Rates of Biomass Production, Growth, and Development of Juvenile Copepod Communities in the Strait of Georgia and the subarctic NE Pacific

by

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ABSTRACT

Estimating the efficiency with which energy is transferred to higher trophic levels demands estimates of adult and juvenile copepod biomass production rates. Adult rates (egg production) are well studied; however, our knowledge of how juvenile biomass production rates (BPR) vary *in situ* remains incomplete and has been limited by difficulties associated with measuring somatic growth rate in the field. This problem may be resolved with the application of new methods that either: a) directly estimate BPR without additional estimates of growth or biomass, and/or; b) improve estimates of development and growth rate that may then be applied to estimates of biomass. The objective of this thesis is to develop a routinely applicable method that may be used to gain greater insight into how production of juvenile copepods is regulated in the field.

I have developed a field method for estimating BPR, development and growth of marine copepods that is based on measuring the *in situ* rate of decay of the crustacean moulting enzyme, chitobiase. The method may be generalized to the community level because the relationship between chitobiase activity liberated into seawater after moult and individual body size was found to be common to three calanoid and one harpacticoid copepod species. This relationship, and the rate of decay of chitobiase in the water column, can be used to estimate the community-level BPR and size-class specific stage durations. Estimates of stage duration based on the turnover rate of chitobiase in the water column were in good agreement (within 5-15%) with both conventional incubation techniques and literature-based estimates. The approach was also used to estimate community-level BPR during a two-year time series in the Straight of Georgia (2004 and 2005) and two broad-scale spatial surveys encompassing shelf, slope and open ocean regions in the subarctic NE Pacific during the spring and late summer of 2004.

This thesis presents the development and field validation of a novel method of estimating rates of juvenile copepod production, development, and growth that may be used to further characterize how variation of these rates is regulated in the field. The results of field studies using this approach suggest that: (i) the chitobiase-based method can be routinely used to estimate production rates that are broadly applicable to copepod communities; (ii) even when food is abundant, poor food quality can negatively influence copepod community production rates over relatively short timescales and; (iii) contemporary predictive models of *in situ* copepod growth rate may not be globally applicable because the relative influence of temperature, individual body weight, and chlorophyll *a* concentration on *in situ* growth rate varies with region and copepod community composition.

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CHAPTER 1.

Introduction

1.1 General introduction

Quantitative estimates of the direction and magnitude of energy flow within a food web require accurate measures of production rate; the product of biomass and growth rate (Downing 1984). In marine ecosystems, researchers have long been able to directly address factors regulating variation of primary and bacterial production rates because of the continued refinement and routine use of several field methods (i.e. ^{14}C; Steeman Nielsen 1952; dilution technique; Landry and Hassett 1982; ^{3}H and ^{3}Leu; Fuhrman and Azam 1982). The rate of biomass production (BPR) for juvenile copepods, however, is not routinely estimated and its variation is not well understood relative to rates of primary and bacterial production. Part of this discrepancy is due to the relative complexity of metazoan growth and difficulties associated with its measurement. Owing to generation times of weeks to months, accurate *in situ* estimates of growth and development rates of juvenile copepods, the dominant metazoan herbivore in marine ecosystems, have proven very difficult to obtain (see Hirst et al. 2005). As a consequence, most studies have been limited to productive coastal systems and/or single populations. The objective of this thesis is to evaluate the utility of a community-level estimate of juvenile copepod BPR which also has the potential to be used to estimate *in situ* rates of development and growth.

1.2 Calculation of secondary production rates

In marine ecosystems, the biomass of planktonic crustacean communities is typically dominated by copepods. Thus, measurement of copepod biomass production rates is necessary for calculating secondary production. The production rate of a population is calculated as:

$$PR = \Sigma B_i * g_i \qquad \text{(Eq. 1.1)}$$

Where, B_i is the biomass of stage i and g_i is the estimated growth rate of that stage. A complete estimate of production for a given species must also include adult production, estimated as egg production (EPR; see Runge and Roff 2000). A community-wide production rate demands estimates of biomass and growth rate for all species-specific stages and their respective egg production rates. Egg production rates dominate the literature because this measurement is logistically simpler than estimates of juvenile growth and development. Estimating juvenile growth rates for all of the species in a community is prohibitive, and most studies have therefore relied on global predictive models (e.g. Huntley and Lopez 1992) or species-specific relationships established in the laboratory (Uye 1982; Escribano and McLaren 1999). Use of these relationships, however, often assumes temperature dependence of *in situ* rates, which under conditions of food limitation may result in overestimates of biomass production.

In general, the contemporary understanding of juvenile biomass production rate is limited to single species studied in ostensibly productive systems (i.e. temperate coastal systems and upwelling regimes). Variability of production rate estimates is largely dependent on variation of *in situ* growth rates. Unfortunately, an understanding of how *in situ* rates of juvenile copepod growth are regulated has remained elusive. Laboratory and field studies have demonstrated species-specific patterns of copepod development and growth. Syntheses of both laboratory and field estimates (e.g. Huntley and Lopez 1992; Hirst and Bunker 2003) have demonstrated that growth rates generalized across species also vary significantly with factors such as temperature, body size, and food concentration. Thus, any evaluation of a new method that purports to measure biomass production must consider the relative influence of factors regulating copepod development and growth rate.

1.3 Factors influencing copepod developmental rates

All arthropods possess a rigid chitinous exoskeleton that must be shed and replaced periodically during development in order to accommodate growth. Measures of arthropod stage duration and development time are used for obtaining estimates of recruitment, mortality, growth, and production rates. Traditionally, studies concerned with the regulation of developmental rates have focused on the relative influence of temperature, body size, and food concentration.

Copepod life cycles are broadly divided into three phases: embryonic, juvenile, and adult. Most of the life cycle is spent in the juvenile phase. This phase can be divided into 6 naupliar (NI-NVI), and five copepodite stages (CI-CV). The pattern of development is defined by the time spent in any given stage relative to the total development time of a species. In general, two patterns of development have been defined: isochronal (e.g. see Peterson 2001 for review) and equiproportional (see Hart 1998 for review). In marine systems, isochronal development is exemplified by *Acartia* spp. and is characterized by development time that is divided into approximately equivalent stage durations. Equiproportional development is characterized by each stage occupying a defined portion of development time relative to the embryonic duration.

Equiproportional development assumes temperature regulation (that is equivalent for all stages) and is described by fitting stage duration estimates from laboratory cultures to Belehradek's equation (Belehradek 1935; McLaren and Corkett 1981; Corkett et al. 1984). Landry (1975) demonstrated that isochronal development of *Acartia* spp. was also temperature dependent. It must be noted that all of these observations were based on laboratory studies of animals growing under food saturated conditions. Therefore, species-specific relationships may be used to estimate *in situ* rates, but only provided that food concentration is not below the threshold required to moult (Vidal 1980b; Crain and Miller 2001) and/or if food quality issues do not hinder development (i.e. Breteler et al. 2005; Koski et al. 2006).

It was formerly argued that the final body size of adult copepods was unrelated to development time (e.g. McLaren 1978; Landry 1983; Huntley and Lopez 1992). Again, however, these studies typically estimated development rates for animals under food saturated field and laboratory conditions. More recent syntheses of *in situ* developmental rates suggest that species with larger adult body weights generally also have longer generation times (Hirst and Sheader 1997; Hirst and Kiorboe 2002).

Food concentration can exert a significant influence on *in situ* moulting rates (estimated as the reciprocal of stage duration). Both laboratory (e.g. Vidal 1980b; Lopez 1996; Davis and Alatalo 1992) and field studies (e.g. Campbell et al 2001a; Crain and Miller 2001) have measured a significant decline in the moulting rate of copepod populations growing at low food concentrations. Crain and Miller (2001) postulated that a threshold weight must be met during the period immediately following moult (early post-moult) if an animal is to successfully moult to its next stage of development. Assuming non-limiting food concentrations and a uniform age distribution within the stage, the distribution of individuals should reflect the relative time spent within each of the inter-stage categories defined by Drach (1939) and identified through changes in jaw morphology. They found that the relative proportion of *Calanus finmarchicus* copepodites in early post-moult increased with both the severity and the duration of suboptimal feeding conditions. Despite this, studies documenting food-limited developmental rates in the field are not common, in part because most have focused on copepod communities developing during productive periods (i.e. spring in temperate waters) when the potential for food limitation is low. Thus, it remains unclear how frequently natural populations/communities of copepods experience food-limited developmental rates.

1.4 Factors influencing copepod growth rates

1.4.1 Temperature

Identifying and quantifying the factors that regulate variability in juvenile copepod growth rates has long been a goal of biological oceanography. Estimating growth rate is necessary for the proper calculation of secondary production. Juvenile somatic growth rate is generally defined as (but also see below):

$$g = \ln (W_2/W_1)/t, \qquad \text{Eq. 1.1}$$

where, g is the weight-specific growth rate estimated as an exponential increase in weight (W_1 to W_2) over a defined period of time (t).

Early field and laboratory studies (e.g. Paffenhoeffer and Harris 1976; McLaren 1978) found that variation in copepod growth rates were best described by variation of temperature. Huntley and Lopez (1992) compiled a large number of field and laboratory estimates and found that 91% of the observed variation in growth rate estimates could be explained by temperature alone. There is a physiological basis for the strong temperature-dependent relationship described by Huntley and Lopez (1992); all estimates of rate processes, whether chemical reaction rates or metabolic rate processes are dependent on temperature in determining a "baseline" for activity. Variations in *saturated* activities relative to temperature are described by a 10 degree rule (Q_{10} parameter), whereby variation of all physiological rates can be expected to vary in proportion to every 10 degree increment of temperature (within a biologically relevant range; see Hochachka and Somero 2002). In retrospect, it may not seem surprising that body size and food concentration played little observed role in determining growth variability in these early studies, as most were conducted under food saturating conditions. Consequently, the only variable that could explain differences was temperature.

1.4.2 Food concentration, quality, and availability

Controlled laboratory experiments have demonstrated that juvenile copepod growth and development rates vary with the concentration, size class, availability, composition and quality of food (Vidal 1980a, b; Berggreen et al. 1988; Davis and Alatalo 1992; Breteler et al. 2005). However, very few studies have demonstrated the relative importance of these dietary factors in the field. Huntley and Boyd (1984) considered the prospect for food limitation (in terms of concentration) at global scales and concluded that suboptimal growth should be more commonly encountered by copepods in oligotrophic open-ocean systems than in coastal systems. However, some studies in ostensibly productive systems (e.g. Campbell et al. 2001a; Liu and Hopcroft 2006a, b) have demonstrated that *in situ* growth rates can be enhanced by amending growth incubations with phytoplankton. In addition, the most recent synthesis of field estimates of growth rates (Hirst and Bunker 2003) demonstrates that phytoplankton concentration (as estimated by chlorophyll *a*) is significantly related to *in situ* growth rates of juvenile copepods. However, comparisons of field estimates to rates predicted by the Hirst and Bunker (2003) model demonstrate that the model severely overestimates growth rates (Liu and Hopcroft 2006a, b).

Thus, in order to quantify the relative influence of dietary factors on *in situ* variation of juvenile copepod growth rates we must re-evaluate food proxies and directly estimate juvenile copepod community growth rates across a variety of systems and conditions. The widespread tendency to attempt to relate field estimates of phytoplankton concentration (using chlorophyll *a*) as a generalized substrate for juvenile growth rate may be partly responsible for the common discrepancies between controlled experiments and paired chlorophyll *a* concentration and field estimates of copepod growth rates (Hirst and Bunker 2003). Ultimately, the use of a state variable such as chlorophyll *a* concentration may not always reflect: 1) the influence of past feeding history on copepod growth rates, which may result in a potential mismatch between concomitant estimates of food availability and growth rate estimates (e.g. Davis and Alatalo 1992; Calbet and Alcaraz 1997; Rey-Rassat et al. 2002b); 2) the non-autotrophic components of copepod diets or; 3) the nutritional value of a potential diet.

Calbet et al. (1996) compared the use of state and rate variables to address the degree of coupling between copepods and phytoplankton and concluded that variation in the transfer of carbon between the two groups was best explained by comparisons of rate processes (i.e. phytoplankton production rates and copepod egg production and respiration rates). Furthermore, they suggested that the response of copepod vital rates to variation of phytoplankton rates was faster in systems under aperiodic physical influences. This study represents an ideal approach to addressing the relative influence of physical and biological variability on *in situ* copepod growth. However, few comparable studies exist. As discussed above, routine methods do exist for the determination of phytoplankton and microbial production rates. However, routine estimates of copepod growth rates (population and/or community level) are not common. This discrepancy exists, in part, because we have yet to develop an application that is broadly and routinely applicable in a field setting.

1.5 Conventional estimates of development and growth

Conventional estimates of development rates for natural copepod populations are either based on cohort analysis or through stage-specific incubations. Landry (1978) was able to identify 11 cohorts of *Acartia clausii* (*Acartia hudsonica*) in Jackle's Lagoon, San Juan Islands, Washington, via estimates of the relative stage distribution in a time series of net samples (Heinle 1966). This information was then used to estimate generation times and stage durations. Like laboratory studies, this field study benefited from: (i) a spatially isolated population that was not subject to advection; and (ii) synchronous development, which allowed for estimates of stage distribution and cohort discrimination. In practice however, these conditions are rarely met in the field.

Alternatively, stage-specific moulting rates have also been used as a method of estimating stage duration. This method involves incubating individuals of the same stage over a defined period of time and counting the number (i.e. estimating the proportion) that have moulted to the next stage of development. Provided that assumptions of a uniform age distribution within the incubated stage are met, stage duration (days) can then be calculated as;

$$D = (1/MR) * t, \qquad \text{Eq. 1.2}$$

where, MR = moulting rate and t is the incubation period (days). This approach has been widely adopted and elaborated upon to estimate growth rates (e.g. Peterson et al. 1991). However, Miller et al. (1984) and more recently, Hirst et al. (2005) have demonstrated that when the assumption of uniform age distribution within a stage is violated, estimates of stage duration can be severely under- or overestimated. Estimates of stage duration relying on moulting rate incubations may be further prone to error when the intermoult period exceeds ~1.2 days (see Miller 1993). For example, Equation 1.2 should yield similar stage duration to a comparable calculation:

$$D = 1/-\log(s), \qquad \text{Eq. 1.3}$$

where, s = proportion surviving or remaining in the incubated stage at the conclusion of the incubation period. However, when an intermoult period exceeds ~1.2 days (see Figure 1.1), estimates using Equations 1.2 and 1.3 will diverge, with progressively greater stage durations predicted using Equation 1.3. The resulting discrepancy arises because the age distribution of animals within a stage does not actually vary with the moulting rate.

To date, logistical difficulties associated with conventional methodologies have meant that field estimates of *in situ* growth and development remain limited to a very few copepod species from only a few environments (Hirst and Bunker 2003). Such studies are generally carried out using incubations (Kimmerer and McKinnon 1987; Peterson et al. 1991). Two general approaches dominate the literature. The "moult rate" method (Peterson et al. 1991) is based primarily on the methods described above; incubation of a specific stage or size class of animals over a defined period of time is used to estimate the stage duration. Growth during that stage is estimated from the change in weight of successive stages sampled from the water column. The "artificial cohort method" proposed by Kimmerer and McKinnon (1987) is also based on incubations of defined size fractions. However, rather than using moulting rates to estimate growth rates, this method relies on direct weight estimates of animals before and after incubation over a defined duration.

Repeated handling of animals is unavoidable with both approaches. Copepod exoskeletons are fragile and even minor damage to antennae and/or setae can render individuals incapable of moulting (Miller et al. 1984; personal observations). Both methods also require that *in situ* food conditions and temperature be adequately reproduced at sea. Enumeration, identification, and direct weight estimates are also required. These considerations result in estimates that are potentially prone to considerable experimental error, and which are highly demanding in terms of labour, cost, and time. Moreover, Rey-Rassat (2004) and Hirst et al. (2005) have recently

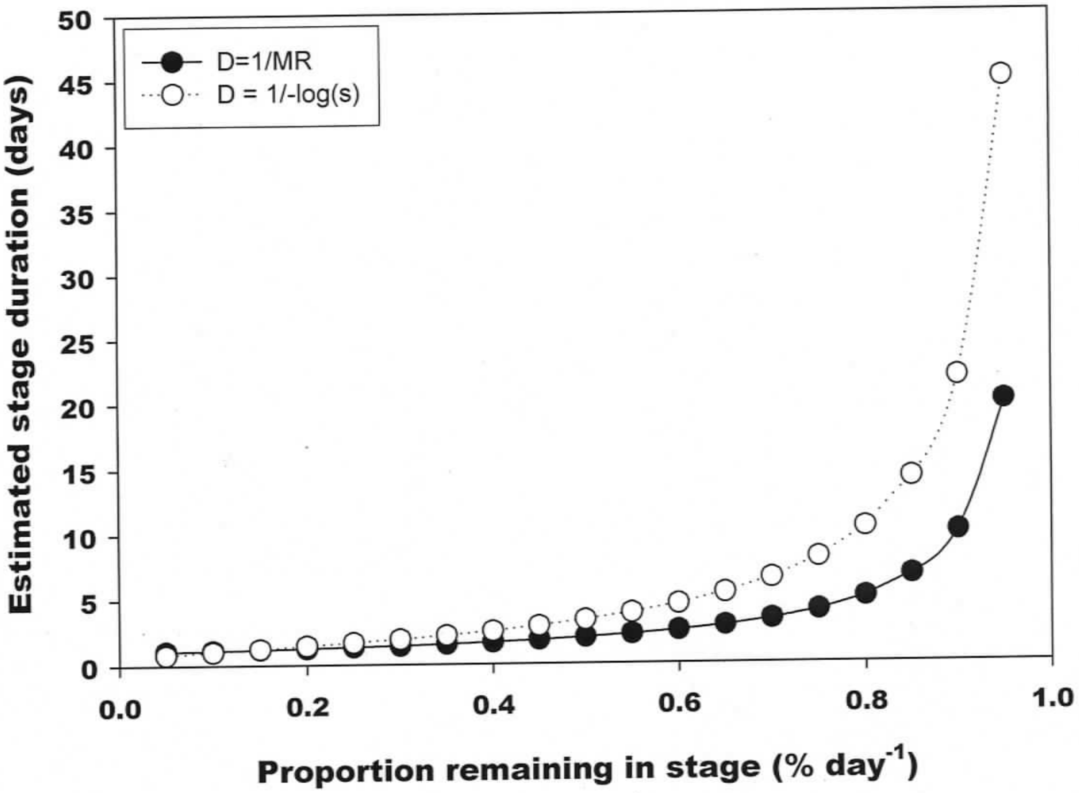

Figure 1.1 Comparison of predicted stage durations using Equations 1.2 (black symbols) and Equation 1.3 (white symbols). Estimates of stage duration using Equation 1.2 were estimated as D = 1/MR, where MR represents the % moulting per day, or equivalently 1-% remaining in stage per day.

demonstrated that both approaches can be further biased due to the underlying assumptions of uniform age distribution, continuous growth throughout development, and a potential mismatch between stage durations and estimates of growth increment. Another potential error for growth rate calculation may arise from the form of growth assumed. As discussed above (Section 1.4.1) growth is assumed to be exponential However, Equation 1.1 (often referred to as an exponential model of growth) differs from the geometric model of growth:

$$g = e^{[\ln(W_2/W_1)/t]} - 1, \qquad \text{Eq. 1.4.}$$

where, g represents the daily weight-specific growth rate. Although Equation 1.1 is used exclusively in plankton growth literature, it represents an approximation of Equation 1.4 which is better suited to estimates of growth over discrete time intervals. The potential for error is illustrated in Figure 1.2 by comparing growth rate estimates across a range of relative growth increments (assuming a constant stage duration of 2 days). It should be noted that growth rates estimated using Equation 1.1 are comparable to those estimated with Equation 1.4 up to a value of ~0.25 day^{-1}. When growth rates exceed ~0.25 day^{-1} Equation 1.4 will yield increasingly faster growth rate estimates than Equation 1.1. This discrepancy may not lead to significant errors of production rates in polar and temperate water, but will almost certainly lead to gross underestimates of production rate in warmer waters.

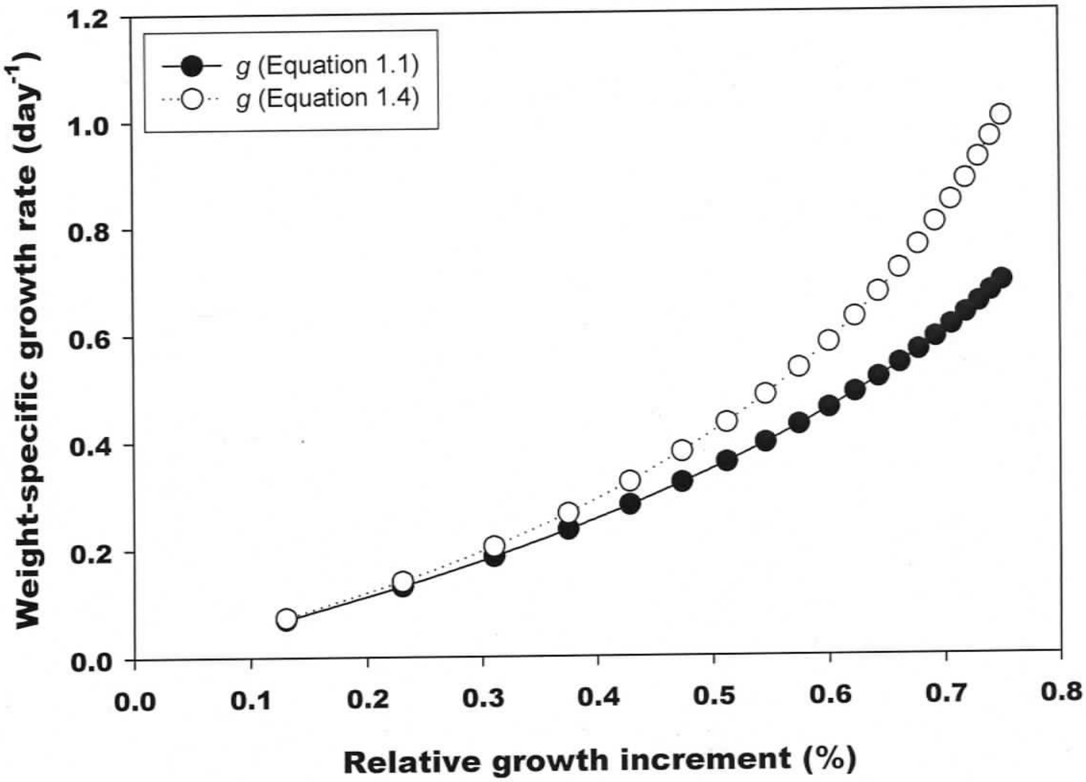

Figure 1.2 Comparison of weight-specific growth rate (day^{-1}) estimated using Equation 1.1 (exponential growth) and Equation 1.4 (geometric growth). The relative growth increment (%) is varied while the stage duration was held constant at 2 days.

1.6 Alternative indices and estimates of juvenile copepod biomass production, development and growth

Overcoming the limitations of traditional approaches for estimating copepod development and growth requires alternative methodologies that are unconstrained by demographic assumptions and which are not logistically limited in their application. To date, the greatest attention has been devoted to the development of methods that yield instantaneous estimates of biomass production, growth and development rates. Such approaches are based on physiological indices that are purported to be related to somatic growth (Runge and Roff 2000), and which can be divided into three broad categories: 1) radiochemical techniques, analogous to ^{14}C or ^{3}H for phytoplankton and bacterioplankton, respectively; 2) changes in nucleic acid content; and 3) enzymatic approaches. All of these approaches attempt to measure (either directly or by proxy) the rate of increase of a single tissue or the turnover rate of a specific biochemical pool related to copepod growth. However, characterizing metazoan growth in this manner is difficult since turnover rates of various structural components (e.g. protein, fat, and carbohydrates) can vary independently. Thus, growth estimates based on either a change in total weight or an increase in a single structural component can potentially obscure true somatic growth.

Radiochemical methods

Radiochemical methods (i.e. Roff et al. 1994) represent a possible solution to this issue, as the synthesis rate of a single (or several) structural component(s) can be followed through time. This approach is appealing because it is analogous to the widely used approaches for measuring the production of phytoplankton (Steeman Neilsen 1952) and heterotrophic bacteria (Fuhrman and Azam 1982). However, copepods differ from phytoplankton and bacteria by virtue of possessing an impermeable exoskeleton that imposes a barrier to diffusion and/or active uptake of dissolved substrates. Thus, the labels must be introduced indirectly, via the incubation of animals with radio-labeled food. Roff et al. (1994) were able to estimate somatic growth rates of *Daphnia magna* by measuring incorporation of radio labels into chitin precursors. However, as with other traditional approaches, this technique demands lengthy incubations and is therefore not practical as a routine measure in the field.

Nucleic acid ratios

Estimates of growth rate that measure changes in the relative concentration of nucleic acids (RNA and DNA) are premised on a constant DNA content (relative to RNA content), which varies with metabolic rate. Statistically significant relationships between *in situ* growth rate and RNA/DNA ratio have been reported for specific copepod populations (e.g. *Calanus finmarchicus*; Wagner et al. 2001). However, the approach is constrained by the fact that it is not broadly applicable either between populations or across communities (Steinhart and Eckmann 1992; Runge and Roff 2000), since the cellular concentration of DNA in copepods has been demonstrated to vary with body size (i.e. Sulkin et al. 1975), growth rate (Ota and Landry 1984), and season (Brodsky and Ureyvaeva 1985). Furthermore, cellular concentrations of mRNA, tRNA, and rRNA, do not co-vary with metabolic rate. Thus, although variation in RNA/DNA ratios may represent a useful condition factor, the technique holds little promise as means of directly estimating somatic growth rate.

Enzymatic approaches

The rate of substrate flux through a specific biochemical pathway can be estimated through enzyme activity. Methods relying on measures of enzyme activity are thus attractive because they are: 1) substrate specific; 2) measured as activities which are defined as actual rates and; 3) often consist of simple and inexpensive assays (Runge and Roff 2000). On a precautionary note, however, Newsholme and Crabtree (1986) warn that typical measures of enzyme activity (V_{max}) are not always representative of the actual rates of product formation because *in vivo* reactions do not normally proceed at maximum rates.

Berges et al. (1990) define a measure of metabolic rate as being an activity that co-varies with the rate that it purports to measure (i.e. enzyme activity varies in proportion to variation in growth). In contrast, simple correlates with factors known to influence growth (i.e. the relationship between temperature and body size) do not confer a measure of growth rate *per se*. Of the eight enzymes studied by Berges (1990) only one, nucleoside diphosphate kinase (NDPK), varied independently with directly measured rates of growth for *Artemia franciscana*. However, this enzyme was not found to vary with growth rate across species (Jones 1995).

Recently, Yebra and Hernandez-Leon (2004) and Yebra et al. (2005) estimated the *in vivo* activity of aminoacyl-tRNA synthetases (AARS) in *Daphnia magna* and *Calanus helgolandicus.* Each enzyme in this complex is specific to a single amino acid and catalyses its placement on tRNA during protein synthesis. Thus, Yebra and Hernandez-Leon (2004) and Yebra et al. (2005) estimated total AARS activity as a measure of the rate of protein synthesis in relation to growth. This approach is appealing, since enzyme assays are run without the addition of substrate. Thus, estimates of AARS activity should represent *in vivo*, rather than maximal rates. However, the results of both studies suggest only a weak correlation between growth rates and AARS activity, which has yet to be shown to have a demonstrated applicability across species. The weak correlation between *in vivo* AARS activity and growth rate may therefore suffer from the same shortcomings as the RNA/DNA technique. AARS activity is a measure of *total* protein synthesis and is not, therefore, exclusive to protein synthesis related to growth but also a reflection of the synthesis of proteins that do not vary with growth (i.e. regulatory enzymes).

Espie and Roff (1995 a, b) considered the activity of the moulting enzyme chitobiase (N-acetyl-β-D-glucosaminidase), one of two enzymes secreted by the crustacean epidermis during the pre-moult phase (apolysis) and which catalyses the partial degradation of chitin from the old exoskeleton. Specifically, chitinase hydrolyses oligomeric and trimeric of N-acetyl-glucosamine ($[NAG]_3$ and $[NAG]_4$), and chitobiase hydrolyses dimers to monomeric NAG, which is then recycled towards synthesis of the new exoskeleton (see Roff et al. 1994). Espie and Roff (1995 a, b) estimated chitobiase activity (CBA) in homogenates of three species of freshwater cladocerans and found that CBA in pooled homogenates varied with the proportion of individuals in apolysis (i.e. index of stage duration) and that CBA varied with body size. However, these relationships differed significantly across species, and CBA in homogenates of *D. magna* overestimated growth rates (as estimated by Roff et al. 1994) by >100 fold. Espie and Roff (1995b) suggested that these overestimates of growth rate may have been due either to an inability to adequately discriminate between digestive and moulting isomers of chitobiase (see Peters et al. 1999) or because optimized CBA is not an absolute measure of chitin degradation rates.

Sastri and Roff (2000) further investigated CBA in laboratory cultures of freshwater cladocerans and assayed CBA in the water surrounding individuals and laboratory populations in a manner similar to Vrba and Macacek (1994). Consistent with Vrba and Macacek's (1994) observations, they found that the CBA liberated into the water by individuals immediately following ecdysis was proportional to body size. They also found that this relationship was similar across three species of freshwater cladocerans: *D. magna*, *D. pulicaria*, and *Ceriodaphnia* spp. By measuring the native activity of chitobiase (CBA_{native}) in laboratory cultures and its rate of decay, they were able to produce estimates of stage durations for the average-sized individual in monospecific and mixed species cultures that were in good agreement with conventional estimates.

Oosterhuis et al. (2000) also measured chitobiase activity and decay dynamics under controlled laboratory conditions and found a positive relationship between CBA liberated by individuals and body weight of the marine copepod, *Temora longicornis*. Using this relationship, they estimated the stage-specific growth increment relative to CBA. Estimates of biomass production rate based on daily estimates of CBA_{native} and CBA decay rate compared well with their conventional estimates of biomass production of synchronously developing cohort of *T. longicornis*. This approach is unique among all others, because it purports to estimate production rate *directly*, thus obviating the need to measure biomass and growth rate.

To date, the chitobiase-based approach (Oosterhuis et al. 2000; Sastri and Roff 2000) represents the first proposed method for estimating biomass production and stage duration that may be potentially applied to natural planktonic communities and used to directly quantify community-level production rates.

1.7 Thesis objectives and structure

1.7.1 Thesis objectives

As discussed above, variation of copepod production rate exerts a significant ecological and biogeochemical influence in marine ecosystems (Buitenhuis et al. 2006). To date, however, a comprehensive understanding of how this rate varies *in situ* has remained elusive. The objective of this thesis is, therefore, to present an alternative method that may be used to directly estimate juvenile biomass production and to potentially help clarify the relative influence of temperature, individual body weight, and food concentration on juvenile copepod development and growth rates in the field. Most development and growth rate studies have typically been conducted under conditions of food saturation in either laboratory cultures or in very productive coastal ecosystems (e.g. Huntley and Lopez 1992). This is due, in part, to the logistical constraints and limited applicability of the various methods employed to date. Consequently, it has been widely assumed that marine copepods generally develop and grow at maximal, temperature-dependent rates (i.e. Huntley and Lopez 1992).

Ultimately, a greater number of *in situ* estimates, and with greater spatial and temporal coverage, will be required to resolve the relative importance of the regulatory factors identified with laboratory cultures in field settings. Furthermore, as the scope of biological oceanography increasingly extends to the consideration of ecosystem-level processes, variation of fundamental rate processes such as zooplankton production must be measured at the community level.

1.7.2 Thesis structure

This thesis consists of five chapters. Chapter 2 provides a field validation of the chitobiase-based estimate (Sastri and Roff 2000) of development and growth rates for marine copepod communities. Although formatted for this thesis, the contents of this chapter have already been published (Sastri and Dower 2006) in the Canadian Journal of Fisheries and Aquatic Sciences. Chapters 3 and 4 report results from field studies which use the chitobiase-based methods to test their efficacy and relative sensitivity as routine measures of biomass production rate that may be used in comparative studies.

Chapter 3 explores the factors contributing to temporal variation of biomass production rates and also community rates of development and growth in the Strait of Georgia, a productive coastal system, over the two consecutive spring phytoplankton blooms. This chapter represents one of several studies that were conducted during STRATOGEM (Strait of Georgia Ecosystem Modeling Project), a larger three year study that has been directed at characterizing variability of physical and biological processes in the Strait of Georgia.

Chapter 4 adopts a large scale approach and considers patterns of spatiotemporal variability in juvenile biomass production rate from three distinct systems (shelf, slope and oceanic) in the subarctic NE Pacific. This study was carried out during the spring and summer of 2005 along the West Coast of Vancouver Island and Line P. These two broad-scale oceanographic surveys are part of the long term sampling series in the southern Gulf of Alaska that continue to be conducted by the Institute of Ocean Sciences (Department of Fisheries and Oceans).

