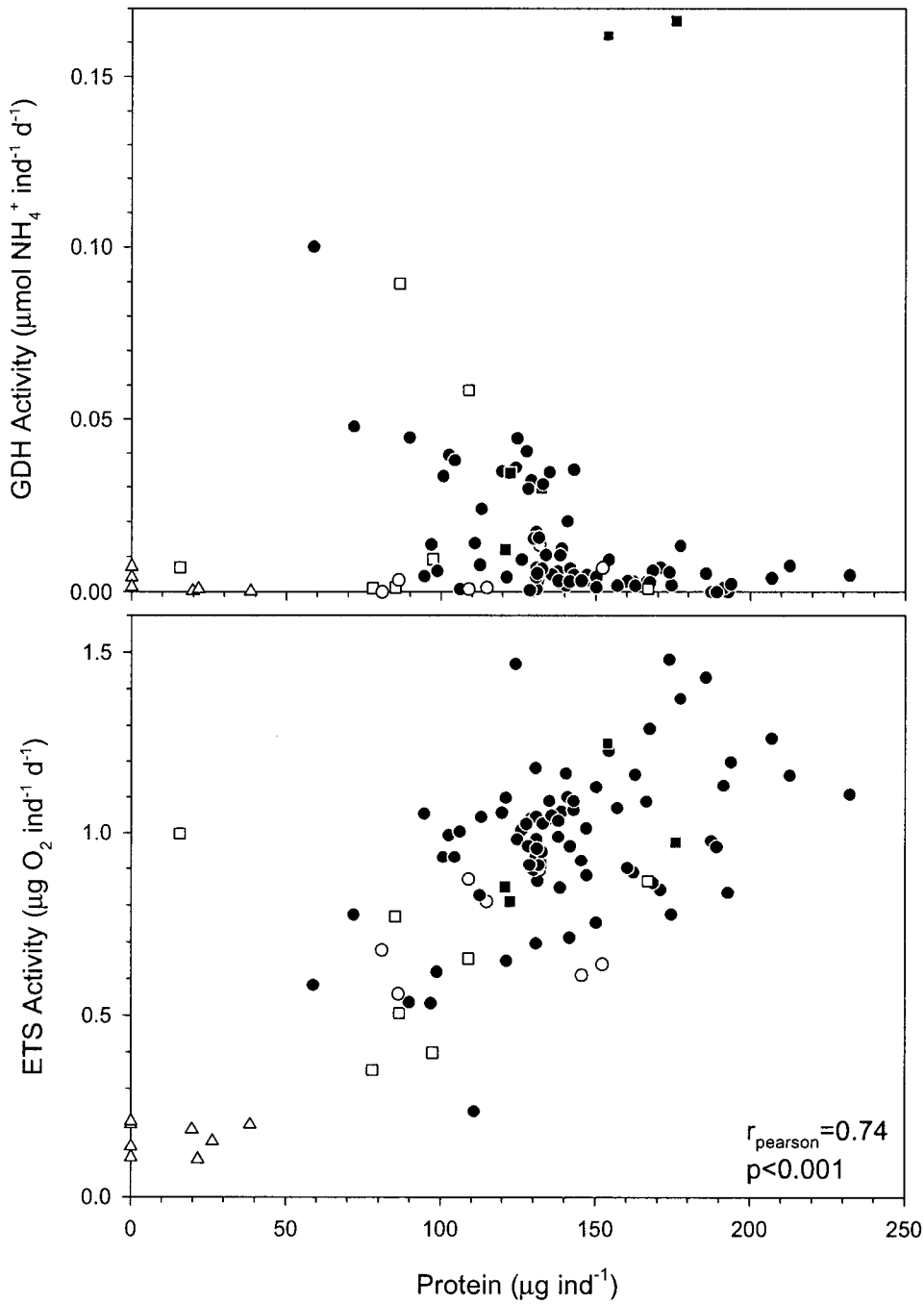

Figure 9. Relationship between protein content and (top panel) GDH activity or (bottom panel) ETS activity in groups of *N. plumchrus* collected from the Strait of Georgia. Symbols as in figure 8.

of "substrate" that is present within a homogenate. Thus, while I will refer to ETS "activity" (in order to maintain a consistent vocabulary), what has actually been measured is a proxy for the amount of substrate in a homogenate.

GDH activity did not change consistently over the annual cycle (Fig 8B). High activity was observed in some adults, and surface dwelling CVs in 2002 only. Approximately half the CVs overwintering at depth had high GDH activity in May-July 2002. Closer examination of GDH activity and protein content of overwintering CVs (i.e. between May 2003 and Feb 2003: Fig. 10) indicates that individuals with less protein early on in the overwintering period tended to have higher GDH activity, while most of the individuals collected later in the season tended to have lower GDH activity.

Since individual-specific ETS activity was correlated with protein content (Fig. 9), it followed protein content fairly closely, with the highest activity occurring in overwintering animals, and the lowest activity observed in adults. After normalizing ETS activity to protein content much of the seasonal signal was removed, leaving very little discernible pattern in ETS activity over the seasonal cycle (Fig. 8C). Surface dwelling CVs did exhibit some of the highest ETS activities, but there was also considerable variability in both the overwintering CVs and adults.

4.3.2 Incubations

Protein content generally declined over the course of the incubations, with the greatest declines occurring after moulting to adulthood had occurred (Fig. 11). Changes in enzyme activities were much less consistent. GDH activity loosely tracked protein content but did not always decline in synchrony (incubations 5 and 6, notably). Changes in ETS activity over the course of the incubations did not change markedly, although there was usually a net decrease with time. In some incubations protein-specific ETS activity increased abruptly during the final time point. Those final time points were comprised almost entirely of spent animals, and thus the increase in ETS activity was due partly to their very low protein contents (e.g. the final time point of incubation 8 had undetectable amounts of protein, which

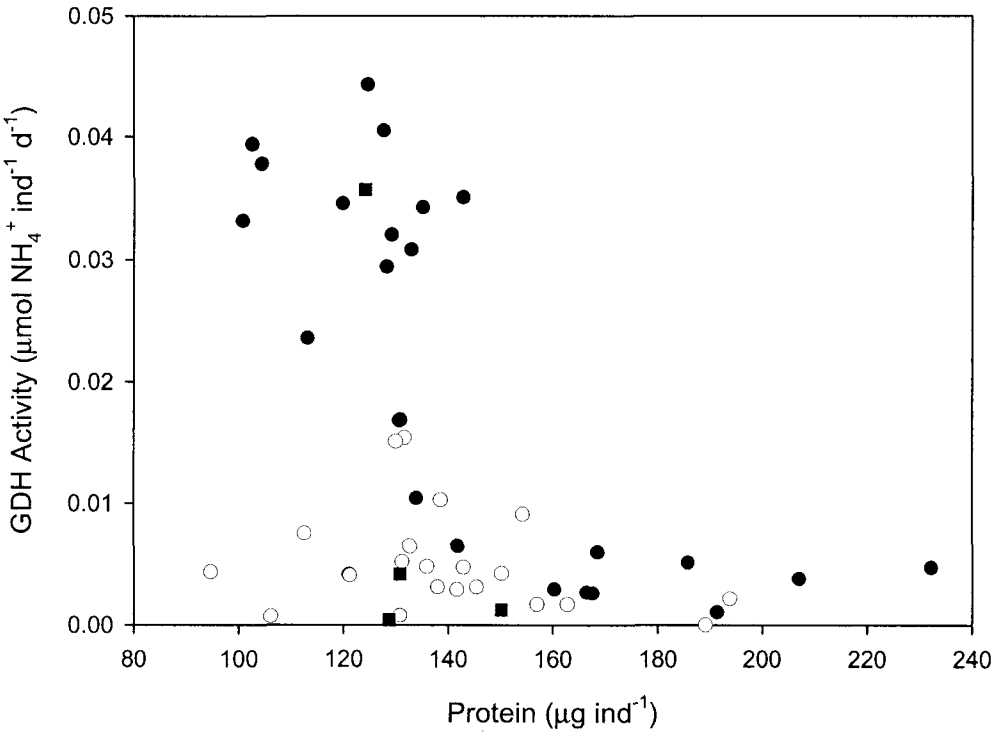

Figure 10. Relationship between protein content and GDH activity for overwintering *N. plumchrus* CV copepodids from the Strait of Georgia, May 2002 - February 2003. Symbols denote collection time: ● = May - July, ○ = August - December, ■ = January & February.

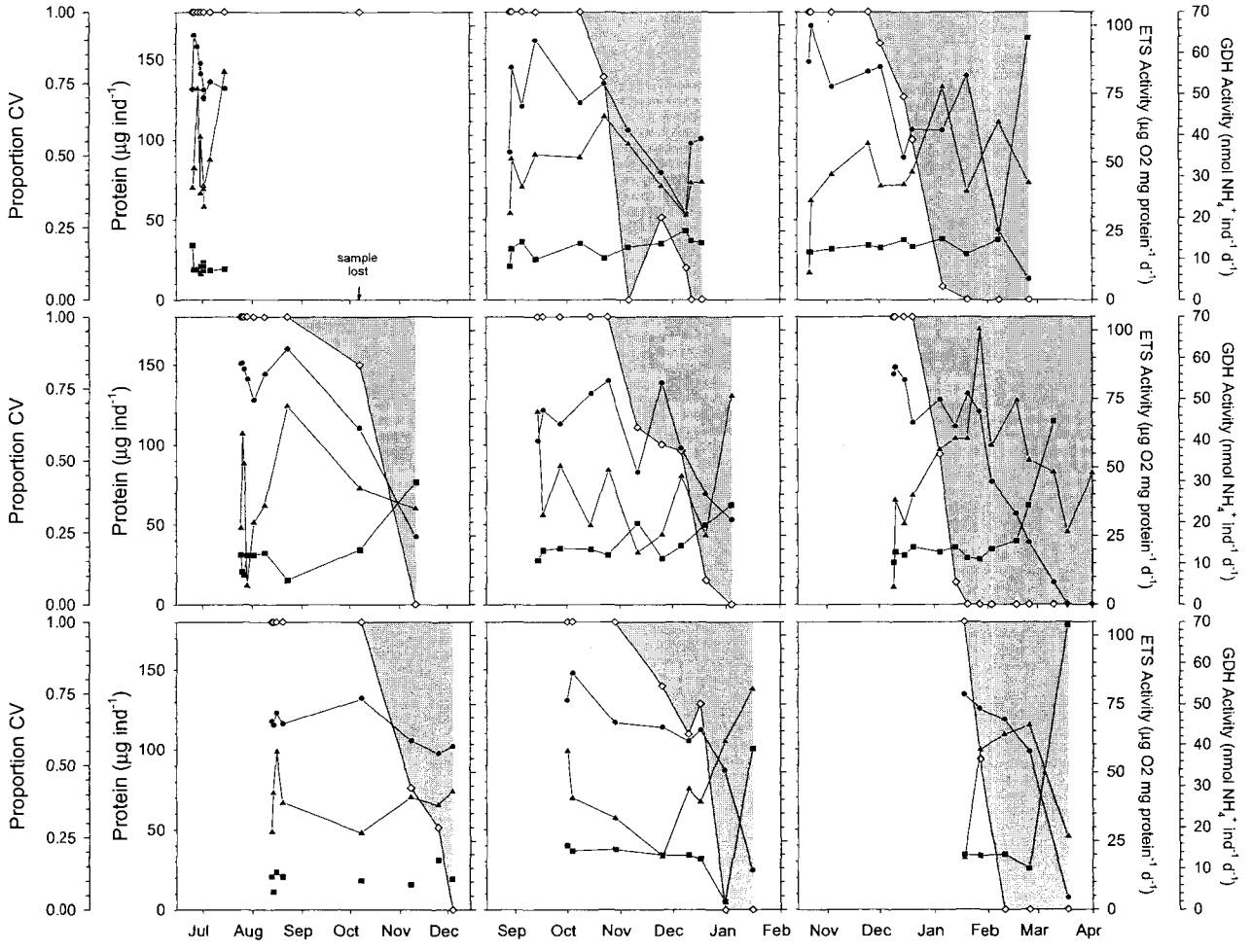

Figure 11. Time course of stage composition, protein content and enzyme activities in *N. plumchrus* incubation experiments. Open diamonds (highlighted by shaded area) indicate proportion of CVs, symbols denote measurement: ● = protein content, ▲ = GDH activity and ■=ETS activity. Note columnwise scale change of abscissa.

would make protein specific ETS activity nominally infinite). Each measurement (i.e. time point) is a single unreplicated value, which probably explains the variability among incubations.

As observed in the *in situ* population, GDH activity was uncorrelated with protein content ($r=-0.06$, $p=0.61$), while ETS activity was positively correlated with protein content in the incubations ($r=0.43$, $p<0.001$; Fig. 12). Since it was necessary to pool all individuals from a single incubation jar, the observed protein and enzyme activities are aggregates of all the developmental stages in a jar when that particular incubation was terminated. The exact stage composition of the "cohorts" varied amongst incubations, making it difficult to infer the extent to which each stage contributed to the observed value. However, dividing each incubation into three groups (all CVs, all adults, and everything in between; i.e. 100%CV, 0% CV and 0%<CV<100%) reveals a decline in protein content as the relative abundance of CVs declines. This is not unexpected, however, given that protein contents declined with time. GDH activity had a wide range in all three groups, and there is some indication of a decline in ETS activity in incubations containing adults.

4.4 Discussion

4.4.1 Protein metabolism

Since the period during which young stages of *N. plumchrus* are present in surface waters is brief (Fulton, 1973), and the sampling frequency of the field population was approximately monthly, active CVs were observed in surface waters only once per year. In April 2002, surface dwelling CVs had comparatively low protein and high GDH activity, while CVs in April 2003 had higher protein content and low GDH activity. This suggests that the individuals sampled in 2002 were actively growing and catabolizing ingested protein (lipid levels were also intermediate: Chapter 3), while in 2003 they had essentially finished structural growth and were pre-dormant.

