NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

STUDIES ON FACTORS AND MECHANISMS DETERMINING INTRAPOPULATION SIZE VARIATION OF MARINE CALANOID COPEPODS

by

Ruben Escribano

A thesis submitted to the
Faculty of Graduate Studies of
Dalhousie University in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
Department of Biology
August 1990

ISBN 0-315-64582-2

CONTENTS

TABLES

TABLE

FIGURES

FIGURE

Abstract

Temporal and spatial variation of body size within copepod species is well-known to be associated with variability of temperature and food supply.

The study of patterns of growth, development and changes in body size of various species reared in the laboratory, allows an interpretation of the mechanisms regulating copepod size. Under satiating food, growth in body length was nearly linear and growth in body mass varied with species. Calanus finmarchicus with variable lipid contents showed exponential growth in total dry weight (TW) and lipid discounted dry weight (SW). In C.glacialis exponential growth was obscured by delayed development of late stages. Eurytemora herdmani with no visible lipids showed a clear exponential growth in TW.

Temperature and food affected body length and body mass differently, thus altering body density. Food effect was more pronounced than that of temperature, and it was expressed in lipid content, influencing TW but not SW. It was hypothesized that food and temperature influence body size by altering timing of development and hence growth rates. Within treatments there was a weak correlation or lack of it between stage durations and body size, suggesting again that timing of development, but not size itself, regulate growth and development rates, which in turn determine size variations.

Analysis of nuclear DNA content of 3 species subjected to food-temperature treatments suggested that genome size has a fundamental role in controlling development rates and indirectly body sizes. There were significant food-temperature effects on nuclear DNA, associated with effects on body size. Body size was negatively correlated to nuclear DNA contents in two of the three species. A negative correlation between nuclear DNA content and development rate in C.glacialis suggested that the role of genome size is more directly related to cell development rates rather than to cell size.

ACKNOWLEDGEMENTS

It is a pleasure to thank my supervisor Dr. Ian McLaren for his guidance and support throughout this study. His encouragement, dynamism and continuous stimulus were most valuable.

I would like to express my recognition and thanks to Dr. Trevor Platt for giving me the opportunity of coming to Nova Scotia and undertake graduate studies. The time working in his lab at BIO was an important part of my preparation for doing research at Dal.

Friendly help and advice from Dr.J-M Sevigny was always welcome during the initial stage of my research.

Finally, I thank to a few good friends who in one way or another have supported me during my four years at Dalhousie.

The research was supported by NSERC grants to Ian McLaren. My graduate studies were completed with support from ICOD (International Centre for Ocean Development) and with the support of a Dalhousie Graduate Fellowship.

CHAPTER 1

GENERAL INTRODUCTION

1.1. Calanoid copepods and secondary production in the ocean

In the marine plankton, copepods have been traditionally viewed as a crucial link between primary producers and economically important fishes (Edmondson et al. 1962, Cushing 1975, Waters 1977). This view has prompted and justified numerous studies of their biology, with notable emphasis on production dynamics (Tranter 1976, Greeze 1978, McLaren 1978, Corkett and McLaren 1978, McLaren and Corkett 1981, Mills and Fournier 1979, Johnson 1981, Tremblay and Roff 1983, Kimmerer 1987, Davis 1987, McLaren et al. 1989d).

Measurements and modelling of plankton production can be approached at different levels. Holistic approaches, such as use of trophic levels biomass (Riley 1947, Steele 1974, Steele and Henderson 1985), attempt to simplify inherent complexity of ecosystems, although such simplifications cannot be evaluated without understanding of the complexity (see Davis 1987).

Alternatively, some size-related functions have been used to construct predictive models of production. Since animal growth is allometric in form (Huxley 1932, Humphrey 1979, Smith 1980, Peters 1983), it follows that physiological rates are also allometrically related to body size. This view has

stimulated the application of size-dependent models for estimates of copepod production (e.g. Mills and Fournier 1979, Tremblay and Roff 1983, McClatchie 1987).

Another different and most complex approach to production, is through population dynamics (e.g. McLaren 1978, Kimmerer 1983, 1987, Davis 1987, Aksnes and Magnesen 1988, Middebrook and Roff 1986, McLaren et al. 1989d). This method involves analysis of life cycles and production of cohorts for single species. Despite the great amount of work required, it has been proposed as the only way to understand processes controlling species populations and their flux of energy (Davis 1987), and to obtain reliable estimations of production rates (McLaren et al. 1989d).

1.2. The importance of copepod size

Either for use in size-dependent equations or for the analysis of single populations, quantitative methods require information on body size, which can be measured in a variety of ways, such as: body mass, energy content, chemical content, or body length. It is evident that accuracy and predictiveness of methods of estimating production will be greatly improved with clearer definitions of those size components that are significant for production, and with a better understanding of the control of these components. Therefore, basic knowledge of the mechanisms influencing sizes of organisms seems crucial to test reliability of such methods, and particularly to

determine if size-dependent equations offer an adequate approach to system production (see Levins 1966).

Firstly, at the species level, there is a need to explain how size variations develop among quite distinct generations in the same populations, and secondly, it is necessary to clarify the relative importance of different components of size and to understand their relationships, such that different ways of measuring body size can be standarized or interchangeable.

Secondly, understanding how size variations develop may also be important in the context of evolution of body size. For instance, body size is often considered to respond adaptively to changing conditions (Haldane 1928, Roff 1981, 1986). Among species, as a consequence, size is involved in "r and k selection" (MacArthur and Wilson 1967, Pianka 1970).

1.3. The influence of temperature and food on body size

In copepods, body size at a certain developmental stage must result from two physiological processes, development and growth. According to much field and experimental evidence, these rates are both influenced by environmental temperature and food availability. For example, it is well-known that adult body size of calanoid copepods is negatively correlated to environmental temperature under natural (e.g. Deevey 1964, McLaren 1963) and experimental conditions (e.g. Heinle 1969, Lock and McLaren 1970). As a general explanation, it might be

assumed that instar duration determines the amount of time available for somatic growth, and because temperature affects this duration, body size is inevitably influenced by temperature as well. However, the situation is not this simple, because temperature also affects growth rate. Furthermore, interactions between body size and temperature also affect temperature dependence of growth rate, such that growth rate becomes independent of temperature as body size increases (e.g. Diel and Klein-Breteler 1986, Vidal 1980a). Thus, temperature would affect growth rate differentially depending on developmental stage and on body size. Another complication comes from the occurrence of resting stages which remain without growing or moulting for extended periods of time (e.g. McLaren 1978, McLaren and Corkett 1986). These resting animals usually occur in older stages, and are attributes of particular species without clear connection to body size.

Although there is controversy about the importance of food as influencing development, growth and hence body size of copepods in nature (e.g. Huntley and Boyd 1984 for discussion), laborious rearing experiments in laboratory (e.g. Klein Breteler et al. 1990), and some observations in the field (e.g. Diel and Klein Breteler 1986), suggest that food availability may be a potential factor affecting size variations. The extent to which such suggestion can be

generalized to natural conditions is still, however, matter of controversy.

Despite the above complications, there are some clues to begin with. In copepods, for instance, final body length results from discrete increments between moults and final body weight from a continuous growth of somatic tissue throughout the stages. Thus, information about temperature and food effects on both growth in body length and growth in body mass might give insights on how size variations develop.

1.4. Genome size and body size in copepods

In closely related species of calanoid copepods some evidence suggests that the same developmental stages might have similar numbers of nuclei (e.g. McLaren and Marcogliese 1983). Determinate nucleus number is even more likely to be found within species. This implies that, given a constant number of cells, variations in body size might result from different cell sizes. This hypothesis, once tested, pushes us to understand how or why cells change in size. In this context, it has been found that the amount of nuclear DNA or genome size presents a great variation among and within species (Mirsky and Ris 1951, Grime and Mowforth 1982, Cavalier-Smith 1985 for review), and that this amount is positively correlated with cell size (e.g. Commoner 1964, Shuter et al. 1983). A negative relationship between cell size and development rate has been reported as well (Bier and

Müller 1969, Bennett 1971). According to Cavalier-Smith (1978, 1985), the cell volume of an organism results from a compromise between conflicting selection for large cell size and for rapid development.

In copepods, this process of adjusting cell size can be hypothetized to be a response to environmental temperature, and this might explain the observed negative correlation between body size and temperature. This process, however, might also be affected by availability of food, since rearing experiments and field observations have shown significant effects of both food quality and food quantity on copepod size (e.g. Klein-Breteler and Gonzalez 1982, Diel and Klein-Breteler 1986, Klein Breteler et al. 1990).

A positive relationship among body size, cell size and genome size might be obscured by body size variations independently from cell size (Cavalier-Smith 1978). Body size may also change by altering cell numbers, and it is not known if size variations induced by temperature and/or food supply result from modified cell sizes or cell numbers. These questions are certainly relevant for a more mechanistic explanation of size variations.

1.5. Scope of this study

In the present study, I intend first to analyze patterns of growth and development of several calanoid species under controlled conditions of food quantity and temperatures, and

how these conditions might influence body weight and body length. Then I explore cellular mechanisms involved in size variation, in order to achieve a deeper understanding of environmental effects on size of marine calanoid copepods.

The thesis has been organized in four major topics. In Chapter 3, copepod size is defined in terms of different measures, for which the magnitude of variance within and among species is analyzed. Chapter 4 describes the course of growth and development during which variation of these measurements of size takes place. Chapter 5 analyses the role of temperature and food quantity in influencing the course of size variations. Finally, Chapter 6 is an attempt to find explanations on size variations at the cellular and molecular level.

CHAPTER 2

GENERAL METHODS AND MATERIALS

2.1. Species studied

Five species have been considered in this research: Calanus glacialis , C.finmarchicus, Pseudocalanus acuspes, P.elongatus and Eurytemora herdmani. These species were selected under the following criteria: a) availability of samples, b) coverage of a relatively broad size range, and c) the species have clearly known and distinct life cycles. It was hoped that these several species might reflect general patterns for the whole calanoid group.

2.1.1. Calanus glacialis

Initially considered as a large form of C.finmarchicus, C.glacialis was described as a new species by Jaschnov (1955), who suggested that it replaced C.finmarchicus in arctic waters. More recent work has demonstrated that despite their morphological similarities, the two species show clearly distinct genetic patterns and genome sizes (McLaren et al. 1988, Sevigny and McLaren 1988).

2.1.2. Calanus finmarchicus

This copepod was once considered as one of the most abundant planktonic species in all northen seas (Wilson 1932,

Grainger 1961), but is now known to be confined to the North Atlantic region (Frost 1974). It is also viewed as the dominant grazer and secondary producer in north Atlantic waters during spring and early summer (Fransz and Diel 1985). It is perhaps the most studied copepod species (Marshall and Orr 1955). It is distinguished from C.glacialis most readily by its smaller size.

2.1.3. Pseudocalanus acuspes

This is a widespread arctic species which is present as a relict in Bedford Basin, Nova Scotia (Frost 1989), where its life cycle has been fully described (McLaren et al. 1989a).

2.1.4. Pseudocalanus elongatus

It is found throughout the temperate eastern North Atlantic Ocean (Frost 1989). Specimens available for this study were originally captured in the Wadden Sea and cultivated in the laboratory for more than 70 generations (Klein Breteler et al. 1990).

2.1.5. Eurytemora herdmani

This species is widely distributed in both coastal and estuarine waters in the northen hemisphere (Heron 1964, Johnson 1966). It shows a more-or-less continuous development of cohorts throughout the year (McLaren 1976) and may play a significant role in estuarine food chains as a contributor to secondary production (Katona 1970).

2.2. Sampling

Live animals were obtained from Nova Scotian waters in 1987, 1988 and 1989, at three locations in Bedford Basin, off Chebucto Head and in the North West Arm (**Fig.2.1**).

Depending on species and stages, 3 types of plankton nets were used: 74 µm mesh for early stages of all species, 260 µm for adult and copepodite stages of E.herdmani, and 450 µm for adult and copepodite stages of P.acuspes and Calanus species. Vertical and oblique hauls were made from various depths. In Bedford Basin, P.acuspes were usually found between 30 and 50 m depth, at Chebucto Head, Calanus species were captured between 20 and 40 m, whereas E.herdmani, were usually obtained between 5 and 10 m in the North West Arm.

Inmediately after capture, samples were diluted in 30 l coolers with water obtained from the surface at the same sampling location. Within 2 hours of sampling, the coolers were placed in a coldroom at 3 ^{0}C. Sorting and identification of species and developmental stages was usually performed in coldrooms at 3 ^{0}C or 5 ^{0}C.

2.3. Culture conditions

Rearing experiments were carried out in temperature-controlled coldrooms. Animals were fed with algal cultures of different species and different quantities depending on copepod stages and treatments. Light conditions were kept constant, usually at low intensities or complete darkness.

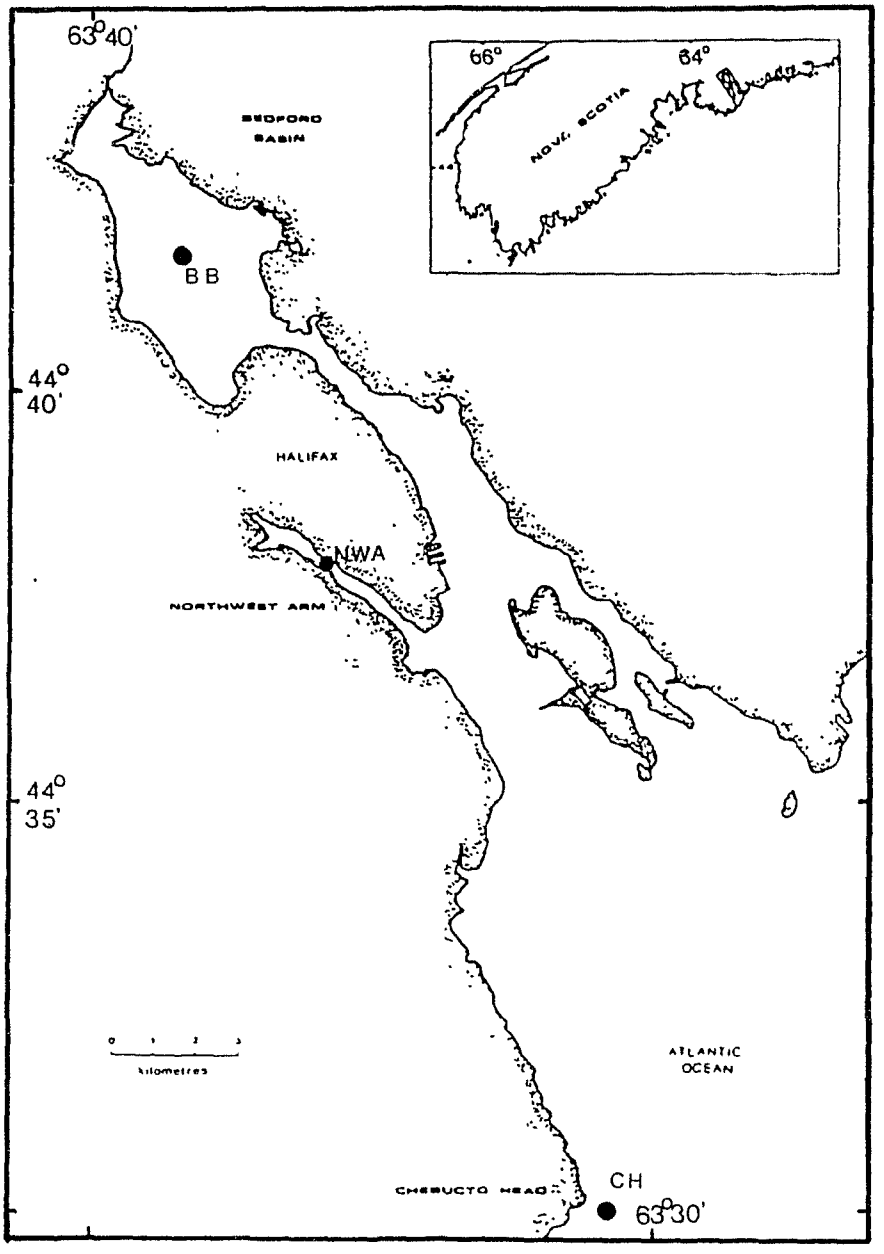

Fig.2.1 Map showing sampling locations. BB: Bedford Basin P.acuspes, NWA: Northwest Arm E.herdmani and CH: Chebucto Head C.glacialis and C.finmarchicus.

2.3.1 Temperature conditions

Depending on the experimental design one or two near-constant temperatures were used, selected from temperature range experienced by the species in nature (**Table 2.1**). This variable was usually measured daily to the nearest 0.1 ^{0}C. Since in some experiments many containers were used (up to 60 at the time), two thermometers were located in different positions to detect possible gradients in temperature. No such temperature differences were observed, but containers were nevertheless frequently rotated in position to avoid undetected gradients.

2.3.2. Food conditions

Some initial rearing experiments were performed under excess conditions of food. Although no measurements of food concentrations were obtained in these experiments, estimations from similar levels of food in later experiments allowed them to be approximated.

Three phytoplankton species were used as food: Isochrysis galbana (3.3-5.8 µm diameter), Thalassiosira weissflogii (ca.15 µm diameter) and Rhodomonas lens (6.3-12.5 µm diameter). These species were reared in the laboratory in F/2 medium according to the method of Guillard and Ryther (1962).

Estimations of algal concentration were performed through cell counting under a compound microscope. This procedure was carried out only in those experiments which involved more than

Table 2.1 Summary of recorded temperatures for rearing experiments in C.glacialis (C.g.), C.finmarchicus (C.f.), P.acuspes (P.a.), P.elongatus (P.e.) and E.herdmani (E.h.). Nominal temperature is the intended value and actual mean with corresponding S.D. is the observed one.

Species	Experiment duration (d)	Nominal temp. (^{0}C)	Actual mean temp. (^{0}C)	S.D.
C.g.	56	9	8.4	0.12
C.g. + C.f.	76	10 & 3	9.7 & 2.8	0.15 & 0.20
C.g. + C.f.	47	10 & 3	9.8 & 3.2	0.25 & 0.21
C.g. + C.f.	135	12 & 3	12.2 & 3.2	0.31 & 0.45
P.a.	12	10 & 3	10.1 & 2.8	0.13 & 0.23
P.a.	80	10 & 5	10.2 & 5.1	0.18 & 0.35
P.e.*	-	15 & 8	-	-
E.h.	29	10	9.2	0.32
E.h.	57	10 & 3	10.2 & 3.0	0.21 & 0.24

* Details for rearing conditions of this species are given in Klein Breteler and Gonzalez (1986).

one level of food. From the culture medium three aliquots of 10-20 µl (by analytical micropipette) were placed on slides and covered with coverslips. After a few minutes the flagellates ceased to move, so that the total number of cells, irrespective of species, could be counted using 40x of magnification.

Some additional information on food concentration was obtained by measuring chlorophyll concentration using a Turner fluorometer (Yentsch and Menzel 1963). Since the ratio chloropyll/carbon changes in great extent depending on physiological condition of cells (Eppley 1972), these measurements were only used to detect deteroriation of food under conditions of low temperature and darkness. For all the experiments involving effects of food, there were two basic levels of food - a high level which was assumed to be in excess of requirements for maximal growth and development rates and a low level which was usually 1/16 fraction of the high one.

In all experiments the medium containing fresh food was changed every 2-3 days. Measurements of chlorophyll concentration indicated that after 3 to 4 days the algae suffered severe deterioration. **Table 2.2.** shows a summary of food levels used.

The relative numerical composition of culture media was, for I.galbana, T.weissflogii and R.lens, approximately 2 : 1 : 1 respectively, for stages CI to adult in all species,

Table 2.2 Summary on food levels used for rearing experiments of C.glacialis (C.g.), C.finmarchicus (C.f.), P.acuspes (P.a.) and E.herdmani (E.h.). Numbers indicate the average for the total number of cells regardless of phytoplankton species.

Species	stages	food level	food (cells/ml x 10^5)	chlorophyll (ug/l)
C.g. & C.f.	CIII-Ad.	High	3.2 - 5.1	12 - 38
		Low	0.2 - 0.3	2 - 8
P.a.	NI-NVI	High	2.2 - 3.1	n.a.
		low	0.1 - 0.2	n.a.
E.h.	NI-NVI	High	2.2 - 3.1	n.a.
	CI-Ad.	High	3.2 - 5.1	n.a.

n.a. = not available; for adult P.a. the same food levels as that of C.g. and C.f. were applied.

whereas for stages NI to NVI these proportions were 1 : 1 of I.galbana and R.lens respectively.

2.3.3. Experimental containers

Two types of experiments were performed: 1) animals in individual containers whose volume was 20 ml for E.hermani and 100 ml for C.glacialis and C.finmarchicus; 2) mass experiments with 1l glass beakers for P.acuspes containing 20 - 100 individuals and 2l glass beakers for C.glacialis and C.finmarchicus, containing 20 - 50 individuals. In mass experiments 2 - 3 beakers for each treatment were used.