A synthesis of the major results is presented in Chapter 5. The chapter concludes by considering some directions for future research of relevance to this thesis that were not addressed in Chapters 2-4.

CHAPTER 2.

Field validation of an instantaneous estimate of *in situ* development and growth for marine copepod communities

2.1 Introduction

Although many studies have measured copepod egg production, estimates of *in situ* development and growth rates of nauplii and copepodites remain rare (see Hirst et al. 2003). This disparity is due primarily to various logistical problems associated with conventional methodologies (reviewed by Runge and Roff 2000). Moulting rates can be estimated through serial sampling in situations where the development of crustacean zooplankton populations is seasonal and synchronous. However, these estimates are: (i) often poorly resolved in space and time; (ii) restricted to individual species; and (iii) highly labour intensive. To estimate development and growth of juvenile copepods at meaningful spatiotemporal scales requires instantaneous estimates of these rates that avoid biases associated with handling and incubations, and which can be applied routinely at sea. Although several radiochemical and biochemical indices have been explored to date (Runge and Roff 2000), none has demonstrated a routine applicability across species.

Recently, a new approach has been proposed, based on the crustacean moulting enzyme, chitobiase (Sastri and Roff 2000). This method is attractive because it is based on a simple enzyme assay of ambient seawater surrounding the moulting community. Chitobiase is one of two chitinolytic enzymes employed by crustaceans during the premoult phase and is secreted into the apolytic space by the epidermis where it catalyses the partial recycling of chitin (Buchholz 1989; Roff et al. 1994).

Vrba and Machacek (1994) first demonstrated that chitobiase activity (CBA) in the medium surrounding an individual following ecdysis is significantly higher than that measured during intermoult, and that the liberated CBA increases with body size. Sastri and Roff (2000) applied Vrba and Machacek's (1994) approach to three species of laboratory-reared freshwater Cladocera and found that the species-specific relationships could all be described by a single body-size relationship. Using this common relationship, and the rate of decay of CBA in the medium, they were able to estimate mean size-weighted stage durations of asynchronous, monospecific, and mixed cultures of freshwater Cladocera. Here we extend this approach to marine copepods, the taxonomic group that dominates virtually all marine mesozooplankton communities. The objectives of this study were to: (i) determine whether the relationship between liberated chitobiase activity and body size is conserved across marine copepod species; and (ii) compare chitobiase-based estimates of copepod community development and growth to conventional estimates for natural communities in coastal British Columbia, Canada.

2.2 Materials and methods

2.2.1 Chitobiase assays and homogenates

Chitobiase activity (CBA) in the water column was assayed following Sastri and Roff (2000) with modifications for seawater. Reactions were initiated by the addition of 0.2 mmol Methylumbelliferyl N-aceytl-β-D-glucosaminide (MBF-NAG; stock dissolved in cellosolve and diluted in citrate-phosphate buffer; CPB) to 2.0 mL of seawater. Reactions were buffered to pH 6.0 by adding 1.0 mL of 0.15 M CPB. The product of this reaction, Methylumbelliferone (MBF), fluoresces optimally at pH 10 or higher, however, carbonate precipitation reduces assay sensitivity. Thus, reactions were terminated after 30-60 minutes by adding 500 μL of a solution of 2N NaOH and 0.4 M EDTA. The final reaction solution was always clear, with a pH between 10.5 - 10.7. Background fluorescence of the substrate was assessed in parallel reactions that were terminated at the initiation of the assay. Background and sample fluorescence were measured with a Turner Designs TD-700 fluorometer equipped with a long wavelength bulb and 300-400 nm excitation and 410-600 nm emission lenses. Raw fluorescence was converted to nmol MBF using 4-Methylumbelliferone (stock dissolved in cellosolve). CBA is expressed as nmol MBF liberated L^{-1} h^{-1}. Reaction temperature was maintained at 25°C.

Frequently, CBA in the water column is close to the level of detection of the assay conditions described above and it is necessary to spike seawater samples with a concentrated source of enzyme in order to reliably estimate decay rates. A homogenate was prepared by homogenizing 30-50 copepods in seawater using a hand-held glass tissue grinder. The homogenate was passed through a 0.2 μm filter and diluted (1:1000 (v/v)) in seawater samples collected for CBA decay rate estimates. In order to validate this approach we compared the CBA decay rates in samples of seawater collected from the same site, time and depth (West Coast of Vancouver Island, June 2005) and which were amended with 100%, 50% and 25% of homogenate. For pH dependence and all CBA decay rate experiments, substrate saturation was always tested by confirming a linear increase in of MBF fluorescence (i.e. fluorescence regressed against time) using parallel reactions which were terminated at 0, 15, 30, 45, and 60 minutes. CBA assays on native CBA samples were terminated after 60 minutes, whereas CBA assays on filtered aliquots with homogenate were terminated after 30 minutes as the concentration of chitobiase was greater.

2.2.2 Chitobiase-body size relationship

Copepods used for individual incubations were collected from the Strait of Georgia and Saanich Inlet, British Columbia (Canada), between March and June 2004 using a SCOR net (55 cm diameter, 236 μm mesh) with a non-filtering cod end to minimize damage to the animals. All moulting incubations were conducted within 48 hours of collection. *Tigriopus californicus* were collected from a splashpool at Ten Mile Point in Victoria, British Columbia. Copepodite stages of *Calanus pacificus*, *Metridia pacifica* and *Pseudocalanus* sp. were identified and then carefully rinsed in sterilized seawater (previously heated to 75°C and 0.2 μm filtered). Individuals were then placed in well-plates containing 2.5 mL of sterilized seawater (for up to 12 hours) at the temperature of collection (8-22°C). At the conclusion of the incubation period, wells were inspected for the presence of exuviae using a dissecting microscope (25X magnification). When exuviae were observed, 2.0 mL of incubation medium was removed for the chitobiase assay (as above), and the prosome length and width of the moulted animal was measured to the nearest 0.05 mm, with an ocular micrometer. All enzyme reactions lasted 60 minutes with the exception of the largest individuals (CV-CVI *C. pacificus*; 30 minutes). Chitobiase activity (CBA) was regressed against prosome length and dry weight. All individual dry weights presented in this study were estimated using species-specific length : weight relationships obtained from the marine zooplankton database maintained by the Institute of Ocean Sciences, Sidney, B.C., Canada.

2.2.3 pH dependence

To determine the extent to which a single chitobiase assay is applicable across species, we compared the pH dependence of a single species (*Neocalanus plumchrus*) to mixed copepod homogenates (*C. pacificus, M. pacifica, Corycaeus anglicus, and Pseudocalanus* sp.). Animals (*Neocalanus* sp., n = 30-35, mixed species, n = ~100) were homogenized in 4.0 mL of sterilized/filtered seawater using a hand-held tissue grinder. Aliquots of sterilized/filtered seawater were spiked with homogenates of either *N. plumchrus* or the mixed species homogenate. Chitobiase reactions proceeded as above, with substrate diluted and reactions buffered using citrate phosphate buffers across a pH range of 4-7 and without buffer (corresponding to a reaction pH of 7.91). Variation of CBA with pH is expressed as % CBA ± standard error.

2.2.4 Chitobiase decay rates under field conditions

Seawater samples were collected from a single deepwater (~400 m) station in the Strait of Georgia (49°15'N, 123°45'W; see Figure 2.1) from 5, 10, 30, and 50 m during 4 cruises bracketing the development and decline of the 2004 spring phytoplankton bloom (29 February, 6 April, 26 April, and 29 May). All samples were screened through 54 μm mesh to exclude crustaceans. At time 0, 15.0 mL of the screened seawater was 0.2 μm filtered and maintained at 4°C. The CBA in this sub-sample is hereafter referred to as native CBA (CBA_{nat}). The screened seawater sample was then amended with 0.2 μm

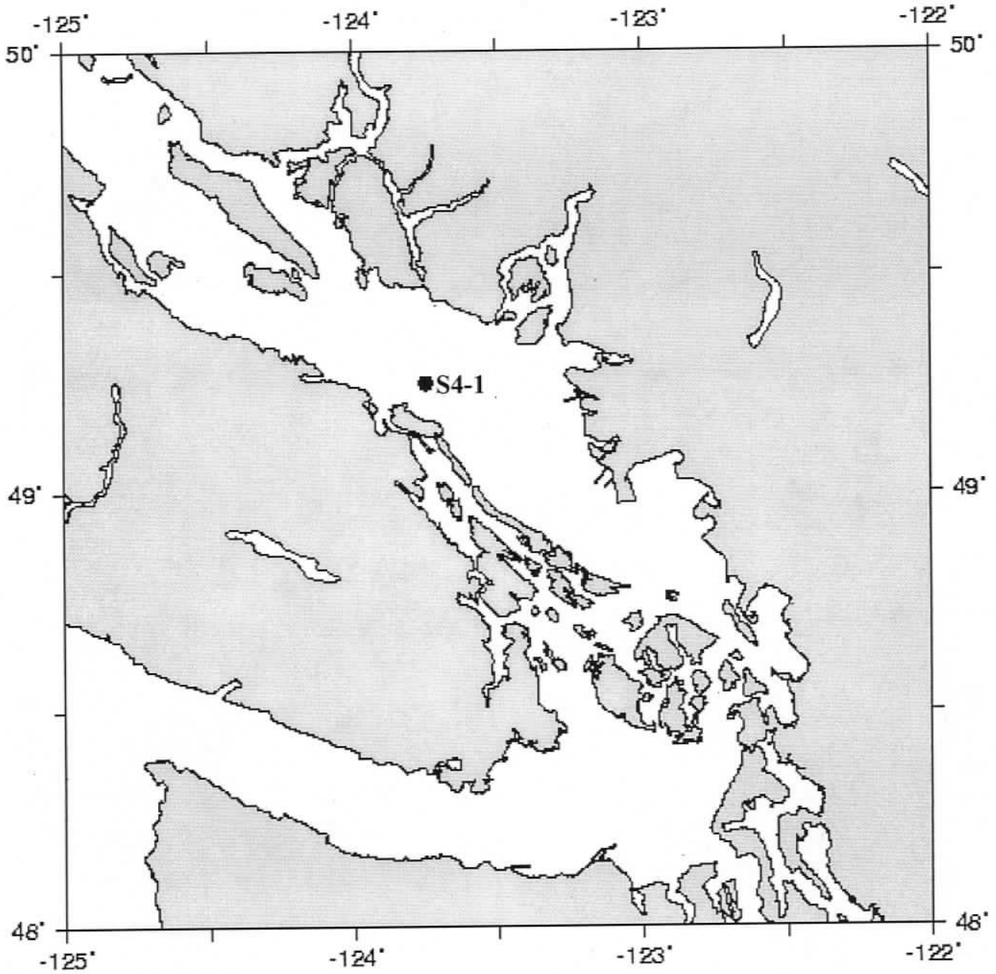

Figure 2.1. Sampling station S4-1 (49°15'N, 123°45'W) located in the Strait of Georgia, British Columbia, Canada. Vertical plankton tows (50-0 m and 100-0 m) were collected, and chitobiase decay dynamics were measured on each sampling trip during spring, summer, and autumn of 2004 and 2005.

filtered copepod homogenate and serially sub-sampled and 0.2 μm filtered every 1.5 to 3 hours for 12 hours. All sub-samples were maintained at 4°C until the conclusion of the 12 hour incubation period. CBA_{nat} was typically assayed within 1 hour of collection, while CBA in sub-samples of amended seawater were assayed concurrently within 4 hours of the conclusion of the incubation period. CBA in 0.2 μm filtered seawater remains stable for up to 60 hours at room temperature (22°C) and longer at 5°C (Vrba and Machacek 1994; Oosterhuis et al. 2000; Sastri 2001). During the incubation period each amended seawater sample was maintained at ambient sea surface temperature.

In order to describe the size-frequency distribution of the copepod community, we collected a series of net casts to correspond to CBA decay rate estimates on each sampling date. Copepods were collected using both a 70 μm mesh ring-net and a SCOR (236 μm) net towed vertically from 50-0 m. All individuals in the largest size fractions (>2.5 mm prosome length) were counted in each sample. For animals <2.5 mm in length, the samples were split up to 3 times (i.e. 1/8 of original sample) using a Folsom splitter.

2.2.5 CBA based estimates of stage duration

Sastri and Roff (2000) estimated the mean stage duration as follows (with some modification of terms);

$$D_{sample} = (\Sigma\, n_i \cdot CBA_i) / (\Delta CBA), \qquad \text{Eq. 2.1}$$

We term this estimate the "sample-based average", where D_{sample} represents the stage duration of the mean-sized individual in the community (as represented by the size frequency distribution determined from a depth integrated net sample). The terms n_i and CBA_i represent the number of animals and the CBA liberated per individual (derived from the CBA-prosome length relationship) in size-class i, respectively. The *in situ* ΔCBA (i.e. change in activity per hour) was estimated by applying the slope (k) of the time versus ln (CBA) relationship to the native CBA (CBA_{nat}) for each depth (i.e. $-k^*$ CBA_{nat}). Thus, $-k^*$ CBA_{nat} is equal to the absolute rate of production of chitobiase per unit time (ΔCBA) by the developing community. This rate may be used to directly estimate biomass production per unit time per unit volume (as per Oosterhuis et al. 2000; see Chapter 3). We do not include the correction term for deviation from equilibrium between production and decay of CBA in the water column, as stable equilibrium is assumed over the course of the seawater incubation period (i.e. 12 hours).

An alternative estimate of the mean community stage duration (D_{CBA}) can be written as;

$$D_{CBA} = CBA_{nat} / \Delta CBA. \qquad \text{Eq. 2.2}$$

Thus if 1/-k = the proportion of total biomass moulting per unit time, then;

$$D_{CBA} = 1/-k = CBA_{nat} / \Delta\ CBA.$$

Estimates of the average copepod community stage duration using Equation 2.2 are valid provided that the following assumptions are met:

i) Copepods dominate the biomass of the planktonic crustacean community, and therefore exert the greatest influence on the dynamics of chitobiase activity in the water column.

ii) Equations 2.1 and 2.2 both assume that the developing community is in steady state (see Sastri and Roff 2000) i.e. the size frequency distribution of the community is unchanging and the mean moulting rate is constant over the 12 hour timescale relevant to a decay rate measurement.

iii) Under steady state conditions, the rate of decay of chitobiase is balanced by its rate of production, therefore, CBA_{nat} is also assumed to be in steady state.

The use of Equation 2.2 also assumes that the specific fractional turnover rate of the CBA pool equals the specific fractional turnover rate of the biomass pool. This assumption is valid provided that;

iv) $CBA_{nat} = (\Sigma\ n_i * CBA_i)$, where $(\Sigma\ n_i * CBA_i)$ is the biomass of all actively developing copepods in the community (estimated from net samples) expressed in units of enzyme activity (using the CBA-prosome length or CBA-dry weight relationships)

v) Thus, CBA_{nat} only varies with $(\Sigma\ n_i * CBA_i)$, and is not influenced by the moulting rate of the community in question (i.e. CBA_{nat} in the water column for two communities of equivalent biomass which are moulting at different rates must be equivalent).

If these last two assumptions are not met, the time value estimated from Equation 2.2 represents the turnover rate of the enzyme pool which then represents the turnover rate of a varying measure of the fraction of biomass actually moulting. Thus, this estimate cannot be interpreted as the average stage duration of the developing community.

Estimates based on Equation 2.2 are not dependent on the contents of a representative net cast. Therefore, this approach may be advantageous because developmental rates can be estimated at the conclusion of a 12 hour seawater incubation, thus allowing repeated estimates over short time intervals. An attempt to address assumptions (iv) and (v) directly is presented in Chapter 3.

Using the CBA-body weight relationship we can also scale the D_{CBA} estimate with body size by modifying Equation 2.2, such that we assume that an entire community is composed of a single weight-class of interest. It is important to note that this estimate maintains the ΔCBA estimated from the field using the following relationship:

$$D_{CBAi} = ((CBA_i - CBA_{avg}) + CBA_{nat}))/(\Delta CBA), \qquad \text{Eq. 2.3}$$

where D_{CBAi} represents the mean stage duration of the size class of interest and CBA_i represents the CBA predicted for that size class from either the CBA : body weight or the CBA : prosome length relationships. The average CBA (CBA_{avg}) is defined as the CBA of the average-sized individual (or fraction of total biomass) molting per hour. Although it is not a rate *per se* (i.e. $\Delta CBA\ h^{-}1$), this value is equivalent to that of ΔCBA. Applying Equation 2.3 to all size classes present in a community generates a relationship whereby stage duration increases with body size. As this approach is dependent on body size and the measured ΔCBA, estimates will not necessarily reproduce species-specific patterns of development (see Peterson 2001), but rather serve as a generalized estimate for all of the individuals in a given size class.

D_{CBAi} was estimated for *Calanus pacificus* copepodites stages II-V using the CBA turnover rates that were measured on each of the 4 sampling dates (see Table 2.1). CBA_i for each stage was estimated using standard dry weight values (see above) and the dry weight against CBA relationship (Figure 2.2.b). These stage durations are presented against the depth-integrated (0-50 m) temperature for each sampling date. For comparison, we also include Vidal's (1980b) stage-specific estimates of optimal intermoult period (IMP) for copepodite stages CII-CV at 8, 12, and 15.5°C.

2.2.6 Conventional moulting rate estimates

On each cruise we also estimated moulting rates using the sieve fractionating method (Peterson et al. 1991). Animals were collected by slowly (~0.5 m s^{-1}) towing a SCOR net (fitted with a 1 L non-filtering cod end) from 30 m to the surface. The contents of the net cast were passed carefully through a submerged cylinder with a 500 μm mesh bottom and diluted in seawater. This 233-500 μm size fraction was then split gently into 6 aliquots. The first 3 aliquots were preserved immediately in 10% buffered formalin seawater and the remaining aliquots incubated at ambient sea surface temperature (see Table 2.2) in 20 L carboys for 48 hours. Carboys were filled with 70 μm screened seawater that was pumped from ~2 m below the surface.

Species-specific stage distributions were estimated by examining the entire contents of each preserved aliquot using a dissecting microscope (25X magnification). Moulting rates were estimated using the relationship presented by Peterson et al. (1991):

$$MR_i = P_{f,i+1} - P_{s,i+1} / P_s, \qquad \text{Eq. 2.4}$$

where, MR_i represents the molting rate of stage i, $P_{s,i}$ and $P_{s,\,i+1}$ represent the proportion of individuals at stage i and stage i+1 at the start of the incubation and $P_{f,\,i+1}$ represents the proportion of individuals at stage i+1 at the end of the incubation period. Stage duration was estimated as the reciprocal of MR_i. These rates were calculated for the species-specific stage dominating the incubations at T_0.

2.2.7 Growth rate estimates

Weight-specific growth rate (g) was estimated as:

$$g = \ln (W_2/W_1)/D, \qquad \text{Eq. 2.5}$$

where, W_2/W_1 represents a stage- or size-specific change in body weight. D (days) represents the time over which the weight change occurred and was estimated from our D_{CBAi} estimates. Stage-specific growth increments for *Pseudocalanus* spp, *Metridia pacifica*, and *Calanus pacificus* were estimated from successive stage weights using length-weight relationships employed in the CBA-body size relationships. These growth increments were pooled and regressed against CBA predicted for each species and stage-specific post moult weight. This relationship was described as:

$$\log (g_{inc}) = 0.864 \log(CBA_i) - 1.78 \; (r^2=0.73, n=19, p<0.0001), \text{ Eq. 2.6}$$

where g_{inc} represents the difference in mass between the premoult size class and the stage present in the water column. Thus, W_1 from equation (5) is equivalent to W_2-g_{inc}. We chose this estimate of growth increment because our CBA-based estimates of D represent the time taken to moult to the stage present in the water column at the time that the CBA decay rate estimates were made.

Growth rates were estimated on a size-class basis and the numerically-weighted average was taken as the average community growth rate. These rates were then compared to the relationship describing juvenile copepod (sac and broadcast-spawners) growth rates presented by Hirst and Lampitt (1998). We applied our estimates of W_1 (corrected to carbon using a conversion factor of 0.45) as the body weight values for the Hirst and Lampitt (1998) global *in situ* model.

2.2.8 Statistical analyses

The natural logarithm of CBA was employed for all decay rate experiments in order to normalize distribution of the data. Homogeneity of the slopes in the decay experiments was tested using ANCOVA as outlined by Zar (1984).

2.3 Results

When observations from the four marine copepod species were pooled, CBA (nmol MBF L^{-1} h^{-1}) was found to be positively related to prosome length (mm) as log (CBA) = 2.939 log(PL) - 0.3325 ($r^2 = 0.889$, n= 44, $p<0.0001$, Figure 2.2a). Chitobiase activity also showed a significant positive relationship with individual dry weight (µg) across the four copepod species. This relationship is described as log (CBA) = 1.149 log (DW) + 1.566 ($r^2 = 0.90$, n= 44, $p<0.0001$, Figure 2.2 b).

Figure 2.3 shows that homogenates of *Neocalanus plumchrus* and the mixed zooplankton homogenate both displayed a pH optimum (expressed as %CBA) of ~6.0.

The time versus ln CBA (nmol L^{-1} h^{-1}) relationships for 3 samples (500 ml) of seawater, each amended with 500 µL of 100, 50 and 25% filtered homogenate are presented in Figure 2.4. The slopes of the different samples were not significantly different $F_{(2,9)}$= 0.0041, $p>0.05$.

Comparisons of CBA-based estimates of the stage duration of the mean-sized individual in the community suggest that estimates based on the D_{sample} method underestimate conventional estimates of stage duration by 53-68% (Table 2.1). The D_{CBA}

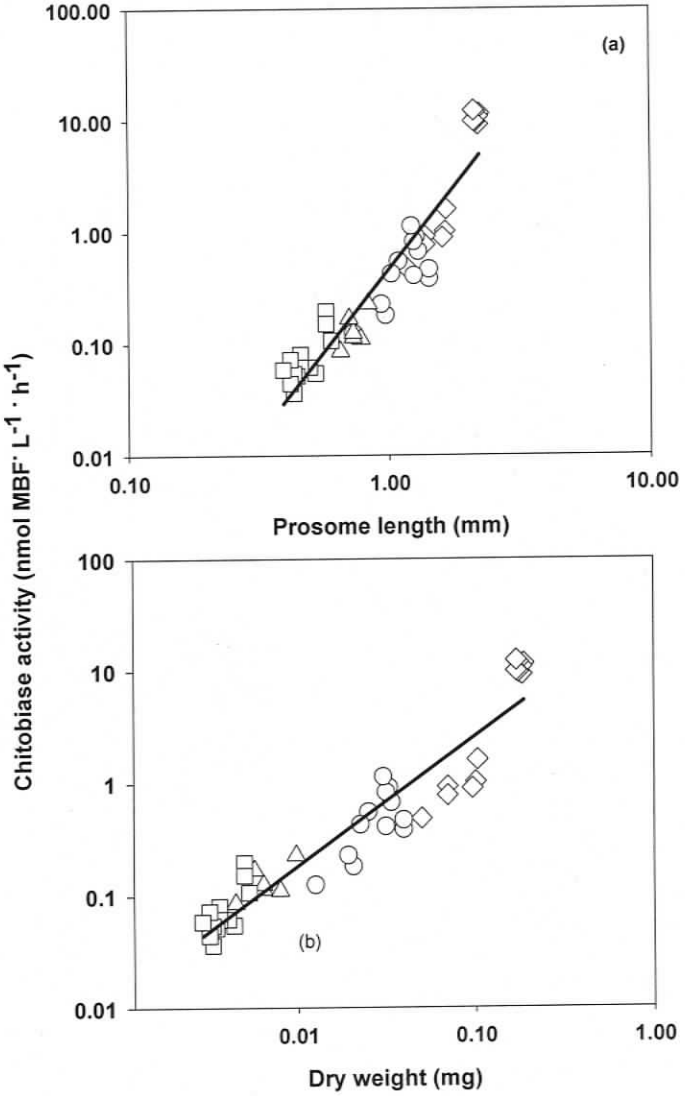

Figure 2.2. a). Generalized relationship between liberated chitobiase activity (CBA) by individual copepods after moulting, and prosome length (mm); log(CBA) = 2.939 log(PL) - 0.3325, $r^2 = 0.889$, n= 44, p<0.0001. b). Generalized relationship between CBA liberated by individual copepods after moulting, and individual dry weight (mg); log (CBA) = 1.149 log (DW) + 1.566, $r^2 = 0.90$, n= 44, p<0.0001. *Calanus pacificus* (diamonds), *Metridia pacifica* (circles), *Pseudocalanus* sp. (triangles), and *Tigriopus californicus* (squares).

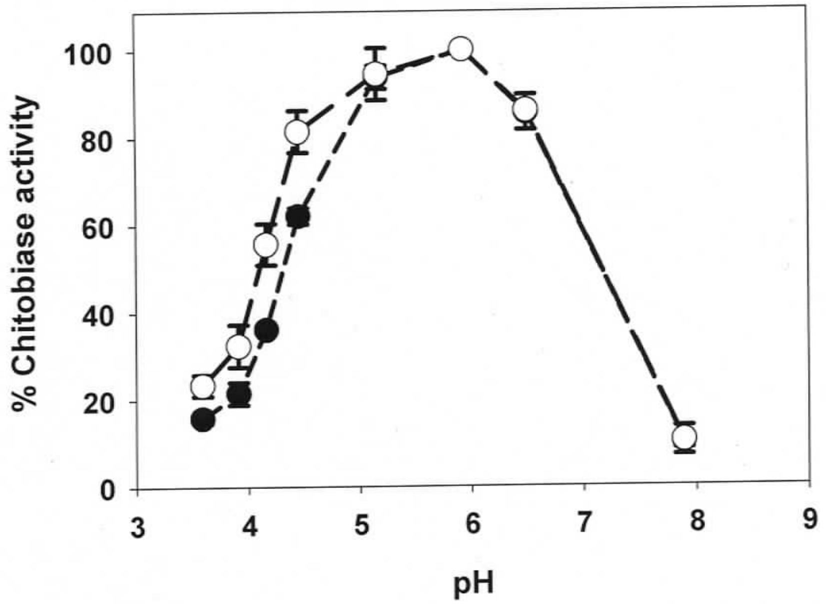

Figure 2.3. Comparison of pH dependence of chitobiase activity (% activity) between homogenates of *Neocalanus plumchrus* (solid symbols; n = 3, mean ± std.error) and mixed copepods (open symbols; *Calanus pacificus, Metridia pacifica, Corycaeus anglicus., and Pseudocalanus* sp; (n=3, mean ± std. error).

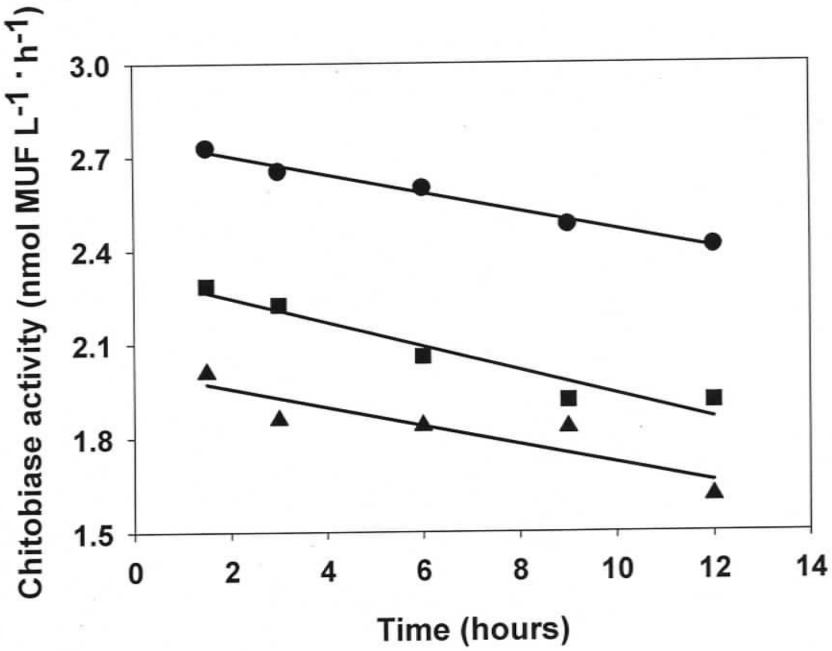

Figure 2.4. Time versus ln chitobiase activity (nmol L^{-1} h^{-1}) relationships for 3 samples (500 mL) of seawater, each amended with 500 μl of 100 (circles), 50 (squares) and 25% (triangles) filtered homogenate. The slopes of each sample are not significantly different ($F_{(2,9)}$= 0.0041, p<0.01).

Table 2.1. Measured values of native CBA (CBA_{nat}), hourly CBA decay rates (Δ CBA h^{-1}), D_{sample}, D_{CBA}, D_{CBAi} and conventional estimates of stage duration (hours) measured on 4 dates in the Strait of Georgia, British Columbia, Canada during the spring of 2004.

Date	CBA_{nat}	Δ CBA h^{-1}	D_{sample} (hours)	D_{CBA} (hours)	D_{CBAi} (hours)	D_{conv*} (hours)
29 February	1.16	0.00722	71.8	161.1	189.2	152.8 (i)
6 April	3.23	0.0354	42.79	91.2	90.7, 94.3	107.2 (ii)
25 April	4.27	0.059	54.24	72.4	72.5	----
29 May	3.87	0.068	21.42	56.9	58.1, 59.5	67.4 (iv)

Note: *stage/species at time 0: i) *Pseudocalanus* sp. CIV; ii) CII *C. pacifcus,* CII *Pseudocalanus* sp.; and; iv) CV *Acartia longiremis*, CIII *Centropages abdominalis*

Table 2.2. Mean community estimates of weight-specific growth rate (day^{-1}) measured on 4 dates in the Strait of Georgia, British Columbia, Canada during the spring of 2004. CBA-based estimates (CBA *g*) represent the numerically-weighted average (from net casts) of growth rates estimated by using size scaled D_{CBA} values (Table 2.1). For comparison, we also include estimates using the temperature and body weight model for *in situ* juvenile copepod growth rates (*g*) presented by Hirst & Lampitt (1998).

Date	Temp (°C)	Ind DW (mg)	CBA *g* (day^{-1})	*g* (day^{-1})
29 February	7.82	0.001489	0.0724	0.090
6 April	8.38	0.004406	0.130	0.087
25 April	9.06	0.019404	0.167	0.082
29 May	10.62	0.003063	0.210	0.108

estimates, however, were generally within 5-15% of conventional stage duration estimates (Table 2.2).

Scaling the D_{CBA} estimates, we estimate the size-class specific stage duration (D_{CBAi}) of *Calanus pacificus* copepodites stages II-V (Figure 2.5). Our D_{CBAi} estimates represent stage durations that vary with both developmental stage and temperature in a fashion similar to the optimal rates presented by Vidal (1980b). Our D_{CBAi} estimates are in good agreement with the temperature-dependent rates estimated from Vidal (1980b). It should be noted that the D_{CBAi} estimates represent the average stage duration for all individuals in the community occupying a defined size class. During our 4 sampling trips, the size classes represented by *C. pacificus* CII-CV were largely dominated by *C. pacificus* and can thus be considered representative of this species.

Table 2.2 compares numerically weighted growth rate averages for each date against the body size and temperature-dependent estimates using the Hirst and Lampitt (1998) equation. Our growth rate estimates suggest that before the spring phytoplankton bloom (29 February), weight-specific growth rates were comparable to weight- and temperature-specific rates predicted by Hirst and Lampitt's global model for juvenile sac and broadcast spawners. During the peak of the bloom (6 April) and the transition toward a "summer" copepod community (25 April and 29 May), however, growth rates of the mean-sized individual in the copepod community meet the expectations of a productive coastal system (i.e. the Strait of Georgia; Harrison et al. 1983) when estimated with the CBA method, but are underestimated by rates predicted by the Hirst and Lampitt global *in situ* model.