Protein contents did not change appreciably during the overwintering period in the Strait of

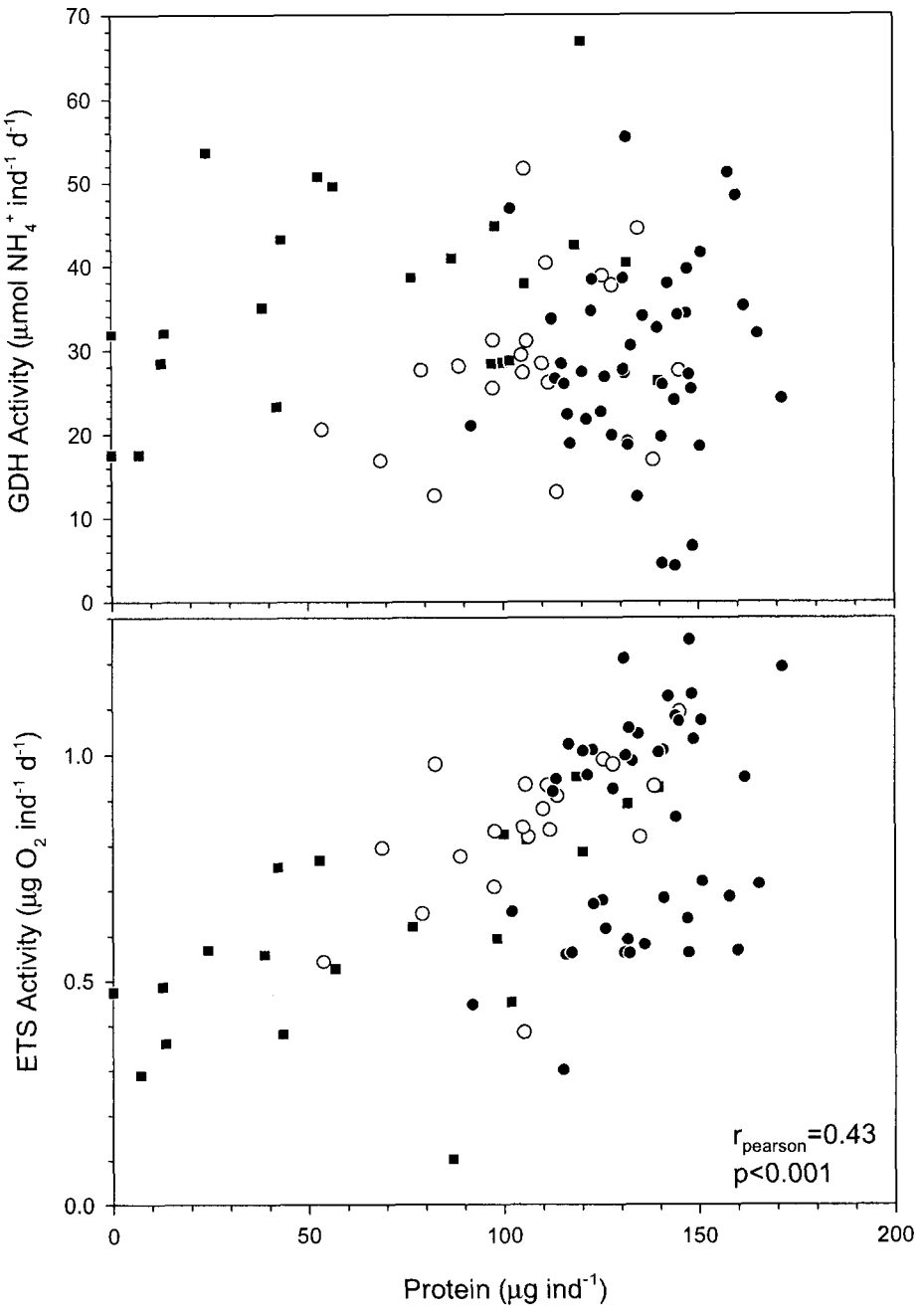

Figure 12. Relationship between protein content and (top panel) GDH activity or (bottom panel) ETS activity in *N. plumchrus* incubations. Symbols denote relative stage proportions in each incubation: ● = 100% CV, ○ = 100%>CV>0%, ■ = 100% adults).

Georgia *Neocalanus plumchrus* population. Curiously, during the earliest part of the overwintering period, GDH activities were highest in measurement groups that contained the lowest amount of protein, but activity was almost universally low towards the end of the overwintering period (Fig. 10). Since GDH "activity" is really a measurement of the amount of enzyme, it is possible that the high activities observed early in overwintering are actually an indication of a supply of GDH left over from growth while near the surface during the spring. However, this is inconsistent with the low GDH activities observed in surface dwelling CVs in April 2003. Also curious is the high protein content observed in some measurement groups early (but not later) on in the overwintering period. This may have resulted from protein catabolism (inconsistent with the low GDH activities observed), or simply from sampling different subpopulations with different nutritional histories. Other studies have observed low ammonium excretion rates in overwintering calanoids (Conover and Corner, 1968). Cowen (1982) observed extremely low excretion rates in overwintering *N. plumchrus* CVs from Saanich Inlet, BC; almost an order of magnitude lower than those of Conover and Corner (1968). The results presented here are consistent with a decline in excretion rates during overwintering, and suggest that GDH activity is a reasonable (albeit noisy) proxy for excretion rate in *N. plumchrus*. However, there is still considerable variability (presumably driven by variability in the physiological states of individuals: Båmstedt, 1988): the high GDH activity early on in the overwintering period suggests a considerable lag in changes of physiology. A similar lag has also been observed in traditional measurements of ETS activity using the Owens and King method (Mayzaud, 1986).

It has been proposed that copepods use protein as a metabolic substrate during overwintering, in order to preserve lipid stores for reproduction (Kirkesæter, 1977 *cited in* Hirche, 1996). It has also been proposed that protein may be used to fuel egg release in *Neocalanus plumchrus* (Evanson *et al.*, 2000). The onset of moulting by the Strait of Georgia *N. plumchrus* population was not accompanied by a large drop in protein content, although a decline was observed in males and spent females (and the "mixed" samples from 2002

indicate a decline as well). GDH activity was higher in gravid females, perhaps indicating some increase in protein metabolism related to egg production.

A more accurate indication of protein use during the termination of overwintering might be expected from the incubation experiments. Each incubation represents an "artificial cohort", and therefore ought to contain individuals with relatively similar nutritional histories. As in incubations designed to observe changes in lipids during the termination of overwintering (Chapter 3), moulting was delayed in the earlier incubations, and progressed towards earlier moulting later on in the season. The incubations therefore represent a somewhat artificial time series of changes in physiology during the termination of the dormant state, that interacts with the state of the individuals when they were captured.

Measurements of protein from the incubations did show a consistent decline, particularly after moulting had begun. However, GDH activity did not change in concert with protein content, as might be expected if active protein catabolism were occurring. Pooling all individuals from an incubation for a single measurement probably obscures stage-specific patterns: different stages (i.e. developing CVs, males, and immature, gravid and spent females) likely use their protein contents in different ways. For instance, females must use some of their protein reserves for the production of eggs (Guisande and Harris, 1995 cite a value of 57-59% protein for in the eggs of *Calanus helgolandicus*), while males may invest far less protein in the construction of their much smaller gonads. Stage specific measurements will be required to clear up this uncertainty.

4.4.2 Respiratory enzyme activity

Båmstedt (2000) suggested that the variability in R:ETS ratios observed by numerous other workers using the Owens and King (1975) method (i.e. observing INT reduction at V_{max}) is an indication that the method is not an accurate measurement of respiration. He found that, in several taxa, his substrate-limited method produced more stable R:ETS ratios. The results presented here are not consistent with a low respiration rate in overwintering animals, as has

been observed in other calanoid copepods (e.g. Conover and Corner, 1968; Ingvarsdóttir *et al.*, 1999), nor is there any coherent seasonal cycle apparent (beyond, perhaps, a reduction in variability centered on the overwintering period).

Packard (1971), citing Chance (1954) and Chance *et al.* (1955) stated that the rate limiting step in the ETS is the oxidation of the coenzyme Q-cytochrome B complex. Chance (1954; interestingly cited as well by Owens and King, 1975) states that there is also evidence that there is no rate limiting step in the ETS. Chance (1965) found that limiting/inhibition reactions were a complex and oyxgen-dependent interaction between substrates, ADP, and phosphate. In any case, all of the aforementioned observations were made on intact mitochondria (and were primarily interested in the production of ATP via oxidative phosphorylation).

Owens and King (1975) used the same method as Packard (1971), but added 0.2% (v/v) Triton X-100, and observed a 200-400% increase in the amount of activity recorded (i.e. INT reduction). Triton X-100 is a mild nonionic detergent that aids in dissolving (solubilizing) membrane phospholipids (le Maire *et al.*, 2000). INT is reduced specifically by complex III of the ETS (Siedler, 1991), and the assay therefore measures the amount of reduction done by complex III in solution. The obvious conclusion is that the increase in activity observed by Owens and King (1975) was caused by solubilizing the mitochondrial membranes and releasing ETS enzymes into solution, releasing them from some limiting condition experienced while incorporated into the membrane.

Regulation of metabolic pathways is a highly complex interaction among numerous physiological requirements and environmental imperatives. There is good evidence that mitochondrial respiration is primarily controlled by adenylate pathways that, in turn, control ETS activity (Tzagoloff, 1982; Hochachka and Somero, 1984), and that ADP concentration limits mitochondrial respiration in certain crustaceans (Chen and Lehninger, 1973). Thus, although one might expect that measurement of ETS activity of intact mitochondria will be

related to actual respiratory rates, measurements of ETS activity (or "substrate use") of isolated enzymes will not. The correlation between enzyme activity (i.e. "substrate") measured with the Båmstedt (2000) method and body size (as measured by protein) is consistent with this hypothesis. The amount of substrate available to reduce INT may simply be a reflection of the size of the animals. Furthermore, enzyme assays done with solubilized membranes may be far removed from actual biochemical function, and may explain the wide range in R:ETS ratios observed by those using the Owens and King (1975) method. Clearly, further investigation will be necessary if enzyme assays are to be used as a proxy for respiration.

The results presented here suggest that metabolic rates of *Neocalanus plumchrus* are relatively low during their overwintering period. No significant protein metabolism was observed prior to moulting, and GDH activity was low over much of the overwintering period. The higher activity observed earlier may represent a lag in changes in the enzymatic machinery of individuals. There is some suggestion, however, that protein metabolism increases in adults.

In the absence of respiration incubations, it cannot be said whether actual respiration rates changed. There was considerable variability in ETS activity of the *in situ* population, but there was comparatively little change during the incubations. Concurrent measurements of lipid contents (Chapter 3) show that lipid was used prior to moulting (both *in situ* and in the incubations), and one might therefore expect that respiration would increase as well, in order to provide the ATP necessary for vitellogenesis (e.g. there is evidence from eels that the respiratory machinery ramps up in order to fuel vitellogenesis: Lokman *et al.*, 2003). The lack of any coherent pattern suggests that either (i) the ETS assay (at least as it was used) provides little information on the true physiological state of the organisms (as has been observed elsewhere: Berges *et al.*, 1993), or (ii) any changes that did occur were masked by individual variability (which can be considerable: Båmstedt 1988) lost by analyzing groups of individuals.

CHAPTER 5

Life history and depth distribution of *Neocalanus plumchrus* in the Strait of Georgia

5.1 Introduction

The depth distribution of overwintering calanoid copepods is variable. In the open ocean, overwintering depths often span several hundreds of meters, and vary both among species and among locales within species. For instance, *Calanus finmarchicus* has been observed to overwinter at depths ranging from several hundred meters to below 1000 m in the open North Atlantic ocean (e.g. Hirche, 1991; Miller *et al.*, 1991b; Heath and Jónasdóttir, 1999; Heath *et al.*, 2000), while the three species of *Neocalanus* that occur in the open Pacific (*Neocalanus plumchrus*, *N. cristatus*, and *N. flemingeri*) overwinter at depths spanning 400 to 2000 m (e.g. Vinogradov and Arashkevich, 1969; Miller *et al.*, 1984; Miller and Clemens, 1988; Kobari and Ikeda, 1999, 2001a, 2001b). In marginal seas, where bottom depths are often shallower than the overwintering depths observed in the open ocean, depth distributions are often compressed. In some cases individuals have been found to accumulate just above the bottom (Williams, 1985; Sameoto and Herman, 1990; Herman *et al.*, 1991), while in others they have been observed to position themselves higher in the water column (Giske *et al.*, 1990; Baliño and Aksnes, 1993). In areas with regional oxygen minima (Santa Barbara Basin and Saanich Inlet), narrow overwintering depth distributions, with high concentrations (10^3-10^4 individuals m^{-3}), have been observed at or near seasonal oxyclines (Alldredge *et al.*, 1984; Mackie, 1984; Osgood and Checkley, 1997).

Overwintering dormancy is widely held to be an adaptive strategy that conserves energy reserves in highly seasonal high-latitude ecosystems. Adaptive arguments (usually centered around avoiding predation) have similarly been used to explain the very deep depth distributions observed in overwintering calanoid copepods (e.g. Fiksen and Giske, 1995). Observed depth distributions are believed to result from three potential mechanisms (Kaartvedt, 1996): (1) differential mortality at depth; (2) selection over time for individuals to "choose" those depths that maximize fitness returns; or (3) use of environmental cues by

individuals to select overwintering depths in order to avoid predators.

There is evidence that individual *Calanus finmarchicus* overwinter at depths where their predators are least abundant (Kaartvedt, 1996, 2000). It is also known that the presence of predators (or cues that could indicate their presence) can alter the behaviour of many zooplankton, including marine copepods (Bollens and Frost, 1989, 1991). However, in Santa Barbara Basin, high mortalities were incurred by *C. pacificus* occupying a very narrow depth stratum above the oxycline (Alldredge *et al.*, 1984). Mortality in overwintering copepods can be substantial (Ohman and Wood, 1996). Although mortality rates are usually much lower than in surface dwelling individuals (e.g., Aksnes and Magnesen, 1983) they are integrated over longer time frames, leading to significant declines in abundance (e.g. Miller *et al.*, 1984). It is not clear at present what mechanism(s) operate (or how they might interact) to structure vertical distributions of overwintering copepods.

The depth distribution of *Neocalanus plumchrus* during its overwintering period is not well described. Studies in the open Pacific have usually sampled only very broad depth strata (e.g. 10^2-10^3 meters: Vinogradov and Arashkevich, 1969; Miller *et al.*, 1984; Kobari and Ikeda, 2001a). Mackie (1985), counting individuals from a submersible in Jervis Inlet BC (November - December), found *N. plumchrus* between depths of 80 and 520 meters, with a modal abundance at ~275 m. In the Strait of Georgia, Fulton (1973; at the same station as this study) used horizontally towed Miller nets to measure the abundance of *N. plumchrus* at six depths in September and December 1968. In September, individuals were collected between ~200 and 400 m with a peak at 300 m, while in December, most individuals were below 250 m (and there was no discernible peak). Gardner (1972; also the same station as this study) observed a similar pattern at the same station in 1971 and 1972, and found CVs, and adults distributed much higher in the water column in January and February.

The objectives of this chapter were to measure the fine scale vertical distribution of *Neocalanus plumchrus* late stage copepods in the Strait of Georgia, and to compare the

observed distribution to those described in historical data as well as with *N. plumchrus* populations from other locales. In order to do this I have combined data from traditional net tows with optical based measurements of depth-stratified abundance, in conjunction with concurrent hydrographic measurements. Given the observed changes in depth, the modelling framework described in Chapter 2 is then applied to show how losses in lipid contents (Chapter 3) and changes in hydrographic properties could effect depth distributions during the overwintering period.

5.2 Methods

5.2.1 Net samples

Net samples were collected as outlined in Chapter 3. Each formalin-preserved sample (that had already been split once at sea) was split again with a Folsom splitter (McEwan *et al.*, 1954), and the number of CV and adults counted under a stereomicroscope. Adult female reproductive stages were defined as outlined in section 3.2.1. The total fraction counted was adjusted such that a minimum of 200 individuals were counted per sample (assuming that at least that many were present in the original sample; the fraction counted varied between 1 and $1/16^{th}$). Early on in the study the net was fitted with a flowmeter, but it was later found that it still revolved after the net was closed, so a constant filtering efficiency of 0.78 (determined empirically from prior studies) was applied.

5.2.2 Optical plankton counter

In addition to the traditional net samples, a Focal Technologies Optical Plankton Counter (OPC) was used to count individual particles *in situ*. The OPC consists of a 2x25 cm sampling tunnel, an array of light emitting diodes (LEDs), and a detector, all mounted within a pressure casing. Details of its operation are provided by Herman (1992). Briefly, light from the LEDs is collimated through a lens into a beam that passes across the sampling tunnel, and is then refocused by another lens onto an array of photodiodes (the detector). The photodiodes convert the light measured by the detector into a proportional electrical signal (Focal Technologies Inc., 1999). As the OPC is towed though the water, particles pass

through the tunnel and occlude the light emitted by the LEDs, creating a pulse (a voltage drop) at the detector. The pulse is separated from the background signal and relayed to a computer for logging. The amount of light occluded (which is proportional to the cross-sectional area of the particle) is proportional to the electrical signal measured at the detector (Herman, 1988). A feedback loop at the detector and an external photodiode monitor the background light level and adjust the intensity of the LED to account for variations in light attenuance. In this fashion, each particle passing through the light beam is counted, and its cross-sectional area estimated as an equivalent spherical diameter. Non-spherical objects (such as copepods) that pass through the beam will therefore present a range of sizes, depending on their individual orientations as they occlude the beam. An external electronic flowmeter (General Oceanics model 2135) was used to estimate flow through the sampling tunnel, and a pressure transducer measured depth. The OPC outputs a timer value, a flow value and a depth value twice per second, and counts are logged immediately after they are registered. Since a slip-ring winch with conducting wire was seldom available, data were usually logged by a custom built autonomous computer logging system, and a battery pack that also supplied power to the OPC.