In order to obtain newly hatched nauplii from P.acuspes and E.herdmani, about 50 mature females were placed in 2l glass beakers under excess of food (as shown in **Table 2.2**) at 10 ^{0}C. After 3 - 5 days cultures were transfered to large glass dishes from which nauplii could be removed under the microscope using fine pipettes. Nauplii were then placed in fresh medium in other containers, usually 0.5 to 1l glass jars.

2.4. Observations and measurements

Rates of development were obtained by observing changes in developmental stages in animals kept in individual containers. Depending on species and temperature, the frequency of observations ranged from 4 to 24 hours. The highest frequency of observations was applied to rapidly

developing E.herdmani at 10 °C, while the lowest one was applied to very slowly developing older stages of C.glacialis at 3 °C. The mean time between two consecutive observations, after finding a molted individual, was used as molting time. This allowed estimations of development rates with an error of no more than 5% .

Changes in body length were obtained by measuring prosome lengths to the nearest 0.01 mm under the microscope using a micrometer scale. Experimental animals were removed from their beakers with glass pipettes, placed on a concave slide with a small amount of water, quickly measured while they rested on one of their sides, and returned to their beakers. A similar procedure was applied to the oil sac in Calanus species.

In order to obtain dry weights, individual animals were first quickly rinsed in distilled water then placed in minuscule, pre-weighed aluminum pans. These pans were dried at 70 °C until constant weight (after 1 - 2 h.), measured to the nearest 0.1 µg using a CAHN electrobalance model G-29. In some cases, before rinsing with distilled water, the animal was squeezed with fine forceps until no lipids could be seen in its body; then the surrounding water containing the lipids was suctioned with a fine syringe. This procedure allowed estimations of lipid-free dry weights.

2.5. Estimation of nuclear DNA content

Estimations of nuclear DNA contents were carried out according to methods described in Robins (1978), Robins and McLaren (1982) and McLaren et al. (1988). Animals were first fixed with ethanol-acetic acid 3 : 1, softened in 50% acetic acid, squashed on randomly numbered slides and then stored in a dessicator for later analysis of DNA. Batches containing 10 or 20 slides and 1 or 2 chick (Gallus domesticus) blood standard were subjected to hydrolysis with HCl 5N at 20 ^{0}C for 45 minutes. Then, Feulgen staining was performed for 2 hours and the squashes mounted in Cargille inmersion oil (refractive index 1.568).

Microspectrophotometry was performed to estimate nuclear DNA contents according to the following procedure: measurements of absorbance for 10 nuclei of each sample and 15 nuclei for each standard were obtained at 570 nm using a scanning microspectrophotometer (Carl Zeiss UMSP-I). Unsystematic movement through the slide was the principal criterion for selecting nuclei. However, selected nuclei had to meet the following requirements: they had to be sufficiently isolated to allow the scanning, they had to show no indications of damage or material losses and they should not have been highly condensed with absorbances of more than 90%, because such highly condensed nuclei underestimate actual values of absorbance (Ruthmann 1970). Assuming a linear relationship between absorbance and DNA, integrated values of

absorbance were converted to amounts of DNA using mean values of chick blood standards (2.5 pg of DNA per nucleus, Rasch et al. 1971).

Following the method for DNA analysis, whole animals were stained with Feulgen, teased in small fragments and then squashed in Farrants medium on randomly numbered slides. These slides were used to estimate the total number of nuclei by counting under the microscope. The total number of nuclei was counted through systematic movement of the slide. Sources of error might involve underestimations due to sometimes dense aggregations of nuclei, or overestimations due to fragmentations of nuclei. Loss of material during preparation of slides is another potential source of error.

2.6. Statistical methods

Data were usually analyzed through parametric statistical tests. Analysis of variance (ANOVA) and covariance (ANCOVA) were applied based on normal distribution of variables. Normality was examined through graphical methods and tested with Kolmogorov-Smirnov tests for goodness-of-fit for continuous variables, while homogeneity of variance among treatments was tested with the Barlett test. Log-transformations were applied in some cases.

To analyse growth curves and other functional relationships, linear or non-linear models were fitted with least squares methods and tested for goodness-of-fit in most

variation. Estimations of means and parameters are usually given with either standard deviations, standard errors or 95% confidence limits.

For hypothesis testing, 5% level of significance was assumed as statistically significant and denoted as **. Highly significant results are considered to be with levels of significance 1% or smaller and they are indicated as ***. Non significant results (P >.05) are indicated as n.s.

Data analysis and graphical representation were performed with statistical softwares for microcomputers SYSTAT, STATGRAPHIC and SIGMAPLOT.

CHAPTER 3

BODY SIZE AND ITS COMPONENTS IN CALANOID COPEPODS

3.1. Introduction

Body size is frequently viewed as an important component in physiological studies of poikilotherms. Size is usually correlated with reproductive success (e.g. Peters 1983) and it may influence physiological rates, such as respiration (Ikeda 1970), development and growth (Vidal 1980a, 1980b), and assimilation rate (Dagg 1976, Lampert 1977).

However, there are various ways of studying body size. In the context of size variations, it may be analyzed as body volume, as linear dimensions of the body, as body mass, as energy content, or as specific chemical components. Selection of the method, or of a particular measurement, may depend on the objectives of specific studies. It is desirable, however, that these measurements and their units be interchangeable, so that comparative studies can be undertaken. These measurements will be referred here as different components of body size.

Variations of copepod size in all its components have long been noted in the literature. Published information, however, deals mostly with description of temporal or regional variation in one or two components (e.g. Bogorov 1934, Deevey 1964, Comita et al. 1966). Little information is available on the relative importance of these components, how they covary

and how they relate to each other. This information is certainly valuable for studies of copepod physiology and production.

Furthermore, data on body size are generally obtained from subsamples containing a few to hundreds of individuals, so that overall means or modes for groups of individuals are reported in the literature, with the consequence that much information on individual variation is being lost. Obviously, patterns of variation within populations or cohorts might be relevant to understanding mechanisms of size variations.

In this study, measurements and estimations of some body components of copepods have been analyzed. All measurements have been obtained from individuals, so that emphasis is given to individual variation of size components. The information given here is based mainly on measurements of body length, dry weight and estimations of the size of the lipid store obtained from rearing experiments. Details on rearing conditions are given in General Methods and Materials; in brief animals were subjected to different treatments of temperature and food levels. Although these treatments did not represent the wide range of conditions in nature, it is hoped that variability in the experimental data will be comparable to some field situations. In any case, the differences from natural conditions will be discussed, as well.

Firstly, different size components will be treated separately, their magnitude of variation analyzed and compared

to values found in nature; and secondly these components will be be related to each other through different mathematical functions.

3.2. Body length

Copepods are small, from less than one to a few mm in length. The body is usually short and cylindrical. The body is composed of five cephalic, six thoracic and five abdominal segments. The head is usually fused with the first thoracic segment forming a unit named the prosome (often called cephalothorax, although this is inappropiate for cyclopoids in which an abdominal segment is incorporated). The abdomen, often called urosome, is narrow, cylindrical and devoid of appendages.

Although there are various ways of measuring and analysing copepod body lengths (see Barnes and Barnes 1953), total length of the body, as noted by Marshall and Orr (1955), is not recommended. The abdomen is often bent and sometimes there is stretching or shrinking between prosome and urosome under the effects of fixation. Therefore, prosome length, is the preferred and most common measurement of copepod length. In the present study, this was measured and only live or recently killed animals were used.

3.2.1. Variations in body Length

Prosome length of adult females reared in the laboratory under various conditions of food and temperature were obtained for 5 species (see **Table 3.1**).

Within species, prosome length of adult females is considerably variable, as shown by coefficients of variation (C.V.) in **Table 3.1**. Magnitudes of variation, as estimated by C.V.'s, show no apparent relation with mean size of species, nor with sample size. The two Pseudocalanus species appear to be the most variable ones, while the others show lower and similar values of C.V. These magnitudes of variation or even greater are also frequently observed in natural conditions. Seasonal patterns of size variation are detected in same locations or among different regions (e.g. Deevey 1960, 1964). It is well-known that size distributions change on a seasonal scale in multivoltine species of copepods. Indeed, these patterns of variation constitute the basis for identifying cohorts in studies of life cycles and production (e.g. McLaren et al. 1989d).

Prosome lengths in **Table 3.1** are, in most cases, in agreement with those obtained from field samples. Marshall & Orr (1955) reported a size range of ca. 2.7 - 5.4 mm for Calanus finmarchicus, but as demonstrated later (Jaschnov 1955) this range probably included both species, C.finmarchicus and C.glacialis. Length range for C.finmarchicus from various locations is ca. 2.1 - 3.2 mm

Table 3.1 Summary of statistics for prosome length of adult females Calanus glacialis (C.g.), C.finmarchicus (C.f.), Pseucalanus acuspes (P.a.), P.elongatus (P.e.) and Eurytemora herdmani (E.h.), reared under laboratory conditions. S.D = standard deviation, C.V. = coefficient of variation, and n = number of individuals.

Species	mean prosome length (mm)	n	Range	S.D.	C.V. %
C.g.	2.892	40	2.625-3.200	0.163	5.64
C.f.	2.538	36	2.031-2.813	0.163	6.42
P.a.	0.901	68	0.725-1.125	0.081	8.99
P.e.	0.876	60	0.680-1.120	0.109	12.44
E.h.	0.764	69	0.650-0.850	0.046	6.02

(Clarke and Zinn 1937, Grainger 1961, Conway and Minton 1975). The larger C.glacialis, on the other hand, shows a size range between 3.4 - 4.3 mm (Jaschnov 1955), which is greater than the size range found in these experiments.

Reported size ranges for P.acuspes and P.elongatus are ca. 1.03 - 1.30 mm (Frost 1989, McLaren et al. 1989c) and ca. 0.83 - 1.55 mm (Frost 1989, McLaren et al. 1989c, Conway and Minton 1975) respectively. Mean values from rearing experiments fall within these ranges (see **Table 3.1**). Mean prosome lengths of females E.herdmani obtained in the laboratory also fall within a size range observed in the field, ca. 0.6 - 0.9 mm (see McLaren and Corkett 1981).

3.3. Body mass

Body mass can be considered a basic measure of body size. In copepods, however, measuring body mass usually means the use of large samples and replicates. This because of a high variance even among individuals of similar body lengths, probably due to nutritional conditions, stage in molt cycle, and reproductive status of animals (e.g. Durbin and Durbin 1978, Tessier et al. 1983, McLaren 1986), but also because estimations of body mass present several technical problems and available methods are not always consistent in comparative studies (Winberg 1971, Beers 1976 for reviews).

Body mass can be estimated as wet weight, dry weight, ash-free dry weight or as content of nutritionally important

elements, such as carbon or nitrogen. Because of a low resolution, due mainly to highly variable content of body fluids, wet weight is generally used only for large samples of zooplankton (Beers 1976). Dry weight is more frequently used for small samples and for single individuals, although it includes parts of the body that might not be important in terms of what has been named, the metabolically effective body weight (Brody 1964). Alternatively, ash-free dry weight and carbon or nitrogen content are recommendable for physiological and trophic studies (Paine 1964).

To obtain information on body mass, I adopted two approaches. 1) Dry weight was chosen as the best integrated measure of individual variation of body size in all its components, regardless of nutritional or physiological status. 2) Because it is well-known that some species contain highly variable amounts of lipids (Corner and O'Hara 1986 for recent review), body mass in the two Calanus species was divided into two components (Harris 1983), total dry weight (TW) and "structural dry weight (SW)". The TW and SW for Calanus spp. were obtained as described below, whereas adult female and male E.herdmani were analyzed only in terms of total dry weight.

3.3.1. Total and structural body mass

According to Harris (1983), the body of a copepod can be divided into two components, "store" and "structural copepod".

In Calanus species, this "store" can be assumed largely to be in the non-structural lipid store, which is an important fraction of the total biomass in older stages of development (e.g. Marshall et al. 1934, Kattner and Krause 1987).

In Calanus species most lipids are stored in a structure termed the "oil sac". Therefore, removing the lipids from this oil sac and weighing the rest of the animal approximates the structural weight (SW). Most lipids in the oil sac can be removed by squeezing the animal with fine needles and forceps (McLaren 1986). Using this technique (see General Methods and Materials), data on SW were obtained for copepodids stages CIII, CIV, CV and adults of C.glacialis and C.finmarchicus. Total weight (TW) was obtained from intact animals.

A summary of data on body dry weight for the three species is shown in **Table 3.2**. As expected, dry weight expressed either as TW or SW is highly variable as indicated by values of standard deviations and coefficients of variation (C.V.), which can reach values up to 40% in some stages. This variability is expressed in all stages analyzed, not showing any clear trend through development in either species.

Ratios of SW to TW for same stages vary from 1 : 1.4 at CIV to 1 : 2 at CV in C.glacialis, and 1 : 1.2 at adulthood to 1 : 1.9 at CIV in C.finmarchicus. The slightly lower ratios in C.finmarchicus suggest that this species accumulates proportionally less lipid, as compared to C.glacialis.

The mean values of adult TW of the larger C.glacialis are ca. 2.2 times greater than for C.finmarchicus. This factor is reduced to ca. 1.5 if the two species are compared in terms of SW. This indicates that, at least in adults, differences in body mass between the two species are partially explained by proportionally greater amounts of stored lipids in C.glacialis.

The small E.herdmani, on the other hand, does not accumulate substantial amounts of visible lipid stores, although TW also shows a high variance (see **Table 3.2**), especially in females, probably due to differential amounts of reproductive material. Using mean values of TW, females appear to be ca. 1.5 time heavier than males.

Comparisons of data for body mass, here obtained, with those from the field can be done mostly in terms of TW, since no information on SW from field samples has been reported, with the exception of some data on C.finmarchicus (McLaren 1986). Reported TW's for females of this species from the North Sea varied between 167.3 - 206.1 µg (Kattner and Krause 1987). A similar TW, 192 µg, has been reported from the Nova Scotian shelf (Williams and Lindley 1980), whereas according to Comita et al. (1966, their Table 1), dry weight of C.finmarchicus fluctuates between 117 and 273 µg on a seasonal scale. Mean TW of female C.finmarchicus, in this study, lies within this range (see **Table 3.2**). Mean adult SW of this species, from my rearing experiments, is also close to that

Table 3.2 Summary of total dry weight (TW) and structural dry weight (SW) for different stages of development in 3 species of copepods reared under various laboratory conditions. n = number of individuals, S.D. = standard deviation, C.V. = coefficient of variation.

			TW (µg)			SW (µg)		
Species	Stage	n	mean	S.D.	C.V.	mean	S.D.	C.V.
C.g.	CIII	6	63.55	23.17	36.5	34.20	11.22	32.8
	CIV	20	99.65	17.85	17.91	71.65	22.32	31.2
	CV	22	226.62	67.36	29.78	110.75	37.33	33.7
	Ad.(♀)	10	421.09	91.73	21.78	221.81	94.59	42.6
C.f.	CIII	6	27.93	7.71	27.6	18.87	3.86	20.5
	CIV	7	85.79	34.58	40.3	45.94	18.20	39.6
	CV	10	91.80	27.07	29.4	53.05	10.15	19.1
	Ad.(♀)	12	189.23	55.06	29.1	154.90	29.03	18.7
E.h.	Ad.(♂)	18	6.08	0.85	14.0			
	Ad.(♀)	16	9.51	2.16	22.7			

C.g = Calanus glacialis, C.f.= C.finmarchicus, E.h.= Eurytemora herdmani, Ad.=adults.

observed in field samples (see McLaren 1986, his Fig.1).

Observed values of TW in nature are more variable for the larger C.glacialis. Females averaged ca. 700 µg in the Greenland Sea (Hirche and Bohrer 1987), whereas an average of 968 µg was observed at Spitsbergen (Båmstedt and Tande 1985). These reported values differ differ considerably from the mean TW shown in **Table 3.2.** This is consistent with smaller prosome lengths obtained in this study as compared to those found in the field (as shown above).

Females of E.herdmani, on the other hand, are heavier in my data than those observed in the field. For example, McLaren and Corkett (1981), observed an average value of ca. 3.84 µg in Nova Scotian waters from a summer sample. This value is only about one third of that shown in **Table 3.2.**

3.4. The lipid store

Calanoid copepods are able to accumulate large quantities of lipids in the oil sac which in some cases occupies most of their body cavity (Marshall et al. 1934, Marshall and Orr 1955). Chemical studies on copepods have shown that wax esters are the major components of these lipids (Nevenzel 1970, Lee et al. 1971, Sargent et al. 1977). These wax esters are recognized as important lipid reserve in many species, as they can reach up to 90% of the total amount of lipids (Kattner and Krause 1987). This storage of lipids occurs mainly in later stages of development and it is particularly notable in

species of high latitudes (Gatten et al. 1979). Changes in lipids through development indicate that most of the lipid reserve is utilized for reproduction (e.g. Gatten et al. 1980, Kattner and Krause 1987). In fact eggs contain high levels of wax esters which are used as fuel during naupliar stages (Sargent et al. 1977, Corkett and McLaren 1978). This indicates that amounts of lipids accumulated during older stages may partially determine brood size and survival of early stages. Thus, knowledge of factors controlling the process of storing lipids is certainly valuable in the context of copepod production.

3.4.1. Estimating size of the lipid store

Biochemical studies of lipids in copepods have described relative composition of different types of lipids present (see Corner and O'Hara 1986 for review). Very few works have dealt with quantitative aspects (e.g. Lee et al. 1974) and even fewer with variability in lipid contents (e.g. Sargent et al. 1977, Laberge 1980, Båmstedt 1988, Klein Breteler and Gonzalez 1988). In this study, individual variation has been analyzed by estimating the size of the oil sac which may be considered an index of lipid levels.

In Calanus species the oil sac is clearly visible under a microscope, its general shapes are shown in **Fig.3.1.** In order to estimate the volume of these structure, three linear dimensions were measured: length (L), the diameter in the

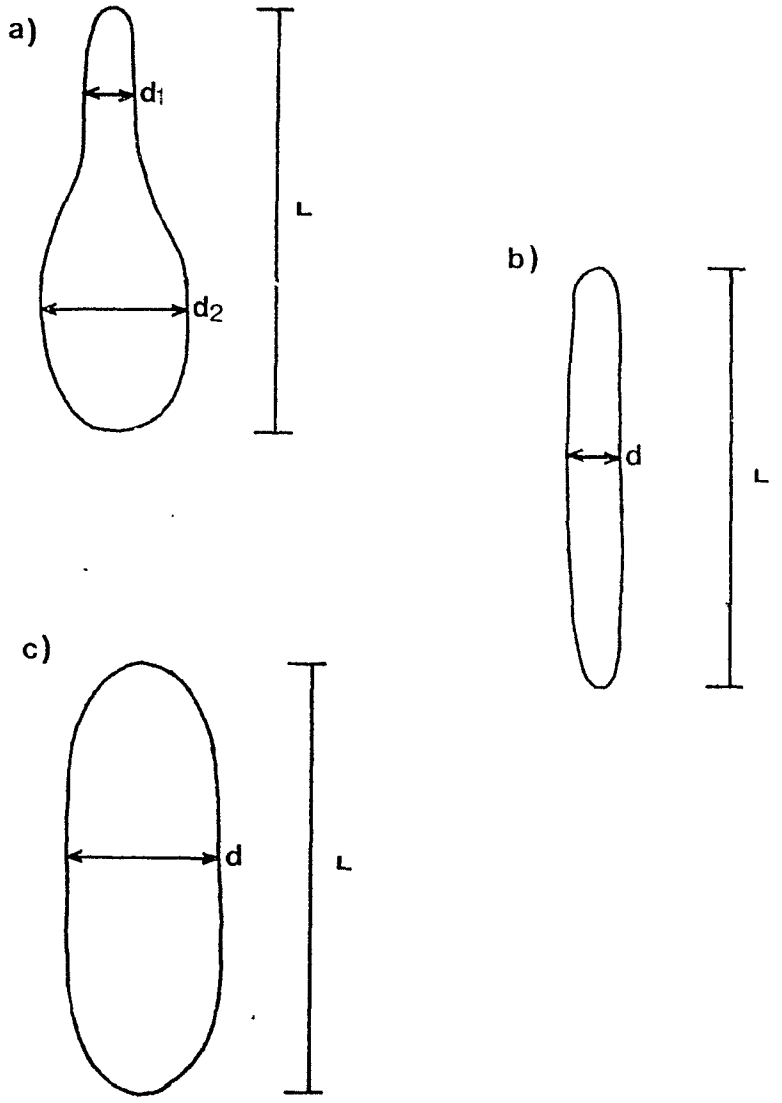

Fig.3.1 General shape of the oil sac in Calanus species showing linear dimensions used to estimate oil sac volume.

widest part (d_1) and the diameter in the narrow portion (d_2). The shape of the oil sac was assumed to approximate an spheroid whose volume (V) can be estimated as, $V = 1/6\ \pi \times L \times d^2$, where L = length, d = diameter. For the oil sac volume, the diameter (d) was assumed to be equivalent to the mean value of d_1 and d_2. Thus volume of the oil sac (OSV) could be estimated as,

$$OSV = 1/6\ \pi \times L \times [(d_1 + d_2)/2]^2 , \qquad (3.1)$$

where L, d_1 and d_2 as defined above, were measured under the microscope at the nearest 0.01 mm. Application of formula (3.1), allowed estimation of oil sac volume for both Calanus species from stages CIII to adult.