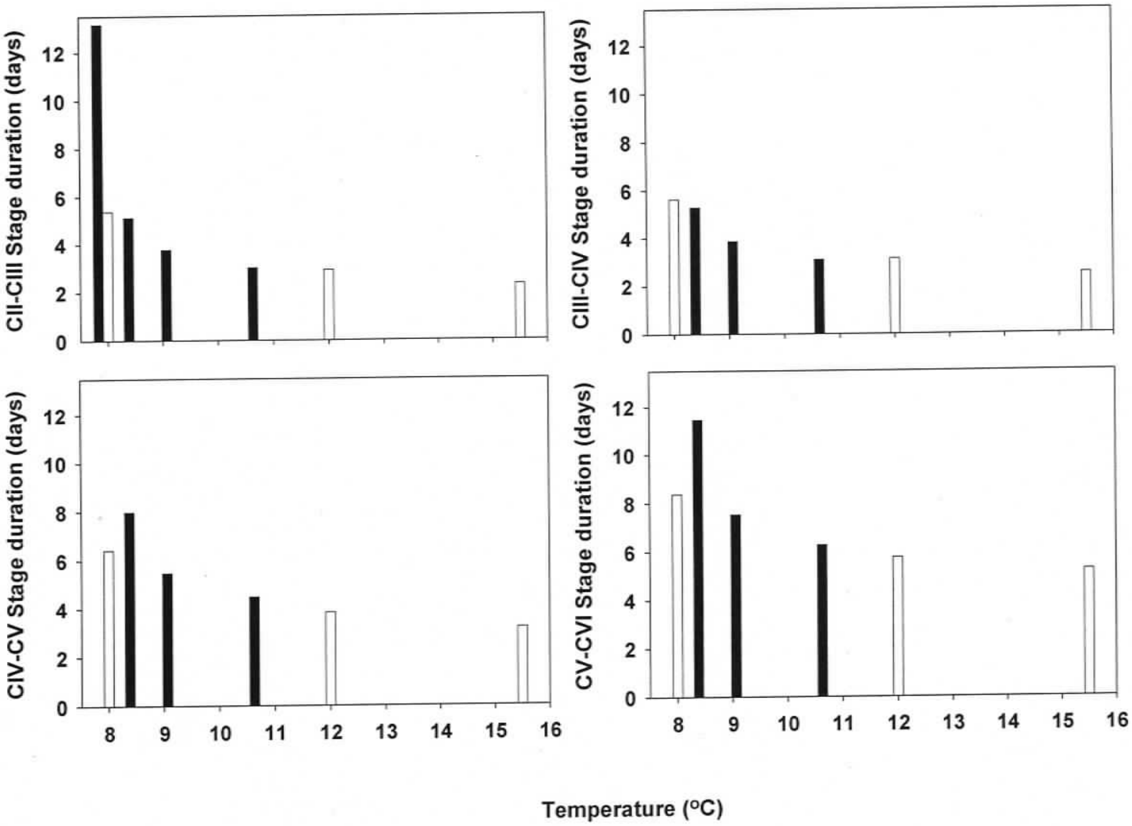

Figure 2.5. Chitobiase-based stage specific stage durations (D_{CBAi}) of *Calanus pacificus* copepodites stages II-V (solid bars). CBA_i for each stage was estimated using standard dry weight values and the dry weight versus liberated chitobiase (CBA) relationship presented in this communication. These stage durations are presented against the depth-integrated (0-50m) temperature for each sampling date. Vidal's (1980b) stage-specific estimates (white bars) of optimal intermoult period (IMP) for copepodite stages CII-CV at 8, 12, and 15.5°C are included for comparison.

2.4 Discussion

2.4.1 Chitobiase-body size relationships

Our results show that CBA can be reliably estimated from prosome length for marine copepods. A simple length-based relationship seems reasonable for the calanoid species examined, given their shared body morphology. We included the splash pool harpacticoid *Tigriopus californicus* in our analysis in order to compare a relatively disparate group to pelagic marine calanoids. However, our results suggest that, despite taxonomic and life history differences, these groups share similar body size dependence with liberated CBA.

Although estimates of development rates based on prosome length are appealing (i.e. prosome length is easy to measure), weight-based estimates are more relevant to growth and production studies. Furthermore, estimates using body weight will more accurately represent CBA in situations where the species composition includes groups whose length-weight relationships are not consistent with the majority of marine copepods. For instance, *Eucalanus* spp. copepodites weigh less than most other calanoids of a similar length. When these groups dominate the biomass of a size class or the mesozooplankton community, individual biomass measures will be a more appropriate descriptor of liberated CBA. Additional considerations such as lipid accumulation may have to be addressed when attempting to predict both liberated CBA and stage-specific growth increment.

2.4.2 pH dependence and homogenates

It is important to note that CBA in the water column is not due exclusively to aquatic arthropods, as the enzyme is also produced by protozoans and bacteria. Vrba et al. (2004) found that pH optima represent a useful means of distinguishing between sources. Our results suggest that a pH of ~6.0 is optimal for single and mixed species of marine copepods, a value that agrees well with Vrba et al. (2004) and Espie and Roff (1995b; pH optima = 5.5, for *Daphnia magna*). In addition to a copepod-specific pH optimum, it is also understood that the other sources of chitobiase activity in the medium are particle bound (>0.2 μm). Thus, in the absence of significant unicellular lysis, dissolved CBA (<0.2 μm) should reflect copepod CBA at pH~6.

Our estimates of CBA decay rates in the water column are based on the rate of decay of a homogenate applied against the native CBA. The slope of time versus ln(CBA) was not significantly different among the three dilutions compared (see Figure 2.4). Estimates of CBA h^{-1}, derived for 100%, 50% and 25% homogenates were 0.257, 0.332 and 0.259 nmol MBF L^{-1} h^{-1}, respectively. These decay rates are similar and suggest that the decay rates estimated from seawater samples amended with homogenate are representative of the *in situ* decay rate of the enzyme. Furthermore, amending samples with homogenates also reduces the time required for the assay to only 30 minutes.

2.4.3 CBA-based versus conventional estimates of stage duration

We were not able to estimate moulting rates on 25 April 2004 as there were no significant changes in the stage-frequency distribution for any of the species incubated. Furthermore, we did not note the presence of exuviae in any of our 48 hour samples.

However, our D_{CBA} estimates were still in good agreement with the conventional estimates for the remaining 3 sampling dates (i.e. usually within 5-15%). In contrast, our D_{sample} estimates did not agree well with any of the conventional moulting rate estimates. Where discrepancies were found, we postulate that either the contents of the net casts were not representative of the copepod community biomass and/or size-frequency distribution, or that the animals were not distributed evenly throughout our 0-50 m sampling profile. Thus, our depth-weighted estimates of CBA turnover may not have been appropriately attributed to the contents of the net casts. We assume that most of the developing copepod community was residing in the upper 50 m of the water column during the spring (Mackas 1992). However, all of our experiments were conducted during daylight, and diel vertical migrators (i.e. *Metridia pacifica*) were therefore likely under-sampled. Nevertheless, our D_{CBA} estimates were in good agreement with conventional estimates and do not suffer from the potential error associated with a dependence on a quantitative net cast.

Another potential source of error associated with our D_{sample} estimates results from attributing the total CBA estimate strictly to the copepod community. We did not estimate the potential CBA contribution from other groups of planktonic Crustacea (i.e. amphipods, euphausids, decapod larvae, etc.), nor do we have a relationship describing the CBA-body size dependence of these groups. Although copepods typically dominate the crustacean mesozooplankton community, other groups may, at times, make significant contributions, especially in productive coastal systems such as the Strait of Georgia (Harrison et al. 1983).

Applying a copepod-specific CBA-body size relationship for D_{CBA} estimates is the most appropriate approach, as these estimates are not necessarily dependent on the contents of the plankton nets and are simply measures of the CBA (biomass) turnover rate in the water column. When we scaled and applied our D_{CBA} estimates to the size-frequency distribution of the contents of our nets, our estimates of mean community stage durations were in good agreement with our conventional estimates. This suggests that our net casts did capture a representative sample of the size-frequency distribution of the community.

Laboratory and field studies have demonstrated that stage durations for most copepod species are neither isochronal nor strictly equiproportional (see Hart 1990; Peterson 2001 for reviews), although the general tendency is for moulting and growth rates to decrease with increasing body size and/or developmental stage. The dependence of these rates on body size suggests that the potential for variation (or even limitation) in growth associated with food concentration may also increase with body size. If this is generally the case, estimates of the stage duration for the average-sized individual in a population (<0.5 mm locally) may only occasionally indicate those slower rates which are indicative of the effects of body size and food availability. Thus, it is useful to be able to scale CBA-based rates against size-class for a given copepod community. Our size-class scaled D_{CBA} estimates (=D_{CBAi}) from Equation 2.3 are premised on the assumption that D_{CBA} represents not only the proportion of animals moulting per hour, but also the hourly turnover rate of total copepod biomass. Thus, in relative terms, it does not matter whether the hourly production of biomass represents x % of the community or that of a single individual.

We explored the validity of this approach by estimating D_{CBAi} for the copepodite stages CII-CV for *Calanus pacificus*, which were present during our 4 sampling trips. Stage durations for CIII-CV are not presented for the first trip (28 February 2004) because the population consisted of CI-CIIIs with only very few CVIs. Our estimate of D_{CBAi} for the CV stage duration during this period was ~35 days. However, we did not consider these animals further, as they most likely had just emerged from diapause, and were not likely to be moulting from CV in the upper water column during February, 2004 (Fulton 1973).

Although these particular estimates agree well with our expectations of a productive (potentially food saturated) environment for late-stage *C. pacificus* developmental rates, they are ultimately representative of the mean-sized individual in that size-class. Where a diversity of species (each with its own developmental pattern) occupy the same size-class, CBA-based moulting rate estimates will represent the community average rather than the species-specific rates. This will almost always be the case for the smaller size classes (<1.5 mm prosome length). However, when a single species dominates the biomass of a larger size-class (e.g. *N. plumchrus* CV's in the Strait of Georgia) the D_{CBAi} estimates, while still influenced by the Δ CBA, will be most representative of that group. Ultimately, however, our objective was to validate a method that can be applied to the entire copepod community. In this respect, our D_{CBA} (Equation 2.2) estimates best satisfy this objective.

2.4.4 CBA-based estimates of growth rate

The true test of any enzymatic growth index is whether enzyme activity at a particular body size varies with growth rate (Berges et al.1990). Yebra and Hernandez-

Leon (2004) have demonstrated that "*in vivo*" activity of aminoacyl-tRNA synthetase provides an index of growth for *Daphnia magna* growing at different rates. While it has not been tested explicitly, variation of optimized CBA liberated into the water appears to be a function of body size rather than growth rate. Our data set represents animals growing at different temperatures (8-22°C), and thus it appears that liberated CBA is best described by measures of body size alone. The chitobiase method assumes that liberated CBA is a function of ecdysis and is proportional to body size across species. Liberated CBA surrounding individuals has previously been demonstrated to be significantly greater following ecdysis relative to intermoult (Vrba & Machacek 1994, Oosterhuis et al. 2000, Sastri and Roff 2000).

Oosterhuis et al. (2000) found that CBA liberated by *Temora longicornis* copepodites was positively related to the growth increment, and were thus able to estimate daily biomass production of a synchronous laboratory culture. Here, we adopt a similar approach using mean dry weights derived from species-specific relationships to estimate a generalized growth increment. Both approaches assume that the weight-specific increment of growth is proportional to the body weight of the preceding stage. The estimates of growth presented here do suffer from recent criticisms of the "moult rate" method addressed by Hirst et al. (2005). One potential error is a mismatch between the estimated stage duration (from moult to moult) and the time over which a growth increment (derived from mean weights at stage) occurs. We acknowledge this potential source of error with our present growth estimates; however, our purpose was to compare those rates to the Hirst and Lampitt (1998) model (a synthesis of "moult rate" method results). For the purpose of this comparison, the mean community growth rates in the

Strait of Georgia were comparable to those predicted by the *in situ* model (Hirst and Lampitt 1998) when growth was <0.10 day^{-1} and greater than those predicted by the model when growth >0.10 day^{-1}. The results of this comparison are consistent with those of Peterson et al. (2002) in which animals observed to be growing at maximal rates may not be adequately represented by a synthesis of *in situ* rates. We are presently investigating the potential to use the CBA method to estimate growth without violating the assumptions of the "moult rate" method.

The CBA-prosome length relationship presented here is based on measurements immediately following ecdysis. Therefore, an appropriate relationship between CBA and the growth increment for which we estimate stage duration can be established. However: 1) we did not collect and weigh the cast exuviae and; 2) prosome length does not increase (or decrease) appreciably during the intermoult period, while body weight does. Establishing an appropriate relationship between liberated CBA and growth increment will require construction of similar relationships to those presented here, with an emphasis on individual body and exoskeleton weights. This would be ideal, as variation in the CBA liberated by an individual appears to be described by body size alone. Thus, a relationship between growth increment and CBA that can be generalized across species would (when combined with the present stage duration estimates) represent a truly instantaneous estimate of copepod community growth rate. Alternatively, weight-specific growth rates can be estimated directly where mean weights at stage are measured. D_{CBAi} estimated for two successive stages (weight classes) would represent the time between successive mean weight estimates and, thus, a true measure of growth rate.

In conclusion, the key advantage of the chitobiase method is that it can be applied *in situ*, at potentially high temporal and spatial resolution, and without the attendant difficulties and errors associated with traditional moult rate techniques (see Miller et al. 1984). Assumptions of asynchronous development and stable age distributions of moulting copepods are also more likely to be met on a community-wide basis (i.e. as opposed to single populations or developmental stages). Furthermore, a general relationship between body size and CBA suggests that mean moulting rate estimates can be easily derived for mixed copepod communities.

We note that this technique can be also extended to produce generalized mortality estimates (i.e. using size/stage duration and size/stage frequency estimates) which may prove of equal importance to estimates of growth and production. Although species and/or stage-specific differences will almost certainly be obscured in estimates of mean community development and growth, we contend that chitobiase-based estimates are valuable when applied to community level studies, in much the same way that phytoplankton ecologists have long relied on the ^{14}C technique to estimate primary production in mixed phytoplankton communities.

CHAPTER 3.

Interannual variability in production, growth, and development rates of the juvenile copepod community in the Strait of Georgia, British Columbia, Canada.

3.1 Introduction

Estimates of copepod production rates typically rely on both egg production and juvenile biomass production rates. Juvenile rates are dependent on estimates of both biomass and size-class or stage-specific growth rate. Estimates of *in situ* rates of egg production are much more common than comparable estimates of somatic growth because the latter are more difficult to measure in the field. As a consequence, studies of community-level rates of juvenile biomass production are rare (e.g. Peterson et al. 2002; McKinnon and Duggan 2003). In practice, conventional methods that rely on live incubations tend to be time consuming and demand repeated handling of animals. These methods may also be prone to error because of assumptions about uniform (within stage) age distributions and the increment of growth (see Chapter 2, Miller et al. 1984; Hirst et al. 2005). Consequently, it has been difficult to address how variables such as temperature, body size, and food concentration/quality interact and regulate the variability of juvenile copepod development and growth and, ultimately, production rates, in the field.

A potential solution to the difficulties and limitations associated with conventional methodology is a *direct* estimate of biomass production rate, as proposed by Oosterhuis et al. 2000. This approach is based on estimates of the rate of production (via decay rate estimates) of the crustacean moulting enzyme, chitobiase. Oosterhuis et al. (2000) demonstrated that chitobiase activity (CBA) liberated by *Temora longicornis* copepodites varied directly with body size and the increment of growth. Thus, they were able to estimate biomass production rate (hereafter BPR) of a synchronously developing culture without concomitant estimates of biomass and growth rate. This approach represents a potentially useful synoptic tool, as other studies (Sastri and Roff 2000; Sastri and Dower 2006) have further demonstrated that CBA liberated into the water is not only proportional to body size, but can also be generalized across species (freshwater Cladocera and marine Copepoda), thus estimating BPR that is representative of *community-level* rates.

Greater insight into *in situ* regulation of BPR variation via estimates of the rate determining steps (development and growth) may be gained through the use of the approach outlined in Chapter 2. Under steady-state conditions, the average stage duration of the developing copepod community may be directly estimated from chitobiase decay rate estimates provided that the critical assumptions stated in Chapter 2 are not violated. Both the Oosterhuis et al. (2000) and the Chapter 2 approach can be routinely applied at sea because they are based on a time series of simple enzyme assays on seawater samples that are incubated for relatively short (~12 hours) periods. Here, the utility and sensitivity of these chitobiase-based methods are tested as instantaneous measures of BPR, development and juvenile growth rates during a time series in the Strait of Georgia, British Columbia, Canada.

The rate of biomass production of juvenile copepods has been difficult to estimate on a routine basis because: a) development at the community level (and often population level) is often difficult to recognize through repeated sampling (see Kimmerer 1987; Kimmerer 1990), and; b) it is difficult to account for *in situ* rates of mortality (Kimmerer

1983). Estimates of *in situ* growth rate and stage duration are used to define biomass production as a rate estimate. Unfortunately, conventional methods are not easily applied at a degree of resolution required for BPR estimates. Thus, most estimates of biomass production rates have relied on growth and often stage duration estimates based on laboratory and/or predictive global models (i.e. Uye 1982; Escribano and McLaren 1999; Roman et al. 2004) that cannot account for the influence of other important factors such as food concentration.

Copepod stage duration is known to be influenced by temperature and body size (Hirst and Lampitt 1998). With the exception of prolonged periods of severe food limitation (i.e. Davis and Alatalo 1992; Campbell et al. 2001a), copepod development rates in the field are not known to be especially sensitive to variability in food concentration. Growth rates, however, can be influenced by temperature, body size and food concentration (e.g. Hopcroft et al. 1998; Hirst and Bunker 2003; Liu and Hopcroft 2006a). While syntheses of large datasets have attempted to quantify the role of temperature and body size (Huntley and Lopez 1992; Hirst and Lampitt 1998), characterizing the influence of food concentration and food quality on development and growth rates has proven much more difficult. The Hirst and Bunker (2003) global synthesis of *in situ* estimates of juvenile copepod growth rates utilize chlorophyll *a* as a food proxy. Although a positive step toward defining the influence of food concentration on growth rate, recent studies demonstrate that, in general, this model severely overestimates growth rates of *Neocalanus plumchrus* and *Metridia pacifica* in the northern Gulf of Alaska (Liu and Hopcroft 2006a, b). Although chlorophyll *a* estimates are routinely used as estimates of food availability in many zooplankton studies, they actually only represent maximal food concentration, since not all phytoplankton are consumed equally by copepods. Size-specific filtration can help discriminate different phytoplankton size-classes, however, important details such as taxonomic composition and food quality (i.e. physiological condition) remain obscured.

Laboratory and mesocosm studies have addressed the potential role of composition and nutrient stoichiometry as aspects of the mesozooplankton diet that may influence development and growth rate (Vrede et al. 2002; Jones and Flynn 2005; Breteler et al. 2005). Field studies in temperate waters have addressed the role of interannual variability in the timing and magnitude of spring phytoplankton blooms in the context of food concentration and population dynamics and growth of *Calanus finmarchicus* populations (e.g. Durbin et al. 1995; Durbin et al. 2003; Head et al. 2000). These studies have reported food-limited rates when the spring phytoplankton bloom is in decline and the water column is heavily stratified. However, they do not explicitly address the potential impact of a copepod diet composed largely of nutrient-stressed phytoplankton.

This study was conducted in the Strait of Georgia (SoG, hereafter). The SoG is a highly productive semi–enclosed, estuarine system that represents an important feeding ground for several species of juvenile salmon and other economically important fish species (Harrison et al. 1983). The diet of larval and (some) juvenile fishes in the SoG is dominated by copepod nauplii and copepodites (Lebrasseur et al. 1969).
Mesozooplankton biomass in the SoG is typically dominated by production of the large, annual, subarctic copepod, *N. plumchrus* (see Harrison et al. 1983 for review). *Calanus marshallae*, *C. pacificus*, *Metridia pacifica*, *Eucalanus bungii*, *Centropages abdominalis*,

and *Acartia longiremis* also represent major components of the mesozooplankton biomass and production.

The objective of this study was to investigate the utility of the chitobiase-based method of directly estimating (and attempting to address factors influencing) interannual variability of biomass production rates (as per Oosterhuis et al. 2000) in the Strait of Georgia. The chitobiase-based method (as per Chapter 2) was also used to compare rate estimates for copepodite stages of the biomass dominant copepod species and to test the relative utility of global relationships as predictive models for estimating an important aspect of secondary production in the Strait of Georgia. The potential importance of food quality in regulating interannual variability in secondary production is also considered. This study was part of a larger project, the Strait of Georgia Ecosystem Modeling Project (STRATOGEM), which is examining mechanisms of physical-biological coupling in the SoG.

3.2 Methods

3.2.1 Zooplankton sampling, identification and enumeration

All sampling was conducted at STRATOGEM Station S4-1 (49°15'N, 123°45'W; see Figure 2.1) situated in the 400 m deep central basin of the SoG. Mesozooplankton were collected using a SCOR type net (57 cm diameter mouth and 236 μm mesh) towed vertically from 100 m and 50 m to the surface at 1 m s^{-1}. Volume filtered by the net was estimated with a TSK flow meter suspended in the mouth of the net. Upon retrieval, the contents of each net cast were preserved in 10% buffered formalin. Samples were split up to 5X (i.e. 1/32) using a Folsom splitter. Copepodites were identified to stage and prosome lengths were measured at 25X magnification using a dissecting microscope equipped with an ocular micrometer. Individual dry weights were estimated using species-specific length : weight relationships obtained from the marine zooplankton database maintained by the Institute of Ocean Sciences, Sidney, B.C., Canada.

3.2.2 Physical, chemical, and biological data

Water column characteristics (temperature and salinity) were measured using a Sea-Bird SBE 19Plus CTD equipped with a WetLabs, WetStar Fluorometer. Seawater was collected from standardized depths: 5, 10, 30, and 50 m. Chlorophyll *a* concentration was estimated in 0.2, 2, and 20 μm size fractions following the protocol of Strickland and Parsons (1972). Seawater samples from 5 m were preserved and stained with Lugols saturated iodine solution. All the identifiable particles were counted under a magnification of 200 to 320X using a Zeiss Axiovert 10 inverted microscope. Nitrate and silicate concentrations (μM) were estimated from 5, 10, 30, and 50 m using a Bran and Luebbe AutoAnalyzer 3.

3.2.3 Calculation of biomass production rates, stage durations and somatic growth rates

Chitobiase decay rates were estimated from 54 μm screened seawater samples (~1.0 L) collected from 5, 10, 30, and 50 m. Sub-samples (~10 mL) were immediately 0.2 μm filtered to estimate the *in situ* chitobiase activity (CBA_{nat}). Following processing of seawater sub-samples for CBA_{nat} assays, seawater samples from each depth were amended with a 0.2 μm filtered copepod homogenate. Seawater samples were then serially sub-sampled every 1.5 to 3 hours for the next 12 hours. At each interval, sub-samples were passed through a 0.2 μm filter and maintained at 4°C until assayed. See Sastri and Dower (2006) for a detailed description of chitobiase assay conditions. All samples were maintained at the ambient sea surface temperature (range = 6.5 – 16.5°C depending on sampling date) during the 12 hour incubation.

The slope (k) of the natural logarithm of CBA in amended samples versus time (for each depth) was applied to CBA_{nat} measurements to estimate depth-specific chitobiase decay rates (i.e. Δ CBA $hr^{-1} = -k * CBA_{nat}$). During the course of the study, differences between incubation temperature (sea surface temperature) and the temperature at each sampled depth (i.e. 5, 10, 30, and 50 m) varied by up to 1.75°C during spring, and by as much as 4.8°C in summer and autumn. A Q_{10} correction factor of 3.22 (Sastri unpublished) was used to correct the measured slopes (-k) and thus estimate ΔCBA at the *in situ* depth-specific temperature. It should be noted that the Q_{10} value of 3.22 is equivalent to the Q_{10} value presented by Hollibaugh and Azam (1983) for the temperature dependence of microbial catalyzed protein degradation. I have also employed a Q_{10} of 3.0 (Sastri unpublished) based on estimated stage durations in the Strait of Georgia. In addition I used a Q_{10} of 2.7 (Hirst and Bunker 2003) to correct individual weight-specific growth rate estimates to a single temperature. I employed the Hirst and Bunker (2003) Q_{10} for adult egg production rates rather than their estimated values for juveniles because they incorrectly estimated a Q_{10} from *in situ* estimates of juvenile growth rate. Temperature dependence must be estimated when temperature is understood to be the only factor influencing growth (i.e. growth rate is saturated by food concentration). Ultimately, all of the Q_{10} values used in this study (and Chapter 4) center on a value of 3.0 which corresponds to the "food-saturated" estimates presented by both Kiorboe and

Sabatini (1995) and Huntley and Lopez (1992) and are likely equivalent. However, in the absence of a definitive study, I have chosen to employ the rate-specific Q_{10}'s for chitobiase decay, chitobiase-based estimates of stage duration, and individual weight-specific growth rate.

Depth-weighted averages of CBA_{nat} and ΔCBA were used to estimate biomass production rate (as per Oosterhuis et al. 2000), mean stage durations of the developing community (D_{CBA}) and size class specific stage durations (D_{CBAi}). Daily biomass production rate (BPR) was calculated by estimating the change in biomass (Δ mg C L^{-1} hour^{-1}) equivalent to the depth-weighted average estimate of ΔCBA by using equation 2.6 (Chapter 2) and applying a carbon conversion factor of 0.45 (Paffenhoeffer and Harris 1976) to dry weight. Thus, the daily biomass production rate can be estimated as:

$$BPR = (\Delta \text{ mg C } L^{-1} \text{ hour}^{-1}) * 24 \qquad \text{Eq. 3.1}$$

$$D_{CBA} = CBA_{nat} / \Delta CBA, \qquad \text{Eq. 3.2}$$

$$D_{CBAi} = ((CBA_i - CBA_{avg}) + CBA_{nat}))/(\Delta CBA), \qquad \text{Eq. 3.3}$$

where, D_{CBAi} represents the mean stage duration of the size class of interest, CBA_i represents the CBA predicted for that size class from the CBA-dry weight relationship or the CBA-prosome length relationship presented in Sastri & Dower (2006), and CBA_{avg} is equivalent to the CBA of the average-sized individual (or fraction of total biomass) molting per hour. Although it is not a rate per se (i.e. $\Delta CBA\ h^{-1}$), this value is equivalent to that of ΔCBA. It should be noted that where I present species-specific stage durations, these estimates are based on the estimated dry weight at stage and CBA dynamics in the water column. Therefore, these rates are not strictly species- and/or stage-specific but, rather, body size dependent rates that are most influenced by the species/stages dominating the size class of interest.

Growth was assumed to be exponential and daily weight-specific growth rate (g day $^{-1}$) was estimated as:

$$g = \ln(W_{i+1}/W_i)/D_{CBAi}, \qquad \text{Eq. 3.4}$$

where, W_{i+1} and W_i are the numerically-weighted mean individual weights of two successive stages. Dry weight was converted to carbon using a conversion factor of 0.45 (Paffenhoeffer and Harris 1976). Thus, growth rate estimates are presented only when two successive stages were present in 0-50 m vertical net hauls corresponding to CBA decay rate estimates in the upper 50 m. During the summer and winter, the *E. bungii* population consisted entirely of copepodite stages (CV-CVI) which were always present at depths between 200-400 m (Dower, unpublished data). Likewise, *C. marshallae* were only present at depth as CVs during the summer and winter (see also Gardner 1977). Thus, rates for late stage (CIV-CVI) copepodites of *E. bungii*, and (CV-CVI) *C. pacificus* and *C. marshallae* were not estimated when they were the only representatives of their species in the early spring. It is assumed that these animals had ascended to surface waters following diapause and/or moulting at depth and did therefore not contribute to CBA decay dynamics in surface waters on the date of sampling. The average water column (0-50 m) temperature during both spring sampling periods was 8.5°C. A Q_{10} of 2.7 (Hirst and Bunker 2003) was used to correct *in situ* growth rate estimates to 8.5°C for various comparisons.

All chitobiase-based weight specific growth rates (n=158) were compared to growth rates predicted by the temperature-dependent model of Huntley and Lopez

(1992), the Hirst and Lampitt (1998) model for juvenile broadcasters and sac spawners, and the Hirst and Bunker (2003) model for juvenile broadcasters. The predicted versus estimated growth rates were compared using linear regression.

All estimates of stage duration and growth rate presented in this study are dependent on estimates made using the relationship described by Equations 3.2 (Equation 2.2; Chapter 2). Although D_{CBA} estimates compared favourably with the independent estimates of stage duration presented in Chapter 2 (Table 2.1), I consider the potential for violation of the assumptions iv and v with 8 estimates of stage duration from the spring of 2004 (n=4) and 2005 (n=4) using biomass estimates spanning the entire size spectrum of the copepod community (see Section 2.2.4 for sampling protocol).

3.3 Results

3.3.1 Water column properties

The average temperature (0-50 m) at station S4-1 varied seasonally. The highest average temperature observed during the study was 12.8°C on August 11, 2004 (Figure 3.1 a). The average temperature over the entire study period was 9.3°C (s.e. = 0.3). The average temperature during both spring bloom periods was 8.5°C (s.e. = 0.12; Figure 3.1 b). The degree of water column stratification ($\Delta\ \sigma_t$) was estimated as the difference between the average σ_t for the upper 10 m and the σ_t at 50 m. The period of greatest stratification occurred during summer (June-September 2004). The high degree of stratification during this period (Figure 3.1 b) was accompanied by high surface water temperatures and an almost complete exhaustion of nitrate in the upper 10 m (Figure 3.1 c). Silicate levels (Figure 3.1 d) were also depleted during the summer (relative to winter levels). These values however, do not represent the minimum concentrations observed during the study period. The minimum silicate concentrations (measured in the upper 10 m) during the study period were 6.0 and 6.56 μmol $\cdot$ L^{-1}, on March 22, 2005, and April 9, 2004, respectively. Both minima coincided with the peak diatom biomass (Figure 3.2 c; relative to ciliates, dinoflagellates, and flagellates).

The degree of stratification was relatively low during March 2004. During April 2004, stratification increased but was also characterized by intermittent mixing. In contrast, a relatively constant degree of water column stratification appears to have been established by early February 2005. Stratification continued to increase until at least March 22, 2005, after which mixing of the nutrient-depleted surface mixed layer with deeper nutrient rich waters occurred.

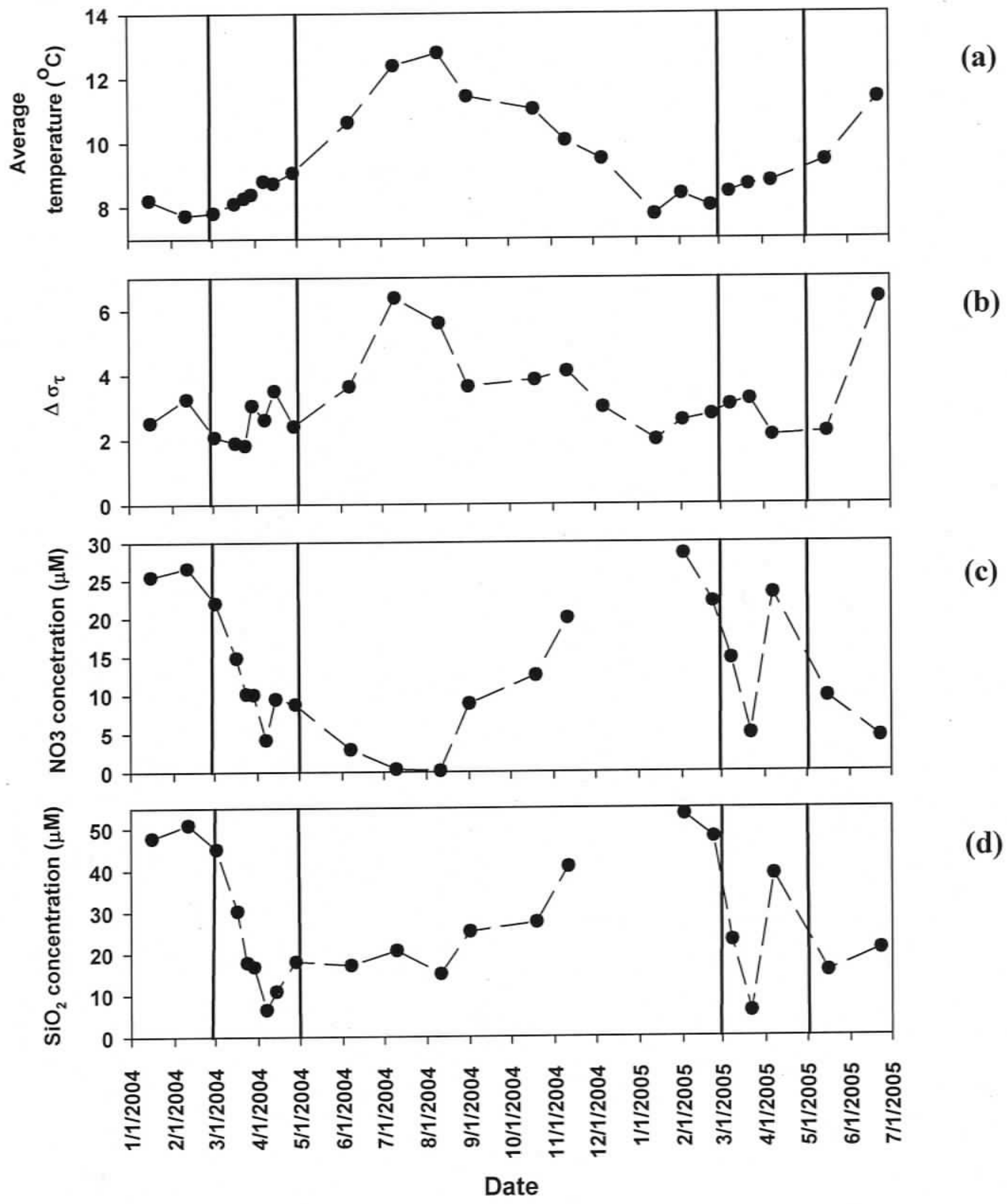

Figure 3.1. Time series (January 16, 2004 to June 23, 2005) of physico-chemical water column properties at STRATOGEM station S4-1: (a) depth-averaged (0-50 m) temperature (°C); (b) stratification parameter ($\Delta\sigma_\tau$) estimated as the difference between the mean σ_τ for the upper 10 m and the maximum σ_τ for the upper 50 m; (c) depth-integrated average NO_3 concentration (μM) for the upper 10 m and; (d) depth integrated average SiO_2 concentration (μM) for the upper 10 m. Solid vertical lines denote spring periods (March 1 to May 1, 2004 and 2005).