Initially, the OPC was mounted on a small frame and profiled vertically, along with a SeaBird model 19 (internally logging) CTD and a WETlabs WETstar fluorometer (Fig. 13). However, given that (i) the tunnel samples a very small amount of water ($0.005 m^2$), (ii) the descent rate was necessarily slow (~1 m s^{-1}), and (iii) particles could only be counted on the downcast (since the frame obstructed the back side of the tunnel), only a small volume of water could be sampled in the time available. For instance, the SCOR net used in this study (mouth diameter of 55cm) sampled approximately 78 m^3 in a 400m vertical tow (assuming a 78% sampling efficiency) while the OPC sampled only 2 m^3 over the same 400m tow. Given that each vertical OPC cast took approximately 30 minutes, and because usually only half a day could be devoted to OPC deployments per trip, only a very small total volume of water could be sampled, even with multiple casts.

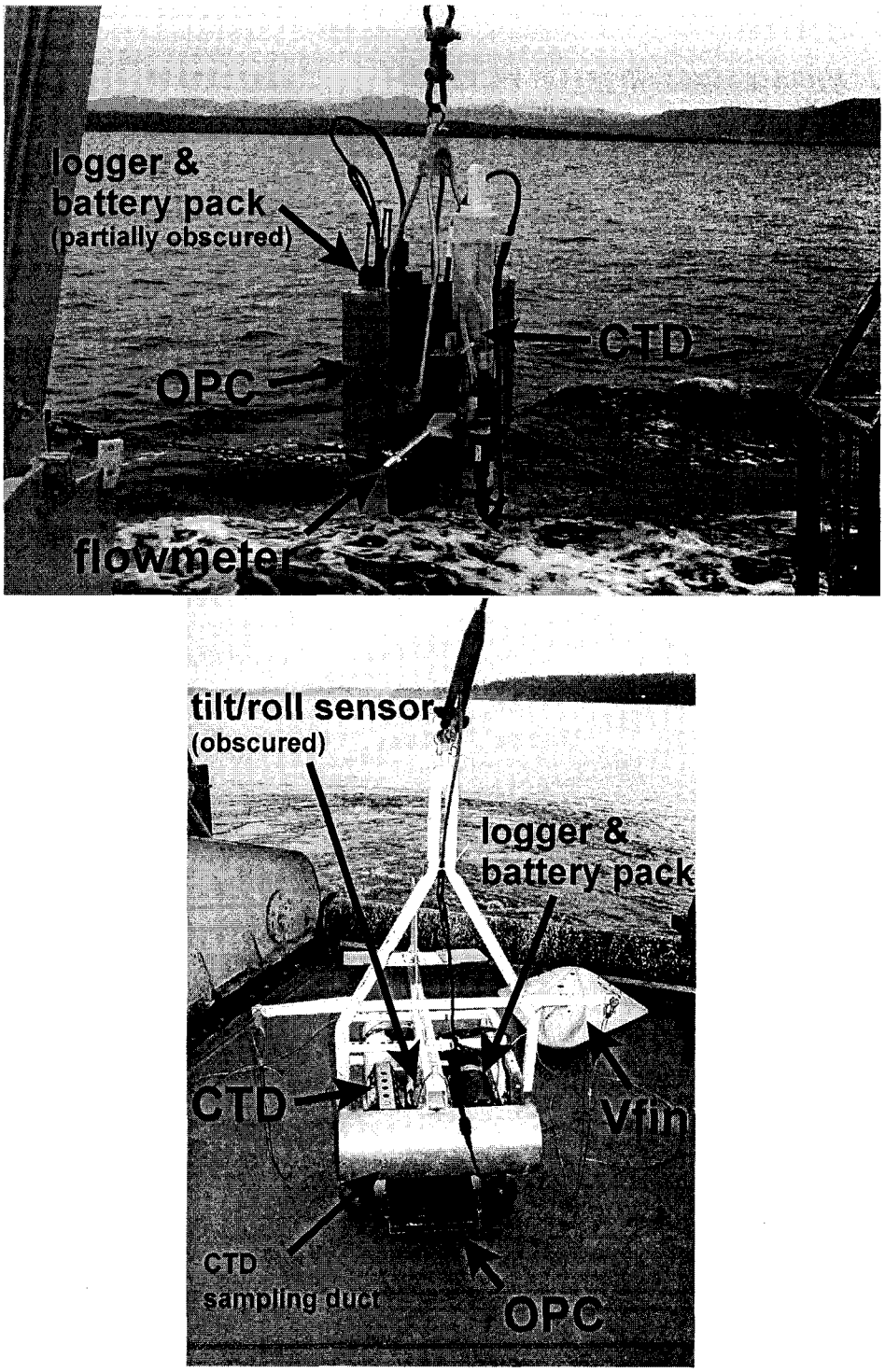

Figure 13. Configurations of the OPC/CTD package in vertical profiling (top panel) and towing (bottom panel) modes.

To get around this problem, the OPC and CTD were mounted on a different frame and towed horizontally during the latter part of the study (05/2002 - 03/2003). A tilt/roll sensor was also added to monitor the orientation of the towing frame and OPC, and a V-fin depressor weight was suspended below the frame to provide additional downward force (Fig. 13). This permitted considerably faster towing speeds (usually about 1.5 - 2 m s^{-1}). The depressor weight partially offset the upward force of drag on the wire and tow body, and helped keep the instrument package at depth for longer periods, thus increasing the volume sampled at each depth. Through trial and error it was found that, with a ship speed of 3 knots, and with a wire-out speed of 1-1.5 m s^{-1} the tow body would be in close (and occasionally intimate) proximity to the bottom with 750 m of wire paid out. Once 750 m of wire had been paid out, the winch brake was applied and the package towed for 10-15 minutes, then retrieved at ~0.5 m s^{-1}. When towing in this manner, the towing frame stopped sinking once the winch was stopped, and slowly lost depth due to drag on the wire and frame until it reached a reasonably stable depth. Once the cable was retrieved, it began to move upward again. Using this arrangement, a considerably larger volume (as much as an order of magnitude more in a 10 m depth bin) of water was sampled.

5.2.3 Data processing

CTD data were processed with software provided by Seabird. As per their operating instructions, conductivity and pressure were low-pass filtered (1 and 0.5 second time constants respectively), and temperature was advanced relative to pressure by 0.5 seconds. Temperature, conductivity and pressure were then binned into 1 m bins, and derived units (salinity and density) calculated.

OPC data were processed using a collection of custom routines written in MATLAB, several processing steps were required. Raw data were read in and checked for counts > 50 s^{-1},which can cause significant coincidence counts (Focal Technologies Inc., 1999). Timer values were occasionally dropped from the data record, and new values were substituted to situate the data in the proper place. Flow (>3.5 m s^{-1}) and depth (values where |depth(t+1)-depth(t)| >3

m) spikes were found, and new values were substituted from linear interpolation between adjacent values. Flow was also smoothed with a 5 s moving average. Flow rates for vertical casts were determined from fall velocities based on changes in pressure, and fall velocities were also smoothed with a 5 s moving average.

For a variety of reasons, a substantial number of OPC casts had to be excluded from the analysis. During March/April 2003, an intermittent ground fault caused spurious counts and incorrect depth and flow values, and several casts from that period were therefore discarded. During postprocessing, it was determined that the electronic flowmeter was unreliable at low towing speeds, and therefore almost all of the towed downcasts had questionable flow values and were not used. As well, on a small number of casts the tow body inverted during the upcast (diagnosed from the tilt/roll sensor, or apparent upon arrival at the surface), and those casts were also excluded. Thus, 66 out of 137 casts were deemed suspect and excluded from the analysis.

Raw data were binned into 10 m depth bins (~40 per 400 m cast). The volume sampled within each bin was calculated (the sum of flow rates times the number of 0.5 s periods within the bin), and the number of counts within the bin tabulated. In order to create a single composite cast for each sampling date, counts and the volume sampled in each bin were then summed across multiple casts from the same date.

In order to compare the OPC-derived abundances with net-derived observations, the binning procedure was repeated using 100 m bins (i.e. depth ranges equivalent to those sampled by the net). Since the OPC package did not always sample the entire water column, 300-400 m net samples were only compared against instances where the OPC had sampled to depths greater than 390 m (November 2001; January, February, March, April and July 2002; and January and February 2003).

5.2.4 OPC calibration

Essentially, the OPC measures the area of the shadow produced by an organism, and converts that measurement to an equivalent spherical diameter (ESD). However, since most zooplankton are non-spherical, and often partially transparent, the digital size produced by the OPC does not usually equal the actual size of the organism (Herman, 1992; Sprules *et al.*, 1998). Thus, in order to generate an empirical size distribution for *Neocalanus plumchrus*, a laboratory calibration was conducted.

In order to generate the calibration relationship, a sample of *Neocalanus plumchrus* was collected from the usual sampling site (200-400 m, August 28, 2003), placed in 0.7μm filtered sea water (FSW), and transported to the lab in coolers with ice packs. The animals were allowed to equilibrate for two days in a dark 9˚C cold room and the OPC was also placed in the cold room and allowed to equilibrate as well (to prevent condensation on the lenses). A flow cell with a 2x2 cm square section was placed inside the sampling tunnel, clamped into place, and connected to a peristaltic pump (Watson Marlow, model 704S/R IP55) operating in a closed loop. The system was filled with FSW, and groups of 20 animals were introduced to the system. The system was run for 5 minutes at a flow rate of approximately 1 m s^{-1} (estimated by timing the movement of the animals through a measured section of tubing). Two different groups of 20 individuals were run through the system. A peak of very small counts was noted in blank runs, which indicated bubbles in the system (Fig. 14). The size range (ESD) of the bubbles did not overlap with that of *N. plumchrus*, so no steps were taken to remove them. The mean (±sd) size recorded in both runs was 2408.5±311.5 μm (Fig. 14), and 95% of the observations fell within a range encompassing 1705 to 3016 μm. That range was therefore taken as the size range that can be expected for *N. plumchrus*.

5.3 Results

5.3.1 Hydrography

Temperature and salinity at depth changed slightly (by ~0.5˚C and 0.5 ppt) during the

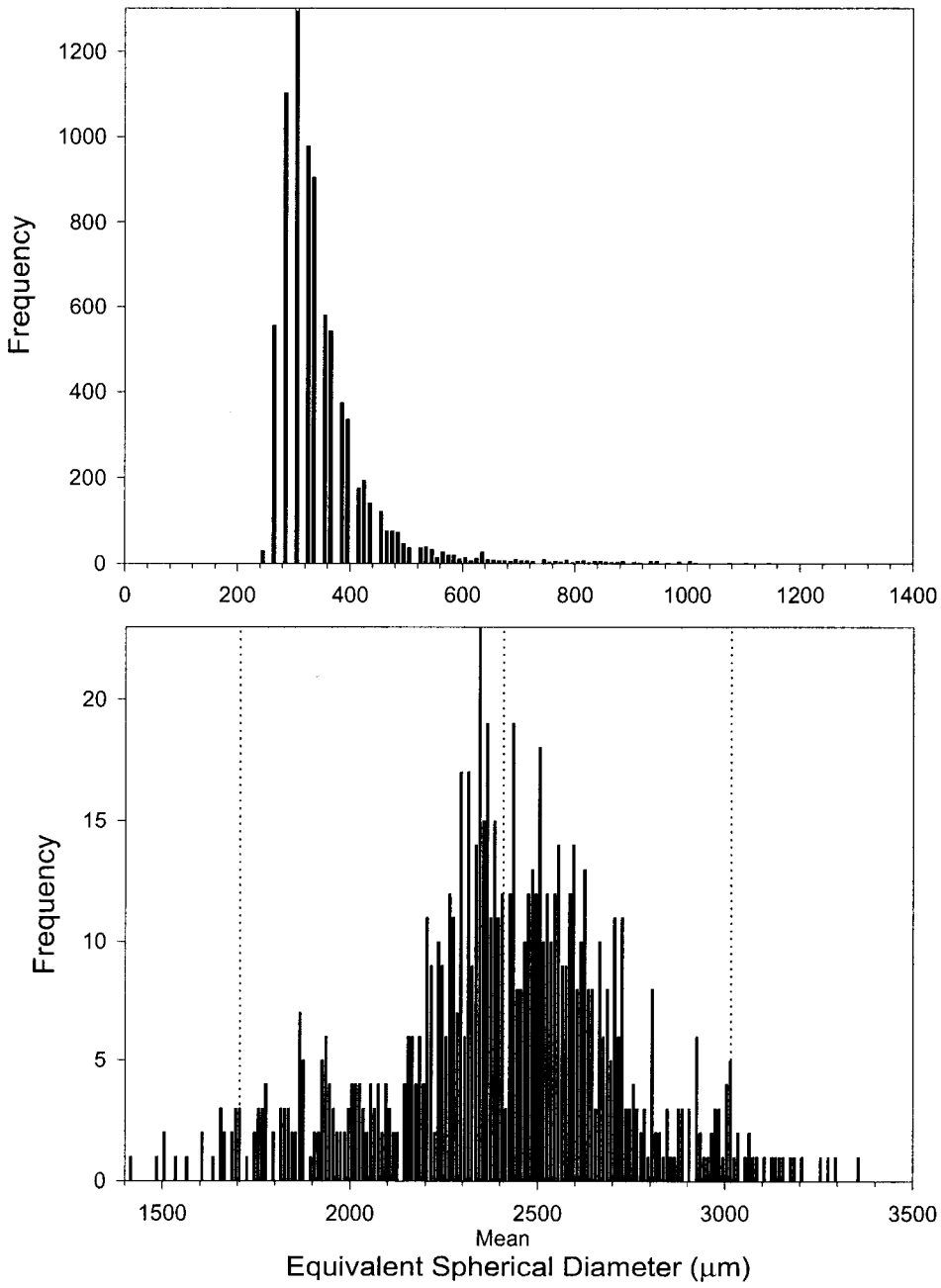

Figure 14. Size spectra from the laboratory OPC calibration. Top panel: Size spectrum from blank runs. Bottom panel: cumulative size spectrum from calibration runs with two groups of 20 *N. plumchrus*. Lines denote the mean size and the range of 95% of the observations. Note that axes are different between panels.