Some consistent variations in the shape of the oil sac were observed. At low levels of lipids it was mostly elongated, as shown in **Fig.3.1b**, whereas at high levels the shape was closer to a spheroid (**Fig.3.1c**). I assumed that equation (3.1) accounted for most of this variation in shape.

3.4.2. Size of the lipid store

Estimated volumes of the oil sac varied between ca. 0.00 - 0.30 mm^3 in C.glacialis and ca. 0.00 - 0.15 mm^3 in C.finmarchicus respectively. The lowest values were found in some adults and stage CIII, while highest lipid levels were

present in stages CIV, CV and a few adults. As expected, the animals were highly variable in lipid content, expressed as oil sac volume (OSV). As shown in **Table 3.3**, great variances are observed in all stages of both species, and in some stages C.V.'s exceeded 100%.

The mean values of OSV in C.glacialis are much greater than those of C.finmarchicus, especially in stages CV and adults (**Table 3.3**). The differences in lipid content at these stages agree with differences in total body mass (TW), as shown above. For example, mean OSV of C.glacialis is ca. 3.1 times greater than that of C.finmarchicus at stage CV, as compared to a factor of 2.2 when differences are considered in terms of total dry weight. The difference TW-SW of C.glacialis is ca. 3 times greater than that of C.finmarchicus, which is consistent with differences in OSV.

3.5. Relationships among size components

Many theoretical and empirical equations relating different measurements of body size have been applied in physiological and trophic studies. Although high correlations may be obtained under given conditions, generality and predictability are usually limited, so that new equations are required for other conditions. Although results obtained here are empirical, I will not attempt to suggest such formulas for general application, but rather intend to use mathematical functions to obtain insight on the important determinants of

Table 3.3 Estimated oil sac volumes (OSV) for different developmental stages of Calanus glacialis and C.finmarchicus reared under various laboratory conditions. n = number of individuals, S.D. = standard deviation, and C.V. = coefficient of variation (%).

Species	stage	n	mean OSV (mm^3 $x10^3$)	S.D.	C.V.(%)
C.g.	CIII	12	8.77	6.74	76.9
	CIV	79	23.31	38.87	142.3
	CV	104	62.39	43.62	69.9
	Ad.	19	86.81	85.20	98.2
C.f.	CIII	12	5.74	2.33	40.5
	CIV	14	21.75	19.27	88.6
	CV	17	20.08	24.53	122.2
	Ad.	15	7.73	8.13	105.2

C.g. = Calanus glacialis, C.f. = C.finmarchicus and Ad. = adults.

body size variations and how different components of size are interrelated.

3.5.1. Length-weight relationship

Length-weight regressions have long been used in zooplankton studies, mostly to estimate body mass directly from measurements of body length (e.g. Cohen and Lough 1981, Uye 1982, Durbin and Durbin 1978). To describe this relationship, various equations have been proposed (e.g. Huxley 1932, Gould 1966, for reviews), among which the allometric function has been widely accepted. This function is mathematically expressed as a power equation,

$W = \underline{a}\ X^{\underline{b}}$, where W = weight or body mass, X = some linear measure of organism size, and $\underline{a}$ and $\underline{b}$ are constants. Its log version, $\log W = \log \underline{a} + \underline{b} \log X$, is more widely presented. However, unless both variables are log-normally distributed, the log-log transformation, may lead to misinterpretations in the analysis of data on relative sizes. Although the proportionality of similarities and differences is preserved after log-log transformation, the distributions of the data are altered (Kermack and Haldane 1950) and often result in overestimated coefficients of correlation (Beauchamp and Olson 1973).

In spite of inevitably highly significant coefficients of correlation between weight and length, there is a greater individual variation in body mass than in body length (e.g.

Durbin and Durbin 1978, Båmstedt 1988). This fact may limit predictiveness of length-weight regressions and lead to large errors in estimates of production (Geller and Müller 1985). Moreover, the function may not necessarily be properly described by a power equation (Kankaala and Johanson 1986). For instance, exponential fitting was found to be more appropiate to describe length-weight relationships in Eurytemora copepodids (Heinle and Flemer 1975), whereas a linear equation best described these relationships in Limnocalanus macrurus (Kankaala and Johanson 1986).

Although log-transformation of data has been critized, as noted above, its application is largely advantageous because log-transformed data usually satisfy underlying assumptions of most parametric statistical tests (Beauchamp and Olson 1973). However, tests for goodness-of-fit to a log-normal distribution of both variables should be previously performed to assure such assumptions are met.

Dry weights, measured as TW and SW, and prosome lengths were first tested for goodness-of-fit to log-normal distributions through the Kolmogorov-Smirnov test for continuous frequencies (**Table 3.4**). Only prosome lengths of E.herdmani showed significant departures from a log-normal distribution.

Length-weight relationships for stages CIII to adult in Calanus species and CI to adult in E.herdmani, were tested for goodness-of-fit to linear, exponential and power models.

Table 3.4 Kolmogorov-Smirnov (K-S) values for testing goodness-of-fit to log-normal distribution of total dry weight (TW), lipid-discounted dry weight (SW), dry weight of the oil sac (OSW), and prosome length of 3 copepod species reared at the laboratory under various conditions of food and temperature. Critical values for K-S were obtained as $1.36/\sqrt{n}$, where n = sample size.

Species	n	TW	SW	OSW	Length
C.glacialis	54	.102	.093	.536	.108
C.finmarchicus	55	.093	.123	.179	.127
E.herdmani	84	.105	-	-	.153**

Fitted curves are shown in **Fig.3.2,,** a summary of regression statistics in **Table 3.5** and residual variation after regression analysis in **Fig.3.3.**

Despite the apparently good fits, variation is considerable as shown by 95% confidence limits in **Fig.3.2.** The deviations are particularly large in the two Calanus species, and this may be partially explained by highly variable content of lipids in animals of similar body lengths. Log transformed residuals, however, show a nearly even distribution around predicted values (**Fig.3.3**) confirming the lack of correlation between the variance and the variables. This also confirms the adequacy of data fit to log-normal distributions and hence the validity of regression analysis on log-transformed variables.

Lipid discounted weight (SW) of Calanus species also was log-normally distributed (**Table 3.4**) and its relationship with prosome length was well described by power equations, as well. Fitted curves are shown in **Fig.3.4,** regression statistics in **Table 3.5,** and residual variation in **Fig.3.5.**

3.5.2. Body length and size of the lipid store

On account of the great variance in lipid content in both Calanus species, the relationship between prosome length and oil sac volume, estimated as described above, was analyzed. It was hoped that body size might account for some of the variability in OSV. In this analysis, data from stages CIII, CIV, CV and adult females of both species were included. The

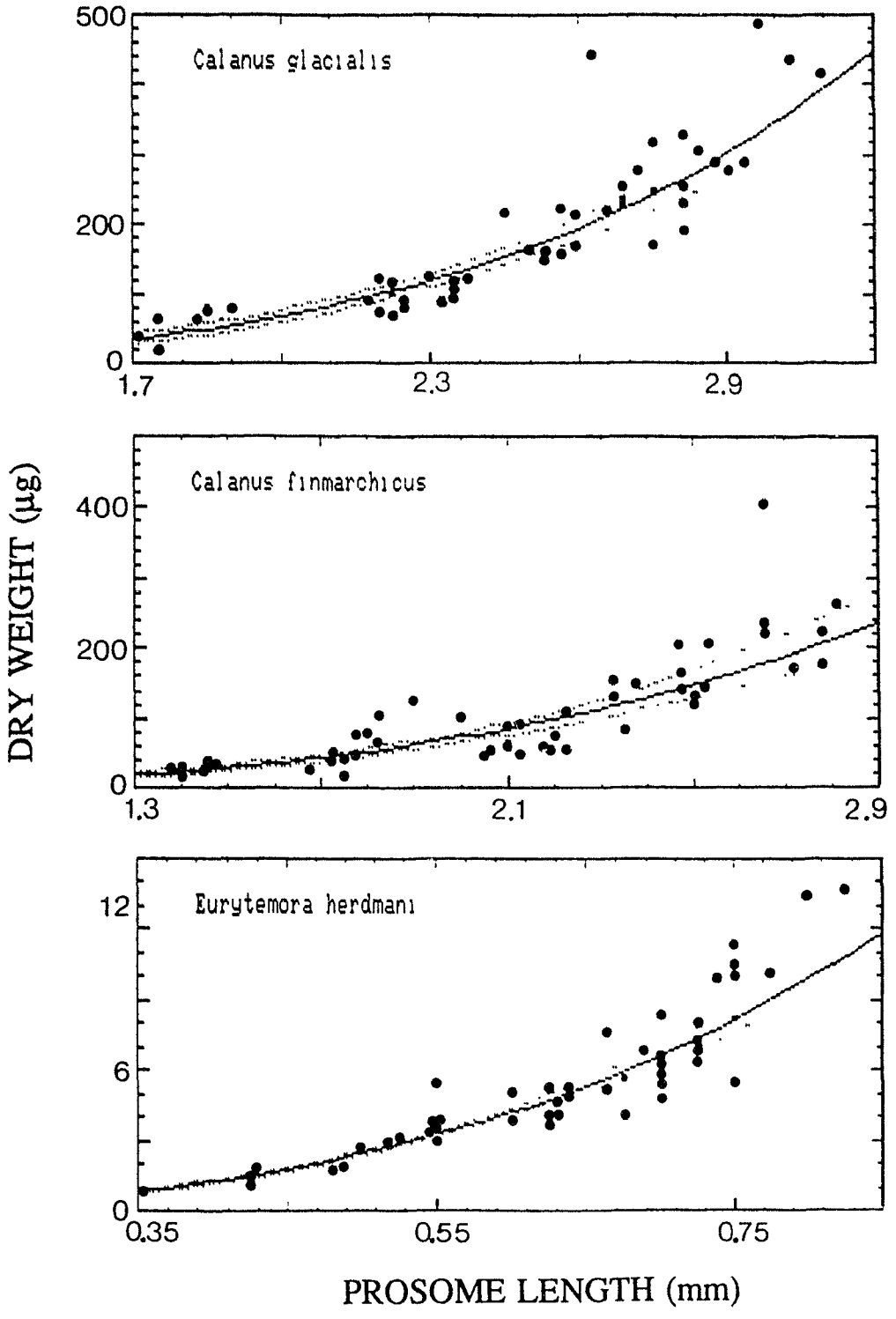

Fig.3.2 Length-weight relationships in 3 copepod species reared in the laboratory. Dotted lines represent 95% confidence limits. Dots may represent more than one value (see Table 3.4).

Table 3.5 Summary of statistics after regression analysis of the length-weight relationships in 3 copepod species reared at various laboratory conditions. TW is total dry weight, SW is lipid-discounted dry weight, L is prosome length, r^2 is the determination coefficient, and C.L. is 95% confidence limits for estimated slopes.

Species	Best fitted model	r^2	C.L.
C.glacialis	$TW = 4.166 \times L^{4.023}$	.814	.535
C.finmarchicus	$TW = 8.255 \times L^{3.145}$	.812	.417
E.herdmani	$TW = 2.70 \times 10^{-8} \times L^{2.949}$	.937	.169
C.glacialis	$SW = 4.756\ L^{3.445}$	.785	.443
C.finmarchicus	$SW = 6.233\ L^{3.113}$	.843	.371

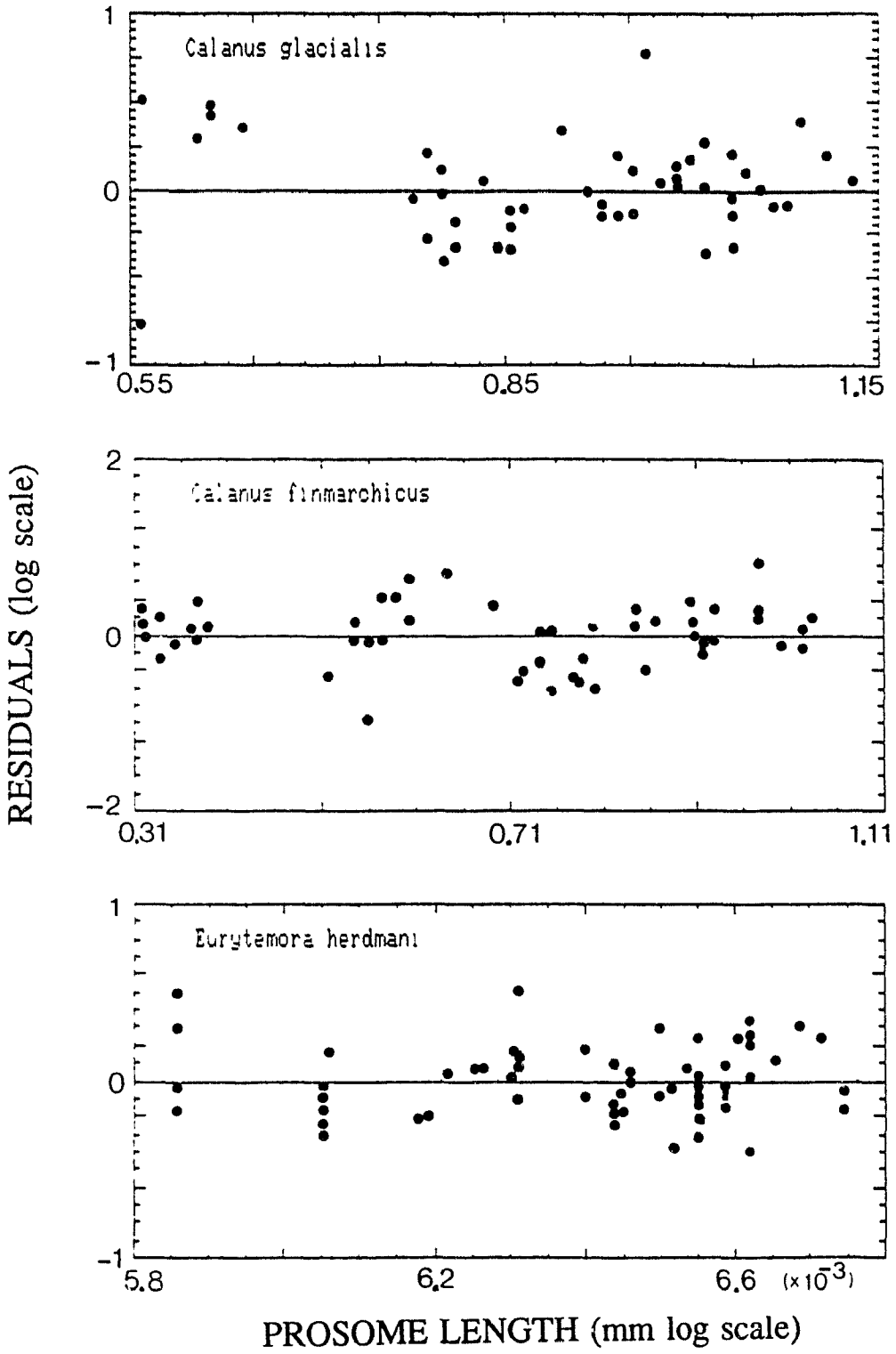

Fig.3.3 Residual variation after regression analysis on the length-weight relationships for 3 copepod species reared under laboratory conditions. Dots may represent more than one value (see Table 3.4).

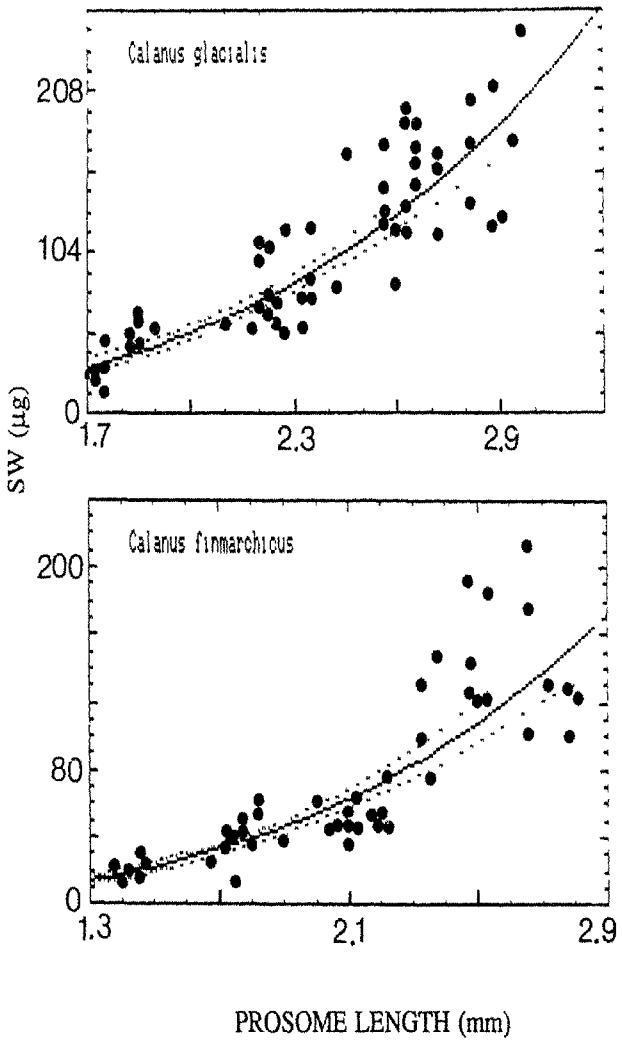

Fig.3.4 The relationship between lipid discounted dry weight (SW) and prosome length in two copepod species reared from stages CIII to adult in the laboratory. Dotted lines represent 95% confidence limits. Dots may represent more than one value (see Table 3.4).

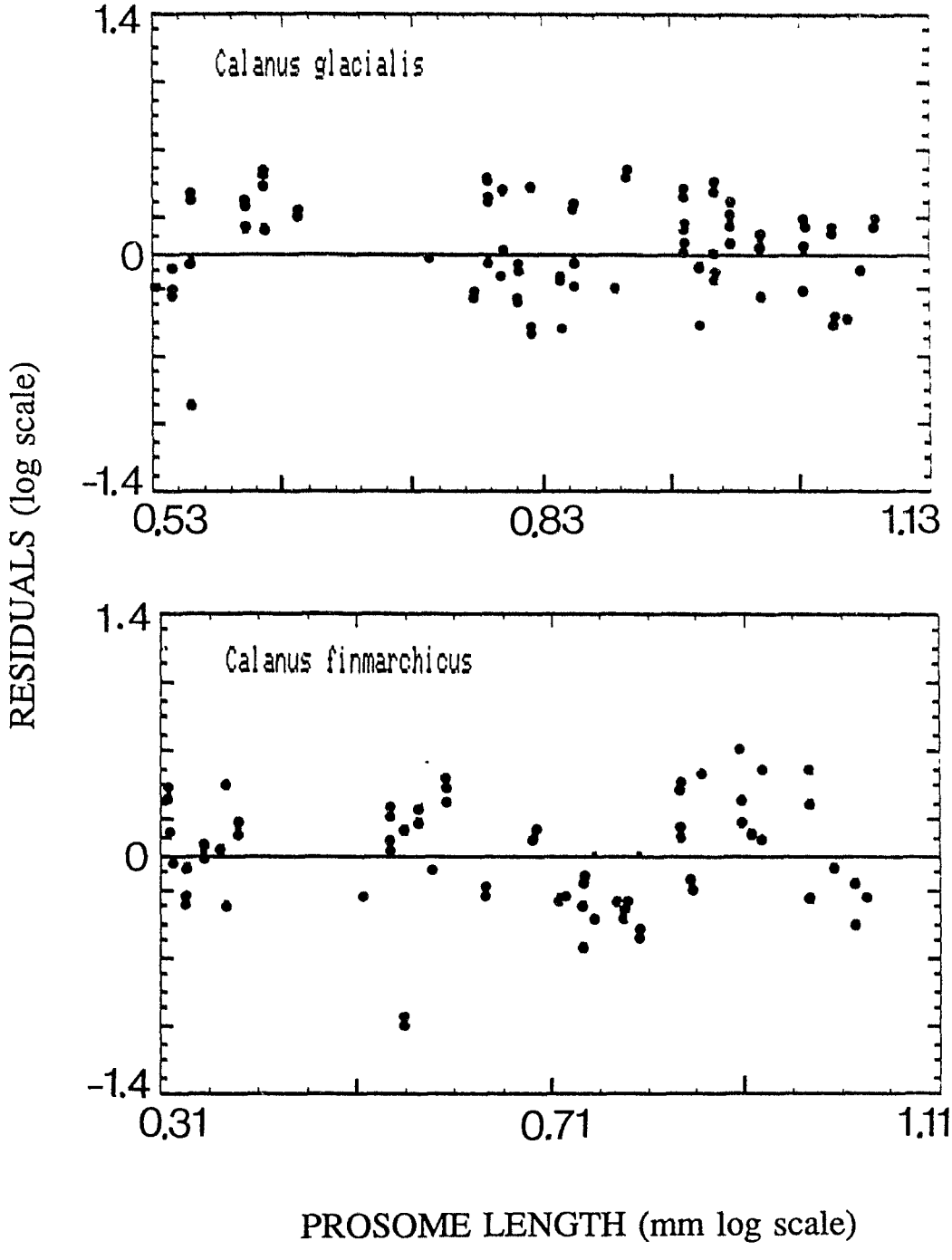

Fig.3.5 Residual variation after analysis of regression on the relationship between lipid discounted dry weight (SW) and prosome length in two copepod species reared in the laboratory from stages CIII to adult.

best fitting model was a power function for C.glacialis, whereas a linear function bettter described the relationship of these components in C.finmarchicus. The resultant equations were,

$$OSV = 0.048\ L^{7.144} \qquad r2 = .637 \quad \text{for C.glacialis} \qquad (3.2)$$

$$OSV = -38.177 + 27.769\ L \quad r^2 = .269 \text{ for C.finmarchicus} \qquad (3.3)$$

However, deviations from these models are extremely large, especially in C.finmarchicus, as shown by 95% confidence limits (**Fig.3.6**) and residual variation (**Fig.3.7**). This variability is indeed expected, since as shown above, there was a great variability in lipid content in all stages for both species, thus prosome length only explains a small proportion of variation in lipid content.