The average depth of the chlorophyll *a* maximum (estimated from fluorescence) during the study was 7.3 m (Figure 3.2 a; s.e. = 1.6). The depth of the chlorophyll *a* maximum was always between 10 m and the surface during both spring bloom periods. The peak chlorophyll *a* concentration (0-10 m depth-averaged) in the >2.0 μm size fraction during each spring phytoplankton bloom was 28.37 and 14.36 μg L^{-1} on March 29, 2004 and March 8, 2005 (Figure 3.2 b), respectively.

3.3.2 Copepod community biomass

The time series of copepod community biomass is presented in Figure 3.2 d. In addition to the biomass dominant species (see Figure 3.3 a and b) these estimates include the biomass of numerically abundant species; *Acartia longiremis*, *Centropages abdominalis*, *Corycaeus anglicus*, *Euchaeta elongata*, *Microcalanus* spp., *Microsetella* spp., *Oncaea* spp., *Oithona* spp., *Paracalanus parvus*, *Pseudocalanus* spp., and *Scolecithricella minor*. Over the course of the entire study period, the maximum biomass in the upper 100m was 21.59 mg dry weight m^{-3} sampled on March 29, 2004. The minimum total biomass (<2 mg dry weight m^{-3}) was measured during January of both 2004 and 2005. Secondary peaks in biomass were measured in the early summer (June through July) of both years, and in 2005 the biomass on June 23 (16.34 mg dry weight m^{-3}) was comparable to the peak biomass observed during the spring (15.69 mg dry weight m^{-3}; March 8, 2005).

The spring peak in copepod biomass of *N. plumchrus*, *C. pacificus*, *C. marshallae*, *E. bungii*, and *M. pacifica* in the upper 100 m was 20.0 and 12.5 mg dry weight m^{-3}, on March 29, 2004, and March 8, 2005, respectively (Figure 3.2 d). Copepod biomass during the spring of 2004 was dominated by *N. plumchrus* and by *C. pacificus* and *M. pacifica* (Figure 3.3). The peak biomass of *N. plumchrus* in surface waters in 2004 was 14.95 versus 2.02 mg dry weight m^{-3} during spring 2005 (Figure 3.3).

3.3.3 Copepod community biomass production, development and somatic growth rates

Biomass production rates for the copepod community increased steadily from 1.88 to 10.27 mg C m^{-3} day^{-1} during the early spring to summer period in 2004 (Figure 3.4a). Maximum BPR during the spring phytoplankton bloom was 9.11 mg C m^{-3} day^{-1} on April 25, 2004. Estimates of BPR for the same period in 2005 ranged from 2.75 and 10.82 mg C m^{-3} day^{-1} (Figure 3.4b). Maximum BPR during the spring was 10.82 mg C m^{-3} day^{-1} measured on April 8, 2005. On February 23, 2005, BPR was 4.79 mg C m^{-3} day^{-1} and declined to 2.75 and 3.86 mg C m^{-3} day^{-1} on March 8, and March 22, 2005 respectively.

Size-class specific estimates of stage duration ($DCBA_i$) were determined for all stages for each of the five main species present in 0-50 m vertical net hauls on the day of chitobiase decay rate estimates. The average, maximum, and minimum estimates of species-specific stage durations are presented in Table 3.1 (all rates corrected to 8.5°C). The surface development of *N. plumchrus* was limited to the spring in both 2004 and 2005. On average, *N. plumchrus* stage duration (CI-IV) was longer in 2005 than 2004. The longest stage durations were 9, 12.7, 22.5, and 46.8 days estimated for stages CI, CII, CIII, and CIV, respectively on March 22, 2005. The shortest stage durations for *N.*

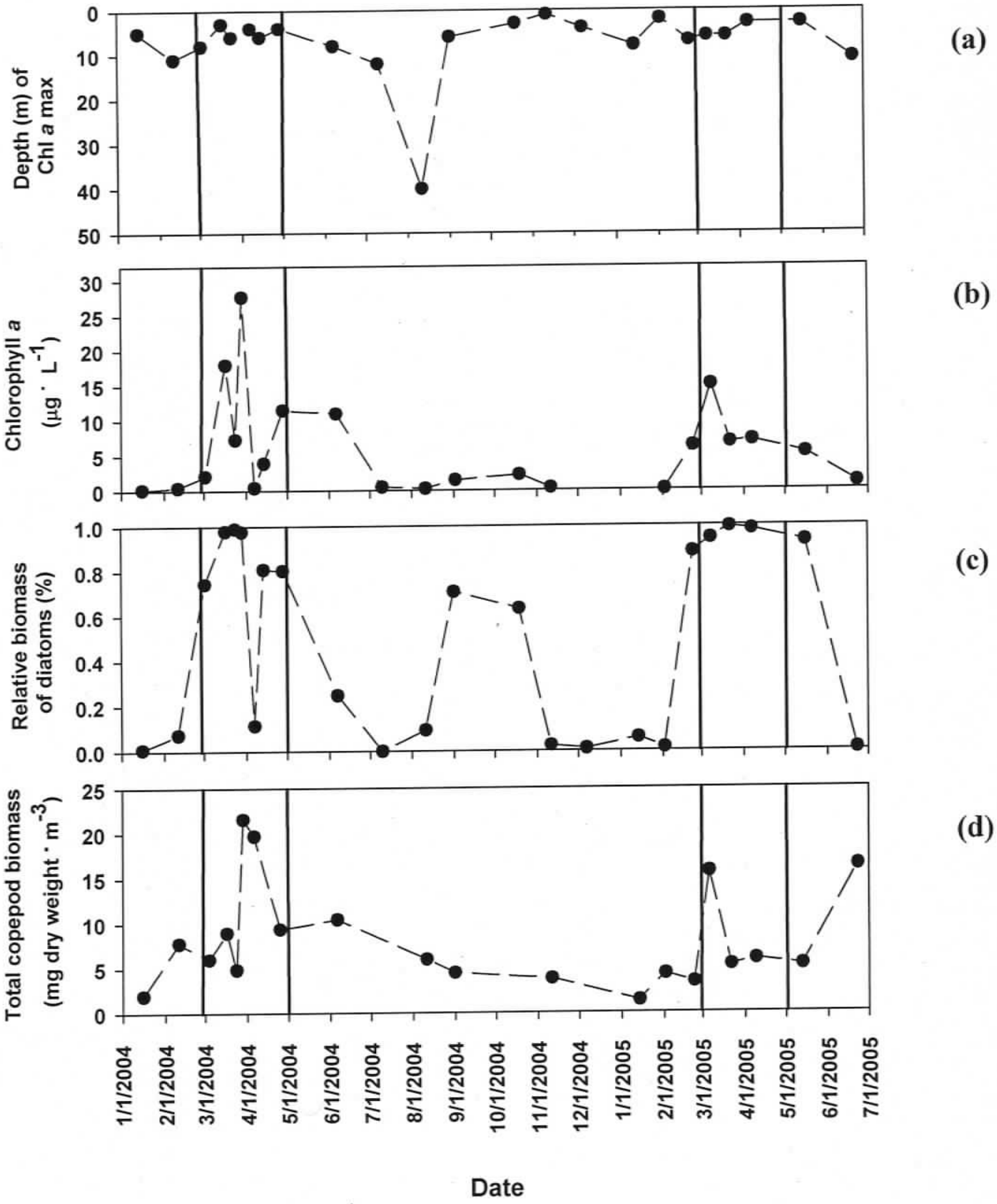

Figure 3.2. Time series (January 16, 2004 to June 23, 2005) of biological properties at STRATOGEM station S4-1: (a) average depth (m) of the chlorophyll *a* maximum; (b) depth-integrated (upper 10m) average chlorophyll *a* concentration (µg L^{-1}) in the >2.0 µm size fraction; (c) relative biomass (%) of diatoms to total biomass of phytoplankton (diatoms, ciliates, dinoflagellates, and flagellates) at 5 m and; (d) total biomass (mg dry weight m^{-3}) of all copepod species present in samples from 0-100 m with a 236 µm mesh SCOR net. Solid vertical lines denote spring periods (March 1 to May 1, 2004 and 2005).

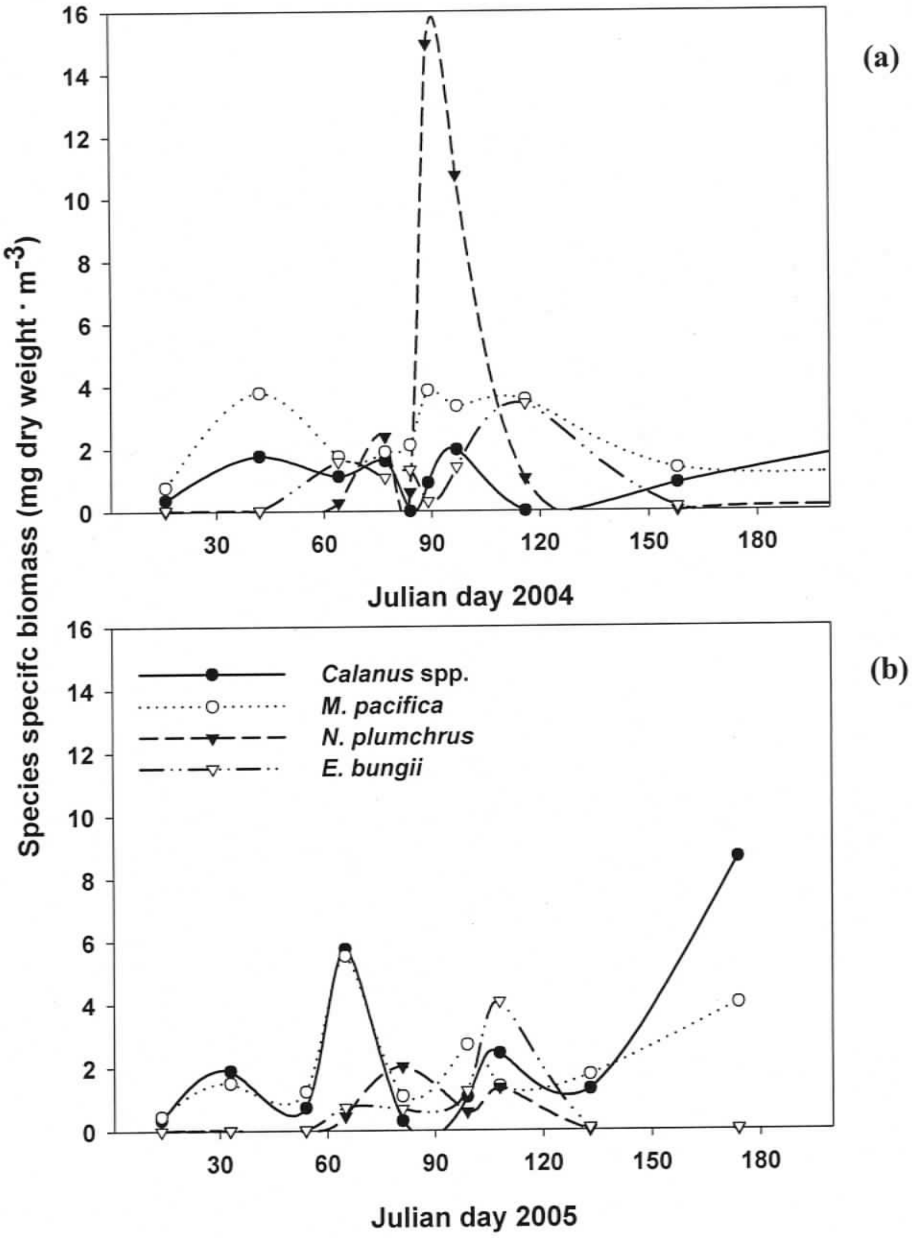

Figure 3.3. Time series of species-specific biomass (mg dry weight m^{-3}). Samples were collected from 100-0 m from STRATOGEM station S4-1. Biomass estimates are presented from (a) January through June 2004 and (b) January through June 2005 (Julian days 0-180). *Calanus pacificus* and *C. marshallae* (combined; solid circles), *Metridia pacifica* (open circles); *Neocalanus plumchrus* (solid triangles); *Eucalanus bungii* (open triangles).

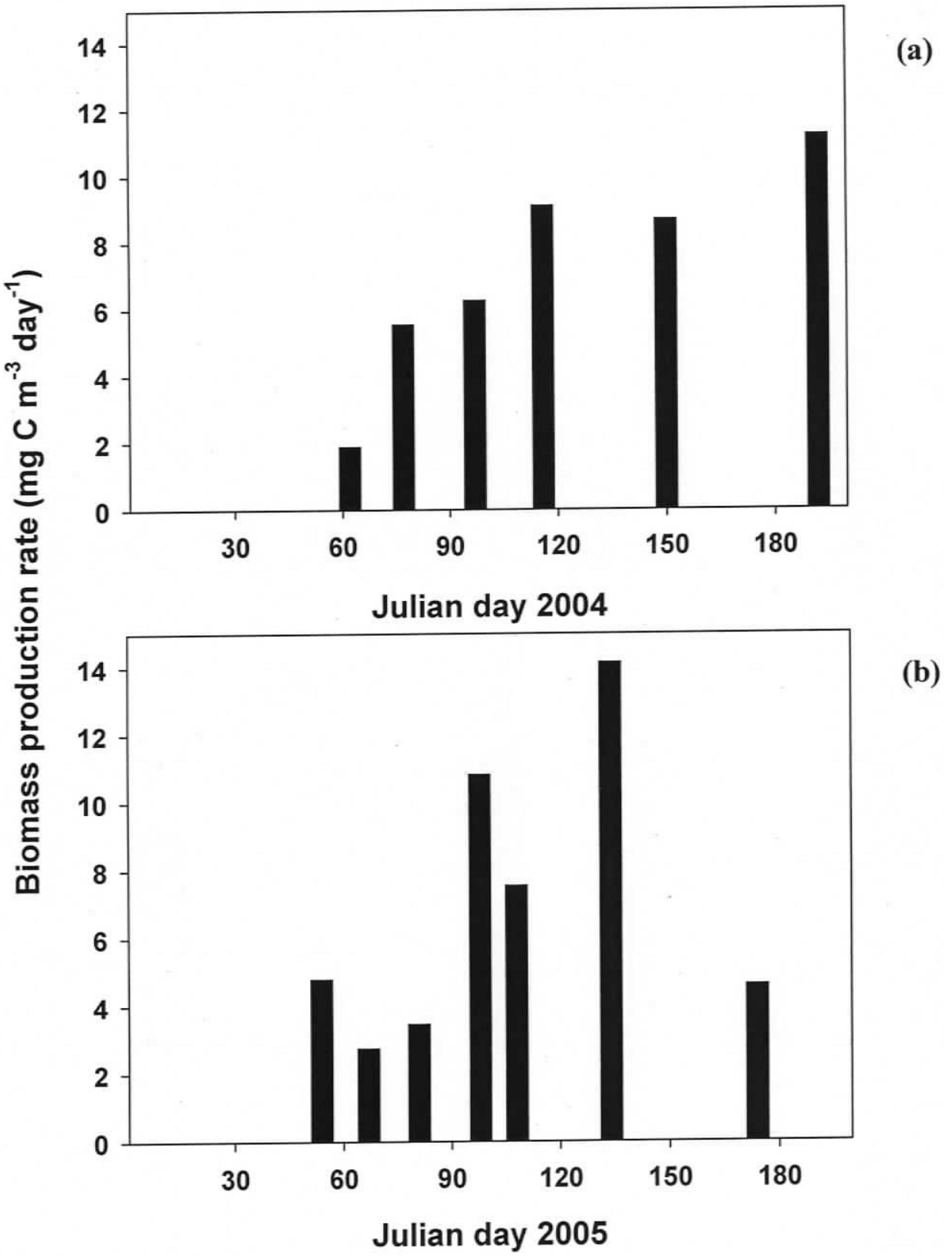

Figure 3.4. Time series of biomass production rate (mg C m^{-3} day^{-1}) estimates at STRATOGEM station S4-1. Biomass production rates are the depth-weighted averages of estimates made from 5, 10, 30, and 50m and presented for (a) February through June 2004 and (b) February through June 2005 (Julian days 30 – 180).

Table 3.1. Mean stage durations (2004 and 2005) and range of stage durations estimated for *Metridia pacifica*, *Calanus pacificus*, *Calanus marshallae*, *Neocalanus plumchrus*, and *Eucalanus bungii*. The number of estimates (n) corresponds to the number of sampling dates on which each species-specific stage was present in the upper 50 m of the water column. All estimates have been corrected to 8.5 °C using a Q_{10} of 3.0.

	2004			**2005**		
	Mean D (days)	**Range of D (days)**	**n**	**Mean D (days)**	**Range of D (days)**	**n**
Metridia pacifica						
CI	4.3	3.4-6.0	7	5.2	3.4-8.7	6
CII	4.5	3.5-6.4	8	5.6	3.4-9.0	8
CIII	4.8	3.7-7.2	7	5.6	3.5-9.0	7
CIVf	7.5	5.0-10.0	2	10.7	10.6-10.8	2
CIVm	5.2	4.0-8.4	7	6.5	3.6-10.1	8
CVf	6.7	4.7-12.9	6	9.1	4.3-12.4	4
Calanus pacificus						
CI	4.2	4.1-4.3	5	5.9	3.4-8.7	5
CII	4.7	4.4-5.3	5	6.8	3.6-10.1	5
CIII	5.3	4.9-5.7	4	8.3	4-12.8	5
CIV	9.7	6.2-18.2	4	10.5	4.6-15.9	8
CVf	9.0	7.5-10.0	2	13.1	5.8-20.3	7
CVm	8.3		1			
Calanus marshallae						
CI				6.5	3.6-9.5	2
CII	5.4		1	7.5	4.1-12.3	4
CIII	6.5	5.2-7.9	3	8.7	4.8-15.5	3
CIV	9.4	7.3-11.4	3	8.0	7.2-8.7	2
CVf	11.6	8.8-13.8	5	8.9		1
Neocalanus plumchrus						
CI	4.8	4.5-5.0	2	5.8	3.5-9.1	3
CII	6.2	6.0-6.4	2	8.1	4.4-13.0	4
CIII	10.3	7.7-11.9	3	14.7	8.1-23.0	4
CIV	18.4	15.8-21.0	2	26.8	12.6-47.7	3
Eucalanus bungii						
CI	4.0	3.6-4.2	3	4.7	3.4-8.4	4
CII	4.6	4.1-5.0	3	5.2	3.8-8.9	4
CIIIf	6.5	5.5-7.6	3	6.4	4.5-10.7	4
CIIIm	6.0	5.1-6.5	3	4.8	4.5-5	3
CIVf	12.6	11.1-13.4	3	12.9	7.4-26.6	4
CIVm	9.6	8.5-10.7	3	9.8	5.7-18.3	4
CVf	30.6	27.2-34.0	2			

plumchrus also occurred during 2005 (April 8, 2005); 3.3, 4.2, 7.8, and 12.2 days for CI, CII, CIII, and CIV, respectively.

Size-class specific estimates of stage duration (D_{CBAi}) and mean weight-at-stage were used to estimate weight-specific growth rates (day $^{-1}$) for each developmental stage of the five species considered. The average, maximum, and minimum growth rates are presented in Table 3.2 (corrected to 8.5°C). On average, *N. plumchrus* growth rate (CI-CIV) was faster during the spring of 2004 than in 2005. The slowest growth rates were 0.056, 0.112, 0.041, and 0.018 day $^{-1}$, for CI, CII, CIII, and CIV, respectively, on March 22, 2005. The fastest growth rates were 0.294, 0.363, 0.161, and 0.057 day $^{-1}$, for CI, CII, CIII, and CIV, on April 8, 2005. On a species-specific basis, the fastest average growth rates were *N. plumchrus> E. bungii> M. pacifica> C. pacificus> C. marshallae*.

To address why such a wide range of moulting rates was observed in this highly productive system (~0.10-0.28 day^{-1} when scaled to 1.0 mm prosome length and temperature corrected to 8.5°C) I considered the role of upper water column stratification and nutrient concentration as proxies for potential nutrient stress of phytoplankton and thus, copepod food quality. Figure 3.5 shows the relationship between depth-averaged nutrient concentration in the upper 10 m and the relative degree of stratification. Nitrate and silicate generally declined with increasing stratification. Nitrate (Figure 3.5 a) was nearly exhausted at the highest observed degree of stratification (July 10, 2004 and June 23, 2005). The average moulting rate was plotted against the degree of stratification (Figure 3.5 a, b, right y-axes). No consistent trend was observed when considering all pair-wise estimates of moulting rate and the degree of upper water column stratification. However, during the spring (i.e. when the relative contribution of diatoms to

Table 3.2. Mean weight-specific growth rates (2004 and 2005) and range of growth rates estimated for *Metridia pacifica*, *Calanus pacificus*, *Calanus marshallae*, *Neocalanus plumchrus*, and *Eucalanus bungii*. The number of estimates (n) corresponds to the number of sampling dates on which a species-specific pair of successive stages was present in the upper 50 m of the water column. All estimates have been corrected to 8.5 °C using a Q10 of 2.70 (Hirst and Bunker 2003).

	2004			**2005**		
	Mean *g* (day $^{-1}$)	**Range of *g* (day $^{-1}$)**	**n**	**Mean *g* (day $^{-1}$)**	**Range of *g*(day $^{-1}$)**	**n**
Metridia pacifica						
CI	0.160	0.088-0.232	2	0.14	0.064-0.280	3
CII	0.176	0.060-0.280	7	0.12	0.078-0.153	6
CIII	0.122	0.060-0.179	7	0.14	0.084-0.237	7
CIVf	0.076	0.076-0.077	2	0.06		1
CIVm	0.064	0.020-0.109	7	0.06	0.020-0.137	7
CVf	0.062	0.028-0.097	2	0.03	0.031-0.034	2
Calanus pacificus						
CI	0.155	0.120-0.177	3	0.122	0.095-0.149	2
CII	0.157	0.113-0.193	5	0.133	0.069-0.211	3
CIII	0.127	0.086-0.146	4	0.109	0.062-0.173	5
CIV	0.083	0.082-0.084	2	0.066	0.028-0.114	5
CVf	0.048	0.030-0.061	3	0.038	0.019-0.092	7
Calanus marshallae						
CI				0.113	0.041-0.185	2
CII				0.181	0.086-0.276	2
CIII	0.101		1	0.078	0.033-0.103	3
CIV	0.070	0.045-0.088	3	0.094	0.071-0.118	2
CVf	0.023	0.012-0.033	3	0.003		1
Neocalanus plumchrus						
CI				0.182	0.056-0.294	3
CII	0.167	0.164-0.169	2	0.209	0.112-0.363	3
CIII	0.094	0.092-0.095	2	0.086	0.041-0.161	4
CIV	0.044	0.03-0.0570	2	0.036	0.018-0.046	3
Eucalanus bungii						
CI	0.295	0.288-0.301	2	0.358	0.317-0.410	3
CII	0.228	0.193-0.272	3	0.273	0.182-0.368	4
CIIIf	0.163	0.161-0.164	2	0.153	0.115-0.186	4
CIIIm	0.136	0.098-0.171	3	0.154	0.109-0.186	3
CIVf	0.087	0.068-0.100	3	0.113	0.095-0.141	3
CIVm	0.091	0.073-0.110	2	0.105	0.100-0.111	3
CVf	0.030	0.030-0.031	2			

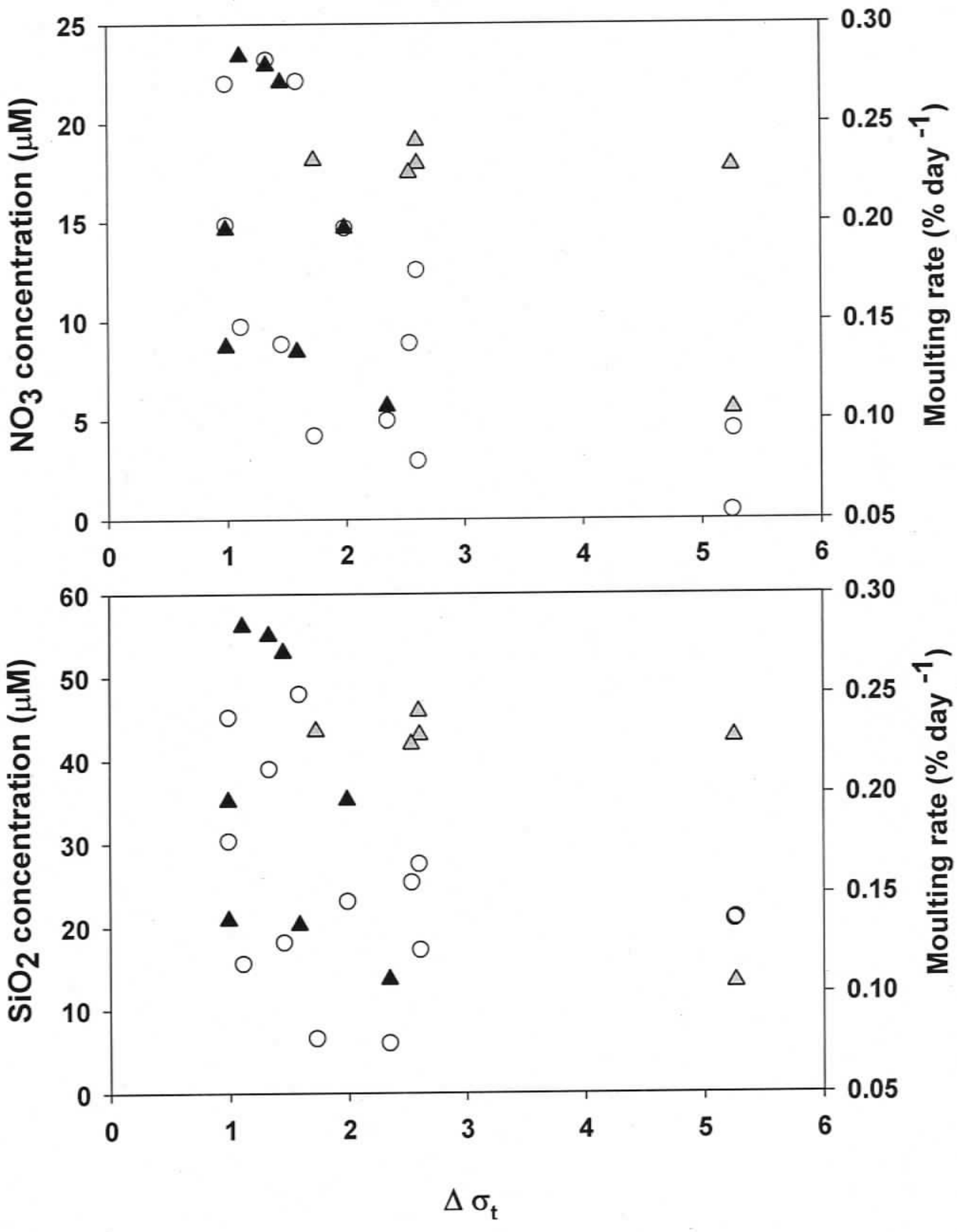

Figure 3.5. Relationship between estimated degree of upper water column (0-50 m) stratification ($\Delta\sigma_\tau$) and nutrient concentration (μM; a = NO_3, b = SiO_2; open circles) in the upper 10 m (left y-axes) and temperature-corrected (8.5°C) moulting rate (% day $^{-1}$; triangles) estimates for a 1.0 mm copepodite (right y-axes). Black triangles represent moulting rates estimated when the relative diatom biomass exceeded 75%. Grey triangles represent moulting rates estimated when relative diatom biomass was less than 75%.

phytoplankton biomass exceeded 75%) copepod moulting rates generally declined with increased stratification and nutrient depletion (i.e. a greater potential for nutrient-stressed diatoms in the diet).

Model predictions of weight-specific growth rate for all *in situ* estimates are presented in Figure 3.6. Although significant, the temperature dependent model of Huntley and Lopez (1992) explained only 3% of variation of the estimated data set (predicted $g = 0.122 + 0.03 *$ estimated g; $r^2 = 0.03$, $p = 0.03$). The comparison to the Hirst and Lampitt (1998) model was also significant (predicted $g = 0.096 + 0.39 *$ estimated g; $r^2 = 0.47$, $p<0.001$) and explained 47% of variation of the estimated data set. The slope of the regression was 0.39, however, resulting in model predictions that consistently underestimated higher growth rates (>0.25 day $^{-1}$). The comparison to the Hirst and Bunker (2003) model was also significant (predicted $g = 0.18 + 1.05 *$ estimated g; $r^2 = 0.44$, $p<0.001$) and explained 44% of the variation of the estimated data set. All predicted values using this model overestimated *in situ* rates. However, the slope of the relationship was 1.05, suggesting that the model was relatively sensitive across the entire range of estimated growth rates.

The assumption (iv; Chapter 2) that $CBA_{nat} = (\Sigma n_i * CBA_i)$ was tested by comparing the CBA_{nat} to $\Sigma(n_i * CBA_i)$ from 4 dates in 2004 (February 23, April 6, April 25, and May 29) and 4 dates in 2005 (March 8, March 22, April 9, and April 18). A positive but statistically non-significant relationship (Figure 3.7 a; $CBA_{nat} = 2.12 + 1.01*(\Sigma n_i * CBA_i)$; $r^2 = 0.29$, $p=0.17$) was found between CBA_{nat} and $(\Sigma n_i * CBA_i)$. Assuming that CBA_{nat} varies solely as a function of the biomass of planktonic Crustacea, I then estimated CBA_{nat} II as the product of the biomass of copepods relative to the total biomass of planktonic crustaceans (i.e. cladocerans, barnacle larvae, decapod larvae, amphipods, and euphausids) and CBA_{nat} (i.e. CBA_{nat} II = %copepod biomass * CBA_{nat}). Again, a positive but non-significant relationship (Figure 3.7 b; CBA_{nat} II = 1.02 + 0.80*(Σn_i * CBA_i); $r^2 = 0.45$, p=0.10) was found between CBA_{nat} II and (Σn_i * CBA_i).

In order for DCBA estimates (and therefore $DCBA_i$) to be valid, the specific fractional rate of moulting of the community in question must equal the specific fractional rate of production of CBA in the water column (i.e. we are not estimating the turnover rate of a pool of CBA that may vary independently of turnover rate of copepod biomass). Therefore, CBA_{nat} should vary positively with biomass (assumption iv) and not be influenced by the rate of production of CBA by the moulting community. To test this assumption, I regressed ΔCBA (estimated as –k * CBA_{nat} and –k * CBA_{nat} II) against the copepod community biomass (estimated from net casts) expressed in units equivalent to CBA (i.e. (Σn_i * CBA_i). As shown in Figures 3.8 a and 3.8 b the relationships between the absolute rate of decay of CBA (Δ CBA hr^{-1}) in the water column and copepod biomass were positive but, again, statistically non-significant (p=0.21 and p=0.08). Figure 3.8c suggests that the relative rate of decay (-k) may be independent of variation copepod biomass (Figure 3.8 c; -k = 0.0092 + 0.0015 * (Σn_i * CBA_i), $r^2 = 0.13$, p=0.43). These three results should be viewed with caution, however, as the sample sizes used were very small (n=8), and the power of the linear regressions was therefore quite low. Thus, in the absence of additional data, a definitive conclusion regarding assumptions iv and v cannot be made at this point.