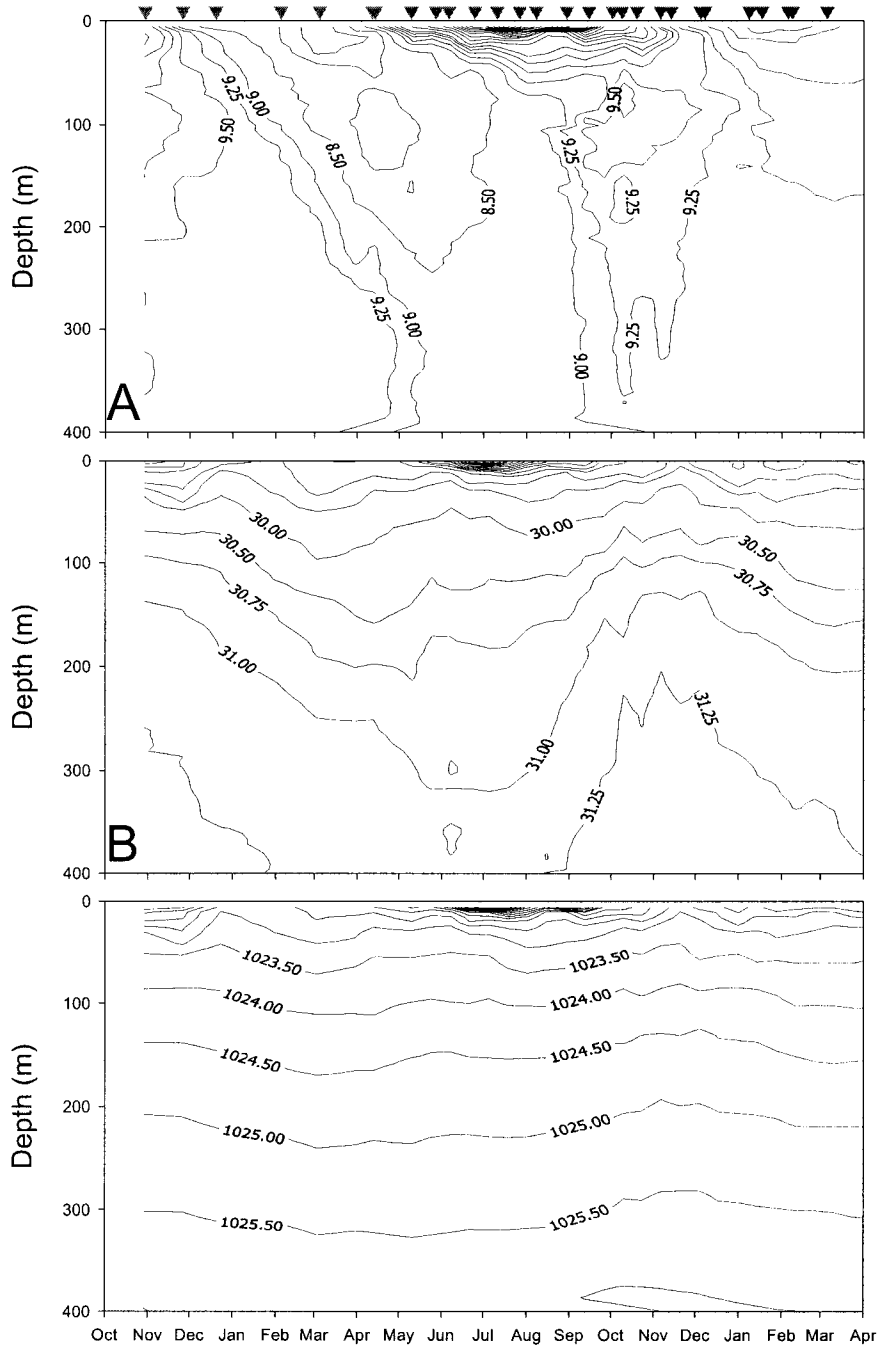

Figure 15. Seasonal variation in (A) temperature, (B) salinity and (C) *in situ* density in the Strait of Georgia (49°15'N 123°45'W) between October 2001 and April 2003. Isolines were fit by linear interpolation of 1 m binned CTD data (5 m vertical nodes, 14 day temporal nodes). Inverted triangles denote sampling dates. CTD data from the UBC/UVic STRATOGEM project (www.stratogem.ubc.ca) were also included to improve temporal resolution.

overwintering period (May 2002; Fig. 15), presumably as a result of late summer deepwater renewal. *In situ* seawater density at all depths below 200 m increased (by ~0.25 kg m^3) from June to December and approximately followed the change in salinity, in spite of a concomitant increase in temperature from October 2002 - February 2003.

5.3.2 Net samples

Large abundances of CVs were observed in surface waters only during April 2002 (5676 ind m^{-2}). At the beginning of the overwintering period (May 2002), high concentrations of CVs were observed at depths below 200 m (23449 ind m^2), with much smaller numbers captured at depths shallower than 200 m (51 ind m^2; Fig. 16). During the overwintering period (defined hereafter as May to December 2002), high concentrations of CVs were present in the two deepest net strata, with very low numbers captured between 100 and 200 m. By November 2002 (and in November 2001 as well) CVs were only found deeper than 200 m. Adult *Neocalanus plumchrus* were first observed in December of 2001, but not until January during the 2002-2003 season. Adults had a broader depth range than did overwintering CVs, and ranged between 100 m and the bottom. Adult males appeared prior to females in 2002, but both males and females were present in January 2003 (though more males were present and they may have moulted prior to females in mid December).

During the overwintering and spawning period, individuals disappeared from the population at rates of 10 $(10^3 \text{ ind})^{-1}$ d^{-1} (Table 3, Fig. 17). Loss rates from the Strait of Georgia were similar to those observed for *Neocalanus plumchrus* populations in the Alaska Gyre (Miller *et al.*, 1984) and the Oyashio region (Kobari and Ikeda, 2001a; Table 3).

5.3.3 OPC measurements

The volume of water sampled per depth bin varied between 5000 - 6.5×10^6 L, the upper range of which approaches the volume the net could be expected to sample over a similar depth stratum (Fig. 18; volumes sampled for each bin are given in Appendix 2). Measured concentrations of *Neocalanus plumchrus* sized particles were roughly consistent with the net

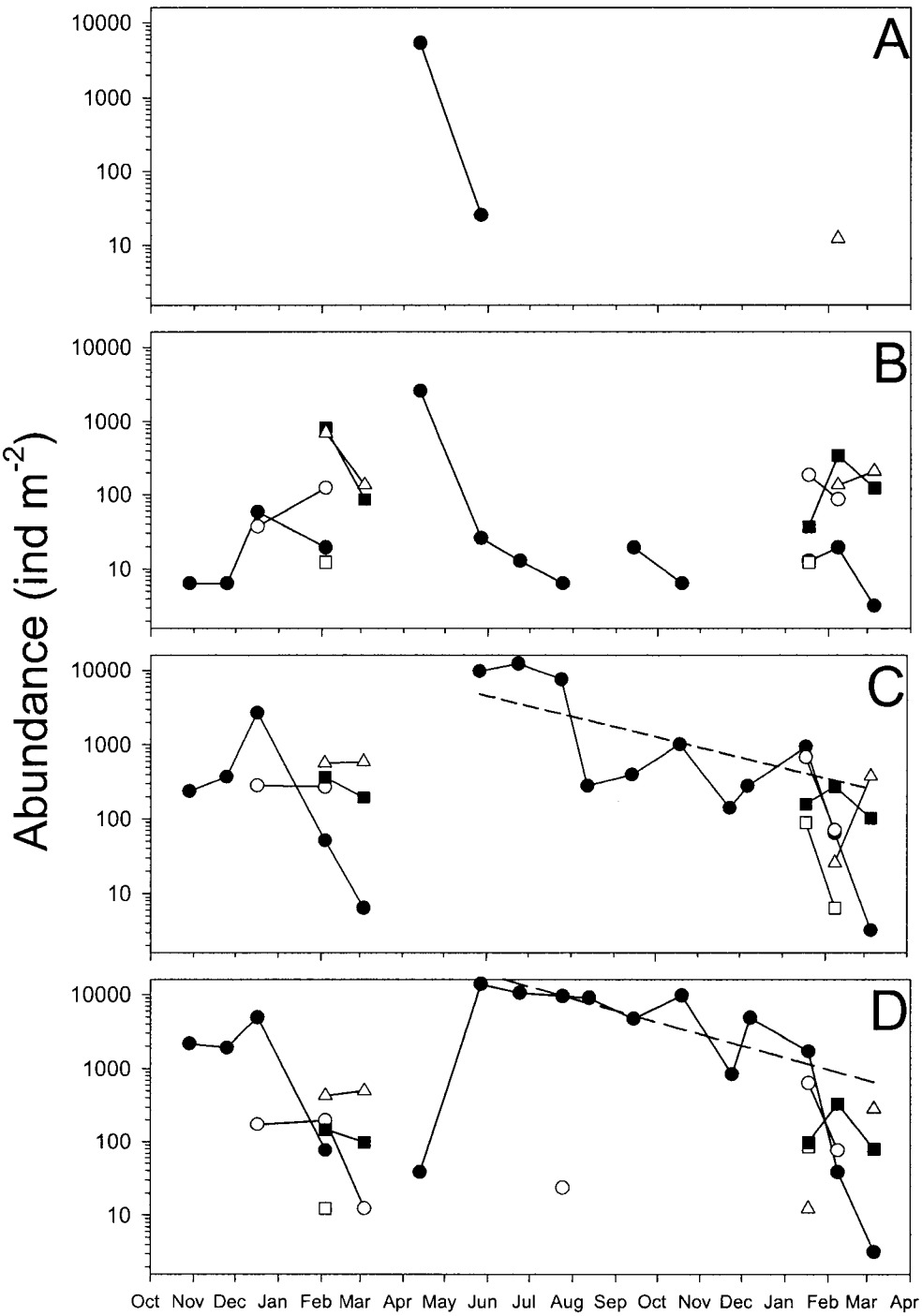

Figure 16. Seasonal variation of abundance by stage in *N. plumchrus* from the Strait of Georgia, October 2001 - March 2003. Each panel is a separate depth stratum (A:0-100 m, B:100-200 m, C:200-300 m, D: 300-400 m), and symbols denote life history or reproductive stage (● = CV, ○ = ♂, □ = immature ♀, ■ = gravid ♀, △ = spent ♀). Dashed lines indicate exponential regression of abundance with time for all stages (see Table 1 for details).

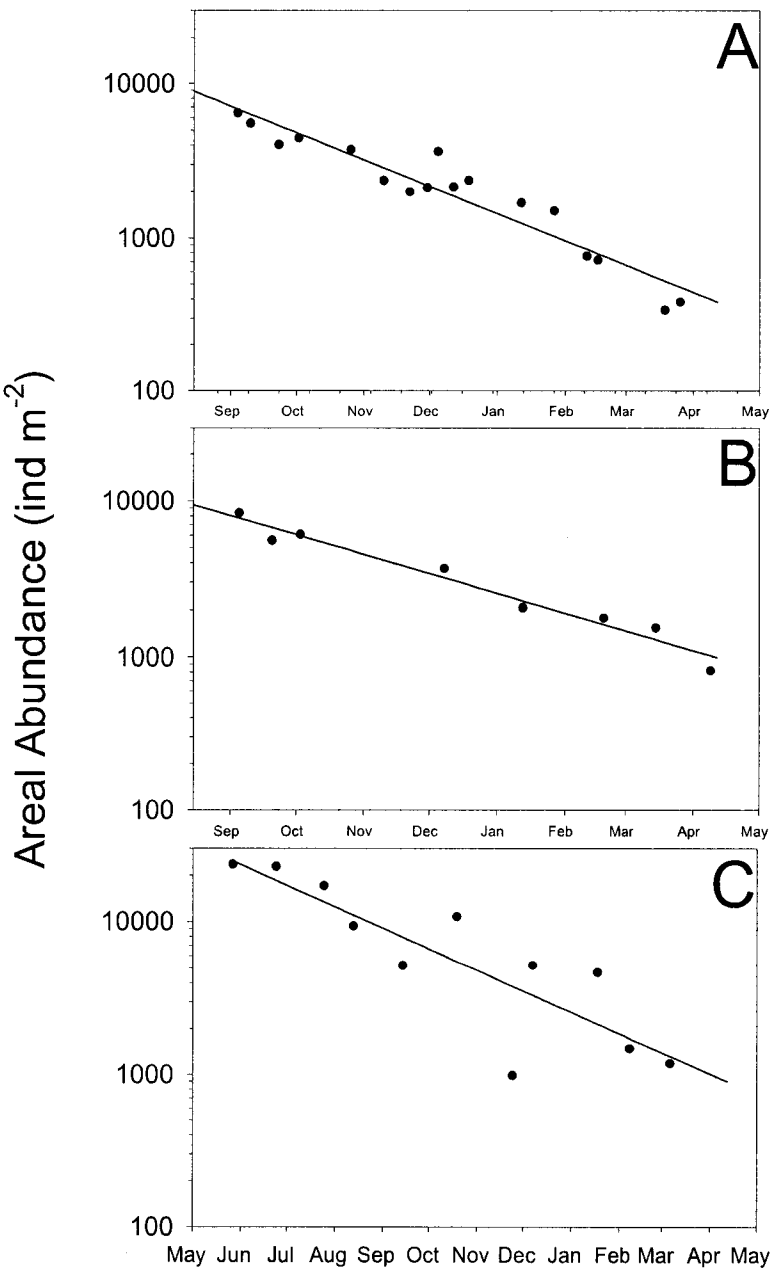

Figure 17. Abundance time series of overwintering *N. plumchrus* from three locations in the North Pacific. Panel A: Station "P" (50° N 145° W; Miller et al 1984), sum of all stages (CV, ♂&♀), from 100-2000 m, from August 1980 to March 1981. These data also include counts of the congeneric *N. flemingeri*, which is usually about 10 % of the abundance of *N. plumchrus* and which has different ontogenetic timing (Miller and Clemons, 1988). Panel B: Station "H" (42° N 145° W; Kobari & Ikeda 2000a), sum of all stages from 0-2000 m, from September 1996 to May 1997. Stage specific data for panels A and B were digitized from Miller et al. (1984; figures 10 and 11) and Kobari and Ikeda (2000a, figure 4) using the DataThief software package (Bas Tummers, The Netherlands, *pers comm*) and summed for each sampling date. Panel C: This study.

Table 3. Loss rates from three populations of *N. plumchrus* in the North Pacific. Loss rates are the exponent (β) from an exponential model (areal abundance=$\alpha e^{-\beta t}$, where t=time in days) for all stages combined. See also figures 16 and 17.

Source	Loss rate	r^2	p_{ANOVA}
Miller et al. (1984)	16 $(10^3 \text{ ind})^{-1} d^{-1}$	0.9	<0.001
Kobari & Ikeda (2001)	9 $(10^3 \text{ ind})^{-1} d^{-1}$	0.92	<0.001
This study			
200-300 m	10 $(10^3 \text{ ind})^{-1} d^{-1}$	0.39	0.03
300-400 m	10 $(10^3 \text{ ind})^{-1} d^{-1}$	0.74	<0.001
All depths	10 $(10^3 \text{ ind})^{-1} d^{-1}$	0.74	<0.001

samples (*N. plumchrus* sized particles shall simply be referred to as "particles" hereafter; Fig. 19). Higher concentrations were measured in surface waters and at depth in April, when CVs were present both at the surface and in the deepest net tows. The highest particle concentrations (244 particles m^3, in the 0-10 m depth bin) were observed in April 2002. High particle concentrations were also observed at depths greater than 200 m in May 2002 (205 particles m^{-3} in the 250-260 m bin),at the beginning of the overwintering period, when *Neocalanus plumchrus* began to appear in abundance in deep net samples (Fig. 16). High particle concentrations (~100 ind m^3) were also measured at the surface at that time, the time that the spring bloom had also begun, as evidenced by an increase in fluorescence (Fig. 19, top panel). As the overwintering period progressed, the upper boundary of the particle depth distribution shifted downwards; the lower portion of the distribution is less certain since the entire water column was not always sampled. During the spawning periods (December 2001 and January 2002; January - March 2003) the particle distribution was more evenly distributed and higher concentrations (~10 ind m^3) were observed higher (up to 200 m) in the water column.

OPC derived concentrations differed from net-based concentrations by as much as one or two orders of magnitude, and the difference varied with depth and time (Fig. 20). In the surface stratum, the OPC tended to count particles that apparently were not *Neocalanus plumchrus* (since the net collected none). In the subsurface stratum (100-200 m), correspondence was sometimes good, but at other times the OPC measured abundance was higher than the net. Correspondence between net and OPC samples was generally best between December and April. At depth (200m - bottom), the OPC and net gave similar measurements of abundance, generally within the same order of magnitude. Examination of the difference between net and OPC measured concentrations and the volume sampled by the OPC shows that the largest departures between the two occurred when the volume sampled was low (Fig. 21). Differences between nets and OPC estimates were usually high for vertical casts, which had considerably smaller sampled volumes than towed measurements.