3.5.3. Body mass and size of the lipid store

In order to work with same units OSV was converted to estimated dry weight of the lipid store (OSW). Since some animals were weighed without lipids (as described above) and others intact, the differences between mean dry weights of animals of the same stage and similar OSV with and without lipids were plotted against mean OSV for the same animals (see Appendix A1, for data obtained). Differences between TW and SW

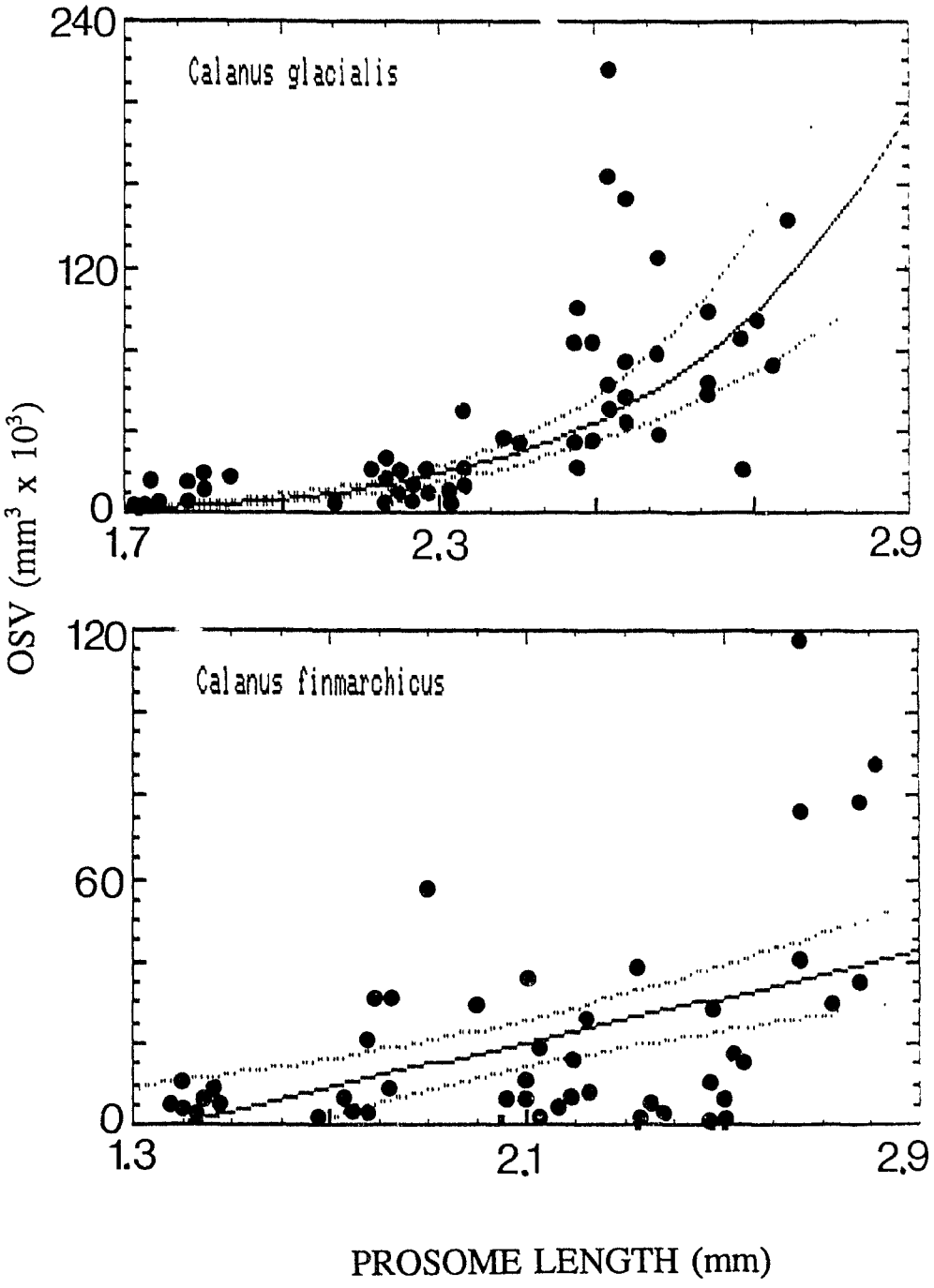

Fig.3.6 The relationship between estimated oil sac volume (OSV) and prosome length in two copepod species reared in the laboratory from stages CIII to adult. Dotted lines represent 95% confidence limits.

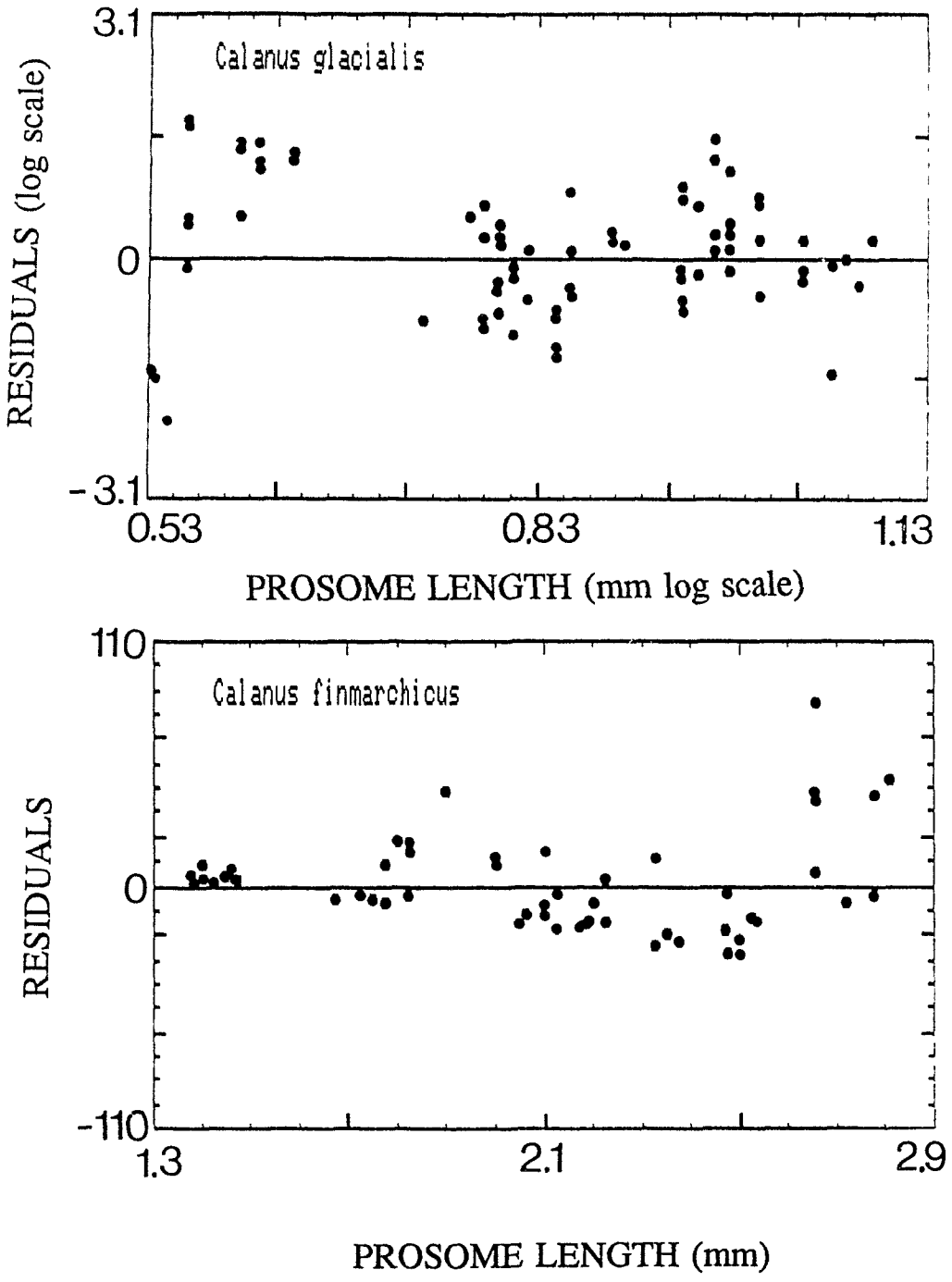

Fig.3.7 Residual variation after analysis of regression on the relationship between oil sac volume (OSV) and prosome length of two copepod species reared in the laboratory from stages CIII to adult.

obviously can be considered estimators of the OSW. The function was best described by an exponential model, forcing the y-intercept to zero (**Fig.3.8**). The resultant equation was,

$$(TW-SW)_i = OSW = (OSV)^{1.104} \quad r^2 = .981 \qquad (3.4)$$

where $(TW-SW)_i$ = difference in mean dry weight between animals with and without lipids within the same group i, OSW = estimator for dry weight of lipid content, and OSV as defined above. Equation (3.4) was, therefore, used to estimate OSW for both Calanus species from OSV estimates, so that the three components, TW, SW and OSW could be analyzed together and in the same unit (µg).

In C.glacialis the relationship OSW-length was found to be well described by a power equation, whereas a linear model better describes this relationship in C.finmarchicus (**Fig.3.9**). The equations obtained were,

$$OSW = 0.035L^{7.887} \quad r^2 = .637 \text{ for } \underline{C.glacialis} \qquad (3.5)$$

$$OSW = -63.212 + 44.120L \quad r^2 = .263 \text{ for } \underline{C.finmarchicus} \qquad (3.6)$$

Equations (3.5 and 3.6), when compared to those describing the relationship OSV-length (3.2 and 3.3), show greater values for most constants suggesting that values of OSW obtained from equation (3.4) might be overestimated, because estimations of

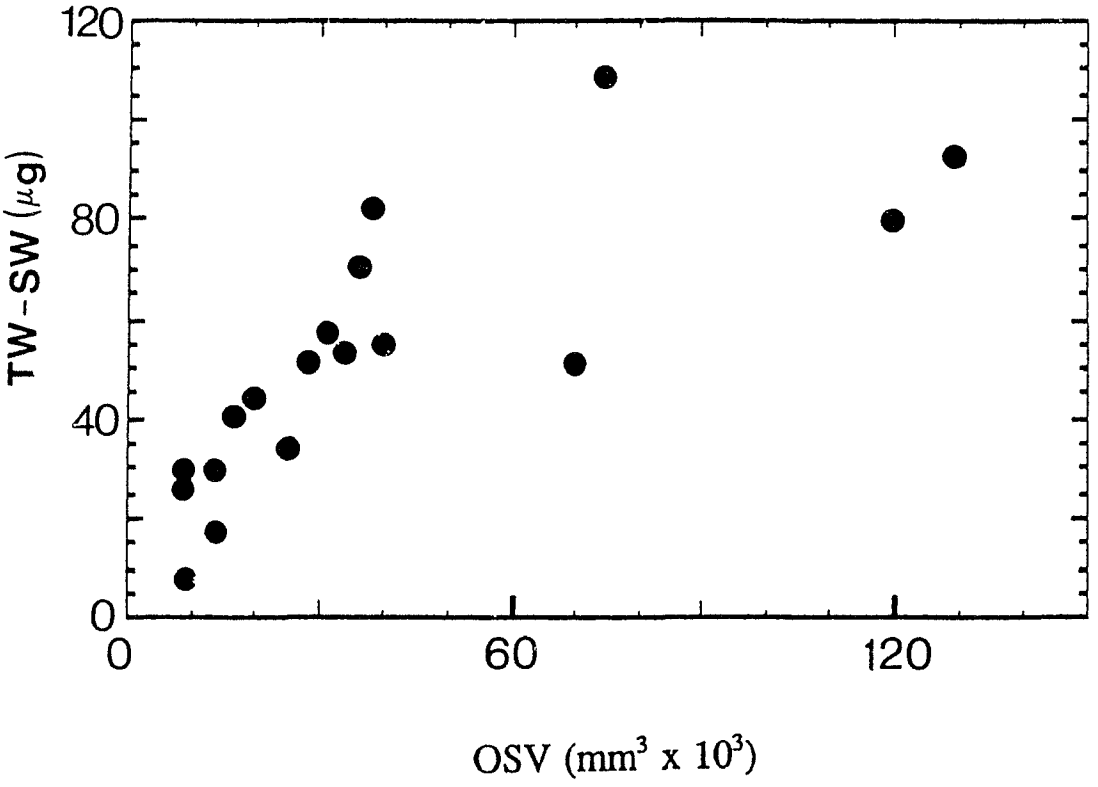

Fig.3.8 The relationship between the difference of mean values of total weight (TW including lipids) and lipid discounted dry weight (SW), and mean oil sac volume (OSV) for the same animals in C.glacialis and C.finmarchicus.

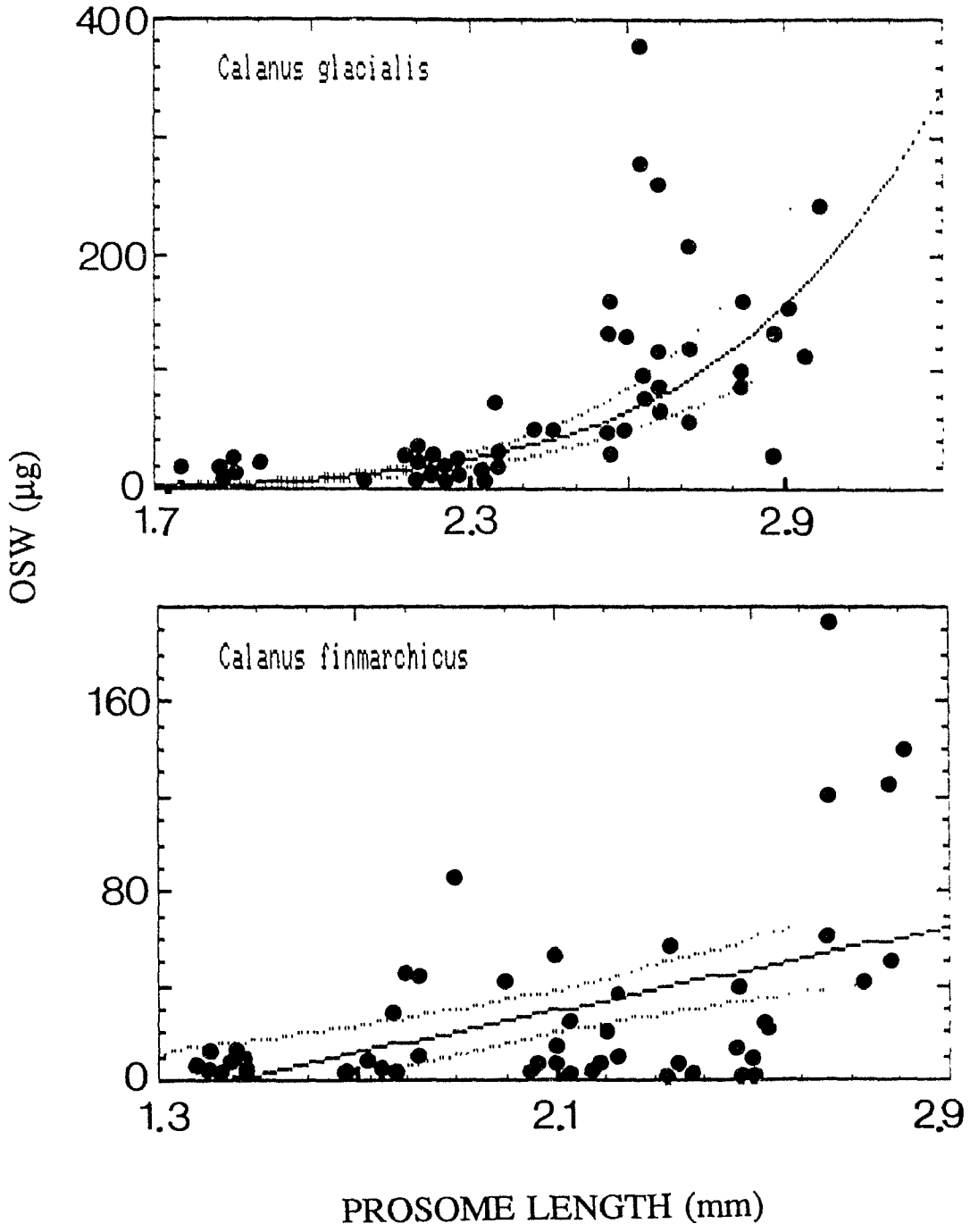

Fig.3.9 The relationship between dry weight of the lipid store (OSW) and prosome length in two copepod species reared in the laboratory from stages CIII to adult. Dotted lines represent 95% confidence limits.

lipid content as weight should have lower values than estimations of lipid volume, since lipid density is supposedly less than 1.

In a similar analysis, OSW was found to be related to SW through a power equation in C.glacialis and a linear one in C.finmarchicus. The best fitted models were,

$$OSW = 0.002\ (SW)^{2.145} \quad r^2 = .712 \quad \text{in C.glacialis} \qquad (3.7)$$

$$OSW = 3.092 + 0.340\ SW \quad r^2 = .209 \quad \text{in C.finmarchicus} \qquad (3.8)$$

The fitted curves are shown in **Fig.3.10**, and residual variation after regression in **Fig.3.11**.