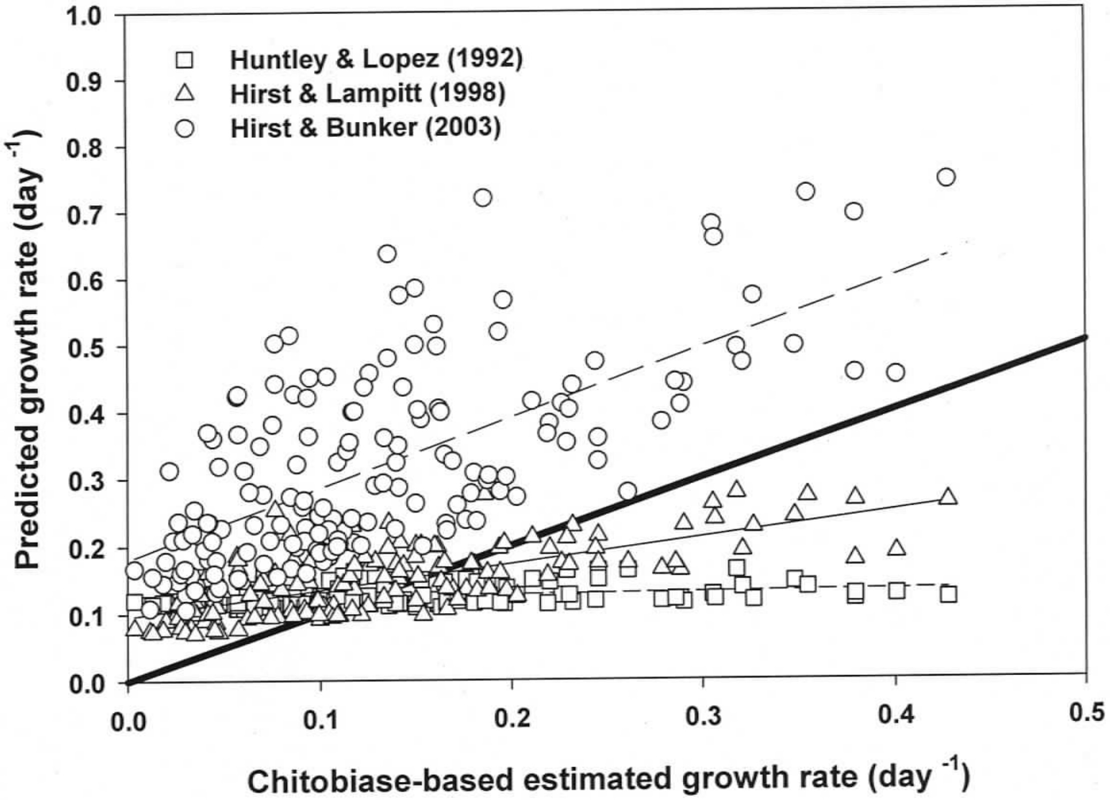

Figure 3.6. Multi-species comparison of chitobiase-based estimates of (n = 159) *in situ* of weight-specific growth rate (day $^{-1}$) versus the weight-specific growth rates predicted by global models. The thick line represents 1:1 agreement between predicted and chitobiase-based estimates of growth rate. All growth rates were estimated at STRATOGEM station S4-1 between February 2004 and June 2005. Square symbols represent predicted versus *in situ* estimates using the Huntley and Lopez (1992) model; regression represented by the line of short dashes line. Triangles represent predicted versus *in situ* estimates using the Hirst and Lampitt (1998) combined juvenile model; regression represented by the thin solid line. Circles represent predicted versus *in situ* estimates using the Hirst and Bunker (2003) juvenile broadcasters model; regression represented by the line of long dashes.

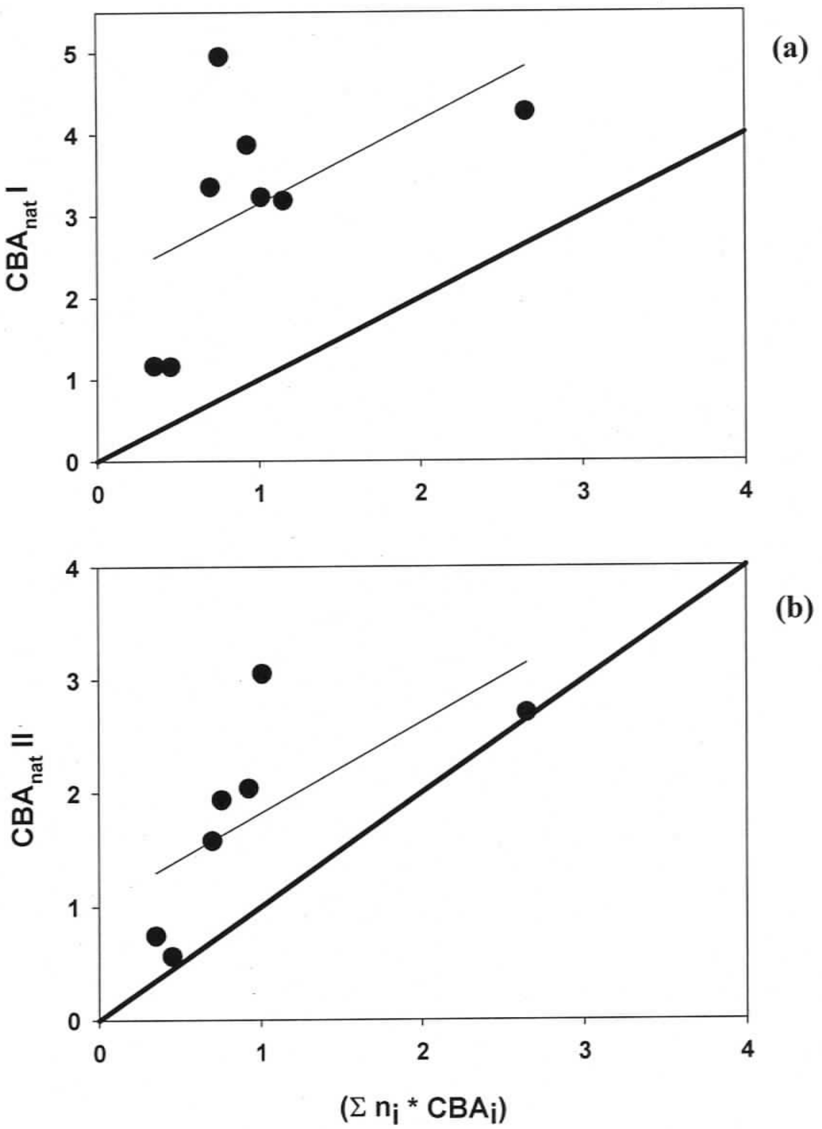

Figure 3.7. Total naupliar and copepodite biomass in the upper 50m estimated from the contents of SCOR and 70μm ring nets expressed in units of CBA ($\Sigma n_i * CBA_i$) versus (a) the depth-averaged CBA (CBA_{nat} I), and versus (b) the depth-averaged CBA corresponding to the relative copepod biomass (CBA_{nat} II) in the upper 50m. Estimates were made at STRATOGEM station S4-1 during the spring phytoplankton blooms of 2004 and 2005. The thick line represents 1:1 agreement between depth-averaged CBA and biomass ($\Sigma n_i * CBA_i$).

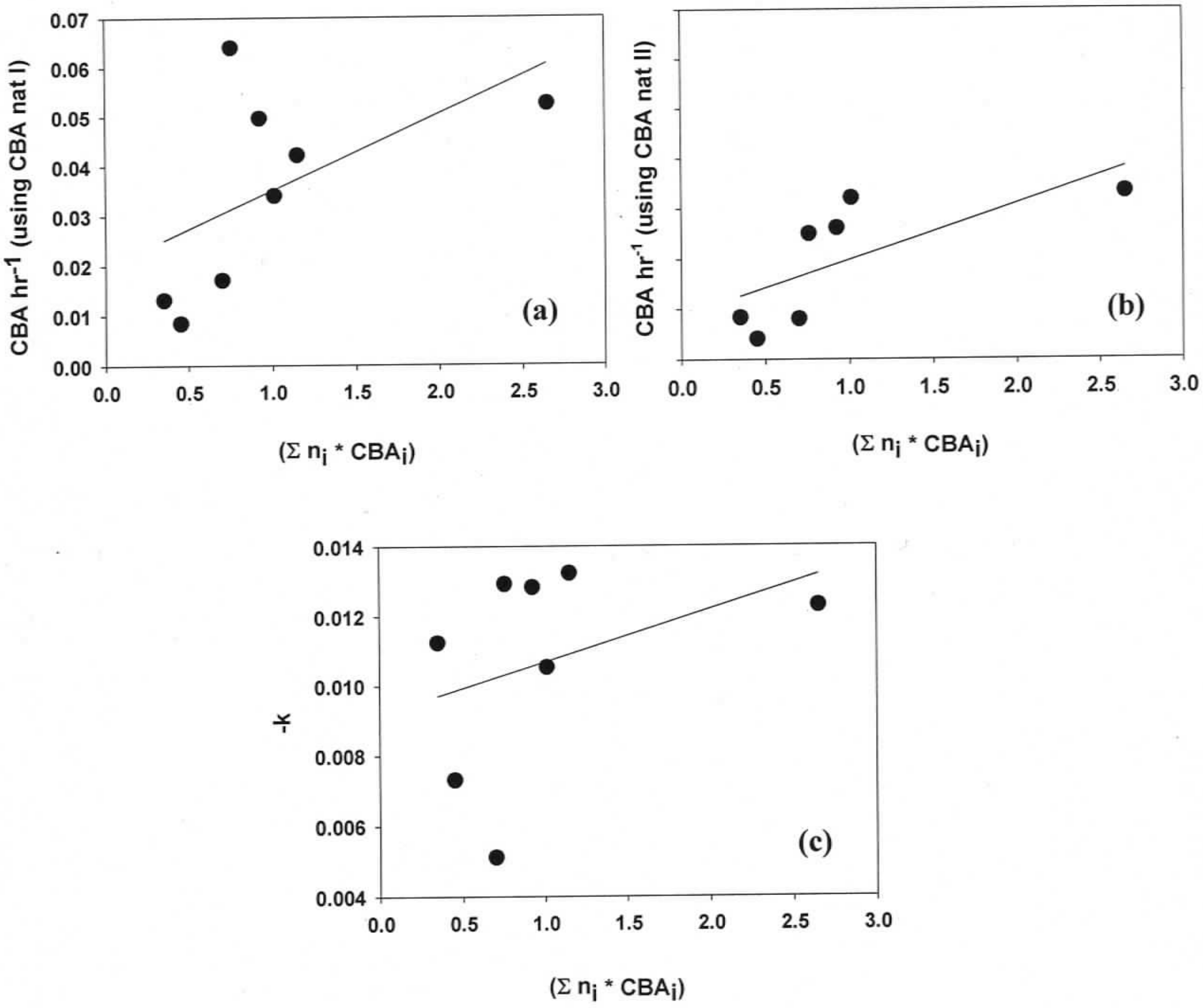

Figure 3.8 Total naupliar and copepodite biomass in the upper 50m estimated from the contents of SCOR and 70μm ring nets expressed in units of CBA (Σ n_i * CBA_i) versus (a) the depth-averaged ΔCBA (calculated as $-k$ * CBA_{nat} I), versus (b) the depth-averaged ΔCBA (calculated as $-k$ * CBA_{nat} II), and versus (c) the depth-averaged decay rate constant (-k) in the upper 50m. Estimates were made at STRATOGEM station S4-1 during the spring phytoplankton blooms of 2004 and 2005.

3.4 Discussion

Local studies of primary production (e.g. Parsons et al. 1969; Stockner et al.1979) characterize the SoG as a highly productive coastal ecosystem. In fact, the Huntley and Boyd (1984) global model of the potential for food-limited growth of marine copepods cites the SoG as an example of a productive coastal ecosystem within which copepods should consistently experience food saturation. Indeed, the SoG has historically supported large resident populations of herring and served as an important feeding ground for several species of Fraser River system juvenile salmon during their first marine year (Lebrasseur et al. 1969).

It has been hypothesized that the interannual variability of salmon returns from the NE Pacific is correlated to first year survival (Francis and Hare 1994). The Optimal Stability Window hypothesis (Gargett 1997; Gargett et al. 2001) suggests that the degree of water column stability may be correlated to juvenile salmon survival. The hypothesis is premised on significant stratification being a prerequisite for initiation of phytoplankton blooms and that prolonged stratification ultimately results in a less productive phytoplankton community. Reduced primary production may negatively affect the availability of food for herbivorous zooplankton. However, more recent laboratory studies have suggested another potential mechanism involving prolonged stratification, wherein nutrient-depleted phytoplankton (especially diatoms) have been demonstrated to significantly retard both moulting and growth rates when consumed by juvenile copepods (e.g. Breteler et al. 2005, Jones and Flynn 2005; Koski et al. 2006).

Although Gargett et al. (2001) concluded that decadal scale variability of the Aleutian Low may not be responsible for observed variability in SoG salmon survival, their model simulations do suggest that small changes in biological rate parameters (e.g. phytoplankton and zooplankton growth rates) may be responsible for the interannual differences in zooplankton biomass observed by Stockner et al. (1979), which are comparable in magnitude to those observed in our study. Stockner et al. (1979) estimated primary production and zooplankton biomass at several stations throughout the SoG during 1975 and 1976. They found that, despite higher primary production in the spring of 1976 versus 1975, zooplankton biomass in 1976 was significantly lower than in 1975. Sampling was conducted on a monthly basis, however, and was not as highly resolved (temporally) as that of the STRATOGEM study during the spring phytoplankton blooms.

3.4.1 Biomass production rates

In 2004, biomass production rates increased steadily from <2 to ~ 10 mg C m^{-3} day^{-1} between February 29 and April 25. During this period the biomass of *Neocalanus plumchrus* in the upper 100 m increased from <1 mg dry weight m^{-3} to dominate the community biomass with ~15 mg dry weight m^{-3} (March 29). By April 25, 2004, most of the *N. plumchrus* population had descended from surface waters to overwintering depths and the biomass of the copepod community was dominated by developing populations of *Calanus pacificus*, *Metridia pacifica*, *Eucalanus bungii*. By early May, the biomass of *Acartia longiremis* and *Centropages abdominalis* also began to increase.

In 2005, BPR was 4.8 mg C m^{-3} day^{-1} (February 23) during the development of the spring phytoplankton, but decreased to 2.75 mg C m^{-3} day^{-1} (March 8) when both the observed phytoplankton and copepod community biomass were greatest. BPR remained relatively low through to March 22 (3.46 mg C m^{-3} day^{-1}). An elevated BPR (10.82 mg C

m^{-3} day^{-1}) was measured on April 8, 2005, by which time the biomass of the copepod community was largely dominated by *E. bungii*, *C. pacificus*, and *M. pacifica*. Clearly, the trends in biomass production rates for 2004 and 2005 were very different. Variation in concomitant estimates of growth rate and stage duration for the biomass dominant copepods were generally similar to the BPR trends. Here, I consider some potential mechanisms responsible for the observed temporal patterns of production rate, with respect to variation of development and growth rates.

3.4.2 Development and growth rates

Liu and Hopcroft (*in press*) present an assembly of the average growth rates of *Neocalanus* spp., *M. pacifica*, *C. pacificus*, and *C. marshallae* corrected to 5°C. With respect to interspecific differences of average growth rate they find that, in the northern Gulf of Alaska, the fastest rate was observed for *C. marshallae*, followed by *Neocalanus* spp., *M. pacifica*, and *C. pacificus* (in that order). A comparison of these same species in the SoG suggests that the fastest average growth rate is achieved by *N. plumchrus*, followed by *C. pacificus, M. pacifica*, and *C. marshallae*. A comparison of the *maximum* observed rates for same species in the SoG suggests that the fastest average copepodite growth rate is also achieved by *N. plumchrus*, followed by *M. pacifica*, *C. pacificus*, and *C. marshallae*. While these results depart from the interspecific patterns observed in the northern Gulf of Alaska, they are consistent with the relative distribution of biomass in the SoG copepod community during a "typical" spring period (e.g. as described by Harrison et al. 1983).

The results in Tables 3.1 and 3.2 are corrected to 8.5°C, and fall within the ranges of species-specific estimates of both stage duration and growth rate reported in other studies (Miller et al. 1993, Peterson et al. 2002; Liu and Hopcroft 2006a, b, *in press*). As noted earlier, stage durations estimated using the chitobiase method represent mean stage durations for a size class, and are not truly species-specific. The specificity that is attributed to stage duration estimates is based on the numerically-weighted average size-at-stage of animals present in the water column on the day that chitobiase dynamics were measured.

The species that dominate the mesozooplankton community in the SoG display several different life history strategies. Up to three annual cohorts of *M. pacifica* were noted during the study period. In contrast to the population studied by Padmavati et al. (2004) in the Oyashio region the SoG population does not appear to undergo a period of winter dormancy. Multiple cohorts of *C. pacificus* were noted in the SoG. However, deepwater CVs were also present during the winter, suggesting that at least part of the population may undergo a dormancy period. Reproduction and development of *C. marshallae* is restricted to surface waters during the spring/early summer and the population over-winters at the CV stage, moulting to adulthood in late winter/early spring. *N. plumchrus* CVs descend to diapause depths of 200-400 m from mid-April to mid-May (Fulton 1973; Gardner 1977). The CVs remain at depth and moult to adulthood by early January. Based on the presence of gravid female *N. plumchrus* in deep-water samples, it appears that females spawn from late January to early April, in agreement with other recent observations of this population (Campbell 2003).

Using a fitted Behlradek's function for *Calanus finmarchicus* (Campbell et al. 2001b), it is estimated that, at 9.3°C (the mean temperature below 100 m in the SoG), development from egg to CI for *N. plumchrus* should take ~13 days. Therefore, recruitment to the surface dwelling CI stage may begin as early as mid-February and continue until early April. In both 2004 and 2005, the first CIs were observed during the last week of February. The peak abundance of *N. plumchrus* occurred in the third week of March for both 2004 and 2005.

3.4.3 Copepod community productivity and water column properties

Mean community moulting rates (scaled to 1.0 mm prosome length) increased steadily from 0.14 to 0.27 day $^{-1}$ between March 3 - April 25, 2004. This is attributed to increases in surface temperatures and food availability. The observation of relatively rapid moulting rates is coincident with developing phytoplankton and zooplankton biomass and production rate estimates. The 2004 spring phytoplankton bloom began in early March. However, due to extremely calm conditions that resulted in early stratification (Collins 2006) the 2005 "spring" bloom actually began in mid-February and peaked by March 8. During the surface development of *N. plumchrus* in 2004 (~March 1 through ~May 1) the spring minima of dissolved nitrate and silicate were observed on April 6. In 2005, the observed nutrient minima occurred two weeks earlier, on March 22. The difference in timing of these events can be attributed to a greater degree of water column stability and an earlier spring phytoplankton bloom in 2005.

The mean community moulting rate on March 8, 2005 was ~0.20 day $^{-1}$. On this date, the chlorophyll *a* concentration was 15 μg L^{-1}, dissolved nitrate and silicate concentrations were 14.7 and 23.1 μM and the estimated degree of stratification was 2.0. On March 22, 2005, the mean community moulting rate was 0.106 day^{-1}, and the total phytoplankton biomass was dominated by diatoms (99.5% of the total phytoplankton biomass). Chlorophyll *a* (> 2μm) concentration on this date was 6.7 μg L^{-1}, stratification was stronger (2.35), and dissolved nitrate and silicate concentrations were 4.95 and 6.0 μM, respectively. Over this two week period, estimates of *N. plumchrus* CI stage duration increased from 4.8 to 9 days, while CII estimates increased from 9.8 to 12.8 and CIII from 18 to 22 days. CVs were not present on March 8; however CIV stage duration was estimated to be ~46 days on March 22. By the next sampling date (April 8, 2005) the degree of stratification had declined to 1.33, and surface nitrate and silicate concentrations had increased to 24 and 39 μM. Estimated CII, CIII, CIV stage durations were 4.2. 7.8, and 12.1 days, respectively. The mean community moulting rate was estimated as 0.28 day^{-1}.

The early stages of the five copepod species considered in this study encountered spring phytoplankton communities composed almost entirely of diatoms in both 2004 and 2005. However, it is hypothesized that, due to greater stratification and earlier development of the spring phytoplankton bloom in 2005, the early copepodite stages may have subsisted on nutrient-stressed (potentially less nutritious) diatoms for an extended period of time. The moulting rates of *N. plumchrus* CIs may be particularly sensitive to food quality as this represents the first feeding stage for the species, and follows a gross morphological change from NVI. In addition, analysis of the fatty acid composition of *N. plumchrus* CVs in surface waters also suggests that these animals may have been subsisting on a nutritionally poor diet (El Sabaawi *in prep*).

Biomass production rates for the copepod community were relatively low between March 8 and March 22, 2005, and the abundance of all five of the biomass dominant species declined. However, with the return of a potentially favorable diet (still composed of diatoms; ~98% relative biomass), the community-level BPR, and growth and moulting rates of the biomass dominant species returned to levels comparable to those observed during spring 2004. As discussed above, recruitment of CI *N. plumchrus* to the developing population ends by early April. Thus, the *N. plumchrus* population was not able to attain the numerical (and biomass) dominance that has been historically observed in the SoG. In fact, spring 2005 saw a significant crash of the developing *N. plumchrus* population. By late May (2004 and 2005), the entire *N. plumchrus* population consisted of CVs that had descended to over-wintering depth. The initial abundance of CV's at depth in 2005 was only 1.4% of the average abundance observed at depth at the beginning of over-wintering between 2002 and 2004.

These observations are consistent with findings from recent laboratory studies of relationships between juvenile copepod development, growth, phytoplankton nutritional status and taxonomic composition (Jones and Flynn 2005; Breteler et al. 2005; Koski et al. 2006). These field observations cannot rule out developmental failure due to the potentially toxic effects of high diatom concentrations (i.e. Ianora et al. 2004) or a general deficiency of required fatty acids in the diet. However, during the spring of 2004 and 2005, both maximal and suboptimal copepod growth and developmental rates were measured when primary production was dominated almost entirely by diatoms. Thus, toxicity alone (unless promoted by nutrient stress of diatoms) does not explain the observed variation. Nor do these results support the notion that a mixed diet is essential for maximal juvenile development and growth. Rather, the deleterious effects of a nutritionally poor diet may be extreme when diatoms dominate.

Characterizing growth rate variability solely as a function of food concentration is difficult, especially when the nutritional value of the diet may elicit growth rate responses that resemble food limitation in an otherwise food "rich" environment. The use of total chlorophyll *a* concentration as a food proxy in models of juvenile growth may erroneously predict food-limited rates when the diet is largely composed of heterotrophs and may predict saturated rates when populations must subsist on a nutritionally deficient diet. The time scales over which growth and/or development rates respond to consistently low food concentrations and nutritional deficiencies are also likely to be different. The effect of diet variability on growth may be more appropriately quantified with the use of autotrophic and heterotrophic production rate estimates or descriptors of condition (i.e. lipid composition; Breteler et al. 2005) in concert with their respective concentrations. Nevertheless, the concentration *and* quality of the copepod diet does have a direct influence on *in situ* rates of growth and development rate.

3.4.4 Evaluation of chitobiase-based estimates of production, growth and development

Peterson et al. (2002) compared production rates from shelf and offshore sites with a copepod community similar to that of the Strait of Georgia, and estimated a combined (female and juvenile) production rate on the shelf of 2.1 mg C m^{-3} day^{-1} during the summer upwelling period. Their estimates of adult and somatic growth rate were not considered food-limited. The production rate estimates presented in the present study only consider juveniles (i.e. adult egg production was not measured). Typically, my estimates of community-level production rates in the SoG are greater (up to 5X) than those measured by Peterson et al. (2002). Assuming that assumptions i-iii (Chapter 2) are met, the discrepancy may be accounted for, in part, by the production rates of copepod nauplii (which Peterson et al. did not measure). Furthermore, although I have assumed that copepods dominate the production of planktonic Crustacea in the SoG (assumption I; Chapter 2), at times euphausids and amphipods can also make significant contributions. Thus, the BPR estimates presented here for the SoG may also include the production of these groups, provided that the relationship between CBA and body size is comparable to that for copepods (Chapter 2). A greater degree of discrimination may be gained by applying the size-class specific growth rate estimates of copepods presented in this chapter to copepod biomass.

It should be noted however, that estimates of the average stage duration (D_{CBA}; using Equation 2.2) used to estimate size-class specific development and growth rates are valid only if all assumptions stated in Chapter 2 are met. Assumptions iv and v are especially critical. For the spring dates considered in 2004 and 2005, it appears that CBA_{nat} does vary with estimates of biomass in the upper 50 m. Although this relationship does vary in a positive manner the regressions were not statistically significant, possibly due to small sample sizes and low power. The relatively weak correlations may also have been due to an inability to determine the fine-scale vertical distribution of animals by using biomass estimated from depth-integrated net samples. CBA_{nat} may also vary, however, because of the moulting rate of the community (assumption v). The absolute production rate of chitobiase (Δ CBA) does vary with copepod biomass. However, because this rate is calculated as the product of the relative rate of CBA decay and

CBA_{nat} (i.e .$\Delta CBA = -k * CBA_{nat}$), it is difficult to determine whether variation in the estimated rate of CBA production is due to variation in biomass, variation in moulting rate, or both. Although positive, a non-significant relationship between the estimated rate (-k) does suggest independence of the rate constant from biomass.

Ultimately, additional data and/or laboratory and field tests of these assumptions will be needed to definitively address the potential for violation of the critical assumptions presented in Chapter 2. Moreover, the CBA_{nat} and decay rate estimates presented here were made during the spring, which corresponds to the seasonal development of the SoG copepod community during which the rates of development and biomass may vary together (i.e. because of patterns in temperature and food availability). Thus, although at present it is difficult to conclude whether assumptions iv and v have been violated, the rate estimates presented in this study do compare favourably with those from other studies and generally vary with estimates of biomass production rate. Therefore, the multi-species estimates of growth rate generated with this approach provide a useful opportunity to test the utility of contemporary predictive models for secondary production estimates in the SoG.

3.4.5 Evaluation of global models of copepod growth

Growth and development rates in the SoG varied on shorter time scales than might be inferred from existing models. As the average temperature during the spring (8.5 °C) did not vary appreciably in either year, the temperature-dependent model of Huntley and Lopez (1992) did not capture the variability (~3%) of copepod growth rates estimated in the SoG. The body-size and temperature-dependent model of Hirst and

Lampitt (1998) captured 47% of the observed variation in growth of the juvenile copepod community. However, this model tended to underestimate all growth rates greater than 0.25 day^{-1}. Thus, the Hirst and Lampitt (1998) model may lead to underestimates of production for copepod communities growing at optimal rates in productive coastal systems (e.g. the SoG). On average, the Hirst and Bunker (2003) model overestimated all growth rates by 337% (ranging from 92 to >700%). However, it did capture 44% of the variation described by this study and the slope of our comparison was ~1. Although it consistently overestimates growth rate, inclusion of a food concentration parameter appears to provide the Hirst and Bunker (2003) model with a greater *relative* applicability to systems in which animals may be growing at maximal or suboptimal rates.

3.4.6 Summary and conclusions

The trophic implications of a crash in the surface development of the 2005 *N. plumchrus* cohort may be significant. The high degree of interannual variability of mesozooplankton biomass observed in the SoG is not without precedent. As discussed above, Stockner et al. (1979) observed a similar pattern in 1975 and 1976. Typically, spring production of mesozooplankton in the SoG is dominated by *N. plumchrus*. Although not considered by Stockner et al. (1979), the low mesozooplankton biomass in 1976 may have arisen because development of the *N. plumchrus* cohort was compromised in a manner similar to that observed in this study. Recent studies describe 2005 as an anomalous year for the Pacific northwest, characterized by delayed winds resulting in a late (~50 days) spring upwelling and low primary production (Kosro et al. 2006; Kudela et al. 2006). A historically low hatching success of the planktivorous

Cassin's auklet (*Ptychoramphus aleuticus*) on Triangle Island, British Columbia, (Sydeman et al. 2006) represents a notable higher trophic level response. Furthermore, zooplankton biomass anomalies extending from northern California to southern Vancouver Island (Mackas et al. 2006) suggest that low mesozooplankton production in the SoG may have been related to larger scale atmospheric and oceanographic anomalies.

In summary, the conclusions of this study are that: 1) biomass production, growth and development rates of juvenile copepods in the field may be influenced significantly by both food quantity and food quality; 2) the time scale of a response in development rate is likely more sensitive to poor food quality than food quantity; 3) therefore, rates of biomass production of juvenile copepods may vary on time scales much shorter than has previously been recognized. Although a similar *in situ* response, attributed to the potentially toxic effects of diatoms on egg production, was recently documented by Halsband-Lenk et al. (2005), few other such field results exist. Thus, it is not yet possible to gauge how common these events are in coastal waters.

Presently, global models of juvenile growth rate do not appear to accurately capture the variation of rate estimates as measured in the field. Refining existing models for their use in production studies demands a greater number of *in situ* estimates that are *representative* of copepod community-level development and growth responses to a wide variety of environmental conditions. The continued development and application of enzymatic methods (e.g. Oosterhuis et al. 2000; Sastri and Roff 2000; Yebra and Hernandez-Leon 2004; Sastri and Dower 2006; Yebra et al. 2006) that are routinely applicable to field studies offers a potential means of addressing this issue.

CHAPTER 4.

Large scale patterns of spatial variability in biomass production rates of juvenile copepod communities in subarctic NE Pacific

4.1 Introduction

Variation in the production of marine copepods exerts a significant influence on the production of higher trophic levels and influences the rate of carbon turnover in marine ecosystems (Sommer et al. 2002; Buitenhause et al. 2006). Copepod production rate is conventionally estimated as the product of biomass and growth rate. However, characterizing how spatial and temporal variability of copepod production rates are regulated in the field has proven difficult. In part, this is due to the logistical constraints associated with conventional measures of growth rate and biomass. Consequently, most production rate estimates of juvenile copepods have been limited to single species in coastal regions (e.g. Uye 1982; Escribano and MacLaren 1999; Rey-Rassat et al. 2004). Where multi-species estimates have been presented, they generally rely on predictive models (e.g. Huntley and Lopez 1992; Hirst and Lampitt 1998; Hirst and Bunker 2003) to estimate community-level production rates (e.g. Roman et al. 2001). Although these models are improving (see Chapter 3), they usually fail to capture variation of *in situ* growth (Liu and Hopcroft 2006 a, b). Predictive global models employ environmental and state variables (i.e. temperature, biomass, and food concentration), which (if solely temperature dependent) must either assume food saturation, or else consider food concentration. Relationships between *in situ* growth rate and phytoplankton concentration may not be appropriate because: (i) this approach assumes complete herbivory; (ii) food quality cannot be directly addressed and; (iii) estimates of rates and state variables can vary on different time scales (Calbet et al. 1996).

Ultimately, only a few studies have directly estimated *in situ* growth rates that are applicable to community-level production rates of juvenile copepods (e.g. McKinnon and Duggan 2003). The continued development of alternative approaches (e.g. Oosterhuis et al. 2000; Sastri and Roff 2000; Chapter 2) may represent a means of routinely estimating *in situ* rates of production that are: i) applicable at the community-level; ii) not dependent on conventional incubations to estimate growth rate, and iii) not (necessarily) dependent on sampling techniques that may fail to adequately census the entire size spectrum and biomass of copepod communities.

Laboratory studies have demonstrated that both growth and moulting rates vary with temperature, individual body size, and food concentration (e.g. Vidal 1980a, b). Huntley and Boyd (1984) considered food limited growth of marine copepods in a variety of systems and concluded that the potential for suboptimal growth was greater in the oligotrophic open ocean than in coastal systems. In fact, observations of food-limited development and/or growth rates under field conditions are surprisingly uncommon for juveniles (Hirst and Bunker 2003). Difficulty in establishing relationships between variation of *in situ* growth rate and food concentration may be due partially to the predominance of estimates from productive coastal systems. Nevertheless, Campbell et al. (2001a) documented food limited rates of moulting and growth for *Calanus finmarchicus* on the Scotian shelf. However, the extent to which food-limited development and/or growth occurs in the field remains unclear (Crain and Miller 2001). Thus, a greater number of field estimates across a variety of systems are needed to properly characterize how temperature, body size, and food concentration (and quality) interact to regulate *in situ* growth rate. Directly addressing the frequency of occurrence of food limited growth and development rates in the field is a first step toward approaching this characterization.

Increasing attention has been devoted toward the development and use of alternative methods for estimating *in situ* growth and development rates of marine copepods that can be used to overcome the logistical constraints of conventional incubation-based methods (e.g. Oosterhuis et al. 2000; Sastri and Roff 2000; Yebra and Hernandez-Leon 2004; Yebra et al. 2006; Sastri and Dower 2006). Here I use chitobiase-based methods (Oosterhuis et al. 2000; Chapter 2) because: i) they can be applied routinely during broad-scale synoptic surveys and; ii) the methodology may be used to directly estimate biomass production and development rates that are generalized across the entire copepod community (i.e. akin to the community wide estimates of rates of primary and bacterial production estimated via ^{14}C and ^{3}H incorporation respectively).

Estimates of copepod community biomass production, development and growth rates were made at stations located in shelf, slope and oceanic regions within the subarctic NE Pacific (the West Coast of Vancouver Island and Line P) during the spring and summer of 2005. Several studies have shown that the spatial pattern of mesozooplankton community composition varies considerably between these three regions (see Mackas and Coyle 2005 for review). Furthermore, this sampling scheme facilitated comparisons between stations that are typically characterized by (i) highly seasonal primary production (i.e. shelf regions) and (ii) an open ocean HNLC region in which primary production is consistently low. The objective of this study was thus to explore the extent to which *in situ* rates of biomass production, development and growth vary among these regions, and to consider whether variation in these rates can be explained by variability in temperature, body size, and food concentration.

4.2 Methods

4.2.1 Cruise track and sampling

Eleven stations were sampled from the CCGS *John P. Tully* between May 26-29, 2005 (WCVI) and June 1-7, 2005 (Line P). During the summer of 2005, 12 stations were sampled between August 17-23 (Line P) and September 7-10 (WCVI). Line P terminates at P26 (145°W 50°W, also known as Ocean Station Papa or Station P; see Figure 4.1). During both the spring and summer sampling campaigns we also sampled a one year old Haida eddy that happened to be situated between P20 and P26.