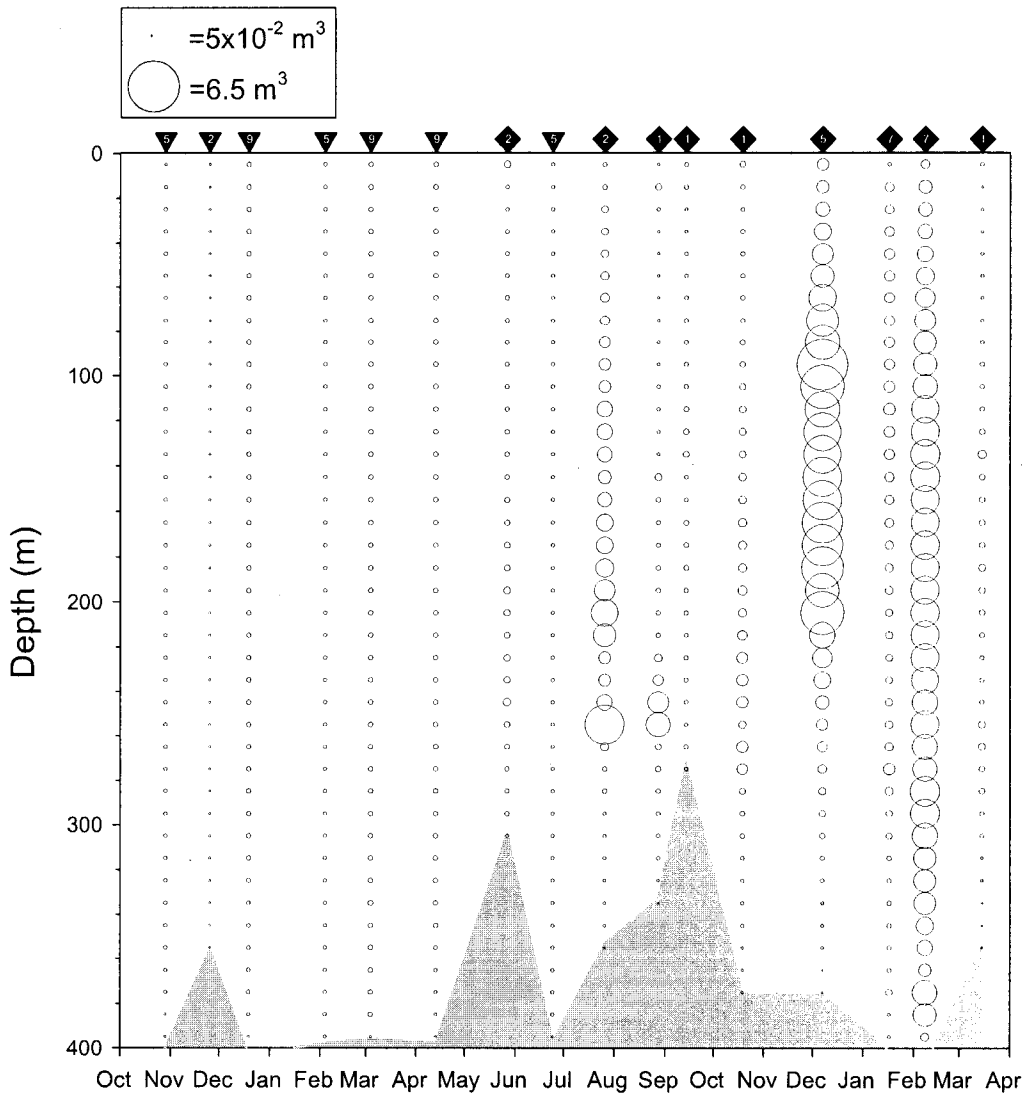

Figure 18. Volume of water sampled (m^3) by the OPC in each 10 m depth bin, October 2001 - May 2003. Bubbles in top panel vary linearly from the minimum to maximum volumes sampled ($5x10^{-2}$ and 6.48 m^3 respectively). Symbols above the top panel denote different cast type (triangles = vertical casts, diamonds = oblique tows), and the number within the symbol denotes the number of casts that were pooled for each date. Grey area denotes regions with no data. Actual data given in appendix 2.

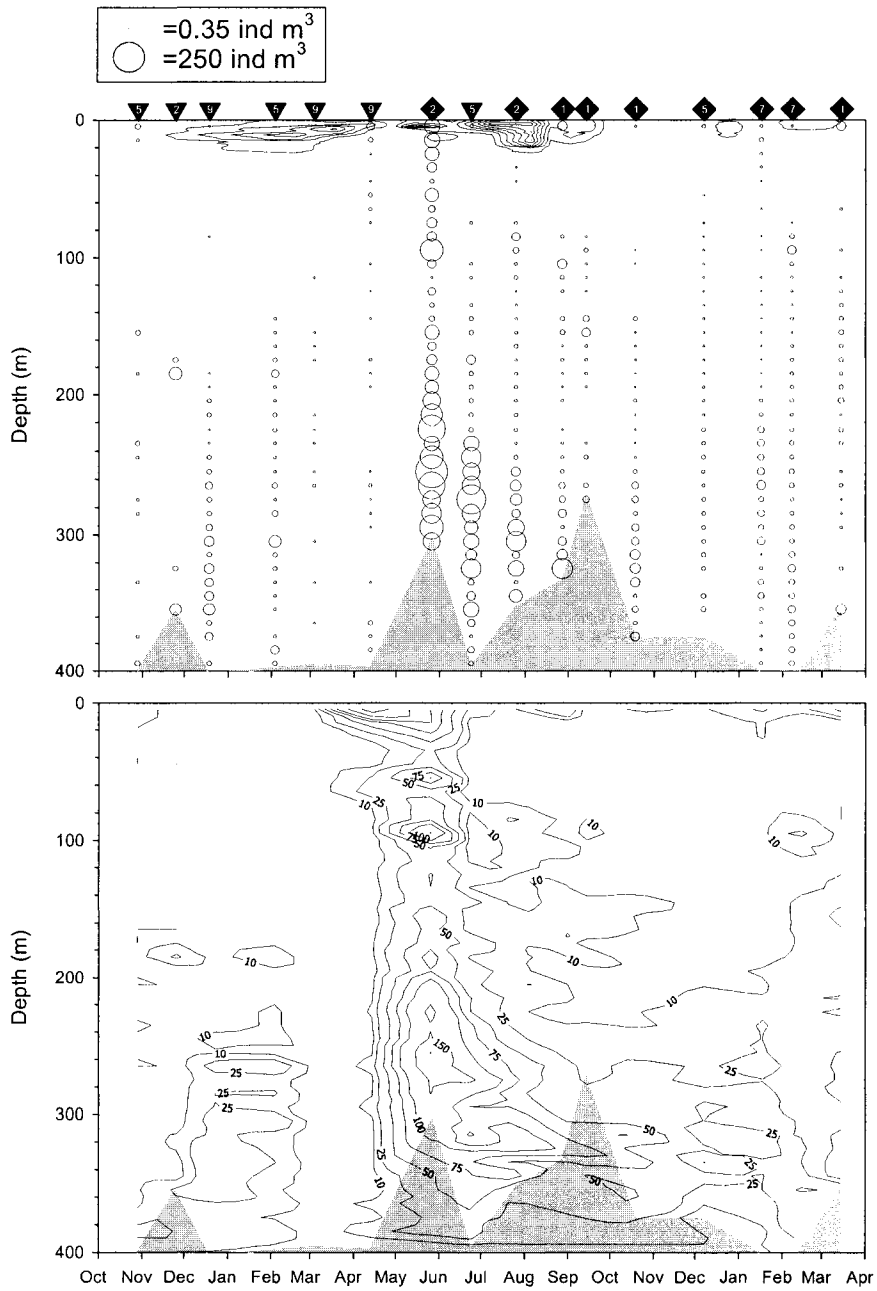

Figure 19. Seasonal variation in *N. plumchrus* sized particles (1705-3016 μm) observed from October 2001 - May 2003, arranged in 10 m depth bins. Top panel: bubble plot of concentration (ind m^{-3}) for each depth bin, abundances have been square root transformed to make the areas of the circles proportional to abundances, and only nonzero concentrations are shown. Contours are of relative fluorescence (volts) from the fluorometer. Bottom panel: contours of particle concentration (ind m^{-3}) from linear interpolation with 5 m depth nodes and 14 day temporal nodes, contours for 10, 25, 50, 75, 100, 150 and 200 ind m^{-3} are shown. Symbols above top panels and grey areas as in figure 18.

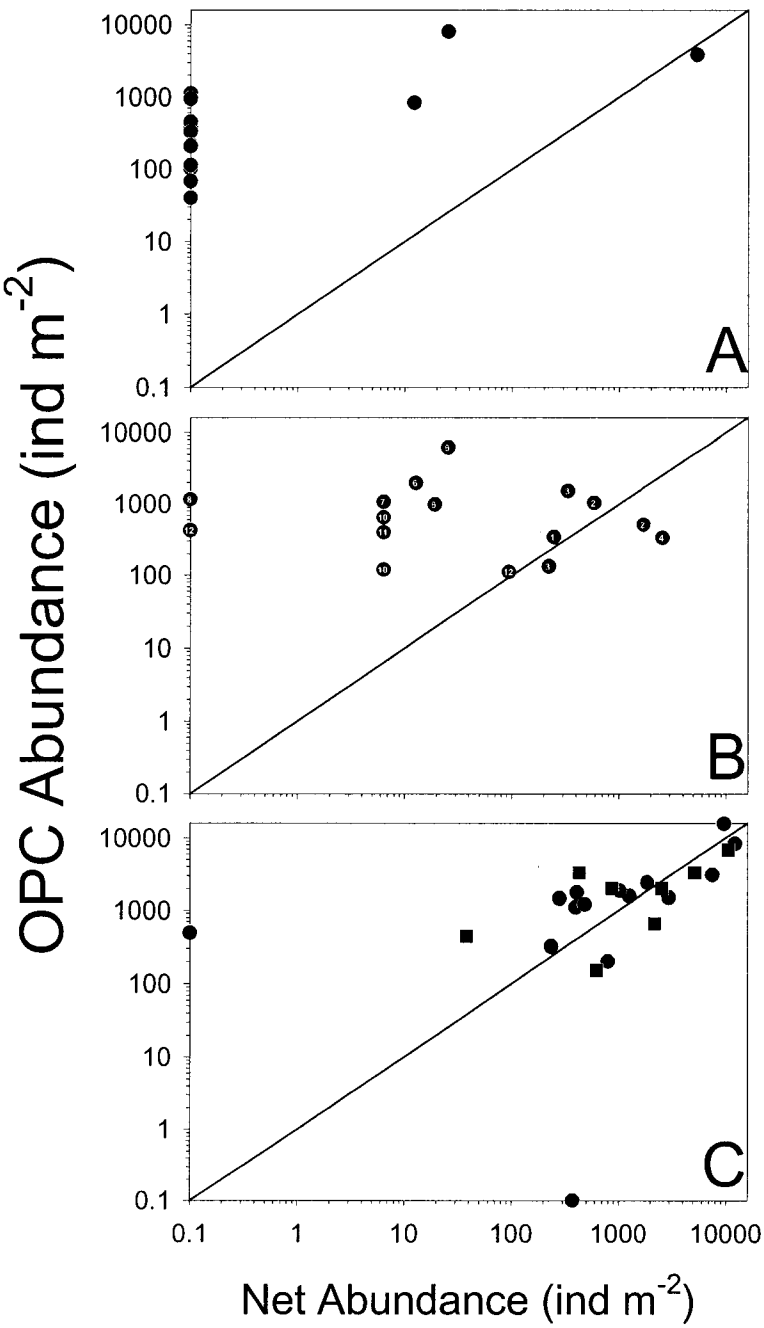

Figure 20. Comparison of OPC and net based estimates of areal abundance for 100 m depth strata. Panel A: 0-100 m stratum. Panel B: 100-200 m stratum (numbers inside the symbols denote the month the sample was taken). Panel C: 200-300 and 300-400 m strata. Only casts that sampled to 400 m were included (see text for details), symbol denotes stratum (● = 200-300 m, ■ = 300-400 m). Solid line is 1:1 line, zero counts have been assigned a value of 0.1 so that they will show on the $\log_{10}$ axes.

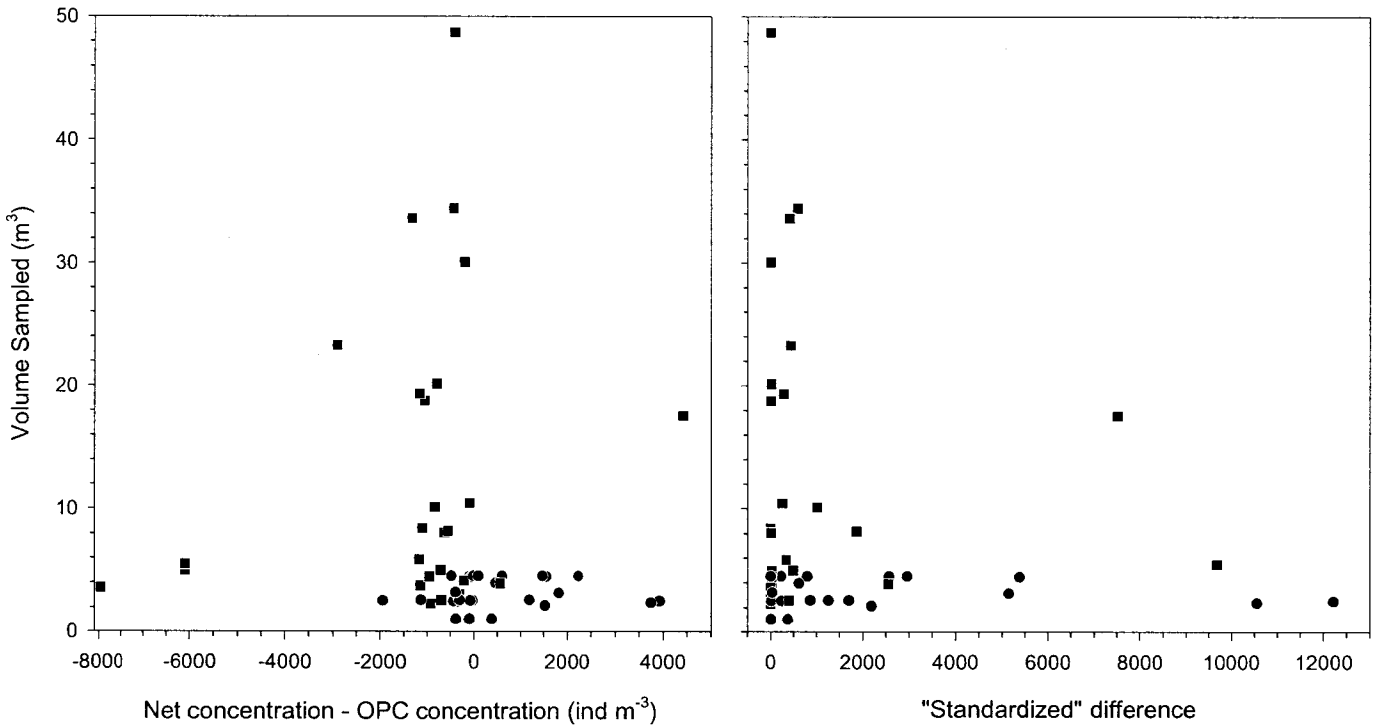

Figure 21. Relationship between net-based versus OPC-based estimates of abundance and volume sampled in each 100 m stratum by the OPC. Symbol denotes cast type (● = vertical cast, ■ = oblique tow). Left panel: absolute difference between estimates. Right panel: difference between estimates standardized by mean estimate size (i.e. [net]-[OPC]/([net]+[OPC]/2)).

5.4 Discussion

5.4.1 Depth distribution during overwintering

The depth distribution and life history pattern shown here are consistent with that described by Fulton (1973). *N. plumchrus* CVs are present only briefly in surface waters, approximately 1-2 months after the springtime increase in primary production occurs. Both net and OPC observations showed individual CVs occurring at depths below 200 m at the start of the overwintering period (May). The depth distribution of the CVs shifted towards deeper depths as the year progressed, with most individuals occurring below 300 m by November. Fulton (1973) suggested that individuals accumulate near the bottom as the overwintering period progresses, while Gardner (1972) observed peaks of abundance at 375 m in November and 350 m in December. Since the OPC did not sample near the bottom during that period, the location of peak abundance cannot be determined from the data presented here.