In using equation (3.4) to estimate OSW and the mean values of TW for each developmental stage (from **Table 3.2.**), it is observed that stored lipids are an important part of the total dry weight of individual in older stages (see **Table 3.6.**). These values are comparable to relative amount of stored lipids obtained from field samples measured through chromatographic techniques (Kattner and Krause 1987), although Båmstedt (1988), reported a range of 20 - 100 µg of total lipids per individuals in adult C.finmarchicus, which is greater than values estimated here. In field samples of C.finmarchicus percent of lipids from total weight of animals fluctuated between 8.4 and 38%, with the highest values in stages CIV and CV (see Kattner and Krause 1987, their Table

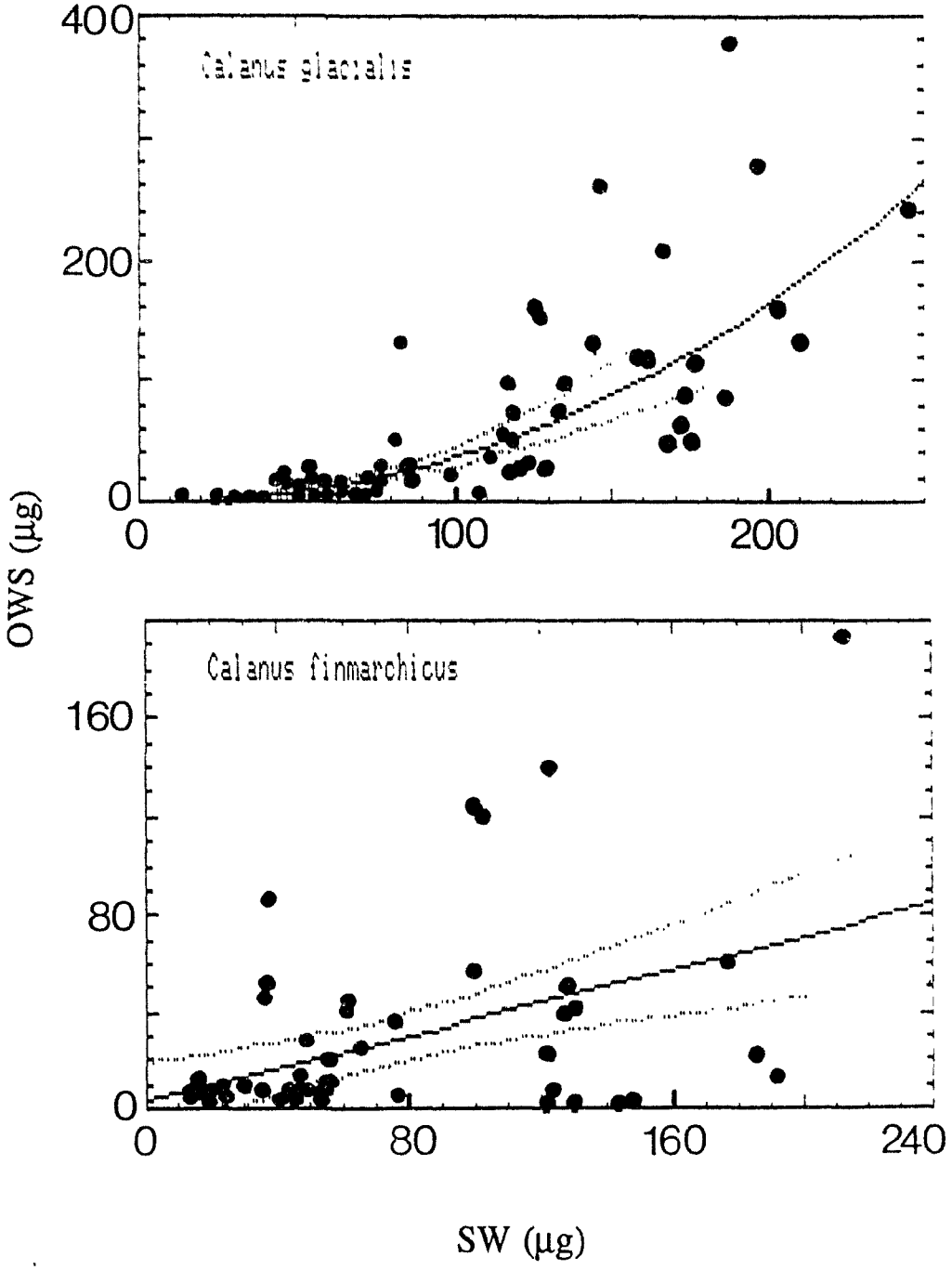

Fig.3.10 The relationship between dry weight of the lipid store (OSW) and lipid discounted dry weight (SW) in two copepod species reared in the laboratory from stages CIII to adult. Dotted lines represent 95% confidence limits.

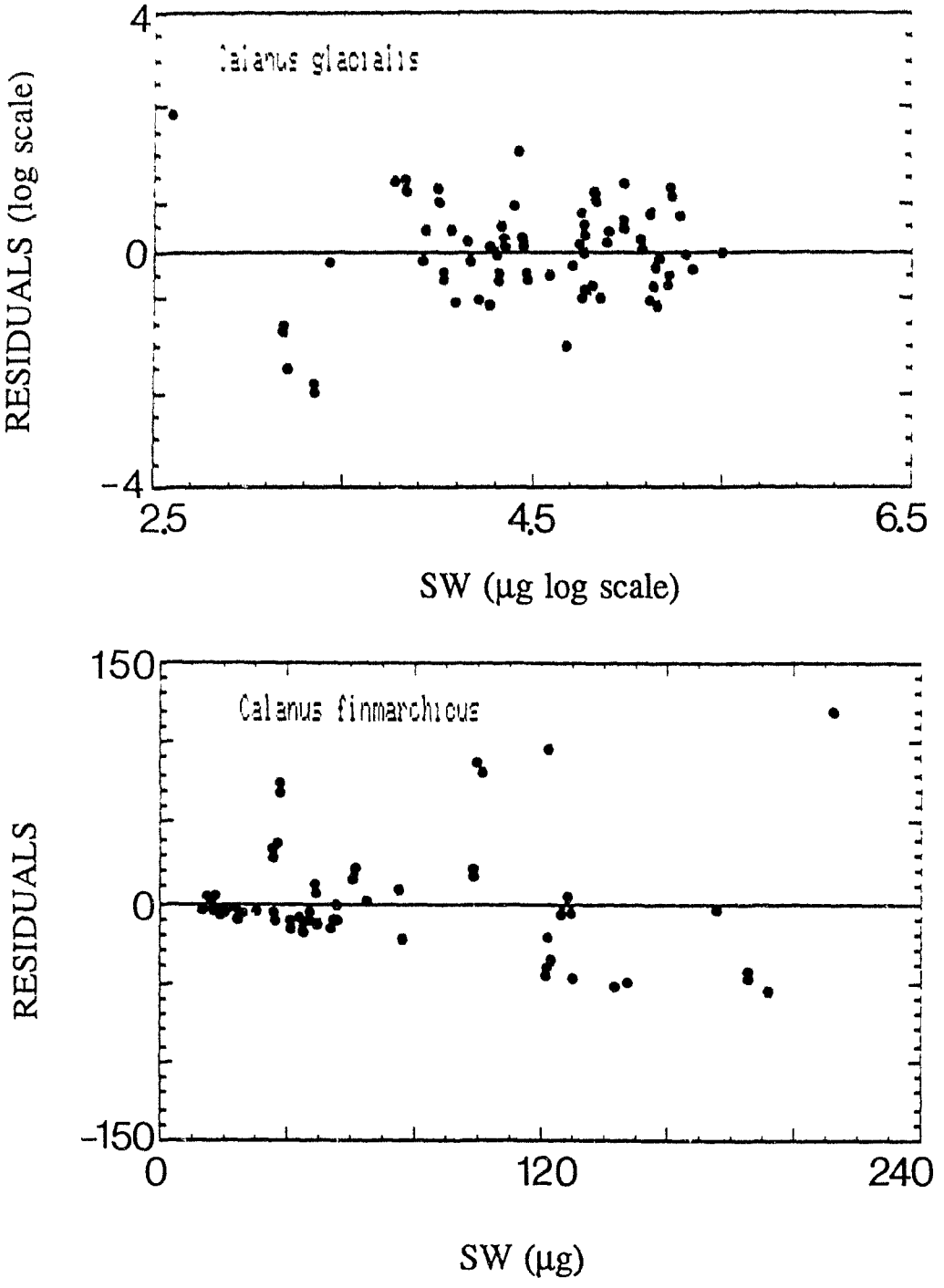

Fig.3.11 Residual variation after regression of dry weight of the lipid store (OSW) on lipid-discounted dry weight (SW) for two Calanus species reared from stage CIII to adult under various conditions in the laboratory.

Table 3.6 Estimated dry weights of the lipid store (OSW) and their proportion from the total dry weight (TW) per individual in copepodids CIII, CIV, CV and adult (Ad.) females of C.glacialis (C.g.) and C.finmarchicus (C.f.).

Species	stage	OSW (ug) ± S.D.	% of TW
C.g.	CIII	10.99 ± 8.22	17.3
	CIV	32.34 ± 56.88	32.5
	CV	95.90 ± 64.60	42.3
	Ad.	138.10 ± 135.27	32.8
C.f.	CIII	6.88 ± 2.54	24.6
	CIV	29.96 ± 26.21	34.9
	CV	27.43 ± 34.22	29.9
	Ad.	9.56 ± 10.11	5.1

2.). Little information on individual variation of lipid content is available for C.glacialis, but according to **Table 3.6.**, this species accumulates greater amounts of lipids per unit of body mass as compared to C.finmarchicus. Estimations of lipid contents at stage CV agree with values found by Båmstedt (1984).

3.6. Discussion

There are genetic components involved in size variation (e.g. McLaren 1976, Corkett and McLaren 1978, Klein-Breteler et al. 1990). However, if some variation is induced by conditions during growth and development, it may be assumed that components with highest variances are most subjected to such conditions. According to coefficients of variation (**Table 3.1**), prosome length is the least variable component, while size of the lipid store is the most variable one. Body weight, on the other hand, although positively correlated to prosome length shows greater variation than the latter, thus body mass and body length respond differently to rearing conditions. This is especially notable when relating weight and length in Calanus species, and although some variation is reduced by using lipid-discounted dry weight instead of total weight, the length-weight relationships still show considerable residual variation, thus limiting their predictiveness.

In E.herdmani where lipids are apparently not important in terms of body weight, there is much less residual variation

from the length-weight relationship and possibly the variation may be reduced in a greater extent when using separate functions for sexes. However, even in non-lipid storing species, weight-length relationships should be used with caution. In cladocerans, this relationship shows a great spatial and temporal variation (Geller and Müller 1985, Kankaala and Johansson 1986). This variation is also observed in copepods (Kankaala and Johansson 1986). Since body mass is more affected by conditions than body length, length-weight equations tend to overestimate body weight, and in some cases may give estimate which are ca. 40% higher than direct measurements (Kankaala and Johansson 1986).

Alternatively, the length-weight relationship might be more useful as an indicator of physiological conditions of individuals, as suggested by Durbin and Durbin (1978). An expected allometric relationship between body mass and body length is based on the assumption that if animals maintain the same shape and density while growing, weight should be related to the cube of any linear dimension, so that $W/L^3 = CF$ can be considered an index of condition, where W is body weight, L is body length and CF a condition factor (LeCren 1951). A condition factor near unity is convenient for comparisons, thus CF is calculated as, $CF = c\ W/L^3$, where c can be estimated by trial and error (LeCren 1951). To obtain CF values near to unity in Calanus species, c was taken as 0.1, whereas in E.herdmani as 0.05. A summary of CF values for the

three species is shown in **Table 3.7**. This factor was found positively correlated to size of the lipid store in C.glacialis (r^2 = .807 P <.001) and in C.finmarchicus (r^2 = .393 P <.001), but showing little or no correlation with prosome length, r^2 = .215 (P <.05) and r^2 = 0.016 (P >.05) in C.glacialis and C.finmarchicus, respectively, thus, suggesting that variations in weight per unit of body length depend principally on lipid content. In E.herdmani, the condition factor CF showed a slight correlation to body weight (r^2 = .622 P <.05) and no correlation to prosome length (P >.05). Thus, in this species CF might vary as a function of other physiological conditions, such as sexual differences and/or reproductive status. From this analysis it may be concluded that, although the exponents for prosome lengths when related to TW and SW (**Table 3.5**) were, in all case, not significantly different from 3 (t-test, P >.05), it is clear that weight per unit of volume is not constant, and this variation is a function of physiological conditions which are probably determined by food supply and other environmental factors (e.g. Durbin and Durbin 1978, Kane 1982, Klein Breteler and Gonzalez 1988).

Large animals should be capable of storing greater amounts of lipids than small ones, thus, a positive correlation among size of the lipid store, prosome length and SW, is not surprising. However, much variation in lipid contents cannot be explained by animal length or structural

Table 3.7 Condition factor (CF) of three copepod species reared in the laboratory under various conditions of food and temperature. Stages CIII to adult female of Calanus species, and CI to adult male and female of E.herdmani are included in calculation of CF's.

Species	Mean ($\mu g.mm^{-3}$)	Range	S.E.	n
C.glacialis	1.083	0.345 - 3.128	0.060	68
C.finmarchicus	0.968	0.341 - 2.170	0.046	55
E.herdmani	1.000	0.652 - 1.653	0.023	84

weight. This level of variation is a common pattern of copepods in field observations (Båmstedt 1988 for discussion). However, if this variation can be explained in terms of condition effects, it may be argued that lipid content is the component most likely to be influenced by growing conditions.

In addition to substantial differences in amounts of storing components, less variability in size components of E.herdmani as compared to Calanus can also be explained in terms of cultivation conditions. The former was first reared from egg to CI under constant conditions of food and temperature, and then subjected only to different temperatures, while the latter were obtained at CIII from the field and then subjected to combinations of food and temperature. Although this experimental design may limit comparisons among species, it also supports the view that most of the variance is explained by food-temperature effects rather than intrinsic or genetic components. Indeed, seasonal variations of body length in Temora longicornis and Pseudocalanus elongatus (Klein-Breteler et al. 1990), did not account for size of the progeny, thus also supporting the hypothesis that size variations are mostly environmentally induced. This hypothesis will be explored in detail in Chapter 6.

CHAPTER 4

THE ROLE OF GROWTH AND DEVELOPMENT IN DETERMINING SIZE VARIATIONS.

4.1. Introduction

Since increase in body size, either as length or mass, depends on the time available for growth (development time) and on the rate of mass accumulation during that period (growth rate), an understanding of influences on development and growth rates should give insights on how size variations develop.

There have been attempts to derive rules for copepod development and its influencing factors. It is well known that environmental temperature is a major influence on development rates in nature. Embryonic duration has been used as a measure of temperature-response of development rate. Some empirical temperature functions have been applied with relative success in these studies (McLaren 1966, Bernard 1971, Heip 1974, Bottrell 1975, Hart and McLaren 1978). Miller et al. (1977) stated that all developmental stages of abundantly fed Acartia spp. might have equal duration, and this was termed the "isochronal rule". Studies of additional species showed that food-unlimited stage duration is not uniform (e.g. Landry 1983), although some species can instead show "equiproportional" development (Corkett 1984), through which

each stage may occupy a fixed proportion of total development time at any temperature. These rules, however, cannot be applied in some overwintering species or cohorts of high latitude copepods, which may have stages that diapause, or otherwise extend for long periods (e.g. Davis 1976). Furthermore, it is not clear to what extent development rates in nature are solely temperature-dependent, since evidence suggests that development may be limited by food availability (Mullin and Brooks 1970, Paffenhöfer 1976, Diel and Klein-Breteler 1986). Despite these complications, equations assuming temperature-dependence have been widely applied to study life cycles and production rates of copepods in nature (e.g. McLaren et al. 1969, 1989d).

Growth rate, on the other hand, has been less studied than development. Temperature affects positively various metabolic processes such as respiration (e.g. Ikeda 1970), ingestion and assimilation (e.g. Anraku 1964). The net result is a positive relationship between temperature and growth rates (Vidal 1980a, Uye 1988), but this relationship may be altered by food conditions and body size (Diel and Klein-Breteler 1986, Vidal 1980a). The effect of food availability on growth under experimental conditions has been well documented (e.g. Mullin and Brooks 1970, Condrey 1982), although the extent to which food limits growth in nature is still an open question (see Huntley and Boyd 1984, Frost 1985).

In understanding copepod growth, a distinction should be made between growth in body mass and growth in body volume or length. Body volume and body length increase discretely through stages of development, six naupliar stages and six copepodid stages, while body mass may increase continuously from the egg to adulthood. Since the two types of growth might be regulated differently, they should be distinguished and analyzed separately. Then their eventual relationships may be more fully understood.

In this study, information is available on growth and development of copepodids of E.herdmani, C.finmarchicus and C.glacialis reared at two temperatures and under excess conditions of food (details on rearing condition are given in Chapter 2). Patterns of growth in body length, body mass and size of the lipid-store will first be described separately, and then attempts will be made to establish their relationships. In addition, the influence of temperature on these patterns of growth will be examined. Development rate, on the other hand, will mostly be analyzed to determine the extent to which it determines intraspecific divergence in body size.

4.2. Growth in body length

Body lengths of newly molted instars at 10 ^{0}C and 3 ^{0}C, from stages CIII to adult females of C.glacialis and C.finmarchicus, and from CI to adult males and females for

E.herdmani were plotted as a function of time to reach a given instar (**Fig.4.1, Fig.4.2** and **Fig.4.3**).

In Calanus species, duration of stage CV is much longer than that of CIV, and changes in length are obviously not continuous through time. This stresses the need of treating growth in body length as a discrete process. Although, body length increases in a nearly linear pattern through time, the use of continuous functions such as linear regressions would obviously be inadequate.

"Brook's Law" implies ca. 25% increase in length between copepod stages (McLaren 1969, Miller et al. 1977, Longhurst 1986). However, if length increments are calculated by following changes in length of individuals, the relative length increase between stages is not constant, but it decreases through older stages. Relative increments in length between stages, here estimated, for the three species are shown in **Table 4.1.**

The gradual decrease in length increments towards late stages (see **Table 4.1**) cannot be derived from patterns shown in **Fig.4.1, Fig.4.2** and **Fig.4.3**, since they suggest a constant growth rate. For instance, fits of linear models are highly significant ($r^2 > 0.70$ for any of the three species). Furthermore, the decrease in growth increments cannot be explained in terms of differential stage durations, instead the extended duration of stage CV, indicates that there is more time available for growth, so that a greater increase on

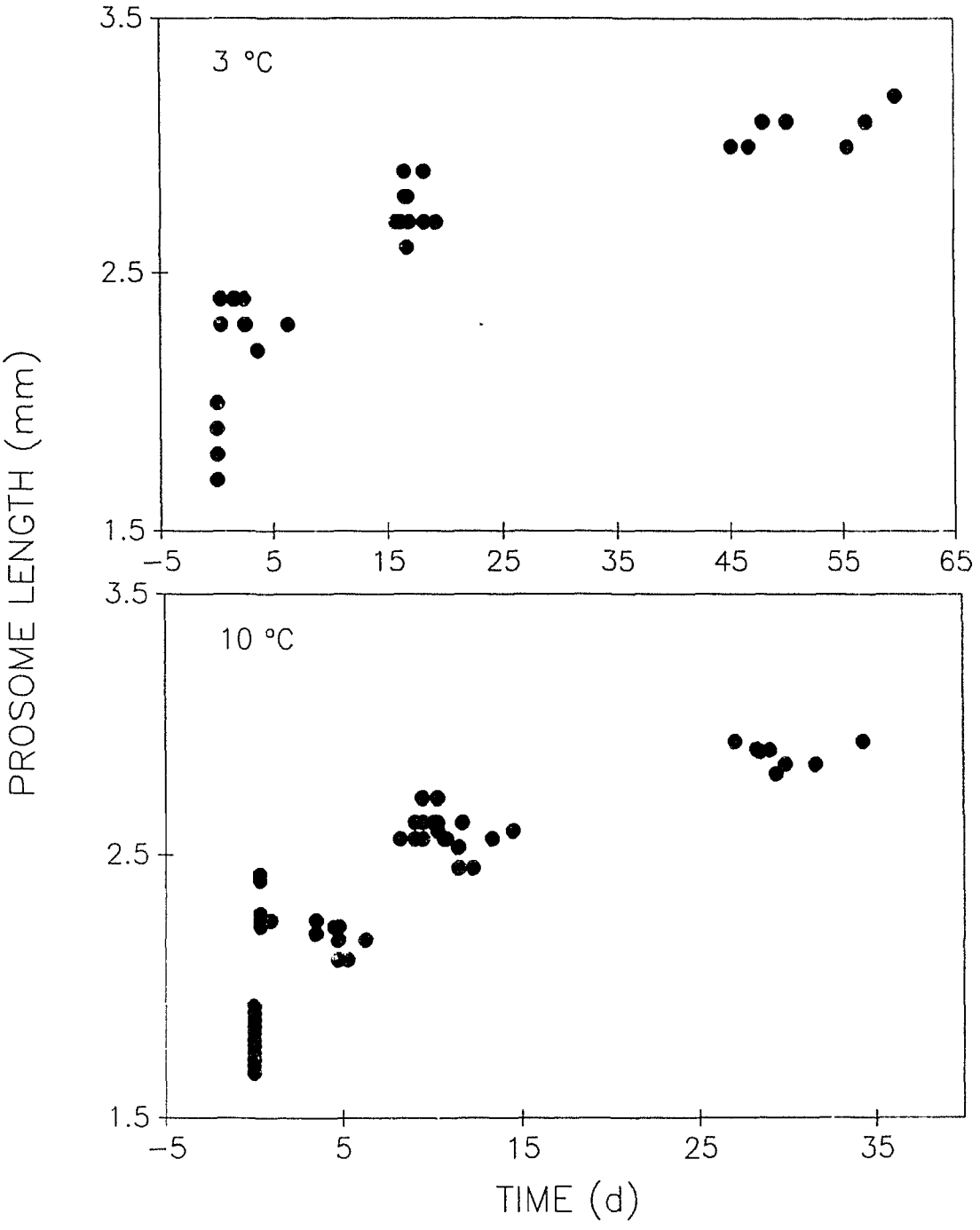

Fig.4.1 Growth in prosome length in C.glacialis reared from CIII to adult at two temperatures under excess conditions of food. Time was estimated from individual observations of molting times.