4.2.2 Sampling protocol

At each station, water column properties were measured and seawater collected using a CTD-rosette equipped with 10 L Niskin bottles. Bottles were tripped at 5, 10, 30 and 50 m. Seawater was collected for estimates of chlorophyll *a* concentration and chitobiase activity (CBA) in seawater. The copepod community was sampled with 0-50 m vertical net tows using 233 μm black mesh Bongo nets with a mouth area of 0.25 m^2. The volume filtered by the nets was estimated from a TSK flowmeter suspended in the mouth of one of the Bongo nets.

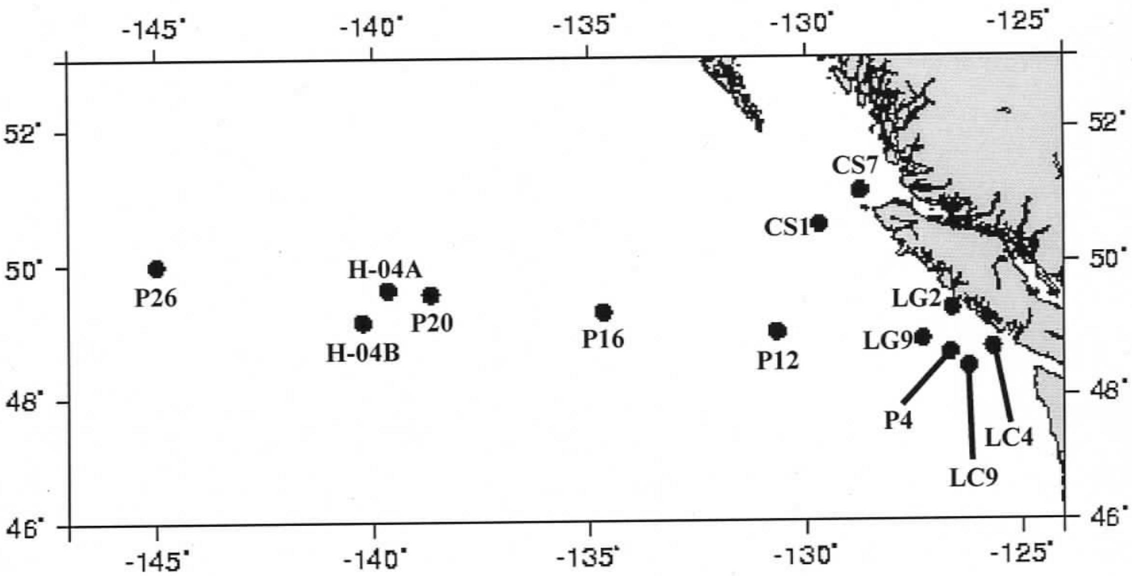

Figure 4.1. Sampling stations along the West Coast of Vancouver Island shelf (LC-4, LG-2, and CS-7), and slope (LC-9, P-4, LG-9, and CS-1). Oceanic stations (P-12, P-16, P-20, H-04, and P26) were sampled from P12 to P26. Vertical plankton tows (0-50 m) were collected, and chitobiase decay dynamics were measured at each station during the spring and summer of 2005. Due to rough conditions, P-16 was not sampled during the spring.

4.2.3 Mesozooplankton identification and enumeration

Upon retrieval, zooplankton samples were preserved in a 10% solution of buffered formalin-seawater. In the laboratory, the contents of each sample were split up to 4X (i.e. 1/16th) using a Folsom splitter. Large and medium sized copepods (*Neocalanus cristatus*, *N. plumchrus*, *Calanus marshallae*, *C. pacificus*, *Metridia pacifica*, *Mesocalanus tenucornis*, *Eucalanus bungii*, *Acartia longiremis*, and *Centropages abdominalis*) were identified to stage. All other copepod species were identified as either copepodites or adults. This group included *Oithona similis*, *O. atlanticus*, *Pseudocalanus* spp., *Clausocalanus* spp., *Calocalanus styliremis*, *C. tenuis*, *Scolecithricella minor*, and *Microcalanus* spp. A minimum of 700 individuals were counted per sample. The prosome length of at least 30 individuals per stage (when sufficiently abundant) was measured at 25X under a dissecting microscope equipped with an ocular micrometer. Dry weights were estimated using length-weight relationships obtained from the Zooplankton Database, maintained by the Institute of Ocean Sciences, Sidney, B.C., Canada. Species specific biomass (dry weight) was estimated as:

$$B_{Sp} = \Sigma N_i * W_i, \qquad \text{Eq. 4.1}$$

where; B_{Sp} is the total biomass of a species (mg dry weight m^{-3}), N_i is the number (individuals m^{-3}) of individuals in a stage/size class and W_i is the numerically-weighted average individual dry weight.

4.2.4 Biomass production, stage duration and growth rate estimates

Rates of biomass production, development and growth of the copepod community were estimated using chitobiase-based methods (Oosterhuis et al. 2000; Sastri and Roff

2000; Chapter 2). A detailed description of these methodologies is presented in Chapters 2 and 3. Briefly, seawater samples (collected from 5, 10, 30, and 50 m) were passed through 54 μm mesh to remove all crustaceans. Each 1.0 L sample was then immediately sub-sampled (~10 mL) to estimate depth-specific ambient chitobiase activity (CBA_{nat}). Each sub-sample was passed through a 0.2 μm filter and maintained at 4°C until the enzyme assay was conducted. Seawater samples were then amended with 1.0 mL of a filtered (0.2 μm) crude copepod homogenate (~40 copepods in 4.0 mL of seawater). Seawater samples were maintained at ambient sea surface temperature and sub-sampled every 1.5 to 3.0 hours for the following 12 hours. Chitobiase assays were run exactly as described in Chapter 2.

The slope (k) of the natural logarithm of CBA in amended samples versus time (for each depth) was applied to the CBA_{nat} to estimate depth-specific chitobiase decay rates (i.e. Δ CBA $hr^{-1} = -k * CBA_{nat}$). During the course of the study, differences between incubation temperature (sea surface temperature) and the temperature at each sampled depth (i.e. 5, 10, 30, and 50 m) varied. A Q_{10} correction factor of 3.22 (Sastri unpublished) was used to correct the measured slopes and thus estimate ΔCBA at the *in situ* depth-specific temperature.

Depth-weighted averages of CBA_{nat} and ΔCBA were used to estimate biomass production rate, mean stage durations of the developing community (DCBA) and size class specific stage durations (D_{CBAi}). Daily biomass production rate (BPR) was calculated by estimating the change in biomass (Δ mg C L^{-1} $hour^{-1}$) equivalent to the depth-weighted average estimate of ΔCBA by using Equation 4.5 (Equation 2.6; Chapter

2) and applying a carbon conversion factor of 0.45 (Paffenhoeffer and Harris 1976) to dry weight Thus, the daily biomass production rate can be estimated as:

$$BPR = (\Delta \text{ biomass}) * 24 \qquad \text{Eq. 4.1}$$

$$D_{CBA} = CBA_{nat} / \Delta CBA, \qquad \text{Eq. 4.2}$$

$$D_{CBAi} = ((CBA_i\text{-}CBA_{avg}) + CBA_{nat}))/(\Delta CBA), \qquad \text{Eq. 4.3}$$

where, D_{CBAi} represents the mean stage duration of the size class of interest, CBA_i represents the CBA predicted for that size class from the CBA-dry weight relationship or the CBA-prosome length relationships presented in Sastri & Dower (2006), and CBA_{avg} is equivalent to the CBA of the average sized individual (or fraction of total biomass) molting per hour. Although not, strictly speaking, a rate *per se* (i.e. $\Delta CBA\ h^{-1}$), this value is equivalent to that of ΔCBA. Again, it should be noted that all chitobiase-based estimates are valid only if assumptions i-iii (Chapter 2) are met; furthermore, valid estimates of stage duration (Equations 4.2 and 4.3) must also satisfy assumptions iv and v. Although, not explicitly tested in this study, conditions satisfying valid estimates of stage duration are assumed.

With the exception of a single station (CS7; summer only), *Calanus pacificus* CVs were present in every sample. This stage is thus used to illustrate spatial and seasonal variation of *C. pacificus* (CIV) growth rates. The CV dry weight used for station CS7 is the average of all weights from the study. For comparisons of community-level growth rates between stations we chose to estimate growth increment as per Chapter 2. Growth was assumed to be exponential and calculated as:

$$g = \ln(W_2/W_1)/D_{CBAi}, \qquad \text{Eq. 4.4}$$

where g is the weight-specific growth rate (day^{-1}). The numerically-weighted average prosome length was used to estimate stage-specific dry weights (W_2). As both CIVs and CVs were not present at all stations, W_1 was estimated using the method proposed by Sastri and Dower (2006). The change in weight between W_1 and W_2, the growth increment (g_{inc}), was estimated as follows:

$$\log (g_{inc}) = 0.864 \log(CBA_i) - 1.78 \ (r^2=0.73, n=19, p<0.0001), \qquad \text{Eq. 4.5}$$

This regression was derived from length-weight relationships for *Pseudocalanus* spp., *Metridia pacifica*, and *Calanus pacificus* (see Chapter 2 for further explanation). Estimating the increment of growth in this manner effectively couples growth to moulting rate. Although this generally holds true for intermediate copepodite stages (i.e. CII, CIII), some studies have observed a decoupling for pre-diapause and pre-adult stages (CIV and CV; Peterson et al. 1991; Rey-Rassat 2004). A decoupling of these rates may also occur between NVI and CI. Hirst et al. (2005) addressed the potential error that this decoupling may impose when using the moult rate method (e.g. Peterson et al. 1991).

For comparisons of the relative influence of temperature, individual body weight, and chlorophyll *a* concentration between oceanographic regions the moult rate method was employed (e.g. Peterson et al. 1991). There are potential sources of error associated with this approach (see Hirst et al. 2005), however, this approach was adopted because species specific variability within a station would not be considered with the use of the generalized growth increment equation (Eq. 4.5, above). Thus, weight-specific growth rates were estimated for *Calanus marshallae*, *C. pacificus*, *Neocalanus cristatus*, *N. plumchrus*, and *Eucalanus bungii* for all successive pairs of stages present in our samples.

4.2.5 Statistical Analyses

Copepod community resemblance was evaluated using the Bray-Curtis dissimilarity index (Clarke and Warwick 1994) which involves a pair-wise comparison of the relative distribution of all species between stations. In this case, total biomass (mg dry weight m^{-3}) for each species in a community was log (X+1) transformed and a dissimilarity matrix developed using PRIMER software (Version 5.0; Clarke and Warwick 1994). Species-specific biomass was chosen, rather than abundance per se because variation in production rate is more likely to influence patterns of biomass than abundance. The significance of clustering within oceanographically distinct regions (shelf, slope, oceanic, and eddy) was tested using Analysis of Similarity (ANOSIM; similar to one way ANOVA). Abundance and biomass estimates for *Metridia pacifica* and *Scolecithricella minor* in our 50-0 m samples are not included in this analysis as both species display high amplitude diel vertical migrations.

The BIO-ENV procedure in PRIMER was used to test which water column properties (temperature, region, season, chlorophyll *a* concentration, and estimated biomass production rate; BPR) most closely matched the spatial pattern of community resemblance as revealed by the Bray-Curtis dissimilarity matrix. The significance of reported correlations was assessed with a Spearman rank correlation (Clarke and Warwick 1994).

The explanatory power of individual dry weight, depth-averaged chlorophyll *a* concentration, and temperature as factors influencing variability of individual growth rates was evaluated using a Spearman Rank correlation (Sigma Stat). All factors (except temperature) were log-transformed and tested against log-transformed individual growth rate for (i) all stations, (ii) shelf stations, (iii) slope stations, and (iv) oceanic stations. The correlations analysis for the oceanic stations was carried out both with and without data from the Haida eddy.

4.3 Results

4.3.1 Copepod community abundance

The relative abundances (in the upper 50 m) of the dominant species for the spring and summer and sampled for both seasons combined are presented for each region in Figure 4.2. In general, total copepod community abundance was greatest on the shelf, followed by the slope, and oceanic stations. Abundance at shelf stations in the spring was dominated by *Pseudocalanus* spp. (41%), *Oithona similis* (29%), *Paracalanus* spp. (9%), *Acartia* spp. (8%); *Centropages abdominalis* (4%), and *Microcalanus spp*. (4%). *C. marshallae* was always present on the shelf but (with the exception of P4) not at slope or oceanic stations. When all slope stations were grouped, *Paracalanus* spp. dominated (33%) followed by *O. similis* (29%), *Pseudocalanus* spp. (21%), *Clausocalanus* (7%), *Acartia* spp. (3%) and *Neocalanus plumchrus* (2%). With the exception of the northernmost station (CS-1), *Pseudocalanus* spp. or *Paracalanus* spp. were the dominant species. At CS-1, *O. similis* was the most abundant species (~70%) followed by *N. plumchrus* (16%) and *N. cristatus* (12%). With the exception of the Haida eddy, *O. similis* was the dominant species (73%) at all oceanic stations, followed by *Neocalanus plumchrus* (8%), *Eucalanus bungii* (~6%), *O. atlanticus, Pseudocalanus spp*., and *N. cristatus* (each <5%). The two *Neocalanus* spp. dominated (~86%) the abundance of the eddy station (H-04). *Neocalanus flemingeri* was not found in any of the surface samples collected during this study. *Acartia* spp. dominated the abundance of the summer copepod community on the shelf (52%), followed by *O. similis* (16%), *Paracalanus* spp. (16%) and *Pseudocalanus* spp. (7%). These three species also dominated the relative abundance of the slope stations (32, 30, and 17% respectively). *N. plumchrus* and *N. cristatus* were largely absent (or rare) from the surface waters and the biomass of these communities was thus dominated by *C. pacificus* and *E. bungii.*

Of particular note is the presence of *Mesocalanus tenucornis* and *Clausocalanus* spp. at many of the stations sampled throughout the survey area. These genera are typical of more southerly copepod communities. The relatively high abundance of *Clausocalanus* spp. was most significant at the southern Vancouver Island shelf and slope stations (LC, LG, and P4).

4.3.2 Copepod community resemblance

Patterns of community resemblance were examined using the Bray-Curtis dissimilarity index. With respect to relative species biomass (log [x+1]), stations tended to cluster among shelf, slope, and oceanic stations (Figure 4.3). The stress value reported for the MDS ordination was relatively low at 0.09. Discrimination of clusters based on these 4 regions (shelf, slope, oceanic, and eddy) was statistically significant (Global R=0.35, p=0.002). Clusters that were significantly different (ANOSIM) were; shelf and slope (p=0.007), oceanic and shelf (p=0.003), and shelf and eddy (p=0.036). Groups of stations that were not statistically different were slope and oceanic (p=0.097), slope and eddy (p=0.13), and oceanic and eddy (0.47). During the spring, oceanic and shelf stations did not cluster together (Figure 4.4 a). The northern slope station CS-1 and southern slope

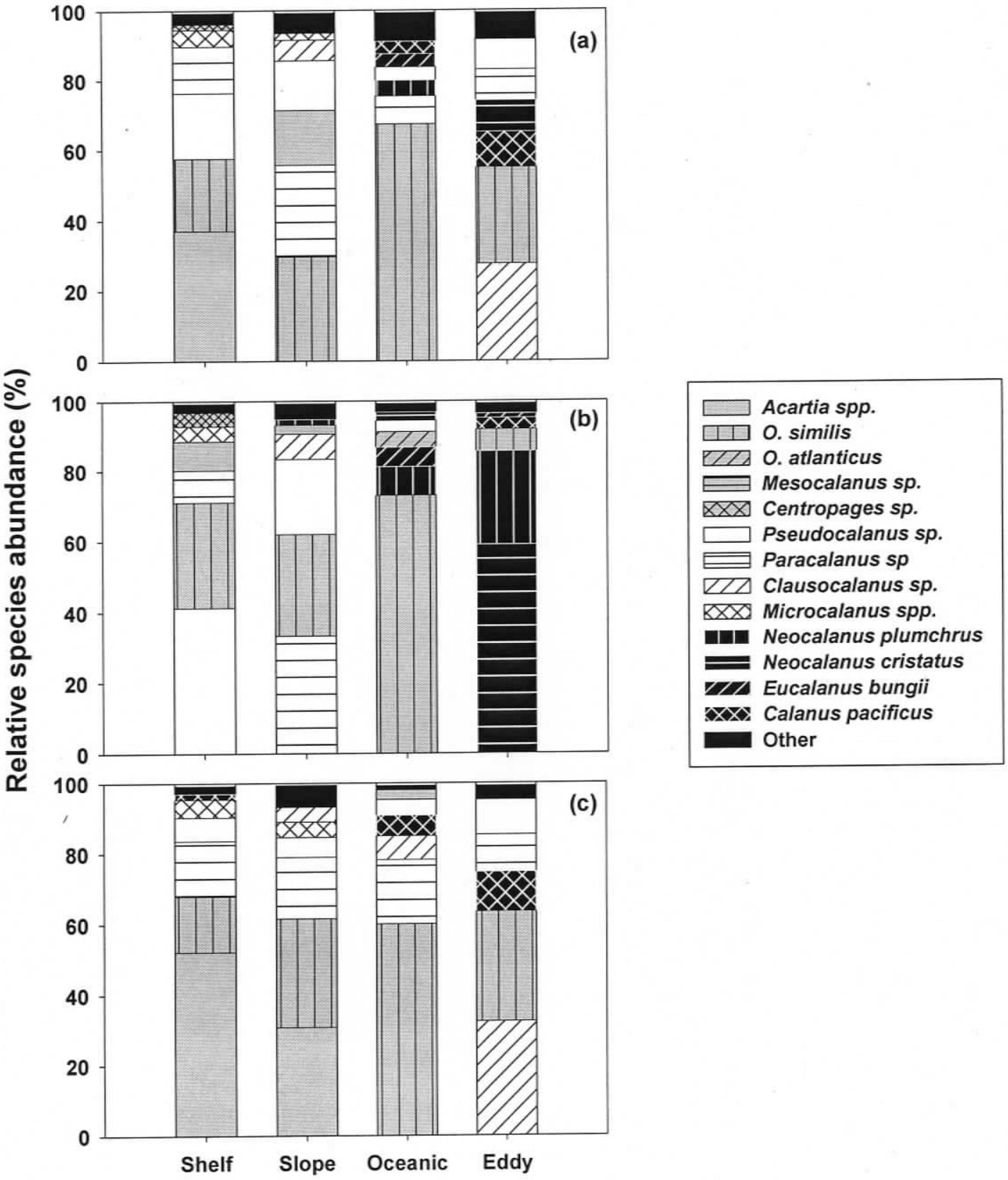

Figure 4.2. Mean relative abundance of species found at stations located on the shelf, slope, oceanic and eddy stations for: a) both seasons combined; b) spring and; c) summer.

station LG-9 clustered with the oceanic stations, while southern slope stations LC-9 and P4 most resembled the shelf stations. In the summer, oceanic stations were more similar to each other than to slope and shelf stations (Figure 4.4 b).

Table 4.1 lists the best results of the BIO-ENV analysis of the degree to which pair-wise community similarity co-varied with the following 4 factors: 1) depth-averaged temperature (0-50 m); 2) biomass production rate (BPR); 3) depth-averaged chlorophyll *a* concentration and; 4) region. The Spearman's rho; r, correlation coefficient, is reported for the best single and combined factors with respect to the entire data set (n=23 stations) and spring (n=11 stations) and summer (n=12 stations). For the combined data set, the strongest predictor of resemblance was a combination of chlorophyll *a*, temperature, and region (r=0.309). For the spring data set, the strongest predictor of community resemblance was temperature and region (r=0.827). In the summer, chlorophyll *a* and region was the best predictor of community resemblance (r=0.622). The best predictor of resemblance including BPR as a variable was BPR and region in the spring (r=0.784) and chlorophyll a, BPR, and region (r=0.560) in the summer.

4.3.3 *In situ* biomass production, development, and growth rates

Biomass production rates for the copepod community varied between 23.40 mg C m^{-3} day^{-1} (P4 spring) and 3.08 mg C m^{-3} day^{-1} (LG-2 summer; Figure 4.5). Rates of biomass production were generally greater at all stations during the spring than in the summer, with the exception of the two northern Vancouver Island (NVI) stations (CS-7 and CS-1) and oceanic station P20, for which rates were moderately greater (<1.5 mg C m^{-3} day^{-1}) in the summer. The lowest rates of biomass production were measured at SVI at slope and shelf stations (LC-4, LC-9, LG-2, and LG-9) during the summer. The range of BPR estimates for these stations was 5.57 (LG-9) to 3.08 (LG-2) mg C m^{-3} day^{-1}.

The mean BPR for the oceanic stations in the spring and summer were 10.85 and 8.87 mg C m^{-3} day^{-1}, respectively. Oceanic BPR estimates varied approximately two times (7.15-14.69 mg C m^{-3} day^{-1}). Among oceanic stations, P12 and the Haida eddy station were the most productive in the spring (14.69 and 13.12 mg C m^{-3} day^{-1}, respectively). During the summer, the Haida eddy station was most productive with 11.46 mg C m^{-3} day^{-1}. The lowest rates of biomass production were measured at P20 (7.15 mg C m^{-3} day^{-1}) in the spring and at P26 (7.81 mg C m^{-3} day^{-1}) in the summer. BPR estimates represent the rate of production of biomass by moulting crustaceans in the water column. While it is assumed that copepods dominate these production estimates, other crustacean groups (e.g. euphausids) were also abundant on southern shelf stations (e.g. >60% of total biomass at P4 in the spring) in both spring and summer and so estimates of production cannot be attributed entirely to the copepod community. The biomass of oceanic stations, however, was dominated (>87%) by copepod biomass).

The *in situ* stage duration for the mean-sized individual in the copepod community varied between 40 and 157 hours (Figure 4.6; D_{CBA} corrected to 11.5°C). Stage durations were shortest (40-45 hours) during the spring at the two northern Vancouver Island (NVI) stations (CS-7 and CS-1) and the Haida eddy. The longest stage durations were measured at SVI at slope and shelf stations (LC-4, LC-9, LG-2, and LG-9) during the summer. The range of D_{CBA} estimates for these stations was 118 (LG-9) to 156 (LC-4) hours. The mean spring and summer D_{CBA} for oceanic stations was 76 and 75 hours, respectively. Oceanic D_{CBA} estimates varied across a relatively narrow range (66-

86 hours). The shortest stage durations in both seasons were estimated for CS-1 (NVI slope) and the Haida eddy. D_{CBA} estimates represent the stage duration of the mean-sized individual in a community. Thus, some stations may have been dominated by smaller individuals (i.e. nauplii and smaller copepodites) that were not well sampled by the nets used in this study. Although standardized for temperature, these estimates are, therefore, not immediately comparable to each other.

Weight-specific growth rates were estimated for *Calanus pacificus* CIV. Growth rates (corrected to 11.5°C; $Q_{10} = 2.7$; Hirst and Bunker 2003) are presented in Figure 4.7. Growth rate estimates are attributed to *C. pacificus* because the estimates are based on variation of the numerically-weighted body mass at stage (CV) and chitobiase decay dynamics at each station. The weight-specific growth rate for *C. pacificus* CIV varied between 0.04 and 0.16 day^{-1} (Figure 4.7; corrected to 11.5°C). Growth rates were fastest (0.14-0.16 day^{-1}) during spring at the NVI slope (CS-1), P4, and in the Haida eddy. The fastest weight - specific growth rates in the summer were estimated at NVI slope station CS-1 and the Haida Eddy (0.15 and 0.12 day $^{-1}$, respectively). The slowest weight-specific growth rate during spring was estimated at oceanic station P20 (0.09 day^{-1}). Among oceanic stations during spring, individuals at station P12 were growing the fastest, at 0.12 day^{-1}. Estimates of weight-specific growth rate at SVI shelf stations (LC-4 and LG-2) were less than half (0.044 versus 0.10 day^{-1}) those estimated during the summer. The weight-specific growth rate at the SVI slope stations were also less than 50% of the rates estimated in the summer relative to spring estimates (0.048 versus 0.11 day^{-1}).

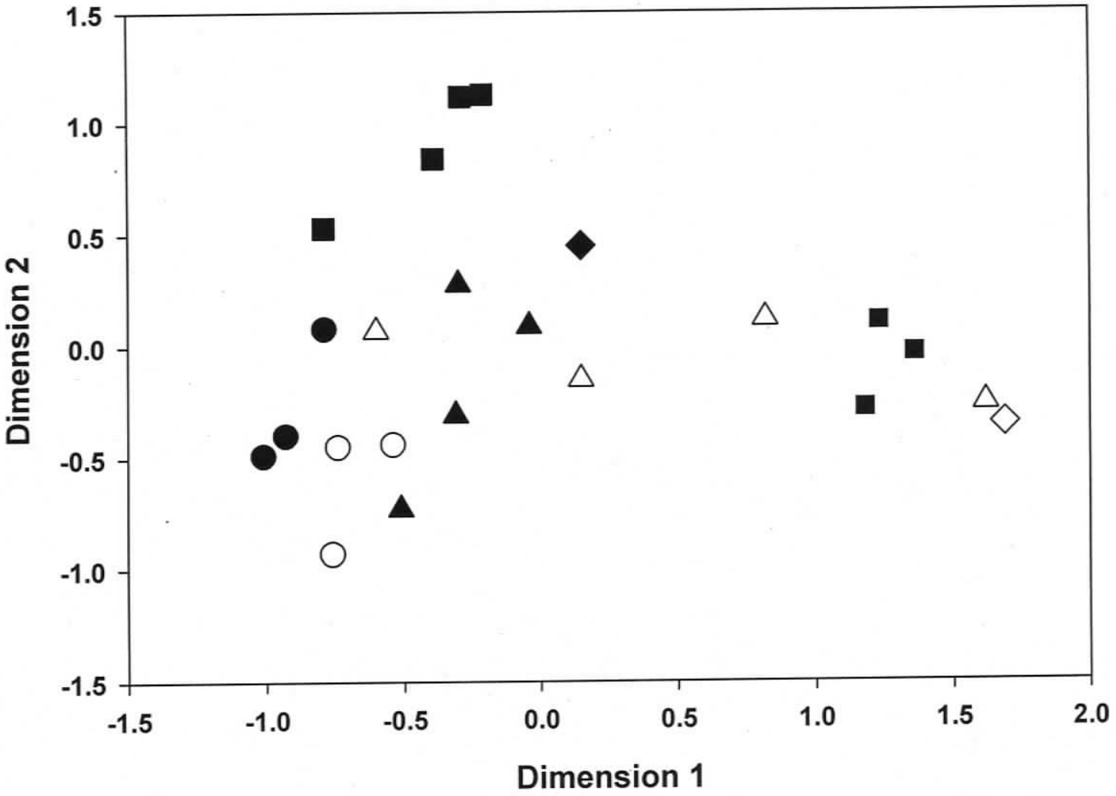

Figure 4.3. Two-dimensional non-metric multidimensional scaling plot of copepod community resemblance from 0-50 m vertical net tows from shelf (circles), slope (triangles), oceanic (squares), and Haida eddy (diamonds) stations sampled in spring (white symbols) and summer (black symbols) 2005 in the subarctic NE Pacific. Stress value=0.09. Ordination produced from Bray-Curtis dissimilarity matrix of log (X+1) transformed species biomass (mg dry weight m^{-3}).

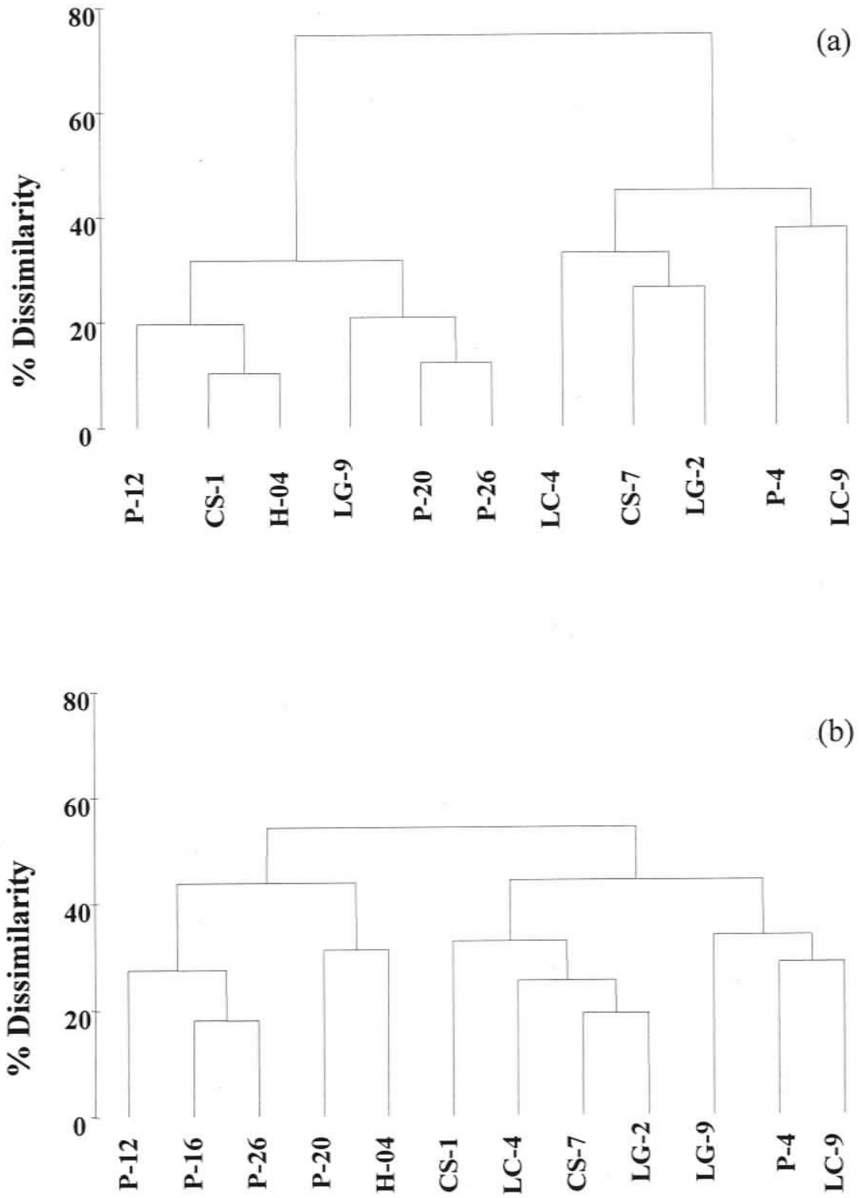

Figure 4.4. Group-average linkage dendrograms of copepod community resemblance from 0-50 m vertical net tows from shelf (LC-4, LG-2, CS-7), slope (LC-9, LG-2, CS-1), oceanic (P-12, P-16, P-20, P-26,), and Haida eddy (H-04) stations sampled in (a) spring and (b) summer 2005 in the subarctic NE Pacific. Group-average linkage produced from Bray-Curtis dissimilarity matrix of log (X+1) transformed species biomass (mg dry weight m^{-3}).

Table 4.1. Rank correlations between Bray-Curtis dissimilarity matrix for all pairwise station comparisons and the best combinations (k=1, 2, 3, and 4) of water column properties. The procedure was run for both spring and summer pooled, and for spring and summer separately. Values in bold represent the best combination (highest rank correlation) for each group.