The deepening of the abundance peak approximately coincided with the change in temperature and salinity beginning in early September, which suggests that the hydrographic changes may, in part, be responsible for the changes in depth distributions. Gardner (1972) suggested that *N. plumchrus* may preferentially inhabit newly formed deep water, or that the inflow may bring in "new" animals to the system. Further, the change in *in situ* seawater density and/or biochemical contents of individual *N. plumchrus* could also alter their buoyancy properties and alter depth distributions for purely biotic reasons. These hypotheses will be dealt with in turn in the following sections.

5.4.2 The effect of deepwater renewal

Deepwater renewal in the Strait of Georgia occurs by two mechanisms (Waldichuk, 1957; LeBlond *et al.*, 1991). Winter deepwater renewal occurs as a result of local cooling in January, February and March, and results in inflows of cool, well oxygenated surface waters to depths of 75 to 200 meters. It is thus is of little relevance to overwintering *N. plumchrus*. Summer deepwater renewal is episodic and tidally modulated, and is caused by gravity flows of warm, salty water spilling over the sill from the Juan de Fuca Strait. Usually, intense tidal

mixing occurs in the numerous channels connecting the Strait of Georgia to the Juan de Fuca Strait, and inward flowing deep water is well mixed with outward flowing Fraser River water. During neap tides, when the tidal mixing is relaxed, deep Juan de Fuca water that is normally well mixed with surface waters overflows the sills at the southern end of the basin, and flows along the bottom into the Strait of Georgia. Evidence for this comes both from the temperature-salinity properties of the water, and measurements of considerable currents at depth in the Strait of Georgia (up to 40 cm s^{-1} northward velocities, after the removal of tidal signals; Leblond *et al.*, 1991). The transport associated with deepwater renewal obviously occurs mostly at depth, and there is little signal associated with transport due to this mechanism above 200 m (Leblond *et al.*, 1991). This suggests one mechanism by which the deepening of the depth distribution may have occurred - an entrainment in the northward moving deep current. Since such currents are episodic (with an approximately fortnightly cycle), they could result in a "ratcheting down" of the depth distributions (however, the monthly sampling conducted here could not detect such a cycle). The observations of a wide depth distribution (between 80 and 520 m, November and December) for *N. plumchrus* in Jervis Inlet in November and December by Mackie (1985) are consistent with this hypothesis. Jervis Inlet is very deep (maximum depth 680 m), and has a 240 m deep sill at its mouth that will prevent deepwater intrusions from Boundary pass (i.e. Strait of Georgia deepwater renewal events) from entering.

The water originating from the Juan de Fuca Strait may also represent a source of *N. plumchrus* to the Strait of Georgia. Gardner (1972) observed an increase in the abundance of CVs between August and November (monthly mean concentrations of 10.04, 11.45, 12.81 and 18.69 ind m^{-3}) and suggested that the increase was the result of individuals transported into the Strait. Published records of *N. plumchrus* abundance in the Juan de Fuca Strait are rather sparse, but Chester *et al.* (1977) did not report significant concentrations. Since the only time that *N. plumchrus* could be expected to be transported into the Strait of Georgia is winter (surface flows are seaward and would not be expected to bring in young stages from offshore), and the overwintering depth of *N. plumchrus* in the open Pacific (below 400 m)

is below the depth of the shelf break (200 m), significant transport of *N. plumchrus* to the Strait in winter is not expected. Anecdotal evidence in support of this supposition includes only very rare occurrence of *N. cristatus* in the plankton samples (<1 sample^{-1} if they were found at all). *N. cristatus* is very abundant at the shelf break, and might be expected to be present in the Strait only if significant transport occurred.

5.4.3 Changes in buoyancy properties

The change in seawater density caused by the inflow of warmer, saltier water in November could also alter the buoyancy properties of the overwintering CVs. The increase in *in situ* seawater density that occurred at all depths would result in the animals becoming more positively buoyant, all other considerations ignored (i.e. if the density of the animals stayed the same, an increase in seawater density would cause a positive density difference). However, the lipid data in Chapter 3 suggests that the lipid contents of individuals decline towards the end of the overwintering period (when the seawater density change is greatest), which would tend to make the individuals less buoyant. The protein data in Chapter 4 suggest that protein contents do not decline, which leads to the tentative conclusion that the amount of "other" (which would also include chitin, and inorganic components, which were not measured) does not decline and offset the loss of lipids.

As discussed in Chapter 2, the extreme sensitivity of the buoyancy model to the choice of parameters does not permit it to be used directly to estimate depths of neutral buoyancy with any degree of accuracy. It does, however, provide a framework within which the effect of changes in seawater density or lipid stores can be examined in a relative way (Fig. 22). As in figure 1, the model parameters have been varied to encompass a realistic range of biochemical contents. The model results show that the change in buoyancy properties due to the increase in seawater density are small relative to those caused by a decrease in lipid content. The change in buoyancy caused by the increase in seawater density between August and December (i.e. the difference between the dashed and solid contours) is equivalent to a change in lipid content of approximately 0.5%. The total amount of lipid use observed in

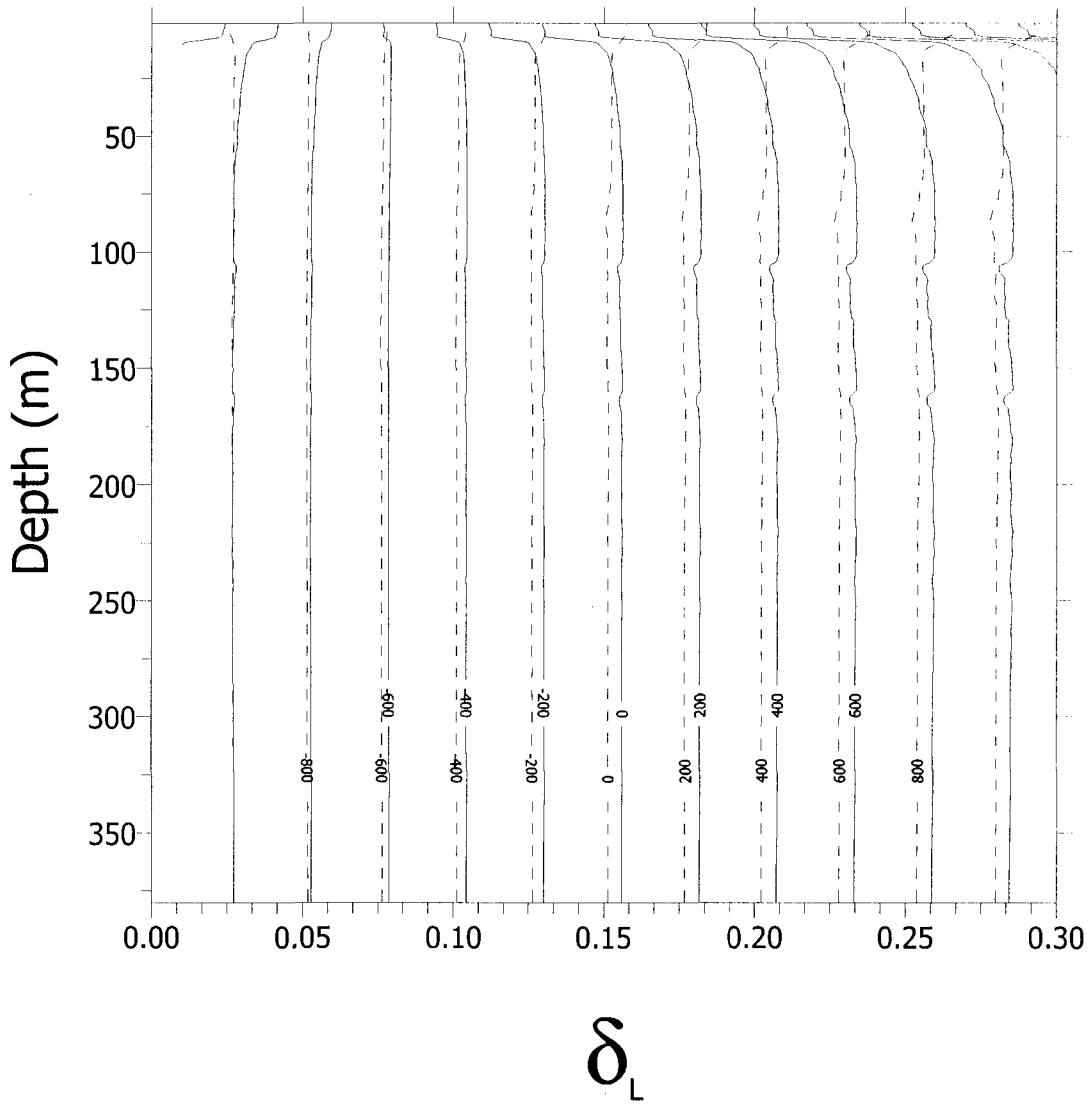

Figure 22. Modeled ascent rates (m d^{-1}, positive upwards) for a model *N. plumchrus* in the Strait of Georgia as a function of lipid content (δ_L, i.e. mass proportion). "Other" content (i.e. δ_O) was held fixed at 0.11, and the density of the "other" component was 1260 kg m^{-3}. Lipid (δ_L) and water (δ_W) contents were altered such that $\delta_L+\delta_W+\delta_O=1$ The value for δ_O was estimated from measurements of wet and dry weight from 89 *N. plumchrus* collected between 200 and 400 m in the Strait of Georgia, July 2000, and estimated as (0.4*dry weight)/wet weight (i.e. dry weight:lipid ratio of 0.4). This gave an estimate for δ_O of (mean±sd) 0.11±0.03. Average water content was 0.72±0.07, which gives an estimate for δ_L for that time of 0.17, which is fairly close to the neutral buoyancy contours in the figure. Contours are of model-derived ascent rates (see fig. 1 for details) for *in situ* seawater density measured on August 28, 2002 (solid lines), and December 4, 2002 (dashed lines).

Chapter 5 (between 22 and 60 % of wax ester contents) would mean a loss in the range of between ~4 and 10 % of total lipids for an individual that began the overwintering period with 17 % lipids by mass (i.e. δ_L; Fig. 22). Given the greater effect of lipid use than changes in seawater density, it might be expected that individuals will move down in the water column as they use their lipid stores. Even with a buoyancy control mechanism, individuals might still be expected to move some distance downwards, if they are not able to determine their depth accurately.

The previous two sections offer two possible explanations for the observed increase in depths over the overwintering period, and they are not mutually exclusive - both could be operating at the same time to produce the observed distributions. The importance of deep water currents could be tested with higher frequency sampling, or North-South transects along the Strait before and after a deepwater renewal event (i.e. before and after a neap tide). Testing for the importance of lipid will be difficult, but could perhaps be done in a laboratory setting with pressure experiments.

5.4.4 The appearance of adults and the upward spawning migration

The timing of the appearance of adults observed in this study was similar to the pattern observed in other studies (Fig. 23). CVs had mostly disappeared from the population by February, males were present in late December or early January, female abundance peaked in February, and the population was mostly females by March (maturity data from this study and Fulton (1973) indicates that most females are spent by March). Females appeared later in this study, but the temporal resolution in both years was poor in both December and January.

It has been suggested recently that the timing of the life history of *Neocalanus plumchrus* changes considerably over long time scales. Mackas *et al.* (1998) observed fluctuations in the timing of peak biomass at Station Papa of order two months, in observations spanning the 1950s to the 1990s. Bornhold (2000) observed fluctuations in the timing of the biomass

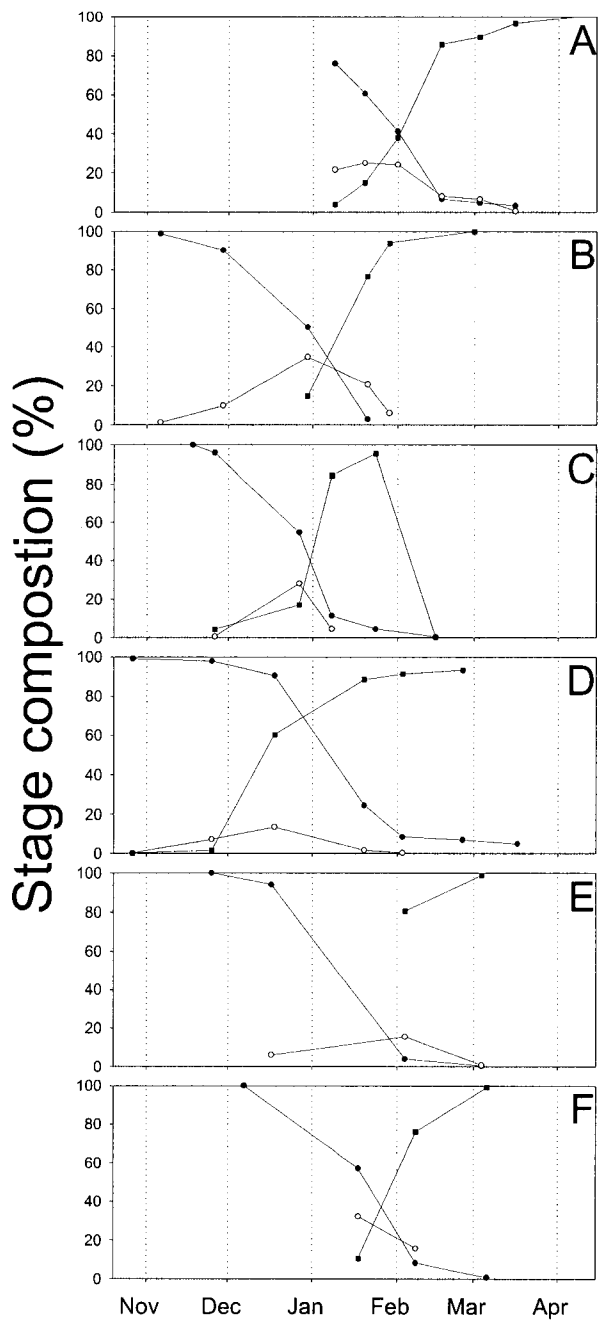

Figure 23. Relative stage composition (all depths) of *N. plumchrus* in the Strait of Georgia over 6 different maturation/spawning periods. Panel A: 1967-1968, data from Fulton (1973), digitized from his figure 2. Panel B: 1970-1971, data from Gardner (1972), digitized from his figure 5. Panel C: 1996-1997, data from Evanson *et al.* (2000), digitized from their figure 1. Panel D: 1997-1998, data from Panton (1998), digitized from his figure 4. Panels E and F: 2001-2002 and 2002-2003 (this study) respectively. Symbols denote life history or reproductive stage (● = CV, ○ = ♂, ■ = ♀). Immature (this study), gravid and spent females (this study, Fulton, 1973) have been combined.

peak of order one month (maximum difference between years was 27 days) in the Strait of Georgia between the 1960s and the 1990s. Bornhold (2000) found some evidence for a move towards earlier timing in recent years, but the pattern in timing can vary by as much as three weeks between adjacent years. Some comparison with the data presented here is possible. 1968 was an "early" year based on the timing of the biomass (peak on May 3rd), but the data of Fulton (1973) shows if anything a slightly later appearance of females (Fig. 23A). 1971 was a "late" year (peak on May 13th), but the data of Gardner (1972) show a slightly earlier appearance of females (Fig. 23B). 1997 was a "late" year (bucking the general trend towards earlier timing; peak on May 9th), but again females appeared comparatively earlier (Fig. 23D). These contradictory observations suggest that changes in overwintering behaviour are not responsible for the observed shifts in life history timing. They also suggest that inferences about changes in timing of less than one month are probably not wise with samples collected on approximately the same time scale.

Adults had a broader depth distribution than did the overwintering CVs, and most adults were found below 200 m, though some were also observed between 100 and 200 m. Fulton (1973) does not mention an upward movement by adults, but the data shown here match those of Gardner (1972; his figure 4) which showed a wide depth range for CVs, males and females in January and February (at the same station sampled in this study). Miller *et al.* (1984) and Kobari and Ikeda (2001a) also observed that males and females appeared to move up to shallower depths during the spawning season in the open north Pacific. A similar partial migration has been observed in *Calanus finmarchicus*, where males have been observed to migrate several hundred meters upwards, presumably to intercept females as they travel to the surface (Heath, 1999). This behaviour could reduce the distance traveled by upwardly migrating nauplii (who might also receive a buoyancy advantage for their upward migration), while also reducing the adults' susceptibility to visual predators by remaining below the euphotic zone.