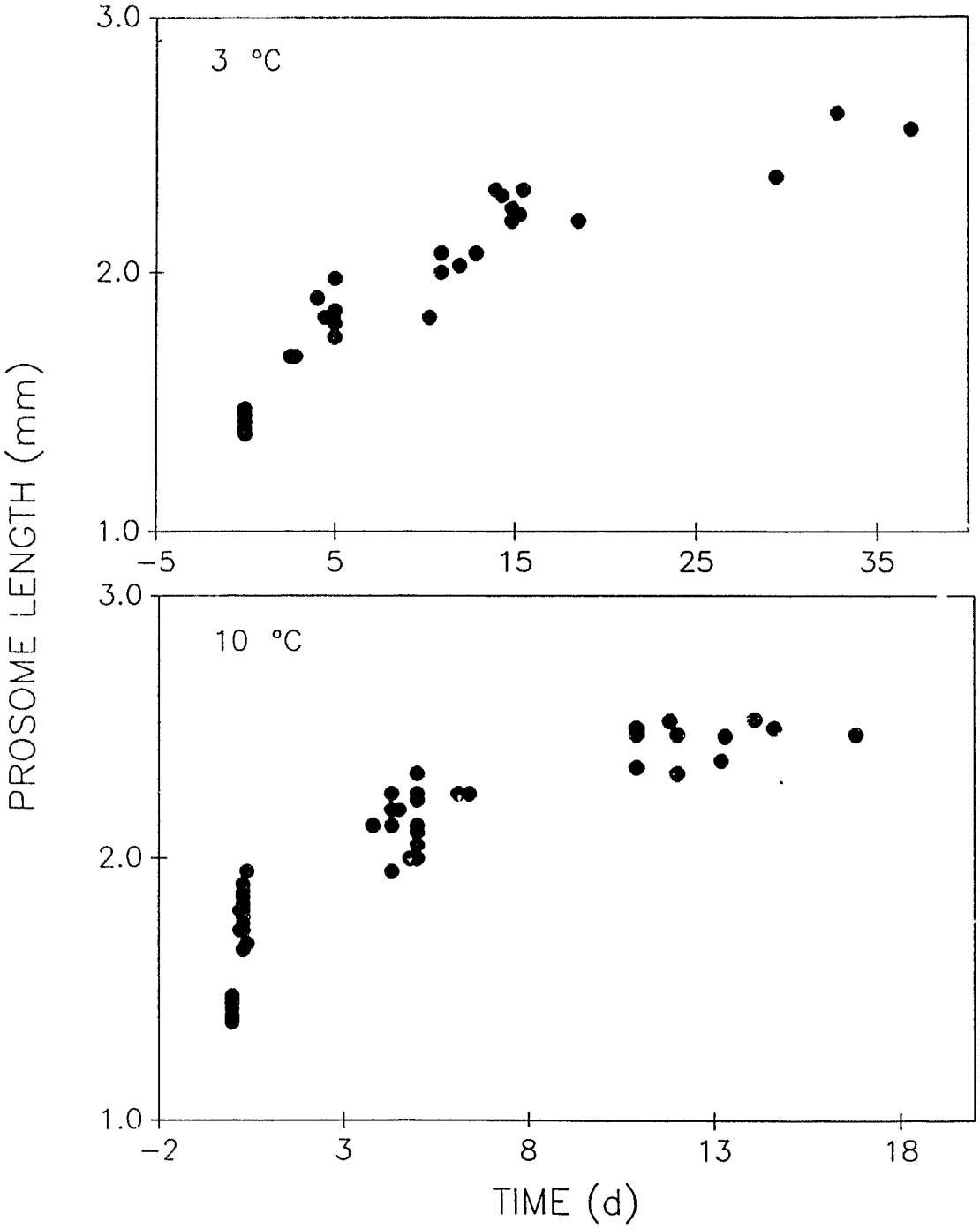

Fig.4.2 Growth in prosome length in C.finmarchicus reared from CIII to adult at two temperatures under excess conditions of food. Time was estimated from individual observations of molting times.

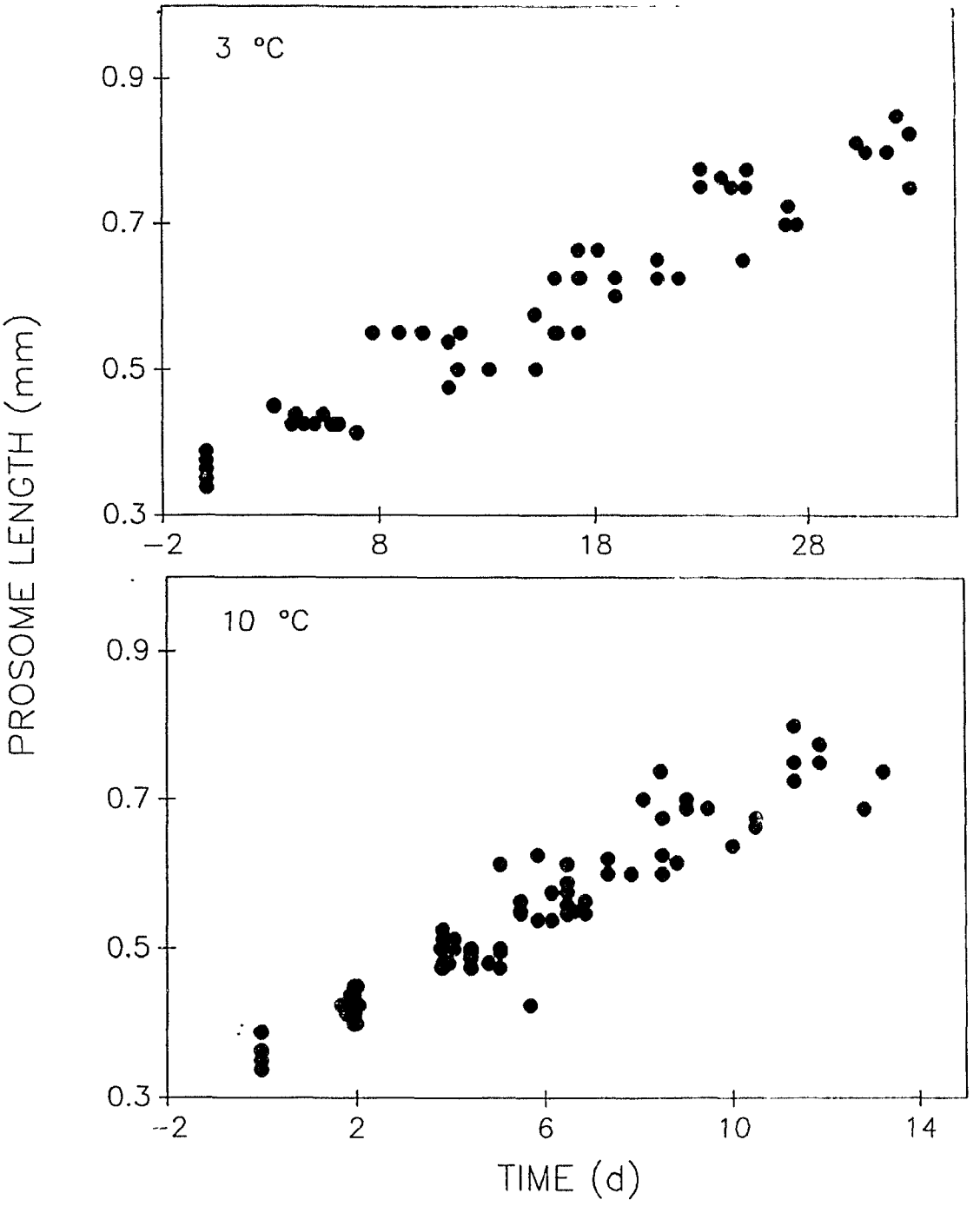

Fig.4.3 Growth in prosome length of E.herdmani reared from stages CI to adult at two temperatures under excess conditions of food. Time was estimated from individual observations of molting times.

Table 4.1. Relative increments of prosome length (k) between stages of 3 copepod species reared at two temperatures under excess conditions of food. k was estimated as length increase (%) relative to the previous stage. Numbers in parentheses represent standard deviations.

spp.	Temp (^{0}C)	%CI	%CII	%CIII	%CIV	%CV
C.g.	3			28.0 (5.1)	18.9 (4.2)	14.6 (4.0)
	10			24.3 (4.1)	15.5 (2.3)	11.6 (3.1)
C.f.	3			27.8 (7.2)	19.9 (6.0)	13.8 (6.3)
	10			25.9 (7.1)	21.1 (4.2)	13.6 (5.0)
E.h.	3	20.4 (4.2)	22.0 (5.1)	12.8 (5.0)	15.1 (4.3)	10.1 (4.0)
	10	18.8 (3.4)	16.9 (3.1)	16.4 (4.4)	13.2 (2.9)	10.9 (2.9)

c.g.=C.glacialis, C.f.= C.finmarchicus, E.h.=E.herdmani.

size might be expected. Therefore, it seems that stage durations or development rate do not not necessarily determine size achieved at a given stage. Moreover, an increment of 25% is clearly not a valid approximation for the variable length increments between stages (**Table 4.1**). Since prosome lengths increase abruptly through time, it seems that in order to obtain more precise estimates of prosome length at adulthood, the length increments for each instar have to be taken into account. Thus, if prosome length for an initial stage (n) is known, length at a certain temperature in the next stage (n+1) can be estimated as,

$$L_{n+1} = L_n + K_n \times L_n \qquad (4.1)$$

where L is prosome length and K is the relative increment in length. For example, in Calanus glacialis if K_1, K_2, and K_3 are relative increments between stages CIII, CIV and CV respectively, then length at adulthood is,

$$L_{Ad.} = L_1 + L_1(K_1 + K_2 + K_3 + K_1K_2K_3 + K_1K_2 + K_1K_3 + K_2K_3) \qquad (4.2)$$

where $L_{Ad.}$= adult length and L_1 = length at CIII.

Using equation (4.2), values for K from Table **4.1**, and mean length (± S.D.) at CIII as 1.805 ± 0.065 mm, the expected mean prosome length of adults at 10 °C is 2.892 mm and at 3 °C is 3.150 mm. These expected values are close to the observed,

2.830 ± 0.180 mm, and 3.0854 ± 0.092 mm, at 10 and 3 ^{0}C respectively.

Equation (4.1) could be considered a function to describe discrete growth, although if length increments between stages are all different to each other, a generalized equation for n stages would be,

$$L_n = L_0 + L_0 \left(\sum_{i=1}^{n} \sum_{j=i+1}^{n} \ldots\ldots \sum_{l=k+1}^{n} k_{i.j}\ldots.lk - n\right) \qquad (4.3)$$

where L_n is body length at a terminal stage (e.g. adult), L_0 is length at an initial stage (e.g. CI). The terms Ki, Kj and Kl are first, second, third or greater order for the additive and multiplicative effect of relative increments between consecutive stages.

4.3. Growth in body mass

The potentially continuous increase in body mass may be described independently of developmental stages. Adopting this approach Vidal (1980a) observed that weight-specific growth rates of two copepod species increased with food concentration and decreased with temperature, age and body size. This implies that, besides being affected by temperature and food, growth rates are also size-dependent. This conclusion, however, has been challenged by observations in other species suggesting that potential growth of copepodids may be

exponential and hence constant through stages (e.g. Miller et al. 1977, McLaren 1986). Exponential growth assumes that food quantity and quality are non-limiting, so that growth would be only affected by temperature. This assumption has been the basis of extrapolation of temperature-dependent growth rates obtained in the laboratory to natural conditions (e.g. McLaren and Corkett 1981, McLaren et al. 1989d).

Total dry weights (TW) were obtained from individuals of Calanus species reared from CIII to adult, and E.herdmani reared from CI to adult. Changes in TW, regardless of developmental stages, have been plotted as a function of time (**Fig.4.4, Fig.4.5**), and tested for goodness-of-fit to an exponential model of growth as,

$$TW = \exp (a + b\ Time) \qquad (4.4)$$

where a and b represent initial TW (at CIII for Calanus spp. and CI for E.herdmani), and the slope of the growth curve respectively. Fitted curves through the Least Square method explain a high proportion of the variation in TW, suggesting that growh in TW of the three species may be adequately described by exponential models (**Table 4.2**).

Residual variation after exponential fits is shown in **Fig.4.6** and **Fig.4.7**. Distributions of residuals in all cases follow a nearly normal distribution (**Fig.4.8, Fig.4.9**). Goodness-of-fit to a normal distributior of residuals was

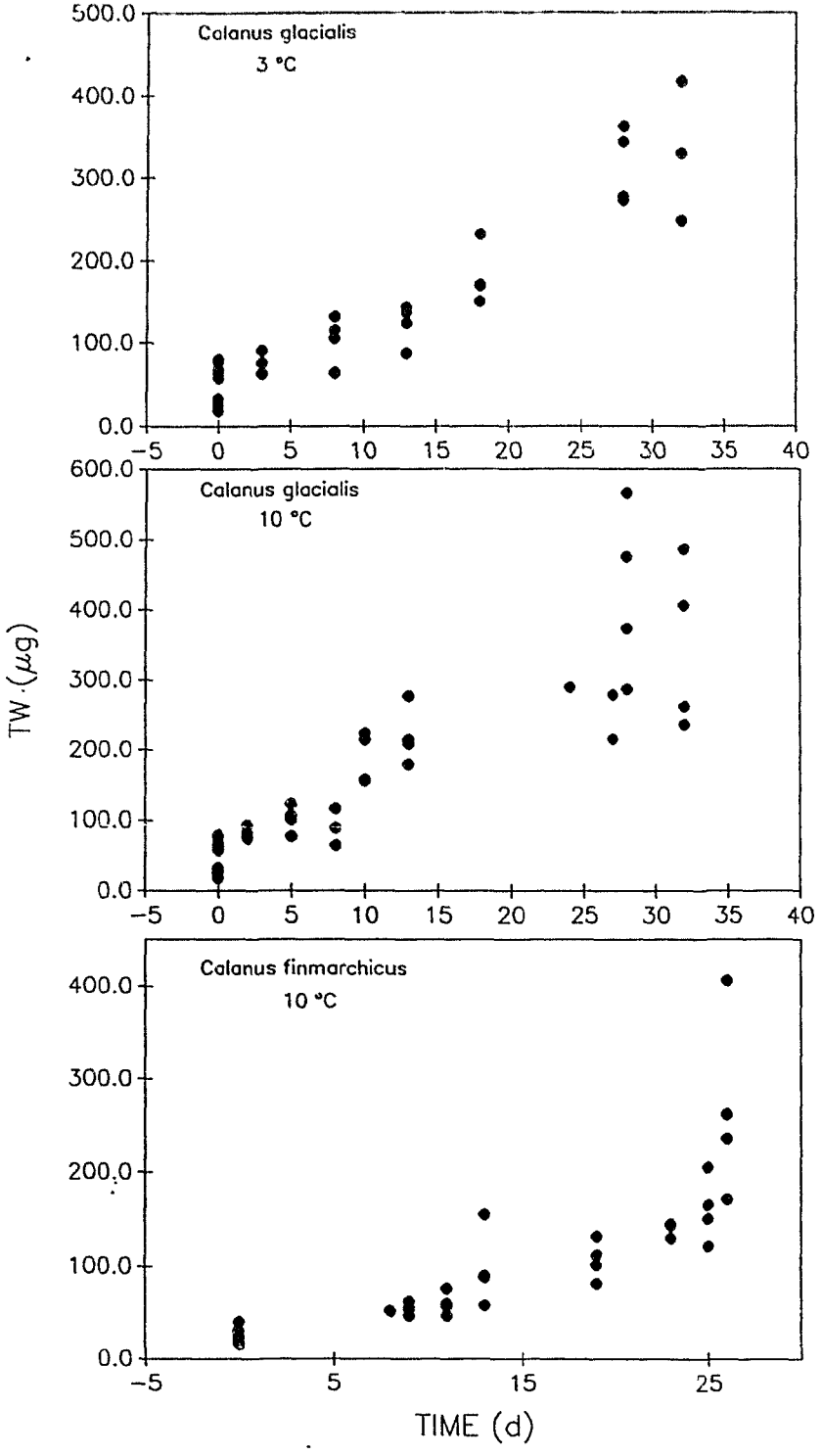

Fig.4.4 Growth in total dry weight (TW) in C.glacialis reared from stage CIII to adult at 3 °C and 10 °C, and C.finmarchicus reared from CIII to adult at 10 °C, under excess conditions of food.

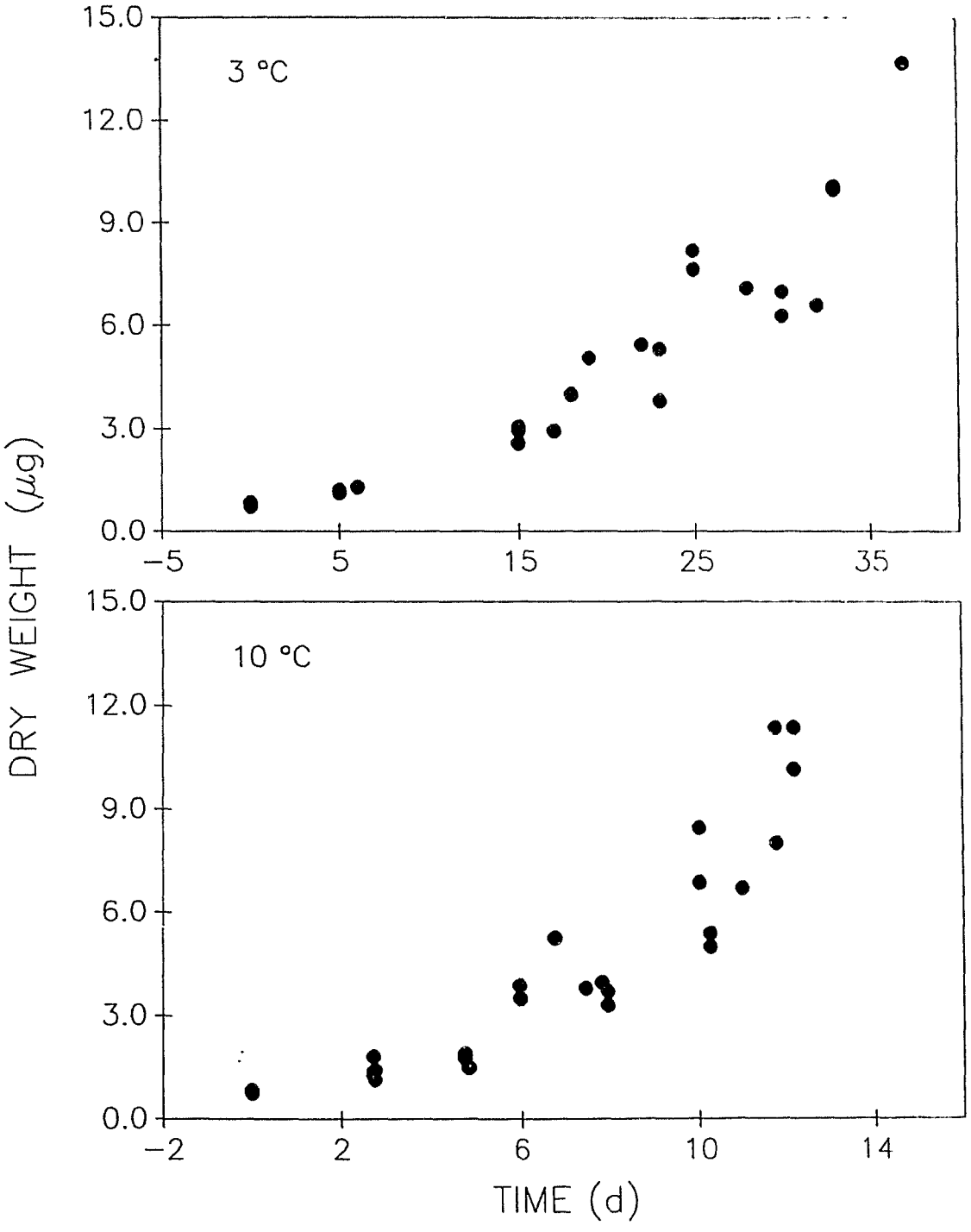

Fig.4.5 Growth in dry weight in E.herdmani from stages CI to adult reared at two temperatures under excess conditions of food. Females and males are included.