Group	***k***	**Best variable combinations**		
Combined	1	Region (0.249)	Chl *a* (0.216)	Temp (0.069)
	2	Chl *a*, Region (0.302)	Temp, Region (0.255)	Chl *a*, Temp (0.243)
	3	**Chl *a*, Temp, Region (0.309)**	Chl *a*, BPR, Region (0.262)	Temp, BPR, Region (0.183)
	4	Chl *a*, Temp, BPR, Region (0.264)	-----	-----
Spring	1	Region (0.807)	Chl *a* (0.095)	Temp (0.039)
	2	**Temp, Region (0.827)**	BPR , Region (0.784)	Chl *a*, Region (0.586)
	3	Temp, BPR, Reg. (0.741)	Chl *a*, Temp, Reg. (0.615)	Chl *a*, BPR, Reg. (0.602)
	4	Temp, Chl *a*, BPR, Region (0.618)	-----	-----
Summer	1	Chl *a* (0.515)	Region (0.430)	-----
	2	**Chl *a*, Region (0.622)**	Chl *a*, Temp (0.586)	Chl *a*, BPR (0.479)
	3	Chl *a*, Temp, Region (0.625)	Chl, BPR, Region (0.560)	Temp, Chl *a*, BPR (0.437)
	4	Temp, Chl *a*, BPR, Region (0.536)	-----	-----

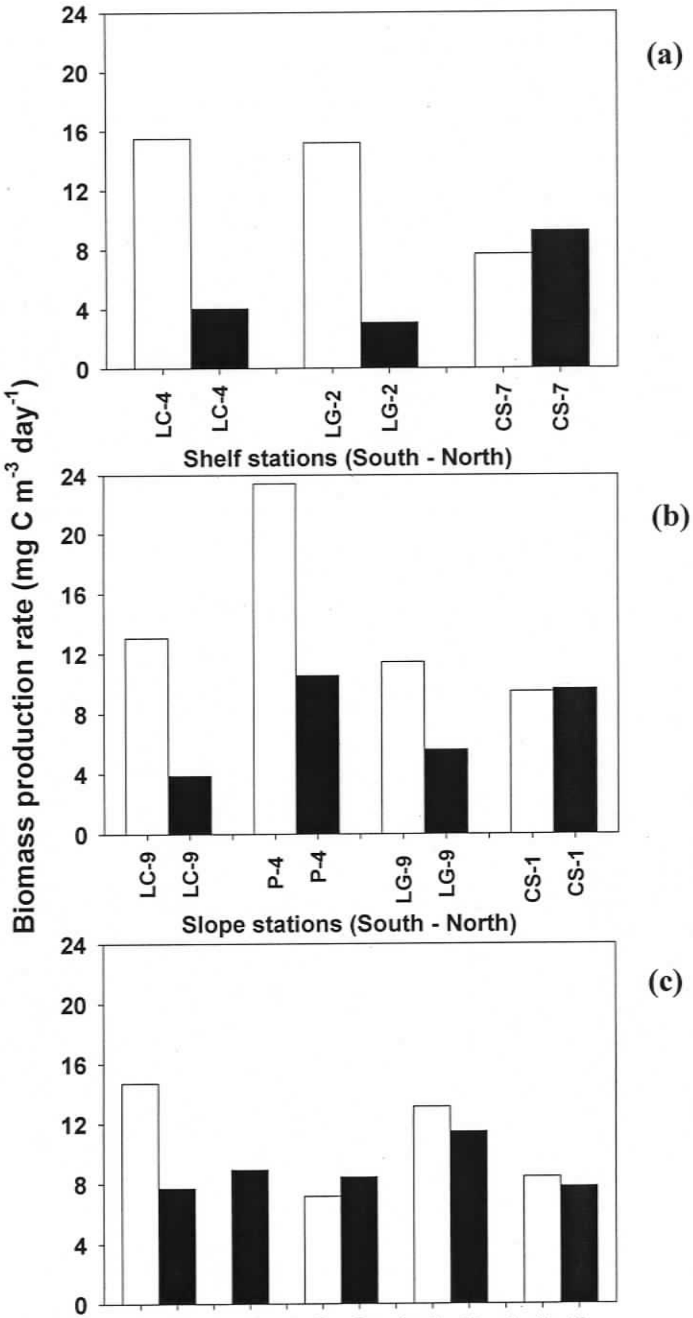

Figure 4.5. Community-level estimates of biomass production rate (mg C m^{-3} day^{-1}) by juvenile copepods at: (a) shelf, (b) slope and, (c) oceanic stations in the subarctic NE Pacific during the spring (white bars) and summer (black bars) of 2005.

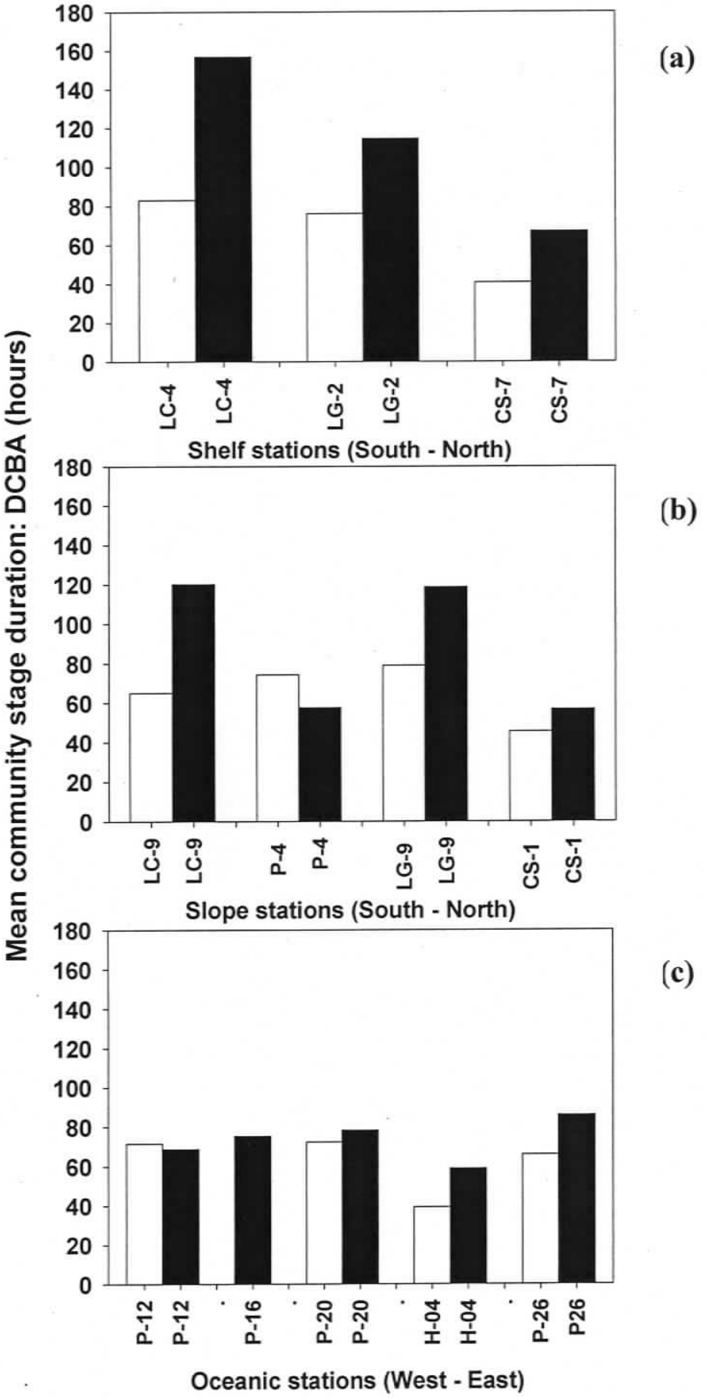

Figure 4.6. Stage duration (hours) estimates for the mean-sized individual in the copepod community (D_{CBA}) for all: (a) shelf, (b) slope and, (c) oceanic stations in the subarctic NE Pacific during the spring (white bars) and summer (black bars) of 2005. All D_{CBA} estimates have been standardized to 11.5 °C using a Q_{10} of 3.0 (Sastri unpublished).

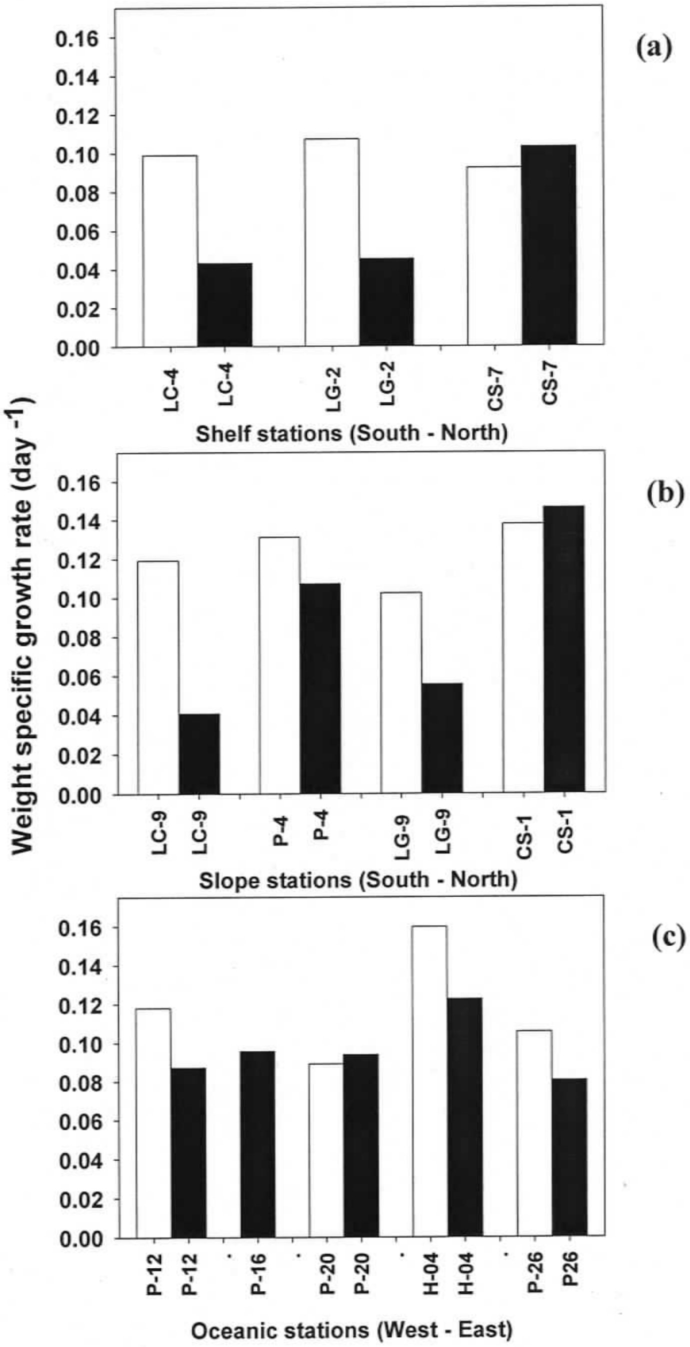

Figure 4.7. Weight-specific growth rate (day $^{-1}$) estimates for *Calanus pacificus* (CIV) for all: (a) shelf (b) slope and (c) oceanic stations in the subarctic NE Pacific during the spring (white bars) and summer (black bars) of 2005.All weight-specific growth rates have been standardized to 11.5°C using a Q_{10} of 2.7 (Hirst and Bunker 2003).

The explanatory power of individual dry weight, chlorophyll *a* concentration, and temperature as factors influencing variation of weight-specific growth rate was evaluated for all large calanoid species. Results of a Spearman rank correlation are presented in Table 4.2. For all regions, whether combined or treated separately, individual body weight was found to be significantly correlated ($p<0.0001$) to weight-specific growth rate. No significant relationship was found between chlorophyll *a* and growth rate when all regions were combined. A marginally significant positive relationship ($p=0.07$) was found between weight-specific growth rate and chlorophyll *a* concentration for the shelf stations, whereas no significant correlation was found with either the slope or the oceanic stations. Temperature was positively correlated with all groups (except slope stations), but only significantly so for the shelf stations. The sign and significance of the correlations between individual body weight, chlorophyll *a* concentration and temperature, and weight-specific growth rate did not change when the Haida eddy was included with the oceanic stations.

Chitobiase decay rates were size-scaled (using equation 4.3) to represent individuals of 0.01 mg and 0.1 mg dry weight. These weights were those used in the Huntley and Boyd (1984) analysis of food-limited growth of marine copepods. Figure 4.8 shows the stage durations estimated for an individual weighing 0.01 and 0.1 mg (dry weight) at each station during each season. For comparative purposes, all stage duration estimates were standardized to 11.5°C. The estimated stage duration for 0.1 mg individuals was always greater than that of individuals weighing 0.01 mg. However, the difference in stage duration between the two weight classes varied spatially and seasonally. At oceanic stations, the stage duration of the larger individual was between 1

Table 4.2. Spearman rank correlations of weight-specific growth rate and individual body weight (mg dry weight), chlorophyll *a* concentration (mg chlorophyll *a* m^{-3}), and temperature (°C). Rank correlations are considered for all stations combined, and for each region considered separately. No distinction is made on the basis of season.

		Combined	**Shelf**	**Slope**	**Oceanic**	**Oceanic w/H-04**
Individual dry weight	Correlation	-0.798	-0.732	-0.704	-0.918	-0.890
	p	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Chlorophyll *a* concentration	Correlation	-0.0682	0.260	-0.056	0.041	0.041
	p	0.374	**0.07**	0.676	0.766	0.741
Temperature	Correlation	0.0325	0.352	-0.126	0.021	0.024
	p	0.672	**0.016**	0.344	0.878	0.845
	n	172	47	58	55	67

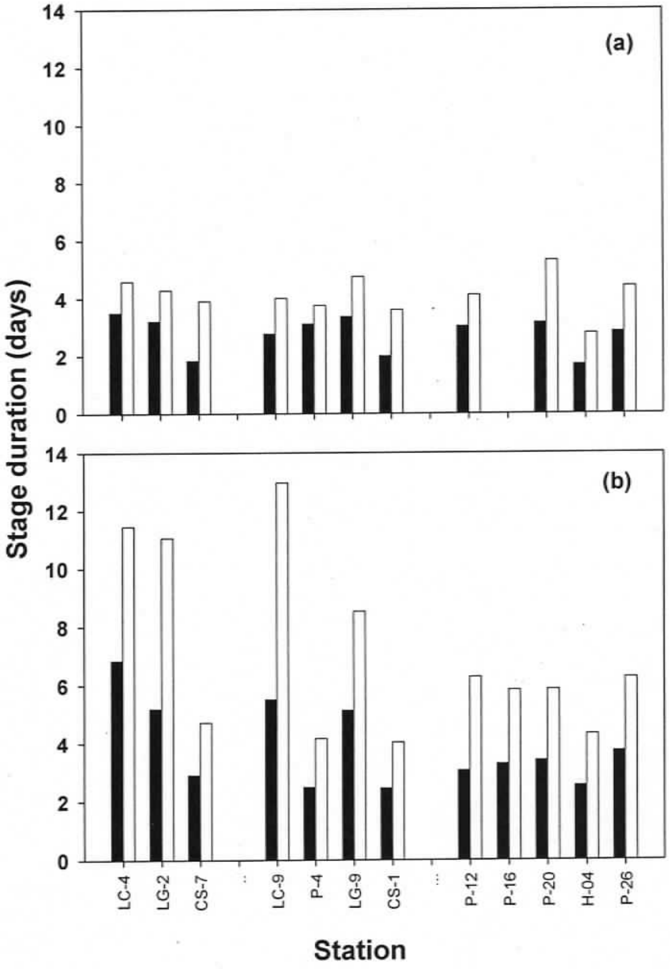

Figure 4.8. Comparison of stage durations (days) for individuals weighing 0.01 mg (black bars) and 0.1 mg (white bars) dry weight during the a) spring and b) summer of 2005 in the subarctic NE Pacific. All stage duration estimates have been standardized to 11.5°C using a Q_{10} of 3.0 (Sastri unpublished).

(P-12 and H-04) and 2 (P-20 and P-26) days longer than that of smaller individuals. During the summer, the difference between the two weight classes was 2.5-3.2 days, except for the Haida eddy station, which remained at ~1 day. During the spring, variation in stage duration due to body size on the shelf and slope was similar to oceanic stations. In the summer, however, exceptional differences were measured at LC-4 (4.6 days), LG-2 (5.8 days), LC-9 (7.4 days), and LG-9 (3.4 days).

4.4 Discussion

This study adopted a community-level approach to investigate two basic questions about copepod production, development and growth rates: 1) do these rates vary across large spatial scales? And; 2) does the relative importance of assumed environmental influences on biomass production rates vary between regions?

4.4.1 Spatial patterns of copepod community resemblance

Communities were defined by their relative compositional similarity and how patterns of resemblance varied with environmental factors (i.e. temperature and chlorophyll *a* concentration). The results of a seasonal and spatial comparison of species composition and community resemblance for this study are generally consistent with previous studies from this region (Mackas and Sefton 1982; Mackas 1984; Goldblatt et al. 1999). Communities clustered into three groups: shelf, slope, and oceanic stations. Although the spatial separation between shelf and slope stations is relatively small compared with the spatial coverage of this study, the difference between these two regions was significant. This is consistent with Mackas (1984) who found that community resemblance decreased more rapidly with separation across the shelf than alongshore. Covering a much larger spatial scale, Mackas and Coyle (2005) also allocated zooplankton communities to the same regions defined by the analysis in this study.

The distinction between the relative community composition of oceanic and slope stations was not significant in this study. In the spring, stations CS-1 and LG-9 tended to be more similar to oceanic stations while, in the summer, these same stations were more similar to shelf stations. This temporal pattern is consistent with Mackas (1992) and Mackas and Yelland (1999) who found that the peak biomass of several species on the shelf preceded the peak biomass of con-specifics at slope stations by about two months. This shift occurs after wind-driven coastal upwelling begins driving surface water (and surface-dwelling copepods) offshore.

4.4.2 Copepod community production, development and growth rates

In general, variation in the estimates of biomass production rates varied with patterns of community resemblance. The results of the BIO-ENV analysis suggest that the best combination of variables varying with community resemblance were region, chlorophyll *a* concentration and temperature. Combinations of variables including BPR estimates were similarly correlated but never among the best variable combinations. With the exception of the Haida eddy station and P12, estimated rates of biomass production during the spring were higher on the shelf and slope relative to the oceanic stations. Rates at the two southern shelf stations (LC-4 and LG-2) were ~2X greater than those estimated for oceanic stations, P20 and P26. Estimates of biomass production rates at oceanic stations during the both the spring and summer were comparable.

BPR and DCBA estimates for SVI slope stations (LC-9 and LG9) in the spring (i.e. before offshore transport) were more similar to oceanic stations than to shelf stations. Both estimates at these same stations in the summer reflect a general reduction production and moulting rate at their adjacent shelf stations (LC-4 and LG-2). Similarly, weight-specific growth rates of *C. pacificus* and a greater body-size dependence of developmental rate was also measured at both the SVI shelf and slope during the summer but not in the spring. Under typical conditions primary production on the shelf is high relative to the slope and oceanic stations (especially HNLC stations; P20 and P26; Mackas and Coyle 2005). Mackas and Coyle (2005) describe high productivity on the shelf from the spring continuing through to the summer. However, the production rate, stage duration, and growth rate estimates on the shelf suggest rates that were comparable to (or lower than) those at the slope stations and the oceanic stations during the summer.

4.4.3 Production, development and growth rates: Shelf and slope regions

Recently, several studies have documented that the onset of wind-driven upwelling in the NW Pacific region was ~50 days late in 2005 (Schwing et al. 2006; Kosro et al. 2006). Biological impacts ranged from extremely low rates of primary production (Kudela et al. 2006) to zooplankton biomass anomalies that extended into the SVI region (Mackas et al. 2006), and hatching failure of planktivorous Auklets (Sydeman et al. 2006). The results presented in Chapter 3 show that during 2005, the copepod community production, moulting and growth rates in the Strait of Georgia were also anomalously low during the early part of the spring phytoplankton bloom. It was hypothesized that a period of prolonged stratification early in the spring led to rapid nutrient depletion in the upper water column and, ultimately, to developmental failure of the biomass dominant *N. plumchrus*, perhaps by feeding on nutrient impoverished diatoms. In addition, analysis of the fatty acid composition of *N. plumchrus* CVs in surface waters also suggests that these animals were subsisting on a nutritionally poor diet (El-Sabaawi and Dower unpublished data).

With reference to the events of 2005, Mackas et al (2006) also point out that although rates of primary production recovered following the delayed upwelling (Kudela et al. 2006), zooplankton biomass anomalies continued into 2006. In the SVI region, these anomalies consisted of an increased abundance of southern zooplankton taxa (California current origin) and a reduction in the relative biomass of the typical boreal zooplankton taxa (Mackas et al. 2006). The results of the present study are consistent with these studies and suggest that once upwelling finally did begin in 2005 (i.e. before our summer sampling period) production rates had decreased, food-limited developmental rates did not improve, and the food-limited signal originating on the shelf was advected offshore.

4.4.4 Production, development and growth rates: Oceanic stations

During both the spring and summer sampling cruises, we sampled a one year old Haida eddy (see Mackas and Galbraith 2002; Whitney and Robert 2005; Mackas et al. 2005; Miller et al. 2005 for details and review) situated well offshore between P20 and P26. In terms of relative community resemblance, the Haida eddy was most similar to the northern slope station (CS-1) during the spring. In the summer, the community resemblance analysis distinguished this station from all clusters as being intermediate between the slope and oceanic stations. Mackas and Galbraith (2002) illustrated the time course over which copepod species indicative of shelf communities persist within a Haida eddy, and the results of this study support their expectations of an eddy of this age (~1-1.5 years). In general, however, with respect to chitobiase-based estimates of production rate, average community stage duration and weight-specific growth rate, the zooplankton community in the Haida eddy was most similar to CS-1 rather than its neighboring stations (P20 and P26). Thus, despite chlorophyll *a* concentrations and a zooplankton community composition similar to the HNLC stations P20 and P26, zooplankton production rates in the Haida eddy were greater than P20 and P26 and also greater than the station closest to its point of origin (CS-1). The results of this study therefore suggest that production of the copepod communities at HNLC stations (P20 and P26) was suboptimal. These results are consistent with the observed acceleration of *E. bungii* developmental rates in response to the iron induced phytoplankton bloom during the SERIES iron enrichment experiment (Tsuda et al. 2006).

Previous work has suggested that primary production in the surface waters of a Haida eddy of this age are not expected to be significantly different from the surrounding waters (Peterson et al. 2005). Nevertheless, Johnson et al. (2005) noted that labile iron concentrations below the surface mixed layer are still high relative to surrounding HNLC waters. Thus, as eddy structure begins to decay, upwelling of deeper iron rich water occurs, and may continue to enhance surface production rates. Chlorophyll *a* concentrations within Haida eddy 2004 were comparable to the surrounding waters (<0.5

mg m^{-3}). Iron enrichment of the surface waters in the Haida 2004 eddy may therefore not have been sufficient (or at occurred at sufficiently rapid rates) to support the development and enhanced production of a markedly different diatom community as observed during SERIES (Marchetti et al. 2006), but may still have enhanced primary productivity of the extant phytoplankton community. Large subaractic copepods in the HNLC waters of the Alaskan Gyre are believed to generally subsist on microzooplankton and sinking detritus (Landry and Lehner-Lefournier 1988; Dagg 1993). Thus, relatively high microzooplankton production rates in an aging Haida eddy could serve to maintain copepod community developmental and growth rates comparable to those observed on the shelf and slope. Alternatively, the diet of oceanic species such as *N. plumchrus* and *N. cristatus* retained in the eddy when primary production was greater (<1 year old) may have translated into enhanced rates of development and growth of the developing offspring sampled by this study. Such a residual effect of the maternal diet (e.g. Rey-Rassat et al. 2002b) is plausible, provided that over-wintering animals are retained within the Haida eddy as it moves further offshore.

4.4.5 Factors influencing spatial variability of growth rates

This study provided an opportunity to compare copepod production rates between different oceanographic regions. With respect to the correlative comparisons of *in situ* estimates of growth rate, it was found that individual body weight was significantly correlated to growth. This is hardly surprising, considering the results of recent syntheses of *in situ* rates of juvenile copepod growth (Hirst and Lampitt 1998; Hirst and Bunker 2003). However, these results place quite a heavy dependence of growth rate on body weight. Some of this dependence may arise from (i) the scaling of stage duration with body size; (ii) the decision to use the largest species for growth rate estimates; and (iii) the use of length : weight regressions to estimate mean weight at stage for growth increments. Nevertheless, the relative influence of body size did vary between regions.

The strongest correlation between growth rate and body size (r = -0.918) was found among the oceanic stations. Neither temperature nor chlorophyll *a* was significantly correlated with growth rate at these stations. This, too, is not surprising, since: (i) temperature in the upper 50 m did not vary appreciably (~9-13°C; also see Whitney and Freeland 1999); (ii) the relative influence of temperature may only be evident when animals are already growing at maximum rates; and (iii) copepod diets in this region are not expected to be composed primarily of phytoplankton (Dagg 1993; Gifford 1993).

The correlation between body size and growth rate was lower (r = -0.704) at slope stations but not significantly correlated to either chlorophyll *a* concentration or temperature. For the shelf stations, growth rate was significantly correlated to individual body weight, chlorophyll *a* concentration, and temperature. Coastal species (e.g. *Acartia* spp. and *Centropages* spp.) may therefore have a greater sensitivity to variation in phytoplankton availability (i.e. Davis and Alatalo 1992; Calbet and Alcaraz 1997) than species dominating the slope and oceanic regions. Peterson et al. (2002) conducted a cross- shelf study of copepod community egg production, moulting, and juvenile growth rates off of the Oregon coast. Based on comparisons of their estimates to global models of *in situ* copepod growth rate, they suggested that predictive models of copepod growth rate may not be universally applicable. Indeed, the results of this study suggest that over large spatial scales, the relative influence of environmental factors on copepod development and growth rates will vary.

Based on laboratory studies (Vidal 1980a, b), it is expected that the effect of food limitation on copepod development and growth rate should increase with body size, as larger individuals require a proportionately greater concentration of food to achieve saturated rates. Furthermore, as rates become progressively more food-limited, regulation by temperature should become less important especially for larger individuals. Using the more conservative measure of food limitation (stage duration) it was found that the difference in stage duration over an order of magnitude in body size for rapidly developing communities was ~1 day (H-04, CS-1). The absolute difference in stage duration between the two size classes was consistently greater for oceanic stations (1.5-2 days), suggesting a body-size dependence of suboptimal development. The most marked seasonal change in the body-size dependence on development was for the SVI shelf and slope stations. All rate estimates suggest that the copepod communities on the shelf and slope were developing (and growing) at relatively rapid rates during the spring. During the summer, however, mean community stage duration was prolonged and the body-size dependence of development increased, suggesting severe food limitation.

4.4.6 Summary

This study estimated juvenile copepod community biomass production, development and growth rates over a large spatial scale encompassing three oceanographically distinct NE Pacific mesozooplankton communities. In general, community-level patterns of biomass production, development and growth are consistent with spatial patterns of community composition. It was found that production rates of the copepod community were severely depressed on the southern shelf and slope of Vancouver Island in 2005. This agrees with recent observations of zooplankton biomass anomalies (Mackas et al. 2006) and a dramatic higher trophic level response (Sydeman et al. 2006) to an anomalously late (and weak) spring upwelling season in the NW Pacific in 2005. This study also provides evidence that the offshore transport (via mesoscale Haida eddies) of micronutrient rich coastal waters to HNLC regions produces localized sites of enhanced zooplankton productivity that can persist for extended periods (>1 year). Finally, the results of this study suggest that the factors influencing variation of juvenile copepod growth rates tend to vary among regions and copepod community assemblages and, thus, that it may not be appropriate to apply contemporary global models of *in situ* juvenile copepod growth rate when comparing copepod production rates across marine systems.

CHAPTER 5.

Summary and Conclusions

5.1 Validation and evaluation of the chitobiase-based methods for marine copepod communities

The primary objective of this thesis was to test the utility of chitobiase-based methods as routine field estimates of biomass production, development and growth rates of juvenile copepod communities. Through application of these methods during a two-year time series and a broad-scale spatial survey I hoped to gain some greater insight into how these important rates are regulated *in situ*.

As stated in Chapter 1, this objective cannot be reasonably approached with conventional methodology. Chapter 2 presented a validation of a method that I developed for routine use in the field. In summary, this method is premised on the assumption that under steady-state conditions, the rate of production of the moulting enzyme, chitobiase, in the water column is equivalent to the moulting rate of the average moulting rate of the copepod community. Necessary tests of this assumption were stated in Chapter 2.

5.1.1 Evaluation of chitobiase-based estimates of developmental rates

I measured the activity of chitobiase liberated into small volumes of seawater by individuals of four copepod species (*Calanus pacificus*, *Metridia pacifica*, *Pseudocalanus* sp., and *Tigriopus californicus*). For all four species, the relationship between chitobiase activity liberated into seawater (immediately following moult) and body size was positive. Furthermore, a single statistically significant relationship described all four species. During four sampling trips in the Strait of Georgia I was able to measure the rate of decay of chitobiase activity in the upper 50 m of the water column. Conventional moulting rate experiments were also run in tandem with chitobiase decay rate experiments. Results of these experiments demonstrate that chitobiase-based estimates of stage duration were in good agreement with conventional estimates (within 5-15%).

Although conventional estimates of stage duration compared well to the CBA-based estimates, it is not yet clear whether all of the assumptions stated in Chapter 2 have been satisfied for the complete data set presented in this thesis. Without conventional estimates of community moulting rate to accompany CBA-based estimates, it is difficult to definitively ascribe the turnover rate of the CBA pool in the water column to the turnover rate of the biomass (via moulting) of the developing copepod community. Under most field conditions the first three assumptions from Chapter 2 should be satisfied; i.e. i) copepods dominate the planktonic crustacean biomass; ii) the copepod community is in steady-state over short periods (~12 - 24 hours), and; iii) the rate of production of CBA is balanced by its rate of decay. Critical to the valid interpretation of CBA turnover rates as estimates of stage duration, however, are assumptions iv ($CBA_{nat} = (\Sigma n_i * CBA_i)$, and v ($CBA_{nat}$ only varies with $(\Sigma n_i * CBA_i)$). If these two assumptions are <u>not</u> met, the time value estimated from Equation 2.2 represents the turnover rate of the enzyme pool which then represents the turnover rate of a varying measure of the fraction of biomass actually moulting. Provided that these last two assumptions are not met, this estimate cannot be interpreted as the average stage duration of the developing community although it may still be used to estimate the change in biomass per unit time (i.e. biomass production rate; D.L Mackas, Institute of Ocean Sciences, personal communication).

Oosterhuis et al. (2000) measured chitobiase decay rates in a synchronously developing *Temora longicornis* culture. Chitobiase activity in the water was found to vary with biomass (i.e. satisfying Assumption iv) however, the estimated decay rate of the enzyme (Δ CBA hr^{-1}) probably varied with both biomass and moulting rate (i.e. violating Assumption v). Application of Equation 2.2 using the decay rates presented by Oosterhuis et al. (2000) yield stage duration estimates that are ~50% of previously reported rates (~1.7 days; Klein Breteler et al. (1994). Under conditions where total biomass is increasing (due to synchronous development), estimates of chitobiase production rates will be influenced by both the average moulting rate and also an elevation in chitobiase liberated by a population composed of increasingly larger animals. As noted by Sastri and Roff (2000), a correction may be applied provided variation of CBA_{nat} is also measured during the decay rate experiments. Unfortunately, Oosterhuis et al. (2000) did not make such measurements in their study. Furthermore, a time course of CBA_{nat} measurements in the field is prohibitive given typical sampling routines.

Ultimately, populations characterized as either synchronous (i.e. a single defined cohort) or steady-state (i.e. resulting from continuous reproduction) represent each extreme of convenient and necessary abstractions used by population ecologists to estimate production and model population dynamics (see Kimmerer 1990). For the purposes of evaluating my proposed developmental rate estimate (Equations 2.2 and 2.3) it is worth considering which of the two abstractions is most likely to be encountered in the field.

First off, the estimate proposed in Chapter 2 is generalized to the community level by virtue of the common body size-chitobiase activity relationships. Second, chitobiase decay rate experiments are made on short (12 hour) time-scales (relative to individual stage durations). Finally, for any given copepod community composed of several populations, both recognizable cohorts and continuously reproducing populations will be encountered. Thus, when all populations are considered together, the size-structure of a *community* will not vary appreciably over the short time-scales defined by both duration of chitobiase decay rate experiments and the average stage duration in that community. Even if all of the populations in a community were characterized as perfectly synchronous, the probability that all populations are developing together in synchrony is extremely low. It is conceivable that a synchronously developing population which dominates the biomass of a copepod community (e.g. *Neocalanus plumchrus*) may force an increase of CBA_{nat} in the water column as a result of a relative increase in the average body size of moulting individuals. However, the influence of increasing body size on variation of CBA_{nat} and ΔCBA can only be recognized on time-scales corresponding to the time that it would take for all individuals in a given stage to moult (several days in temperate waters). Therefore, as consequence of the short time-scales corresponding to my decay rate experiments, a steady-state approximation is valid.

I attempted to test assumptions iv and v with estimates of community biomass and CBA_{nat} made during 8 sampling dates in the SoG (see Chapter 3). The results of this analysis are equivocal. Although the data do suggest that CBA_{nat} does vary with ($\Sigma\ n_i$ * CBA_i), the sample size was very low and the relationships were not statistically significant. As discussed in Chapter 2, some of this discrepancy may be attributed to a lack of vertical resolution of biomass estimates with respect to vertical net casts. It is also important to note that on every occasion (i.e. in all of the estimates presented in this thesis) the measured CBA_{nat} was always *greater* than the corresponding estimate of biomass from net casts. Thus, at least for the data set presented in this thesis, it is unlikely that the turnover rate of CBA_{nat} represents the turnover rate of a *fraction* of the actual biomass pool. However, it is not yet clear whether variation in the absolute concentration of enzyme produced per unit time is due to variation of biomass, variation of moulting rate, or variation of both. The rate constant of decay (k) appears to be independent of variation of biomass, but the results cannot be interpreted as conclusive without the addition of more data. Nevertheless, the potential for violation and subsequent misinterpretation of results obtained using Equation 2.2 demands a more explicit treatment of CBA decay dynamics.