5.4.5 Losses from the population

"Mortality" rates (i.e. loss terms assuming an exponential decrease) were very similar between the 200-300 and 300-400 net sampling strata, though there was a large decline in abundance in the 200-300 m stratum between August and September that was not well described by the exponential model (Fig. 16C). This may represent individuals moving deeper in the water column (i.e. deeper than 300 m), rather than actual mortality *per se*. It is possible, however, that the rates calculated here are overestimates of actual mortality, since deepwater intrusions could displace the population northward over the course of the overwintering period. Overall, the overwintering mortality rates in the Strait of Georgia population were low, compared with mortality rates estimated for naupliar and copepodid stages. For instance, Aksnes and Magnesen (1983) and Ohman and Wood (1996) both reported mortality rates of ~100 $(10^3 \text{ ind})^{-1}$ d^{-1} for copepodid stages in *Calanus finmarchicus*, and the small calanoid *Pseudocalanus newmani*. Similarly, a modelling study of *C. finmarchicus* by Miller and Tande (1993) obtained stable populations with mortality rates of 20-80 $(10^3 \text{ ind})^{-1}$ d^{-1}. This is consistent with the use of a deep overwintering habitat in order to reduce predation risk. However, as discussed above, there is also the potential for interactions with physical and/or endogenous factors.

5.4.6 Comparison of net and OPC abundance estimates

Whereas *Neocalanus plumchrus* copepodids were only observed in surface waters on three occasions, considerable numbers of particles (~20-1100 ind m^{-2}) were often recorded in the surface stratum (0-100 m) by the OPC. Those counts most likely resulted from flocculates and detritus, that have been observed to bias OPC counts in the surface waters of other areas, particularly when phytoplankton are abundant. (e.g. Heath *et al.*, 1999; Halliday *et al.*, 2001). Detritus production is usually seasonal and related to vernal primary production (Turner, 2002), and spurious counts might therefore be expected to occur mainly in spring and summer. The reasonable correspondence between OPC and net abundances observed in winter (between December and April), and poor correspondence thereafter (May to November) in the 100-200 m depth range is consistent with elevated levels of flocculates

sinking down from the euphotic zone during that time.

During the overwintering period, OPC based estimates of abundance at depths greater that 200m were reasonably similar to the concentrations measured with the net. Since *Neocalanus plumchrus* is the dominant copepod at depth in the Strait of Georgia during most of the year (Harrison *et al.*, 1983), one might expect that OPC counts during that period should be less likely to be confounded by other factors. Furthermore, net-based estimates during this period should also be as representative as can be expected since net clogging should be at a minimum, and non-swimming (i.e. dormant) copepods will be less likely to exhibit escape responses to the net (the behaviour of dormant *Neocalanus plumchrus* is described in section 3.3.2).

As has been demonstrated here and in other studies (Herman, 1991; Sprules *et al.*, 1998; Halliday *et al.*, 2001), nets and optical methods each have advantages and biases. Nets have variable filtering efficiency (which can change during a single tow) and can be avoided by actively swimming plankton. Net samples must also be manually counted and identified. In contrast, the OPC generates ready-made size-frequency data, but samples a relatively small amount of water, necessitating long sampling periods (when sampling depth can be controlled) or the pooling of multiple casts, as was done here. Perhaps the most important difference is that the OPC makes no discrimination about species, and by using a size range that encompasses what might be expected for *Neocalanus plumchrus*, other species of similar size are ignored (although, given the degree of dominance of *N .plumchrus* in the mesozooplankton community, the contribution of other similarly sized particles appears to have been low at depth).

The difficulties encountered with the OPC in this study were largely procedural (a large proportion of the data had to be discarded due to quality issues). Despite this, the data presented here do attest to the utility of the OPC, and highlight that sample volumes need to be considered in designing a sampling program around an OPC (particularly when large, rarer forms are the focus of interest). The strength of the OPC would seem to be is its utility

in large scale studies of community biomass and size structure (e.g. in towed applications), where suitably large volumes of water may be sampled.

CHAPTER 6

Summary and conclusions

6.1The role of lipids in buoyancy regulation

The modelling study presented in Chapter 2 indicates that the lipid stores of large calanoid copepods are probably not used in buoyancy regulation. This conclusion is based primarily on the finding that any depth of neutral buoyancy is inherently unstable (caused ultimately by the higher compressibility of lipids than seawater). Rather, the presence of large proportions of lipids actually represents a functional constraint that must be dealt with, if an individual is to maintain its position in the water column during overwintering, or at any other time. Results from the model in the Faroe-Shetland Channel case (Chapter 2) suggest that ascent rates can be negligible if copepods are able to diagnose their buoyancy reasonably well. In the Strait of Georgia, with its shallower water column, this may not be an option for *Neocalanus plumchrus*. Neutral buoyancy appears likely to occur only over a very narrow range of biochemical contents (given the assumptions of the model) and the density properties of the deep water may change fairly rapidly during deepwater renewal events. The increase in depth observed over the overwintering period in the Strait of Georgia is also consistent with a loss of buoyancy due to the catabolism of lipids during overwintering.

6.2 Changes in physiology during the life history of *Neocalanus plumchrus*

Lipid stores in *N. plumchrus* appear to be composed primarily of wax esters, and no significant lipid transformations were observed during the overwintering period. Rates of lipid use in both the *in situ* field population and the laboratory incubations were generally low (0.3 to 1% d^{-1}), and only measurable towards the end of the overwintering period. Overall, substantial proportions of these lipid reserves (22 - 60% of total wax ester reserves) were consumed during the overwintering phase, presumably to fuel gonadogenesis prior to moulting.

Protein contents remained relatively stable in overwintering CVs , before declining during

moulting to adulthood, and were variable in active, surface dwelling CV copepodids. Measurements of glutamate dehydrogenase activity were consistent with protein catabolism by actively growing CVs and adults, but not in overwintering individuals. This suggests that protein is not an important metabolic substrate during the overwintering phase. Activities of respiratory enzymes were highly variable throughout the life history. A review of the assumptions upon which the ETS technique rests suggests that ETS activity is not expected to be related to respiration rates.

Individuals that were incubated in the laboratory moulted in advance of the *in situ* moulting period observed in the field, after a 1 to 2 month post capture lag. Individuals collected early in the overwintering period postponed moulting, relative to individuals collected closer to the time of the *in situ* moulting period, which suggests that the timing of moulting in *Neocalanus plumchrus* could be driven by endogenous clock. It does not, however, rule out the effect of an external cue used to modulate life history. Individuals captured later in the season (at the time when lipids contents were observed to be declining slightly) were more "ready" to moult, and may have been using some unknown time cue to modulate their physiology. Some plasticity in dormancy is perhaps to be expected, given that the deep water of the Strait of Georgia can at times be an energetic environment (e.g. currents speeds of up to 40 cm s^{-1} during deepwater renewal events) . It would appear, however, that the disturbance of collection, or maintaining individuals in the laboratory did lead to premature termination of the dormant state.

Diapause can be divided into several different stages, each characterized by differing physiological states. Although the various states are best described for insects (Mansingh 1971; Tauber et al), they have recently been put into a calanoid copepod context by Hirche (1996). In a classical diapause sequence, there is a period of preparation (during which metabolic reserves are laid down), followed by a period of induction where regular metabolism begins to slow. Next is the refractory period, during which dormancy is deepest. Towards the end of the diapause period there is an activation phase during which metabolism

begins to increase (in preparation for emergence from diapause), and finally termination. A summary of observations made by Hirche (1996) have been updated for *Neocalanus plumchrus* in Table 4.

6.3 Life history of *Neocalanus plumchrus* in the Strait of Georgia

The field data collected in this study are largely consistent with the pattern described by other studies. *N. plumchrus* is present in surface waters for only a brief period during March to May (young stages were not enumerated, but spent females were observed in February and March, and CVs were only observed in the surface in April and May). By May, a large population of overwintering CVs occupied depths below 200 m, and the depth distribution did appear to shift downwards over the overwintering period. Males usually appear before females in December, and females have mostly completed spawning by February. There was some indication of an upward movement by adults to spawn, which has been observed in other populations of *N. plumchrus*.

6.4 Directions for future research

The buoyancy modelling presented in this thesis highlights a potentially understudied mechanism by which plankton may alter their vertical positions. If buoyancy can be intentionally altered, only very small changes in the relative proportion of biochemical constituents are necessary to produce large changes in the buoyancy properties of an individual, and may represent a significant energetic saving over swimming. Clearly, much more work is required to determine whether or not changes in buoyancy are an efficient means to move vertically in the water column. Buoyancy regulation has not been demonstrated in the free-living Copepoda, and the energetic cost of such regulation is also not known for any taxon. A small number of laboratory pressure experiments (i.e. with pressure cases) would allow one to test which of several potential methods used by copepods to alter their buoyancy (review of the literature suggests ionic replacement) could be responsible. Such an approach and would also allow direct measurement of the energetic costs involved.

Table 4. Summary of physiological and behavioural changes during diapause in insects and copepods (from the reviews of Mansingh, 1971, Elgmork and Nilssen, 1978 and Hirche 1996), updated for *N. plumchrus* (this study). Rows 1-3 are adapted from Hirche (1996; fig 1), with some supplementary material added from the original references.

	Preparatory	Induction	Refractory	Activation	Termination
Insects (Mansingh, 1971)	Accumulation of food reserves	Gradual cessation of RNA and DNA sythesis, lowering of metabolism	Succession of RNA and DNA synthesis and mitosis in all tissues but gonads. Maximum cold-hardiness	Development as prior to diapause	Endocrine reactivation, resumption of RNA and DNA synthesis
Cyclopoid copepods (Elgmork & Nilssen 1978)	Gut emptied, increase in number and size of oil droplets, accumulation of individuals near bottom	Increase in dormancy (i.e. quiescence)	Deep torpor: incapacity to develop, low metabolic rate, resistant to anaerobiosis, reduction of gut epithelium	Torpidity diminished (i.e. probability of emergence from sediments increases)	Resting stages leave the sediment
C. finmarchicus (Hirche 1996)	Arrested development, lipid accumulation, downward ontogenic migration, probable cessation of feeding	Reduced digestive tract, reduced metabolism, feeding stops	Resistance to anaerobiosis, torpidity, incapacity to develop	Capable of development, gonadogenesis	Moulting, ascent.
N. plumchrus (this study)	Downward migration, accumulation of lipid reserves.	Gradual reduction in GDH enzyme concentration, move to deeper depths, undetectable lipid and protein metabolism, maturation following disturbance (collection) is prolonged.	Very low concentrations of GDH, undetectable protein or lipid metabolism, most individuals below 300 m, maturation proceeds after disturbance after 2-3 months.	Increase in lipid metabolism, gonadogenesis begins, maturation proceeds more rapidly following disturbance.	Moulting, partial return migration, spawning. Protein and lipid metabolism high
period:	April - June	June - August	August - October	September -December	December - January

Numerous outstanding questions about the ecophysiology of overwintering copepods remain. Although it was apparent that there were changes in the respiratory capacity, they did not change in a consistent way. More detailed measurements of the respiratory machinery are necessary to determine what caused the observed pattern, and such work would perhaps suggest better enzyme-based proxies for respiration rates. Diapause in insects is primarily regulated by the endocrine system (Hodek, 2002), and copepods are likely similar. Systematic measurements of endocrine activity have not been done in marine copepods, though the measurements of ecdysteroids made recently by Johnson (2003) in *Calanus pacificus*, show how they are used over a moult cycle. Any biological clock could also be under some type of neuroendocrine control, and awaits discovery.

The cues that prompt the onset and termination of dormancy have not been determined with much confidence. The main problems in elucidating what those cues are and how they work are methodological. It has not been possible to induce diapause *in vitro*, and transferring dormant individuals to a lab setting appears to alter their behaviour and physiology (as shown here). Successful inducement of dormancy *in vitro* would allow using an experimental approach to determine what cues are important, which would be potentially more powerful than the mensurative experiments and speculative modelling approaches used to date (given the annual life cycle of most marine calanoid copepods, this is not recommended as a good topic for a graduate thesis). Mesocosm experiments might also be useful as they may replicate *in situ* conditions sufficiently enough to permit a reasonably natural life history pattern, and allow some manipulations to be done.

The Strait of Georgia has been episodically studied since the early part of the 20th century, and numerous studies of its physical and biological oceanography were done in the latter half. Curiously, little of the zooplankton work in that area has found its way into the primary literature, in spite of some detailed surveys in the 1960's and 1970's. As well, the physical mechanism for the downward shift of the depth distribution of *Neocalanus plumchrus* described in Chapter 5 could be readily tested with a few well timed field surveys.

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APPENDIX 1: Conversion of volume proportions (α) to mass proportions (δ)

The volume proportion $\alpha_o=V_o/V_c$ may be rearranged (since $V=m/\rho$) to:

$$m_C = \frac{m_O \rho_C}{\alpha_O \rho_O}$$

and substituted into the expression for mass proportion ($\delta_o=m_o/m_c$):

$$\delta_O = \frac{\alpha_O \rho_O}{\rho_C}$$

In this case, ρ_c is the density of the model copepod as given by the model of Visser & Jónasdóttir (1999), which varies with depth. The calculated value of δ_o thus decreases with depth, a consequence of the volume proportion being fixed in their model. Visser & Jónasdóttir (1999) measured parameters at atmospheric pressure, so the value of δ_o at the minimum depth (10 m) was used. For their "case 1" situation ($\alpha_o=0.2$, $\rho_o= 1080$ kg m^{-3}), δ_o varied from 0.2106 (surface) to 0.2095 (maximum depth). For "case 2" ($\alpha_o=0.1$, $\rho_o= 1260$ kg m^{-3}), δ_o varied from 0.1210 (surface) to 0.1202 (maximum depth).

APPENDIX 2: Volume sampled (m^3) in each 10-m depth bin, arranged by sampling date.