Table 4.2. Exponential equations of growth in total dry weight (TW) of 3 copepod species reared at two temperatures and under excess of food. Equations were fitted through Least Square Method. Coefficients of determination (r^2) are highly significant ($P < 0.001$)

Species	Temp. (^0C)	n	Exponential model	r^2
C.glacialis	3	34	TW = Exp(3.946 + 0.064 time)	0.795
	10	42	TW = Exp(4.111 + 0.064 time)	0.647
C.finmarchicus	10	36	TW = Exp(3.247 + .081 time)	0.889
E.herdmani	3	24	TW = Exp(-0.154 + 0.076 time)	0.954
	10	26	TW = Exp(-0.241 + 0.209 time)	0.935

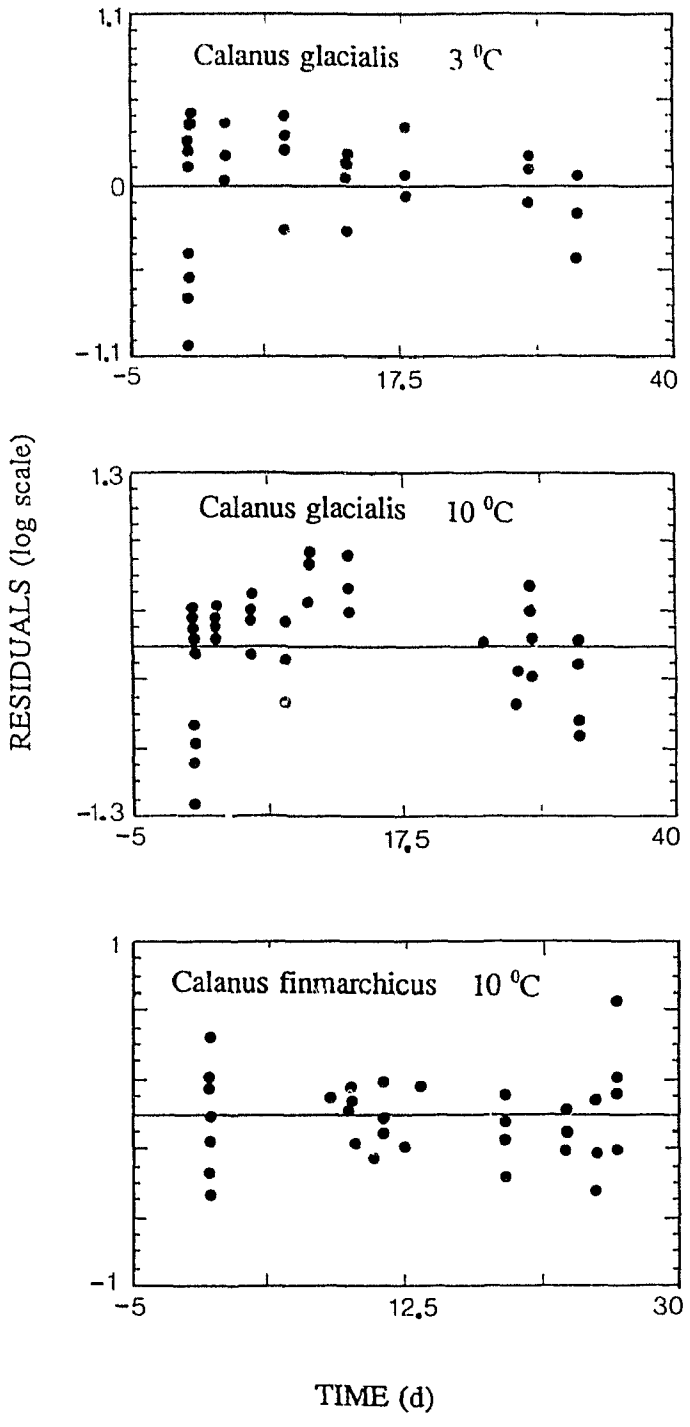

Fig.4.6 Residual variation after exponential fitting of growth in total weight (TW) in C.glacialis reared from CIII to adult at 3 °C and 10 °C and C.finmarchicus reared from CIII to adult at 10 °C, under excess conditions of food.

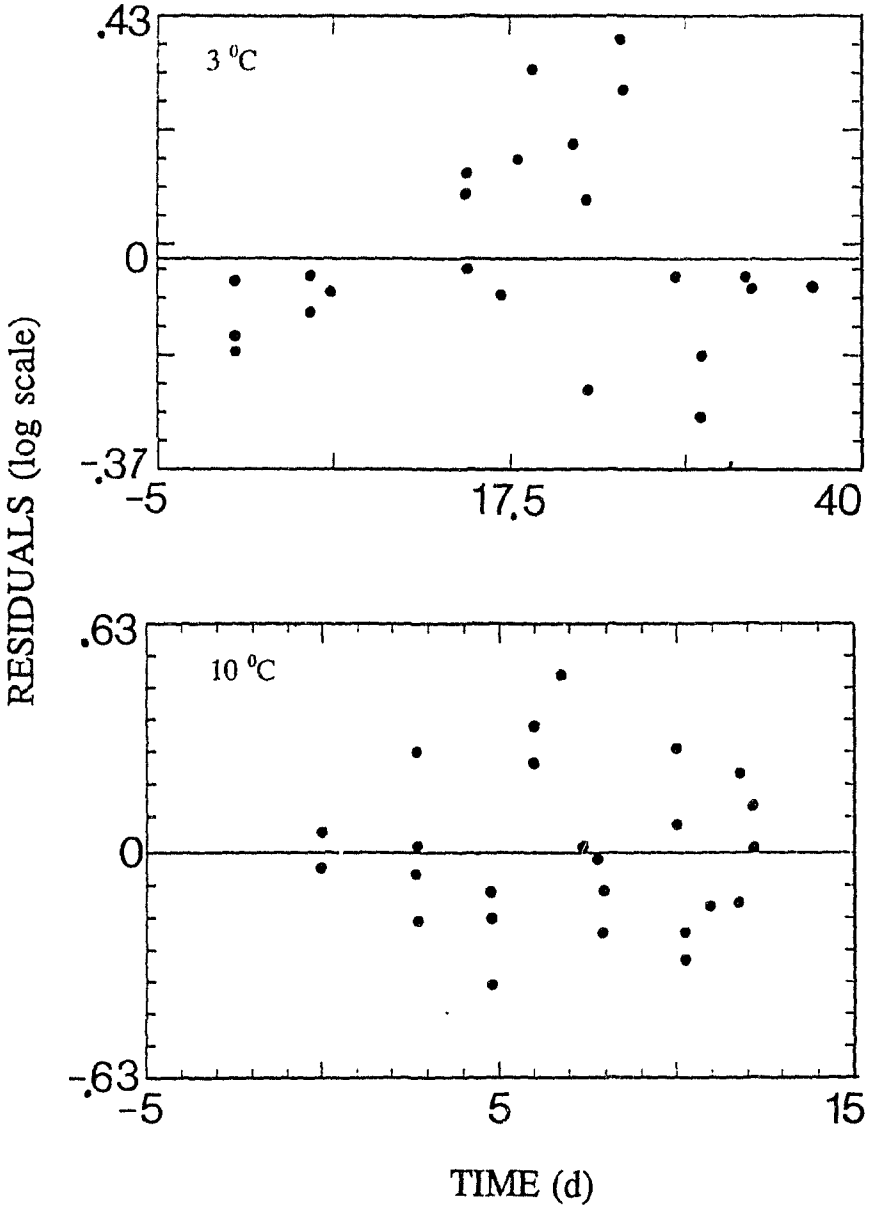

Fig.4.7 Residual variation after exponential fitting of growth in dry weight of E.herdmani reared from CI to adult at two temperatures under excess conditions of food.

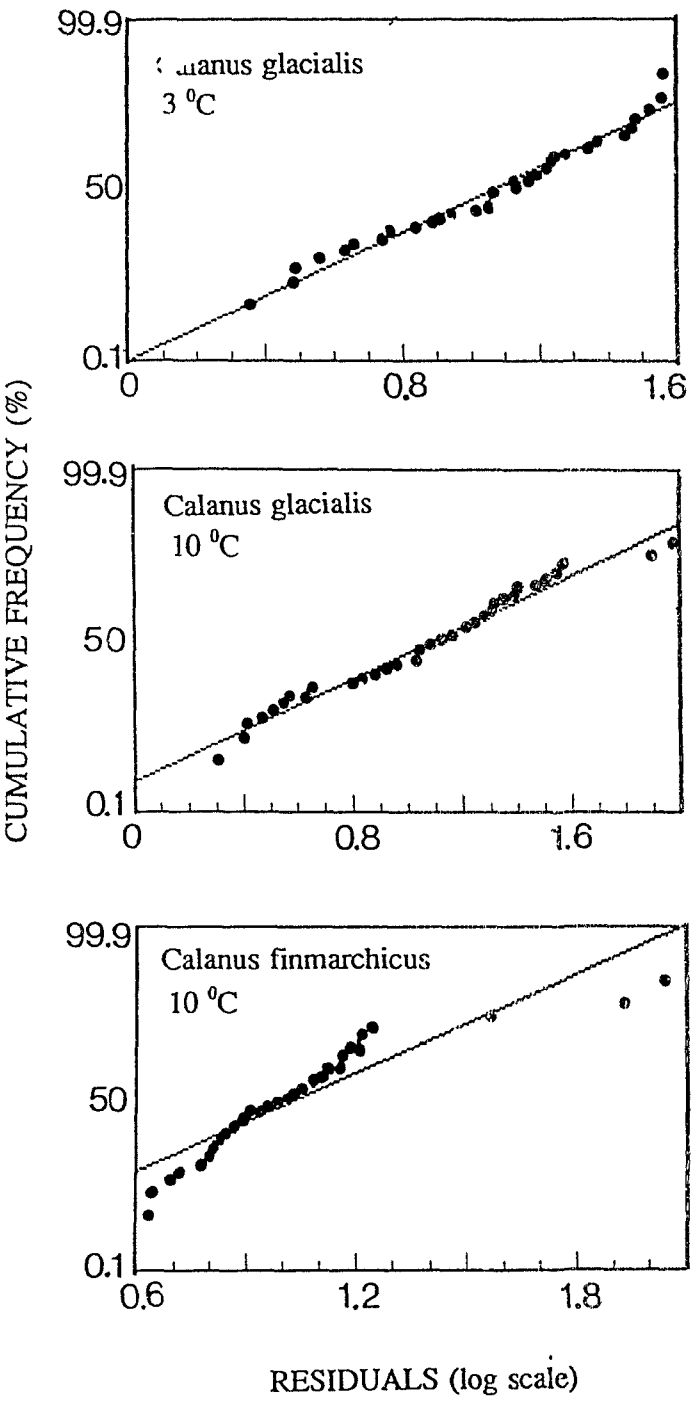

Fig.4.8 Normal plots of residuals obtained from an exponential fitting of growth in total dry weight (TW) of C.glacialis reared from CIII to adult at 3 °C and 10 °C, and C.finmarchicus reared from CIII to adult at 10 °C, under excess conditions of food.

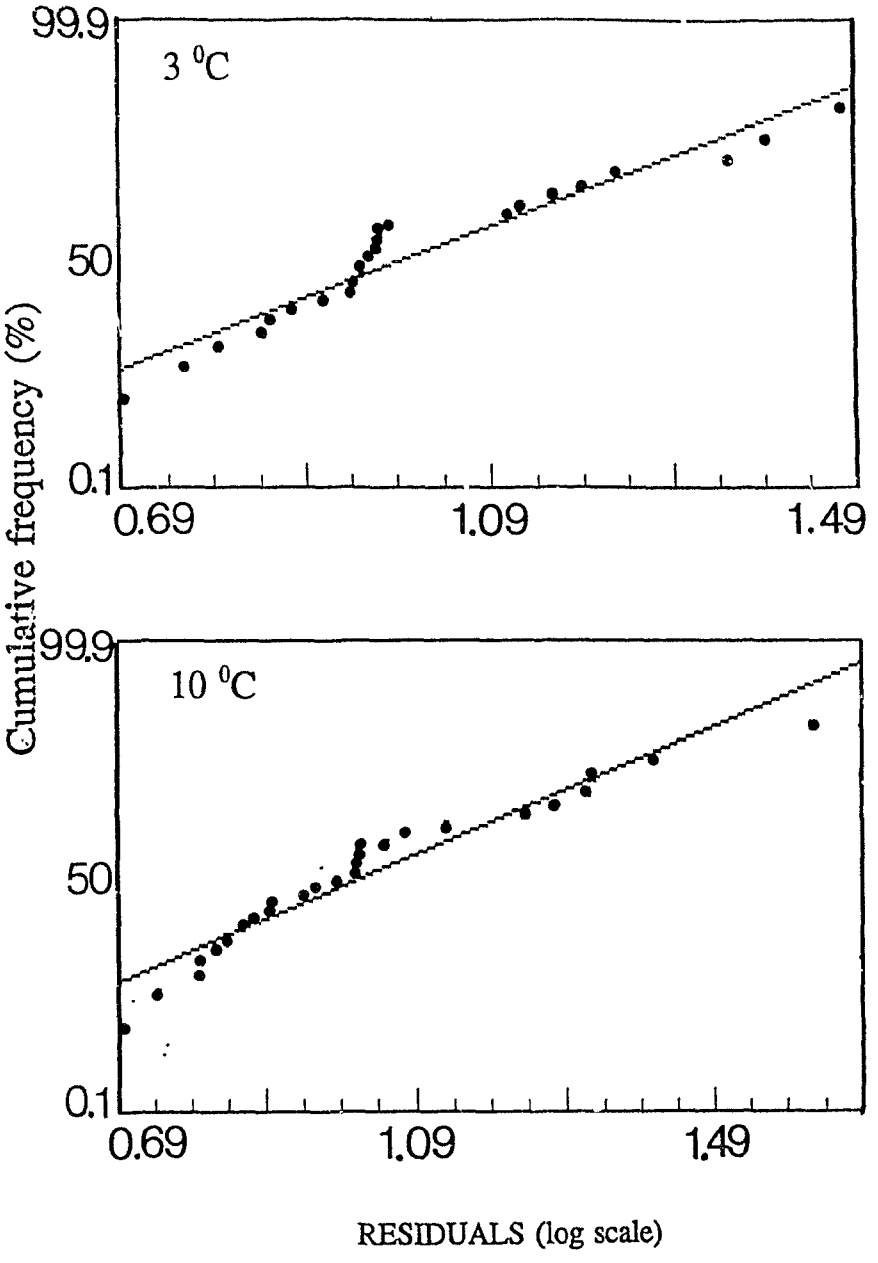

Fig.4.9 Normal plots of residuals obtained from an exponential fitting of growth in dry weight of E.herdmani reared from CI to adult at 3 °C and 10 °C, under excess conditions of food.

tested through the Kolmogorov-Smirnov test for continuous variables. No significant departures from a normal distribution were detected (K-S test < .20, P >.05)

Despite the greater scatter in data for Calanus species as compared to E.herdmani, residual variations after regression are not significantly different among species (F-ratio < 2.0, P >.05), suggesting that exponential models are equally valid for the three species. A slight decline of the growth curve in late development of C.glacialis should be noted, however (**Fig.4.4**). This is possibly because animals were resting in stage CV during that period. Indeed, duration of stage CV extended for more than two months in some individuals at the low temperature, suggesting a general reduction of metabolic rates. This is less pronounced in C.finmarchicus whose pattern of growth is more nearly exponential, and much less pronounced in E.herdmani which seems to be a clearly continuously growing species.

A greater scatter of points in Calanus species than in E.herdmani, is possibly because CIII's of the former individuals were obtained from field samples and may have been in various stages of development, whereas the latter constituted a more uniform cohort since they were all reared from egg to CI in same conditions of food and temperature. More variation in total dry weight of Calanus species can also be explained in terms of a high individual variation in lipid contents (see Chapter 3). Some of the residual variation in

E.herdmani may be explained in terms of sexual differences, since males and females were plotted together. Morphological differences between sexes could be observed at stage CIII and also females were heavier than males.

4.3.1. Growth in structural body mass

Individual variation in total dry weight (TW) of Calanus species may be partially explained by highly variable lipid contents, which account up to 40% of total dry weight in some stages (see Chapter 3). Therefore, removal of lipid stores might reduce some of the residual variation found in exponential models of growth of TW. Harris (1983) suggested that growth rate of Calanus copepodids may be independent of the "structural weight", but is a power function of the size of the lipid store. This implies that under conditions of sufficient food reserves from the lipid store, animals would grow continuously and possibly in an exponential pattern. This hypothesis was supported by McLaren (1986), who concluded that specific growth rate of C.finmarchicus under field conditions may be constant, if stored lipids are discounted.

Following procedures detailed in Chapter 3, "structural" dry weights (SW) for stages CIII to adult of both Calanus species were obtained under excess conditions of food and at 3 ^{0}C and 10 ^{0}C. Like growth in total dry weight (TW), SW also follows a pattern that is well described as exponential in

both species (**Fig.4.10**). Exponential equations are shown in **Table 4.3.**

As shown by scatter of points (**Fig.4.10**) and also by correlation coefficients (**Table 4.3**), although there is even more residual variation in the growth of SW of C.glacialis, there is a considerable reduction in residuals in C.finmarchicus compared with growth of TW variation (see also **Fig.4.11**). Patterns of growth of both, TW and SW in C.finmarchicus seem to be well described by exponential models, whereas in C.glacialis there is much variation not explained by exponential models, especially in growth of SW.

4.3.2. Growth in size of the lipid-store

Kattner & Krause (1987) observed exponential increases of total lipids from CI to CV in field samples of C.finmarchicus during the spring season in the North Sea. They also observed lower amounts of lipids in mature males and females than in copepodid CVs. The decrease in lipids at maturity in females has been attributed to lipid investment in egg production (Marshall and Orr 1955, Lee et al. 1972), and also to use during the molting process from CV (Gatten et al. 1980).

Since measurements of size of the lipid store in the present experiments were made through time regardless of developmental stage, in mature individuals lipid content is expected to be highly variable depending on reproductive status. This variability was especially high in C.finmarchicus

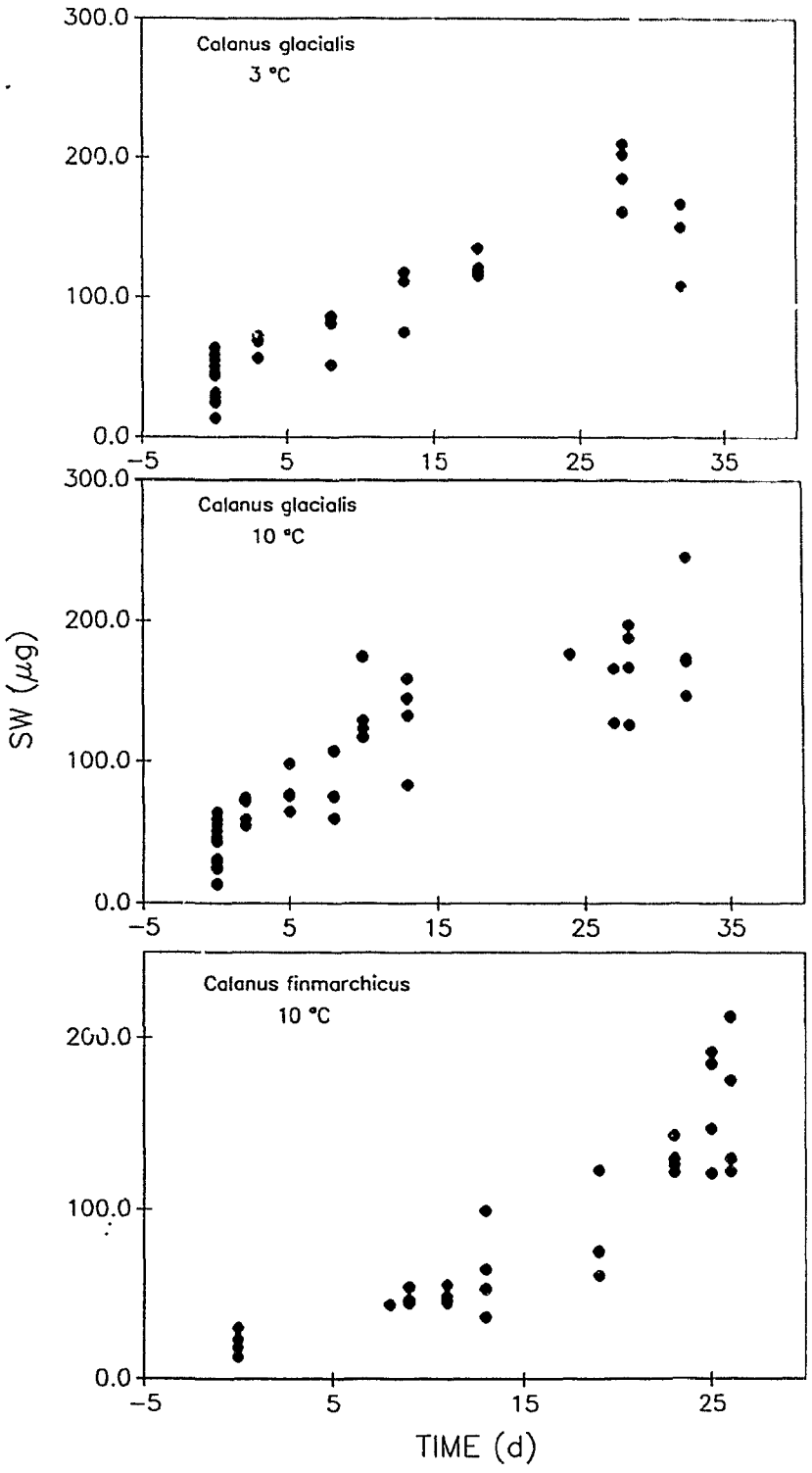

Fig.4.10 Growth in lipid-discounted dry weight (SW) in C.glacialis reared from CIII to adult at 3 °C and 10 °C, and C.finmarchicus reared from CIII to adult at 10 °C under excess conditions of food.