The results of a simple set of field and laboratory experiments may be used to address the critical assumptions of Equation 2.2. Provided that a copepod community is actively developing in the field, the equivalence of CBA_{nat} and $(\Sigma\ n_i * CBA_i)$, could be tested with highly resolved CBA_{nat} estimates (e.g. every 1 or 2 m over the upper 20m of the water column) accompanied by vertical net casts over the same depth range. In the laboratory, cultures of copepod populations in steady-state, fed *ad libitum*, and maintained at different temperatures should develop at different rates. Thus, if assumptions iv and v are violated, we should expect CBA_{nat} to vary with development rate. Alternatively, estimates of CBA_{nat} for populations developing at different rates (e.g. high and low temperature treatments) should vary purely as a function of biomass, while estimates of –k and thus ΔCBA should vary with temperature and conventional estimates of moulting rate.

Chapter 2 also outlined a method of scaling chitobiase decay rates with individual body size, such that size-class specific estimates of stage duration and growth rate can be estimated. This relationship should prove to be of considerable utility to zooplankton researchers as it can be used to address the body-size dependence of moulting and growth rates on food concentration under food conditions (see Chapter 4).

In addition to concerns raised regarding the validity of treating the turnover rate of the chitobiase pool and developing copepod biomass as equivalent, is the potential influence that predation-based mortality may impose on estimates of the rate of chitobiase production. Chitobiase activity associated with the moult cycle is elevated in individuals that are in pre-moult or apolysis (Espie and Roff 1995). This period may comprise between 20 and 50% of the intermoult phase (Espie and Roff 1995; Crain and Miller 2001). Thus, if an individual is not consumed whole (i.e. the exoskeleton is damaged prior to ingestion) chitobiase present in the apolytic space will be liberated into the water (and thus contribute to estimates of its rate of production). Across several copepod species, mortality rates are greatest for naupliar stages NI and NII (~0.1-0.3 day^{-1}; Eiane and Ohman 2004) and the general trend is for mortality rate to decrease with increasing body size (Hirst and Kiorboe 2002). Mortality rates for stages NIII through CVI are typically less than 1% day. Therefore, assuming that all mortality is predation based and is constant for all individuals within a stage, and that during a "mortality event" the exoskeleton is sufficiently damaged before ingestion such that all of the chitobiase in the apolytic space is liberated into the water, then, estimates of moulting rate based on chitobiase decay rate measurements may be elevated by between 0.002 to 0.005 day^{-1} (% in apolysis * daily mortality rate) for stages NIII through CVI. For example, accounting for mortality-based production of chitobiase would reduce a chitobiase-based moulting rate of 0.2 day^{-1} (120 hour stage duration) to between 0.195 (50% in apolysis) and 0.198 day^{-1} (20% in apolysis). Therefore, mortality for NIII-CVI stages may be responsible for reducing stage duration estimates by between 1.2 and 3 hours relative to the "uncorrected" chitobiase-estimate of 120 hours. With respect to stages NI and NII, predation-based mortality may significantly influence stage duration estimates given the conditions described above. However, the individual body size, total biomass, and therefore chitobiase liberated by individuals of these stages is low, relative to all other stages. Moreover, any mortality-based production of chitobiase attributed to stages NI and NII assumes that exoskeletons are damaged before ingestion. The potential for "sloppy feeding" by larval fish on early naupliar stages of copepods may only be addressed by examining the condition of larval fish gut contents.

5.2 Temporal patterns of juvenile copepod community production, development and growth rates

Variability in juvenile copepod community biomass production (BPR), development and growth rates were estimated on 16 occasions in the Strait of Georgia using the method developed and presented in Chapter 2. The majority of community-wide estimates were made around the time of the spring bloom in 2004 and 2005. In 2004, the average rate of development and growth for the biomass-dominant copepods in the mesozooplankton community followed historical expectations based largely on temporal patterns of biomass. Chitobiase-based estimates of size-class specific rates of development and growth rate fit well with species-specific estimates of these rates from other regions in the NE Pacific. In a relative sense, BPR estimates in 2004 tended to vary with expectations of temperate coastal copepod communities before, during, and after spring phytoplankton blooms.

However, in 2005, a rapid decline in abundance and biomass of the copepod community in the Strait of Georgia was detected during the peak of the spring bloom. Biomass production rates, mean community and size-scaled estimates of moulting rates estimated with the chitobiase method also declined rapidly as the abundance of all species dropped significantly.

Production of the spring copepod community in the Strait of Georgia is dominated by the large subarctic calanoid copepod *Neocalanus plumchrus* (Fulton 1973; Harrison et al. 1983). During the spring of 2005, surface abundance of this species fell far below the numbers estimated in 2004 (or historically). The abundance of individuals that survived to descend to over-wintering depths was <2% of those measured in 2002, 2003, and 2004. Failure of the developing *N. plumchrus* cohort was attributed to a prolonged period of strong upper water column stratification that promoted the early development of the spring phytoplankton bloom and a rapid depletion of macronutrients by diatoms. Mixing of deep water with surface waters was limited, and copepods developing in these surface waters were likely forced to feed exclusively on nutrient-depleted diatoms. In addition, analysis of the fatty acid composition of *N. plumchrus* CVs in surface waters also suggests that these animals may have been subsisting on a nutritionally poor diet (El Sabaawi and Dower, unpublished data). Although retarded juvenile development due to feeding on nutrient-deplete diatoms has been demonstrated in the laboratory (e.g. Breteler et al. 2005), this represents the first direct observation of this phenomenon in the field.

The results of this study are important because copepods constitute a key component of the diet of many juvenile fish, including the juvenile salmon that spend part of their lives in the Strait of Georgia. Year class returns of salmon have been shown to be significantly related to their survival during this period (Francis and Hare 1994). This study also suggests that copepod moulting rates (which, historically, have not been believed to be limited by food concentration) may respond to variability in food quality at time scales much shorter than previously considered. As demonstrated by this study, this can have significant implications for species such as *N. plumchrus*, whose reproductive period is temporally limited and defined by feeding success during the previous spring.

Although relationships between food quality and copepod egg production, development, and growth rate have been demonstrated under laboratory conditions, the overall importance of food quality to copepod communities in the field remains unclear. Copepod diets (and food availability) in seasonal coastal systems such as the Strait of Georgia have the greatest potential to vary in terms of food concentration, dietary composition, and food quality over relatively short periods of time. Addressing the dependence of development, growth, and ultimately production rates on food is therefore contingent on the use of an appropriate proxy that can incorporate the *relative* influence of concentration, composition, and quality. Thus, future studies must consider the relevant timescale over which physiological condition and copepod metabolic rate processes respond to diet variability. This may prove difficult; however, as the results of

Chapter 3 would suggest that field studies of this sort may demand a weekly sampling resolution.

5.2.1 Evaluation of chitobiase-based estimates of biomass production rates

Due to the relative scarcity of estimates of biomass production rate estimates for entire communities, it is difficult to evaluate the magnitude of estimates made using the chitobiase method. Indeed, most (if not all) estimates of biomass production rates in the literature which have directly estimated growth rate have not accounted for the biomass of the entire copepod community (i.e. adults, copepodites, and nauplii for all species). It is reassuring that the chitobiase-based estimates were generally greater than those of comparable studies, most of which consider only a few species in the larger community. However, other factors must now also be considered: i) the production of other groups such as amphipods, decapod larvae and euphausids will undoubtedly be included in chitobiase-based estimates; and ii) a true field validation of the production rate estimates demands accurate estimates of biomass.

The production estimates presented in this thesis assume that copepods dominate production of the crustacean component of the mesozooplankton community. However, it is acknowledged that other groups can contribute significantly. Thus, establishing relationships between body size and CBA is a necessity for these other important groups. With respect to biomass, Huntley and Lopez (1992) argued that variation about estimates of biomass were greater than the variation about growth rate, and thus more attention needs to applied to accurate estimates of biomass. Most estimates of biomass rely on net sampling techniques. As discussed in Chapter 2, resolving the fine-scale vertical distribution of biomass using nets remains difficult. Moreover, most studies employ nets with mesh ≥ 200 μm, thus leaving a significant portion of the total copepod biomass (and also production) unaccounted for (Hopcroft et al. 2002). A recent study (Stehle et al. 2007) found that zooplankton biomass estimated from a vertically towed Longhurst Plankton Recorder (LHPR) was on average 5X greater than the biomass estimated from Bongo nets, a tool that is still widely used to sample copepods. Moreover, biomass (and abundance) using and LHPR type sampler may be used to address vertical distributions with a single cast. In the absence of consensus on how best to estimate biomass, comparison of production rates using chitobiase-based estimates of growth may be a first step forward in the continued validation of these methods.

In an effort to quantify the potential contribution of non-copepod crustaceans to the BPR estimates I have assumed that (i) $CBA_{nat} = \Sigma\ CBA_i*n_i$ (see Chapter 2) and (ii) that all crustaceans (copepods, euphausids, amphipods, decapod larvae, barnacle larvae, ostracods, and cladocerans) share the same chitobiase-body weight relationships (see Chapter 2). Re-evaluating BPR estimates based on the relative contribution (%) of each group to the total CBA_{nat} pool as estimated from 100 m vertical SCOR net casts from the Strait of Georgia (Figure 5.1 a and b) suggests that this contribution is significant at times and that the contribution of each group can be partitioned from the BPR estimates presented in Chapter 3. I have also estimated BPR using chitobiase-based individual weight-specific growth rate estimates and total biomass estimated for copepods present in each 50 m SCOR net cast collected in the Strait of Georgia

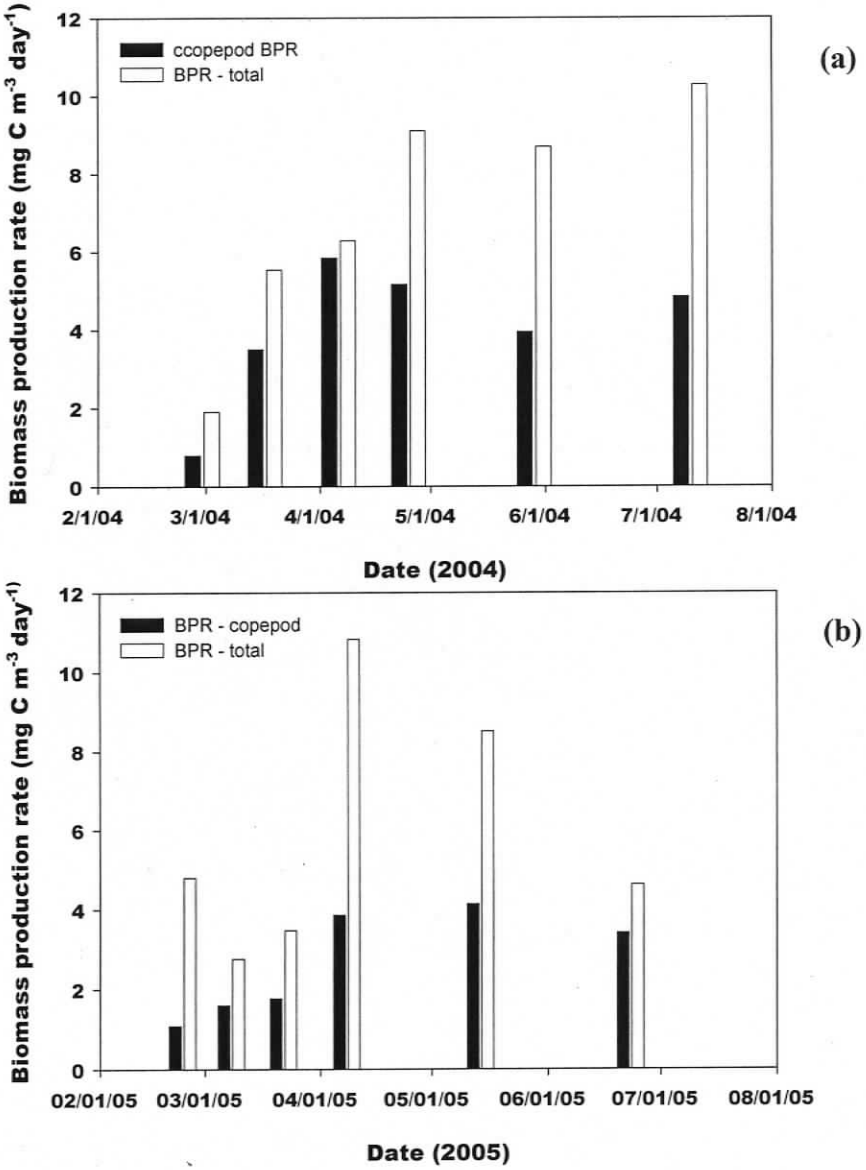

Figure 5.1 Comparison of biomass production rate estimates for the SoG copepod community (black bars) and for all planktonic crustaceans (white bars) during the spring of 2004 (a) and 2005 (b).

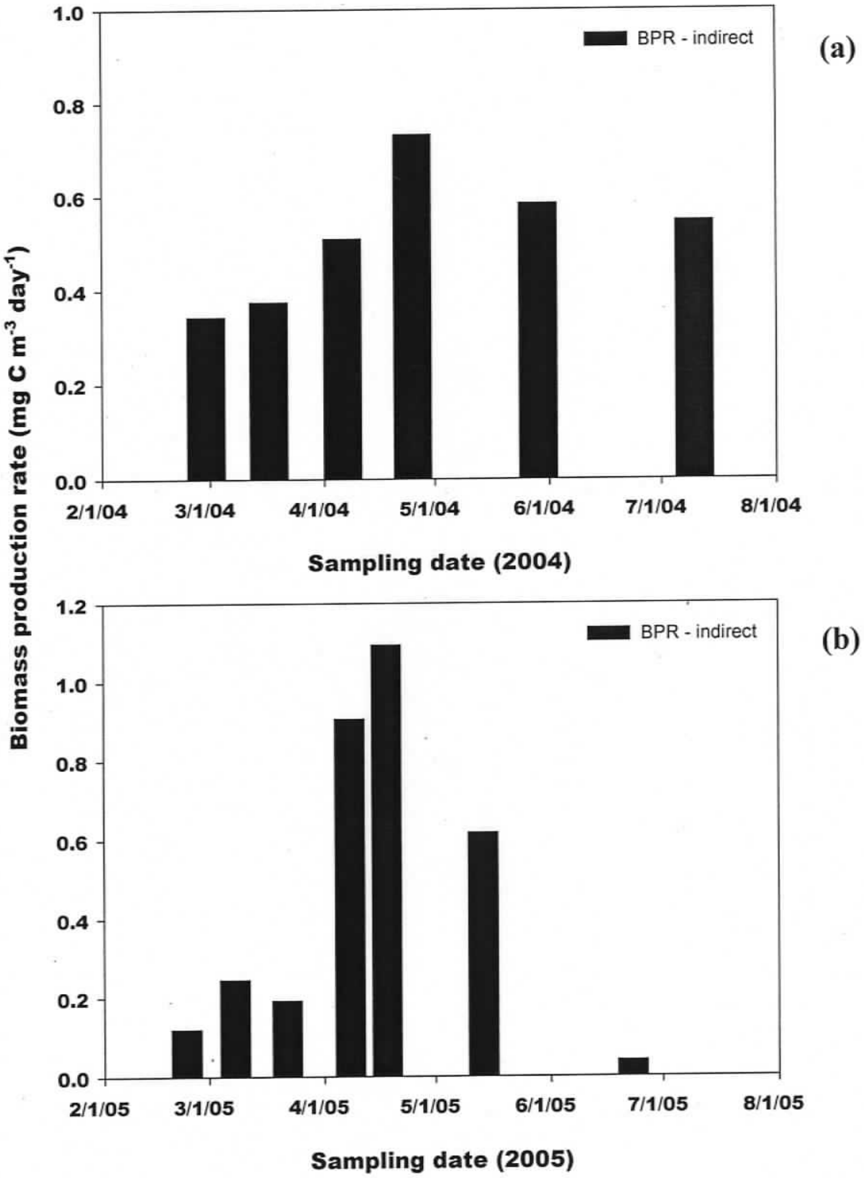

Figure 5.2 Biomass production rate (mg C m^{-3} day^{-1}) estimates for the SoG copepod community estimated as the product of chitobiase-based weight-specific growth rate and total biomass estimated from 50 m SCOR net casts during the spring of 2004 (a) and 2005 (b).

("indirect" BPR; Figure 5.2).Comparing both direct and indirect copepod-specific BPR estimates suggests that the temporal patterns are similar. Moreover, a direct comparison the estimates for each sampling date (Figure 5.3) suggests that the two estimates are well correlated (Direct BPR = 5.25 * (Indirect BPR) + 0.846, $r^2 = 0.52$, $p = 0.011$). The results of this comparison provide further evidence (see Chapter 3) that the assumptions regarding the validity of chitobiase-based estimates of stage duration stated in Chapter 2 have not been violated. The difference in magnitude between the direct-BPR and indirect-BPR estimates suggests that SCOR net casts are consistently underestimating biomass or are unable to account for the vertical distribution of animals within thedepth range sampled.

The primary criticism resulting in my statement of assumptions (see Chapter 2) for valid interpretation of the chitobiase-based stage duration estimates surrounds the key assumption that the turnover rate of the chitobiase pool is equivalent to the turnover rate of copepod biomass in the water column (when the copepod community is in steady-state). This criticism does not apply to the direct biomass production rate estimate (BPR), however, as it is simply a measure of the absolute rate of production of chitobiase in the water column. The ratio of biomass production rate to biomass (P/B) in the water column is often used as an expression of the relative turnover rate (% day^{-1}) of copepod biomass in the water column (akin to a generalized growth rate). Therefore, the P/B expression represents a useful means of assessing the equivalence of biomass turnover rate and that of the chitobiase pool.

I have made comparisons of 'direct P/B' versus 'indirect P/B', using the data presented for direct BPR estimates (as in Figure 5.1) in the Strait of Georgia (Chapter 3) and indirect estimates of BPR (as in Figure 5.2). With respect to 'direct P/B', I have estimated biomass either directly from CBA_{nat} (corrected for relative copepod biomass; Figure 5.4 a) or used the biomass estimated directly from the contents of a 50 m vertical SCOR net cast (Figure 5.4 b).

In general, turnover rates estimated either 'directly' or 'indirectly' vary together, increasing from late winter through spring and early summer for both 2004 and 2005. These trends are consistent with the patterns BPR and stage duration presented in Chapter 3 (see also Figures 5.1 and 5.2). Furthermore, the 'direct' and 'indirect P/B' estimates varied together positively, regardless of whether the biomass value used for 'direct P/B' was based on CBA_{nat} (Figure 5.4 a) or biomass estimated directly from the contents of the nets casts (Figure 5.4 b). The results of linear regressions analysis are as follows:

For Figure 5.4 a: Direct P/B = [0.56 * Indirect P/B] + 0.064, $r^2 = 0.68$, $p = 0.001$.

For Figure 5.4 b: *Direct P/B = [6.47 * Indirect P/B] + 0.095, $r^2 = 0.66$, $p = 0.0013$.

These comparisons suggest that: 1) the turnover rate of the chitobiase pool does vary consistently with the turnover rate of copepod biomass (Figures 5.4 a and b); 2) CBA_{nat} does vary with copepod biomass (Figure 5.4 a) and; 3) since the slope of the relationship between 'direct' and 'indirect' P/B estimates using the same biomass value (SCOR net contents) is >1 there is a consistent upward discrepancy between biomass estimated by the CBA_{nat} and the nets. This final point is noted several times throughout the thesis. This discrepancy may have arisen because: 1) the nets consistently underestimate the actual biomass; 2) nauplii and smaller copepods are not accounted for; and 3) CBA_{nat} is a volume-specific (concentration) estimate, whereas biomass estimates from net casts are necessarily averaged over the depth-range sampled and thus, cannot account for vertical aggregation that would be evident in variation in the vertical profile of CBA_{nat}. A greater degree of resolution on this issue might be gained by estimating the relative contribution (i.e. %CBA) of copepods to the CBA pool using 50 m rather than 100 m net casts. However, during the growing season it is reasonable to assume that most of the actively developing biomass is present in the upper 50 m (Mackas 1992).

5.3 Spatial patterns of juvenile copepod community production, development and growth rates

In 2005, chitobiase decay dynamics were measured in the water column at 12 stations in the NE subarctic Pacific. All stations were sampled in the spring (late May to early June) and again in the late summer (late August to early September). Distances between station pairs ranged from 40 to 1406 km. An analysis of community resemblance grouped the stations into three oceanographic regions: shelf, slope, and oceanic stations. A mesoscale anticylonic Haida eddy was also sampled. This eddy was approximately 1 year old and situated in oceanic waters about 900 km west of its coastal point of origin. In general, biomass production rates were highest at shelf stations (relative to slope and oceanic stations) in the spring, the exception being the relatively high production rates estimated at the Haida eddy situated offshore. In the summer, productivity in the Haida eddy remained high relative to oceanic stations, while production rates estimated on southern shelf and slope stations were among the lowest measured in the study. In summary, patterns of community similarity between stations were significantly correlated

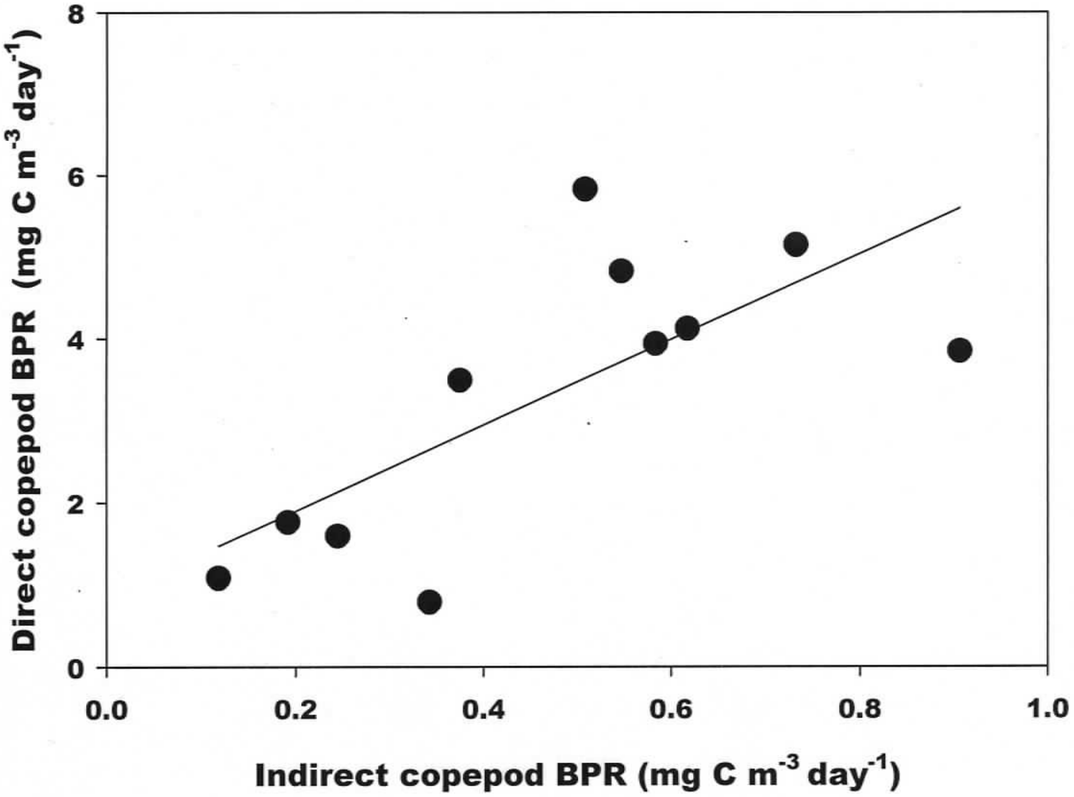

Figure 5.3 Comparison of direct biomass production rates (BPR) based on the relative contribution of copepods to the total CBA_{nat} versus indirect biomass production rates estimated via chitobiase-based individual weight-specific growth rates and the contents of 50 m SCOR net casts in the Strait of Georgia.

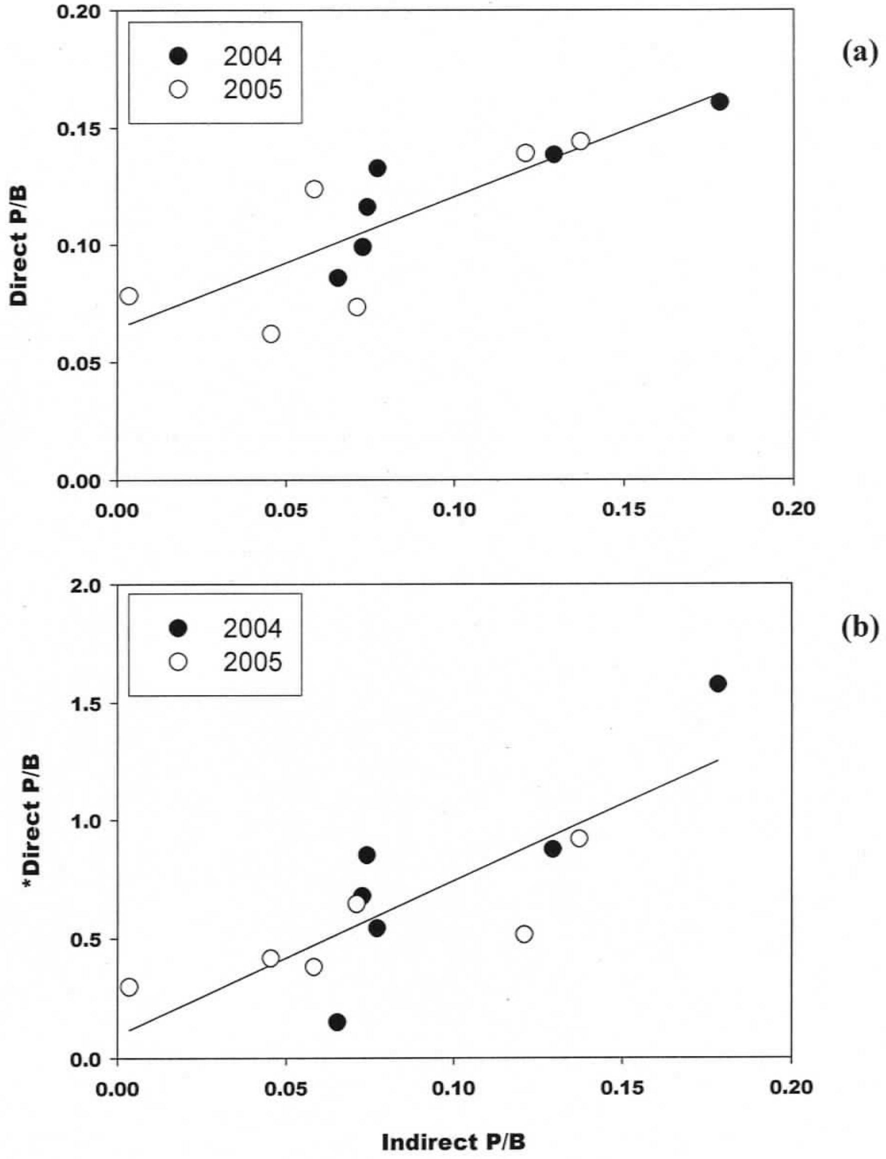

Figure 5.4 Comparison of biomass production rate to biomass ratios (P/B; relative change in mass per unit time). Direct P/B represents BPR estimated directly via chitobiase production rates attributed to copepod biomass estimated (a) directly from CBA_{nat} and (b) estimated directly from the contents of 50 m net casts. Indirect P/B represents BPR estimated indirectly via chitobiase-based estimates of weight-specific growth and biomass estimated directly from 50 m SCOR net casts in the Strait of Georgia in 2004 and 2005.

to temperature, chlorophyll *a* concentration, and region. Although not included in any variable combinations of the best correlations with community resemblance, combinations including biomass production rate were also significantly correlated.

Comparisons of *in situ* developmental and growth rates between regions suggest that the relative influence of temperature, individual body weight, and food concentration on copepod community development and growth rate varies from region to region and that it may therefore be inappropriate to estimate production rates of copepod communities by applying single predictive models of growth rate to biomass across of a range of oceanographically distinct systems. Thus, future efforts to describe the variability of *in situ* rates of copepod development and growth must also consider community composition. In particular, the development and growth rates of copepod communities characterized as "coastal" appear to be more responsive to variation of phytoplankton concentration than are oceanic communities. Due to these apparent differences, the use of region-specific or community-specific half saturation constants (akin to phytoplankton species-specific nutrient uptake rates) may be a more appropriate means of addressing and modeling the variability of *in situ* copepod development and growth rates with respect to phytoplankton concentration.

Chapter 4 compared oceanographic regions in which primary production is typically high, yet seasonal (shelf stations), with a region where primary production is consistently low (i.e. the HNLC region of the subarctic NE Pacific). As the relative importance of phytoplankton in the diets of marine copepods varies among these regions, future attempts to relate food concentration to juvenile copepod growth and development rates should consider using particulate organic nitrogen and/or carbon as a food proxy (i.e. Huntley and Boyd 1984) rather than chlorophyll *a*.

Variation of *in situ* copepod growth rates is often cited as one of several factors contributing to the observed spatial patterns of copepod biomass. However, to my knowledge, no studies have directly addressed spatial autocorrelation with respect to production rates. Chapter 4 examined spatial patterns of biomass production rates at relatively large spatial scales and across oceanographically distinct regions. However, in the future, direct estimates of copepod community production rates made at a relatively high spatial resolution in a well-studied region (e.g. the shelf off the West Coast of Vancouver Island) may be used to address (i) the relative importance of production rate to spatial patterns of biomass and (ii) the optimum spatial scale at which studies of secondary production should be conducted.

5.4 Future directions and conclusions

There is a growing trend in biological oceanography to consider functional-types and ecosystem-scale processes (e.g. Legendre and Rivkin 2005; Le Quere et al. 2005). Although population-level studies will remain necessary, community-level processes must be addressed where possible if we are to gain greater insight into ecosystem scales of response. To date, most ecosystem-level studies have focused primarily on microbial groups (i.e. unicellular autotrophic and heterotrophic producers). Compared to mesozooplankton, these groups are easier to sample and their metabolic rates have been routinely measured at the community level using well established methods (e.g. ^{14}C for primary production and ^{3}H for bacterial production). Establishing a comparable approach for measuring mesozooplankton production rates presents opportunities to address the role of marine copepods at an ecosystem scale in conjunction with these better studied communities.

We now recognize that most copepods are not obligate herbivores (i.e. Sommer et al. 2002) and that their direct grazing impact on phytoplankton represents <10% of global primary production (Calbet 2001). The relative composition of the copepod diet (i.e. heterotrophic versus autotrophic), and how that diet varies in space and time, has a direct influence on the efficiency of energy (carbon) transfer within a given ecosystem. Considerable interest has been devoted to this area of late, with most studies focusing on the use of stable isotopes and fatty acids as tracers and proxies for diet composition. Although such approaches represent necessary tools for defining trophic interactions (e.g. Gurney et al. 2001), they do not provide quantitative estimates of the rate of trophic transfer. Thus, incorporating direct estimates of marine copepod growth rates in feeding studies (e.g. Dam et al. 1994) represents a quantitative means of characterizing the variation and regulation of ecological efficiency in different ecosystems.

Since marine copepods represent the major intermediaries between higher and lower trophic levels, *in situ* estimates of copepod mortality are of considerable interest. Field studies and syntheses (Hirst and Kiorboe 2002) indicate that the greatest source of copepod mortality is predation. However, *in situ* estimates of copepod mortality rates are even less common than are field estimates of development and growth rates. Unfortunately, the use of horizontal life-table approaches (i.e. following a defined cohort through time) is rarely applicable in marine systems because we cannot easily account for the potentially confounding influence of advection. Vertical life-table approaches

(Asknes and Ohman 1996) may offer one solution to this issue. However, most studies using the vertical life-table approach have relied upon temperature-based estimates of stage duration (i.e. Behelradeks relationship), which does not account for other sources of variation in developmental rate (i.e. food limitation). Thus, accurate estimates of copepod mortality also demand direct *in situ* estimates of stage duration and stage distribution.

Application of the chitobiase method may be used to estimate the *in situ* rates of development necessary for mortality estimates. However, such estimates would be representative of the species dominating a given size class, rather than species-specific stage-based estimates. Thus, mortality estimates generalized to an entire copepod community would likely obscure species-specific or group-specific patterns (i.e. broadcast versus sac spawning copepods). Nevertheless, the use of the chitobiase method in conjunction with vertical life-table methods may provide a rapid means of characterizing how patterns of copepod mortality are related to temperature, size class and food availability and how they vary at large spatial scales.

The objective of this thesis was to develop and test a routinely applicable estimate of copepod production, development, and growth rate that is broadly applicable and sensitive to variation of these rates *in situ*. This thesis constitutes the development, validation, and first field applications of such a method. The results of the field applications (Chapters 3 and 4) of this method are quite promising and suggest that this method is broadly applicable and sensitive to variation of juvenile copepod production rates in the field. The continued refinement and application of this method under a wide variety of environmental conditions may be used to determine how copepod production rates are regulated in the field, and to gain greater insight into how variation of these rates influence trophic transfer processes in marine food webs.

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