	Sampling date				
Depth (m)	10/29/2001	11/25/2001	12/19/2001	02/04/2002	03/04/2002
0 - 10	0.184	0.099	0.408	0.249	0.370
10 - 20	0.233	0.095	0.448	0.244	0.444
20 - 30	0.256	0.098	0.447	0.255	0.456
30 - 40	0.250	0.103	0.453	0.251	0.445
40 - 50	0.250	0.095	0.445	0.249	0.453
50 - 60	0.252	0.105	0.455	0.264	0.450
60 - 70	0.251	0.101	0.450	0.247	0.448
70 - 80	0.251	0.098	0.451	0.252	0.453
80 - 90	0.245	0.100	0.442	0.253	0.452
90 - 100	0.247	0.099	0.452	0.252	0.446
100 - 110	0.252	0.099	0.451	0.256	0.441
110 - 120	0.249	0.104	0.452	0.256	0.458
120 - 130	0.258	0.095	0.451	0.259	0.459
130 - 140	0.247	0.104	0.448	0.252	0.451
140 - 150	0.252	0.098	0.455	0.256	0.451
150 - 160	0.245	0.099	0.445	0.252	0.442
160 - 170	0.253	0.101	0.447	0.251	0.448
170 - 180	0.245	0.099	0.457	0.252	0.454
180 - 190	0.252	0.102	0.448	0.253	0.452
190 - 200	0.253	0.099	0.455	0.255	0.446
200 - 210	0.249	0.099	0.453	0.248	0.454
210 - 220	0.248	0.098	0.446	0.245	0.443
220 - 230	0.251	0.105	0.449	0.255	0.451
230 - 240	0.251	0.096	0.454	0.255	0.450
240 - 250	0.255	0.100	0.452	0.256	0.451
250 - 260	0.248	0.102	0.445	0.252	0.449
260 - 270	0.254	0.096	0.461	0.268	0.451
270 - 280	0.252	0.103	0.446	0.255	0.452
280 - 290	0.245	0.100	0.441	0.257	0.447
290 - 300	0.248	0.100	0.447	0.260	0.445
300 - 310	0.255	0.099	0.459	0.247	0.455
310 - 320	0.251	0.102	0.447	0.254	0.451
320 - 330	0.250	0.098	0.404	0.259	0.445
330 - 340	0.253	0.058	0.320	0.271	0.463
340 - 350	0.250	0.051	0.298	0.279	0.442
350 - 360	0.243	0.037	0.298	0.253	0.445
360 - 370	0.206	4.855	0.301	0.254	0.429
370 - 380	0.192	20.868	0.295	0.247	0.361
380 - 390	0.113	0.000	0.196	0.268	0.330
390 - 400	0.102	19.663	0.106	0.211	0.110
400 - 410	0.005	0.000	0.015		

	Sampling date				
Depth (m)	04/13/2002	05/27/2002	06/24/2002	07/26/2002	08/28/2002
0 - 10	0.410	0.656	0.218	0.313	0.188
10 - 20	0.444	0.275	0.248	0.426	0.600
20 - 30	0.452	0.268	0.248	0.652	0.244
30 - 40	0.452	0.288	0.252	0.708	0.175
40 - 50	0.454	0.319	0.251	0.819	0.150
50 - 60	0.450	0.312	0.256	0.883	0.163
60 - 70	0.448	0.350	0.248	0.971	0.175
70 - 80	0.454	0.354	0.247	1.036	0.200
80 - 90	0.443	0.360	0.247	1.204	0.206
90 - 100	0.453	0.399	0.254	1.375	0.231
100 - 110	0.445	0.413	0.248	1.372	0.238
110 - 120	0.454	0.390	0.250	1.894	0.263
120 - 130	0.451	0.419	0.251	1.905	0.250
130 - 140	0.452	0.453	0.253	1.773	0.219
140 - 150	0.454	0.457	0.251	1.478	0.713
150 - 160	0.438	0.508	0.245	1.667	0.425
160 - 170	0.452	0.553	0.254	1.997	0.375
170 - 180	0.455	0.561	0.247	2.015	0.381
180 - 190	0.452	0.567	0.250	2.219	0.425
190 - 200	0.442	0.657	0.252	2.452	0.453
200 - 210	0.453	0.604	0.248	3.349	0.351
210 - 220	0.446	0.561	0.245	2.725	0.362
220 - 230	0.454	0.617	0.251	1.424	0.819
230 - 240	0.452	0.643	0.254	1.393	1.180
240 - 250	0.445	0.796	0.252	1.849	2.549
250 - 260	0.452	0.566	0.249	4.884	2.955
260 - 270	0.464	0.499	0.250	0.855	0.557
270 - 280	0.454	0.399	0.249	0.452	0.518
280 - 290	0.440	0.377	0.247	0.330	0.458
290 - 300	0.446	0.432	0.250	0.279	0.376
300 - 310	0.455	0.241	0.250	0.232	0.286
310 - 320	0.452		0.253	0.201	0.245
320 - 330	0.453		0.252	0.185	0.182
330 - 340	0.447		0.250	0.180	0.125
340 - 350	0.413		0.257	0.164	
350 - 360	0.287		0.245	0.071	
360 - 370	0.243		0.252		
370 - 380	0.197		0.249		
380 - 390	0.158		0.235		
390 - 400	0.084		0.108		
400 - 410					

	Sampling date				
Depth (m)	09/14/2002	10/19/2002	12/07/2002	01/17/2003	02/08/2003
0 - 10	0.306	0.572	1.336	0.241	0.896
10 - 20	0.338	0.352	1.411	0.946	1.513
20 - 30	0.200	0.319	1.695	0.964	1.588
30 - 40	0.225	0.336	2.061	1.086	1.728
40 - 50	0.250	0.352	2.507	1.191	1.890
50 - 60	0.275	0.380	2.832	1.161	2.160
60 - 70	0.325	0.407	3.379	1.137	2.356
70 - 80	0.344	0.412	4.035	1.196	2.499
80 - 90	0.381	0.495	4.291	1.184	2.685
90 - 100	0.400	0.522	6.482	1.158	2.825
100 - 110	0.413	0.638	5.488	1.223	2.952
110 - 120	0.469	0.682	4.377	1.257	3.357
120 - 130	0.538	0.731	4.721	1.207	3.447
130 - 140	0.576	0.753	4.715	1.170	3.592
140 - 150	0.300	0.797	4.841	1.058	3.583
150 - 160	0.322	0.792	4.839	0.998	3.484
160 - 170	0.486	0.891	5.084	0.954	3.439
170 - 180	0.505	0.913	5.123	0.919	3.577
180 - 190	0.466	0.913	5.313	0.847	3.472
190 - 200	0.402	0.946	4.209	0.806	3.512
200 - 210	0.346	0.880	5.447	0.784	3.574
210 - 220	0.319	1.067	3.151	0.746	3.439
220 - 230	0.365	1.226	2.358	0.724	3.409
230 - 240	0.333	1.254	2.001	0.713	3.281
240 - 250	0.291	1.303	1.552	0.777	3.118
250 - 260	0.253	0.792	1.286	0.800	3.428
260 - 270	0.326	1.288	1.110	0.879	3.042
270 - 280	0.316	1.195	0.976	1.277	2.973
280 - 290		0.632	0.767	0.849	3.748
290 - 300		0.471	0.687	0.617	3.595
300 - 310		0.433	0.526	0.555	3.166
310 - 320		0.312	0.375	0.446	2.727
320 - 330		0.265	0.286	0.394	2.680
330 - 340		0.231	0.216	0.378	2.650
340 - 350		0.199	0.226	0.387	2.082
350 - 360		0.173	0.196	0.345	1.800
360 - 370		0.158	0.092	0.345	1.460
370 - 380		0.104	0.060	0.613	3.005
380 - 390				0.231	2.836
390 - 400				0.210	0.857
400 - 410				0.013	0.320

depthbin	Sampling date 03/15/2003
0 - 10	0.300
10 - 20	0.125
20 - 30	0.145
30 - 40	0.153
40 - 50	0.188
50 - 60	0.191
60 - 70	0.241
70 - 80	0.268
80 - 90	0.309
90 - 100	0.342
100 - 110	0.392
110 - 120	0.443
120 - 130	0.599
130 - 140	0.870
140 - 150	0.587
150 - 160	0.529
160 - 170	0.586
170 - 180	0.573
180 - 190	0.661
190 - 200	0.636
200 - 210	0.546
210 - 220	0.489
220 - 230	0.358
230 - 240	0.360
240 - 250	0.330
250 - 260	0.783
260 - 270	0.693
270 - 280	0.536
280 - 290	0.545
290 - 300	0.350
300 - 310	0.285
310 - 320	0.185
320 - 330	0.172
330 - 340	0.132
340 - 350	0.116
350 - 360	0.043

APPENDIX 3: Particle concentration (m^{-3}, size range 1705 - 3016 μm ESD) in each 10-m depth bin, arranged by sampling date.

	Sampling date				
Depth (m)	10/29/2001	11/25/2001	12/19/2001	02/04/2002	03/04/2002
0 - 10	21.684	0.000	2.452	4.018	5.406
10 - 20	12.878	0.000	0.000	0.000	0.000
20 - 30	3.902	0.000	0.000	0.000	0.000
30 - 40	0.000	0.000	0.000	0.000	0.000
40 - 50	0.000	10.520	0.000	0.000	0.000
50 - 60	3.964	0.000	0.000	0.000	0.000
60 - 70	0.000	0.000	2.220	0.000	0.000
70 - 80	0.000	0.000	2.216	0.000	0.000
80 - 90	0.000	0.000	4.526	0.000	2.212
90 - 100	0.000	0.000	0.000	0.000	0.000
100 - 110	0.000	0.000	0.000	0.000	0.000
110 - 120	0.000	0.000	0.000	0.000	2.183
120 - 130	0.000	0.000	0.000	0.000	0.000
130 - 140	0.000	0.000	0.000	3.964	0.000
140 - 150	0.000	0.000	2.197	3.899	0.000
150 - 160	8.157	0.000	0.000	3.970	4.529
160 - 170	0.000	0.000	0.000	3.985	2.231
170 - 180	0.000	10.152	2.190	7.934	4.402
180 - 190	3.962	29.438	4.465	19.742	0.000
190 - 200	0.000	0.000	2.199	7.841	0.000
200 - 210	0.000	0.000	6.628	4.036	0.000
210 - 220	4.028	0.000	6.720	8.164	2.259
220 - 230	0.000	0.000	4.457	11.780	2.218
230 - 240	15.962	0.000	4.409	11.752	2.220
240 - 250	3.925	0.000	15.483	15.623	2.217
250 - 260	0.000	0.000	8.992	3.965	4.458
260 - 270	0.000	0.000	32.509	41.092	4.436
270 - 280	3.963	0.000	17.947	15.689	2.212
280 - 290	4.087	0.000	24.916	27.262	0.000
290 - 300	0.000	0.000	26.838	15.357	0.000
300 - 310	0.000	0.000	32.674	44.548	2.196
310 - 320	7.969	9.809	29.066	19.684	0.000
320 - 330	4.003	10.234	42.129	19.302	0.000
330 - 340	3.951	0.000	46.827	25.834	2.158
340 - 350	4.006	0.000	40.316	17.901	2.262
350 - 360	8.218	26.991	46.909	11.851	2.249
360 - 370	4.855	23.239	23.239	19.694	4.662
370 - 380	20.868	16.921	16.921	8.108	0.000
380 - 390	0.000	20.451	20.451	22.399	0.000
390 - 400	19.663	18.874	18.874	9.485	0.000
400 - 410	0.000	0.000	0.000		

	Sampling date				
Depth (m)	04/13/2002	05/27/2002	06/24/2002	07/26/2002	08/28/2002
0 - 10	243.956	95.967	18.308	3.199	58.667
10 - 20	65.283	101.922	0.000	2.345	0.000
20 - 30	13.286	67.074	8.067	0.000	0.000
30 - 40	2.212	27.801	0.000	2.825	0.000
40 - 50	4.407	34.512	0.000	4.885	0.000
50 - 60	24.456	102.571	3.913	0.000	0.000
60 - 70	33.486	37.110	0.000	5.147	0.000
70 - 80	8.815	70.582	12.134	11.584	0.000
80 - 90	4.515	72.265	4.056	30.719	4.848
90 - 100	2.205	155.218	0.000	22.545	0.000
100 - 110	2.248	53.303	4.032	12.395	25.263
110 - 120	2.203	35.857	8.008	12.674	7.619
120 - 130	4.431	52.482	15.909	9.447	8.000
130 - 140	2.214	50.739	7.907	6.768	13.714
140 - 150	4.401	41.557	31.847	4.736	14.035
150 - 160	0.000	72.883	12.268	9.596	16.471
160 - 170	4.428	59.643	15.773	14.522	8.000
170 - 180	6.589	64.127	44.603	8.934	15.738
180 - 190	4.420	88.213	20.009	11.717	7.059
190 - 200	2.262	74.614	35.713	13.868	2.208
200 - 210	2.208	120.948	28.171	9.258	11.395
210 - 220	4.482	138.920	48.976	16.144	13.798
220 - 230	6.609	166.865	47.717	11.942	4.883
230 - 240	4.428	149.285	78.623	15.080	10.171
240 - 250	0.000	157.062	83.388	25.963	18.830
250 - 260	2.214	204.805	96.283	50.984	20.305
260 - 270	10.773	196.372	108.113	67.837	39.498
270 - 280	2.203	157.910	160.530	75.183	32.834
280 - 290	4.549	153.664	105.104	57.661	24.038
290 - 300	11.213	132.027	72.054	75.220	31.942
300 - 310	4.396	128.713	92.032	98.940	48.922
310 - 320	2.211	67.234	67.234	119.142	69.400
320 - 330	2.209	107.337	107.337	113.523	99.026
330 - 340	2.235	75.926	75.926	44.397	48.157
340 - 350	4.841	73.893	73.893	97.783	
350 - 360	0.000	73.590	73.590	28.059	
360 - 370	12.362	55.618	55.618		
370 - 380	5.075	44.161	44.161		
380 - 390	19.010	46.894	46.894		
390 - 400	0.000	9.298	9.298		
400 - 410					

	Sampling date				
Depth (m)	09/14/2002	10/19/2002	12/07/2002	01/17/2003	02/08/2003
0 - 10	0.000	12.238	8.232	29.024	8.927
10 - 20	0.000	2.841	0.709	11.631	0.000
20 - 30	0.000	0.000	0.590	11.407	0.000
30 - 40	0.000	0.000	0.000	5.523	0.579
40 - 50	0.000	0.000	0.000	3.358	0.000
50 - 60	0.000	0.000	0.353	0.862	0.463
60 - 70	0.000	0.000	0.592	2.638	1.273
70 - 80	0.000	0.000	0.991	0.836	2.802
80 - 90	10.492	0.000	2.563	1.689	18.996
90 - 100	15.000	1.914	4.782	3.454	33.269
100 - 110	4.848	1.567	3.827	2.452	14.904
110 - 120	6.400	2.933	3.884	3.979	8.340
120 - 130	5.581	0.000	3.389	1.657	5.512
130 - 140	1.737	5.309	1.909	0.855	5.011
140 - 150	16.681	17.555	4.545	1.891	10.604
150 - 160	21.714	8.838	4.133	4.008	9.184
160 - 170	18.533	10.101	3.541	3.145	10.179
170 - 180	5.935	4.381	5.075	3.264	10.065
180 - 190	19.293	2.191	6.023	4.723	11.810
190 - 200	4.977	9.514	5.940	11.163	18.510
200 - 210	5.774	5.682	9.547	7.654	16.507
210 - 220	6.276	6.560	12.375	10.723	13.085
220 - 230	2.741	9.784	13.572	23.466	15.840
230 - 240	12.029	8.772	12.493	26.635	19.508
240 - 250	13.747	13.809	21.259	25.740	13.469
250 - 260	11.849	16.414	14.780	26.260	20.419
260 - 270	15.328	34.169	21.628	34.147	20.379
270 - 280	22.124	31.809	19.460	25.062	14.462
280 - 290	26.892	26.892	29.971	31.812	18.410
290 - 300	48.798	48.798	21.839	29.167	22.250
300 - 310	41.534	41.534	24.714	39.616	24.318
310 - 320	76.818	76.818	15.981	17.926	29.331
320 - 330	41.481	41.481	28.005	10.162	33.580
330 - 340	64.993	64.993	9.256	29.065	31.325
340 - 350	35.094	35.094	31.034	23.243	38.903
350 - 360	51.990	51.990	20.360	23.217	53.884
360 - 370	56.992	56.992	10.900	11.584	37.659
370 - 380	28.935	28.935	0.000	14.684	33.610
380 - 390			4.325	4.325	32.442
390 - 400			9.534	9.534	26.835
400 - 410			0.000	0.000	15.638

Depth (m)	Sampling date 03/15/2003
0 - 10	56.616
10 - 20	0.000
20 - 30	0.000
30 - 40	6.524
40 - 50	0.000
50 - 60	0.000
60 - 70	4.144
70 - 80	0.000
80 - 90	0.000
90 - 100	5.842
100 - 110	2.553
110 - 120	6.774
120 - 130	10.014
130 - 140	12.644
140 - 150	22.162
150 - 160	30.268
160 - 170	23.877
170 - 180	15.700
180 - 190	13.612
190 - 200	11.002
200 - 210	16.470
210 - 220	8.172
220 - 230	30.712
230 - 240	11.108
240 - 250	21.231
250 - 260	8.942
260 - 270	7.214
270 - 280	9.324
280 - 290	7.333
290 - 300	11.433
300 - 310	17.521
310 - 320	5.418
320 - 330	5.809
330 - 340	0.000
340 - 350	0.000
350 - 360	46.664