Table 4.3. Exponential equations of growth in lipid discounted dry weight (SW) of 2 copepod species reared at two temperatures and under excess of food. Equations were fitted through Least Square Method. Coefficients of determination (r^2) are highly significant ($P < 0.001$).

Species	Temp. (^0C)	n	Exponential model	r^2
C.glacialis	3	34	SW = Exp(3.779 + 0.049 time)	0.710
	10	42	SW = Exp(3.919 + 0.046 time)	0.647
C.finmarchicus	10	36	SW = Exp(3.247 + 0.080 time)	0.908

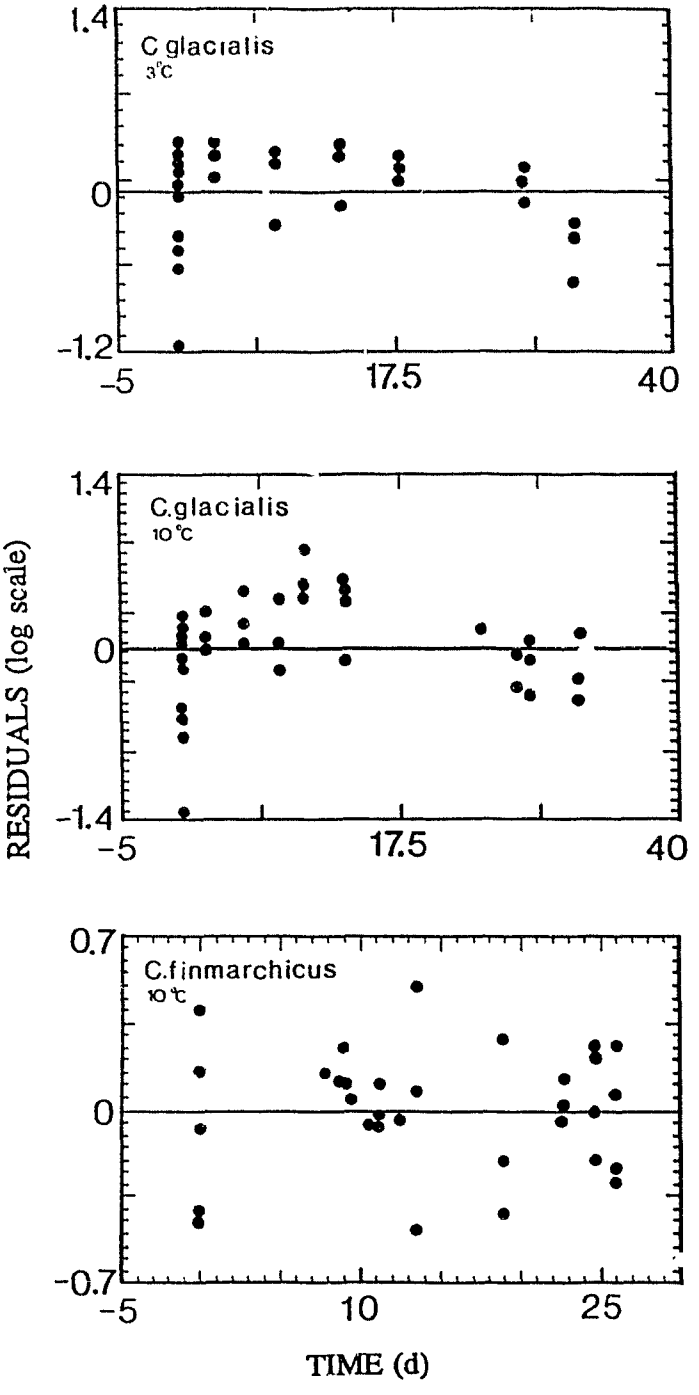

Fig.4.11 Residual variation after exponential fitting of growth in lipid-discounted dry weight (SW), in two Calanus species reared from CIII to adult at two temperatures, under excess conditions of food.

reared at 10 ^{0}C, where no clear pattern of growth in dry weight of the oil sac (OSW) can be observed (**Fig.4.12**), although a linear regression is significant (r^2 = .189, P <.01).

In C.glacialis, on the other hand, OSW correlates more strongly with time from CIII to adult at 10 ^{0}C and 3 ^{0}C (**Fig.4.12**). Exponential models are significant (r^2 = 0.570 for 3 ^{0}C and r^2 = 0.538 for 10 ^{0}C, P <.05), although, a high variability is evident from residual variation after exponential fit (**Fig.4.13**). Residuals do not show an even pattern around the predicted values, suggesting a non-normal distribution of errors. Log transformation of OSWs did not substantially reduce variability, or improve distribution of residuals.

4.4. Development rate and body size

It is often assumed that instar duration determines increments in size between developmental stages of copepods (e.g. Longhurst 1986), and that factors influencing this duration would ultimately regulate body size achieved at maturity. This assumption implies that size and development rate should be negatively correlated. In fact, it is a general rule that small species develop faster than large ones (e.g. Pianka 1970, Peters 1983).

Even though among copepod species development rate is, broadly speaking, inversely related to body size (e.g. McLaren

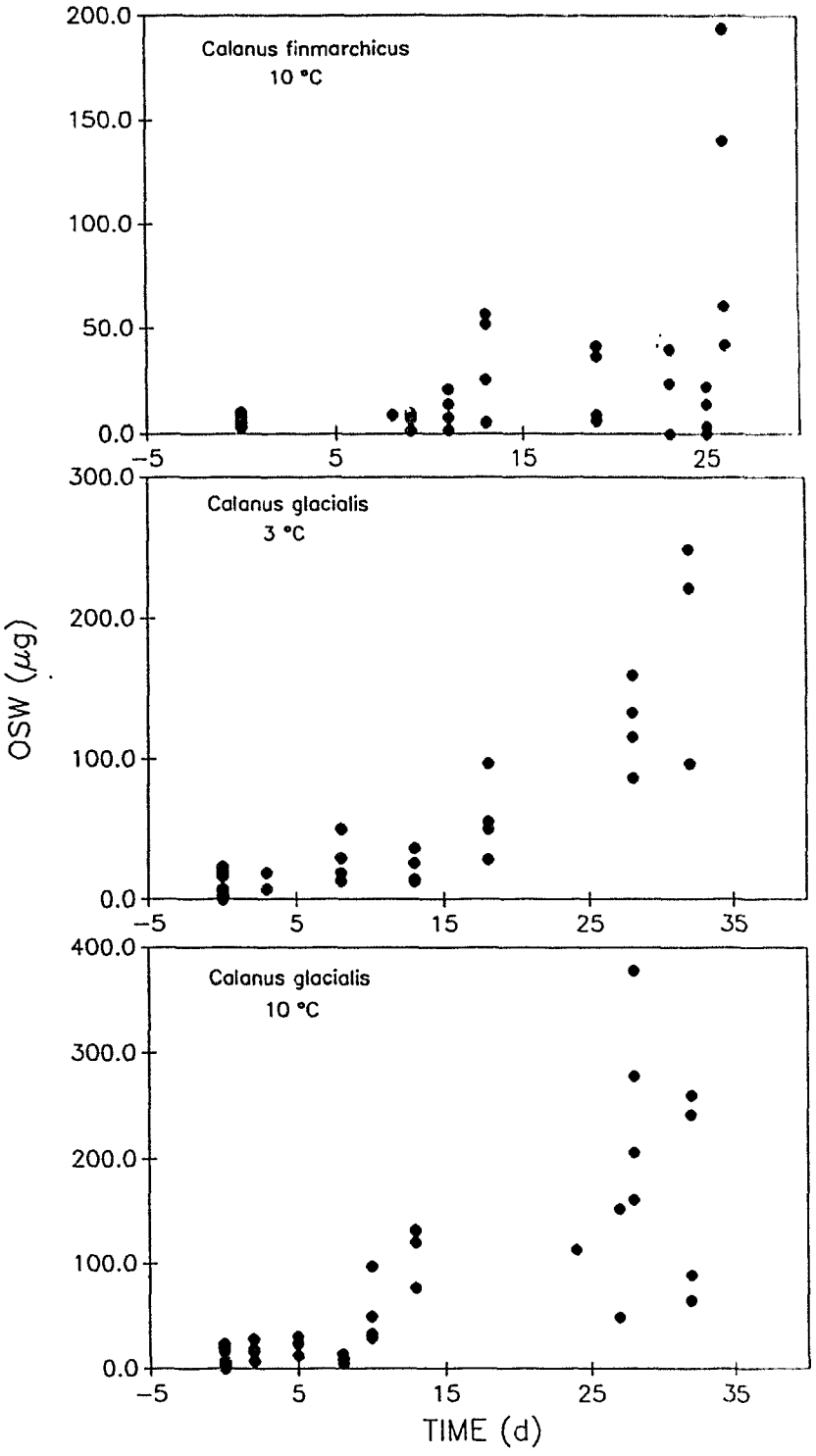

Fig.4.12 Growth in estimated dry weight of the oil-sac (OSW) in C.glacialis reared at 3 °C and 10 °C, and C.finmarchicus reared at 10 °C, under excess conditions of food, from stage CIII to adult.

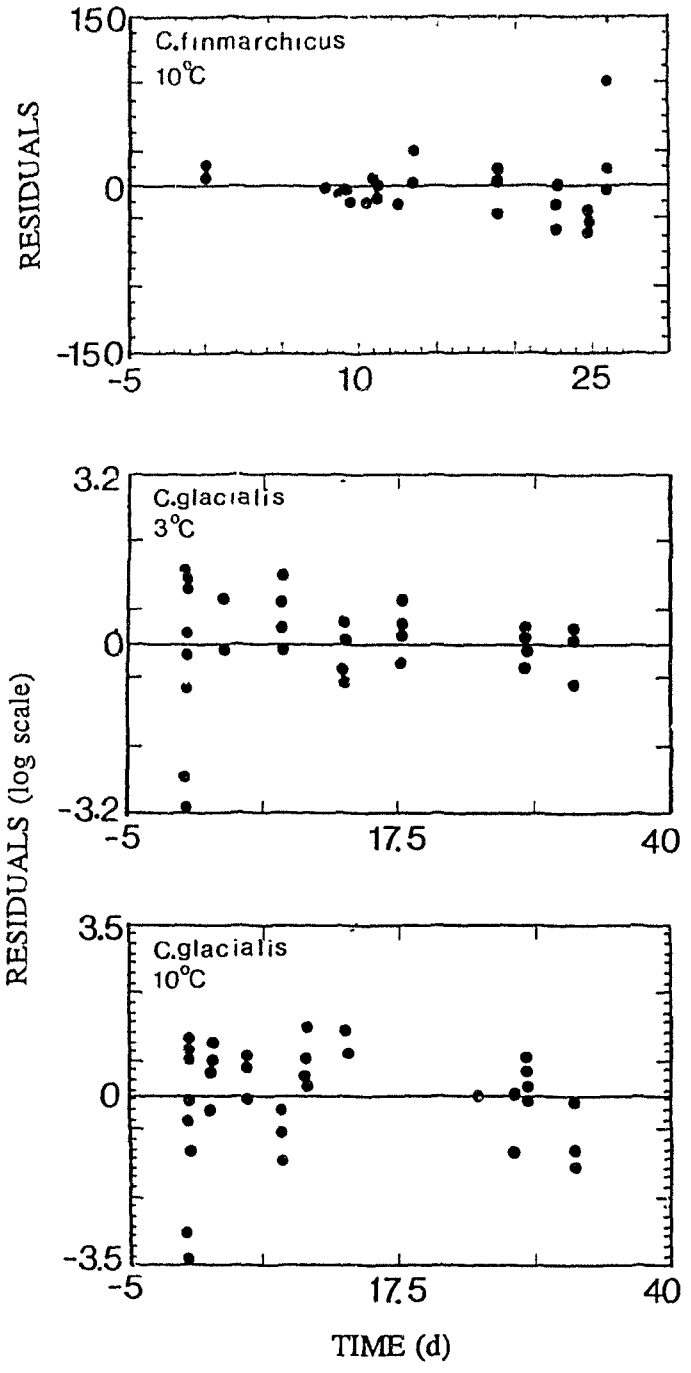

Fig.4.13 Residual variation after linear regression and exponential fit of growth in estimated dry weight of the oil sac in two Calanus species reared from stage CIII to adult (linear regression was fitted to growth of C.finmarchicus at 10 °C and exponential model to growth of C.glacialis at 3 °C and 10 °C.

et al. 1988), there is no clear evidence that intrapopulation variations in size are related to changing stage durations. Since molting rates are quite variable among individuals, even under controlled conditions (e.g. Peterson and Painting 1990), development rates are usually obtained by estimating the median development time for each stage; i.e. the time when 50% of the population has molted. This method obviously does not consider individual times, which eventually determine size variations. The presence of abnormal "laggards" (McLaren et al. 1989b) is a common problem in rearing copepods. These "laggards" alter distributions of development times, such that log transformations are required to normalize data. However, untransformed data of individual variation of development times may be more useful to examine their eventual influences on body size.

In this study, estimations of molting times from individuals of known body sizes (lengths and masses) allow us to examine to what extent size at the end of a given stage depends on the duration of that stage.

4.4.1. Stage duration and body length

In an initial experiment, about 100 field-sampled copepodid stage CIVs of E.herdmani were measured in prosome length and randomly sorted in individual containers with excess of food at 10 ^{0}C and 3 ^{0}C. As soon as they reached maturity, their lengths were measured again. Analysis of

covariance (ANCOVA) revealed no temperature effects on adult length ($F_{1,96}$ = 0.144, P >.05), significant differences between sexes, a significant influence of initial length (at CIV) on size at adulthood, and no influence of the duration between CIV and adult on adult length (**Table 4.4**). Plotting of adult prosome lengths as a function of the time between CIV and adult illustrates the absence of correlation (**Fig.4.14**).

In a second experiment with E.herdmani, molting times and changes in lengths were obtained from frequent observations of individuals from CI to adult at 3 ^{0}C and 10 ^{0}C. At both temperatures there was considerable variation in stage durations (**Table 4.5**). Also, stages CIV and CV tended to extend longer than did earlier stages, especially in females.

As observed in the first experiment, adult length correlated to length of earlier stages. However, no correlation was found (partial correlation tests, P >.05) between length increments between stages and stage duration (**Fig.4.15, Fig.4.16**).

The influence of development rate on body length was also studied in C.glacialis at 3 ^{0}C and 10 ^{0}C. In a first experiment individual CIII were reared to CV at 10 ^{0}C with excess of food, so that CIV duration was recorded. the absence of correlation of size with CIV duration is self evident (**Fig.4.17**).

Table 4.4 Analysis of covariance testing dependence of adult prosome length on length at CIV, on duration between CIV and adult, and differences between sexes, in E.herdmani reared from CIV to adult under excess conditions of food. An initial test for homogeneity of slopes revealed a significant interaction between temperature and stage duration ($P > .05$). Since temperature did not affect either adult length or length increment, it was not included in this analysis.

Source of variation	d.f.	F-ratio	P	
Length CIV	1	4.813	< 0.05	**
Duration CIV-Adult	1	3.534	> 0.05	n.s.
Sex	1	65.992	< 0.001	***
Error	96			

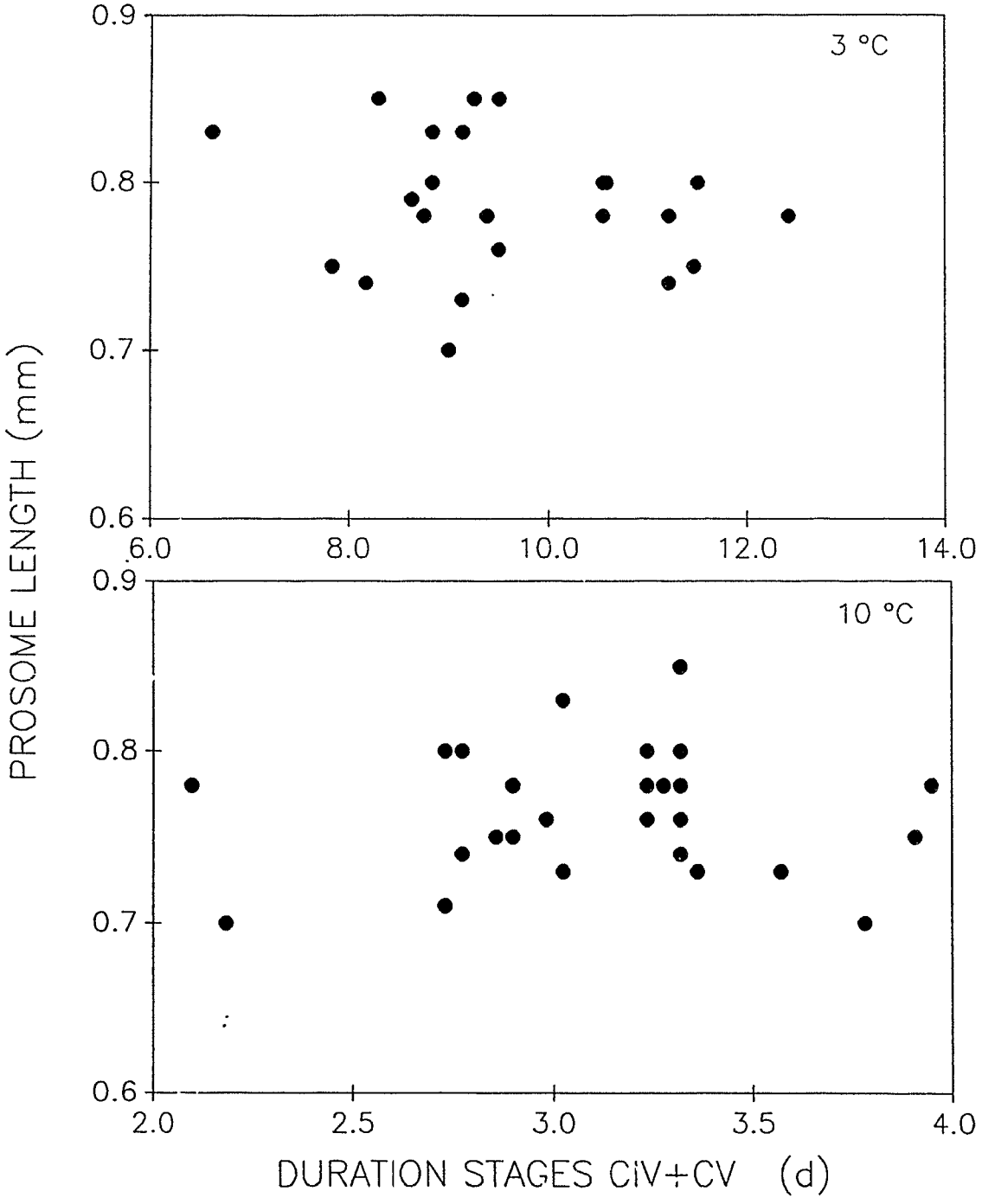

Fig.4.14 Prosome length of adult males and females as a function of stage CIV and CV durations in E.herdmani, reared at at two temperatures under excess conditions of food.

Table 4.5. Mean stages durations of copepodids CI to adult of E.herdmani reared under excess of food at two temperatures.Data were obtained from individual observations (between 10 and 20 individuals for each mean value).

	Mean stage duration (d) + S.D.			
Stage	3.0 °C		10.0 °C	
CI	5.19 ± 0.39		1.96 ± 0.15	
CII	5.28 ± 0.45		2.22 ± 0.41	
CIII	5.05 ± 0.54		1.95 ± 0.27	
	F	M	F	M
CIV	6.35 ± 0.53	5.23 ± 0.55	2.43 ± 0.29	1.69 ± 0.15
CV	8.64 ± 0.73	5.67 ± 0.58	2.84 ± 0.05	2.38 ± 0.40

F = females, M = males

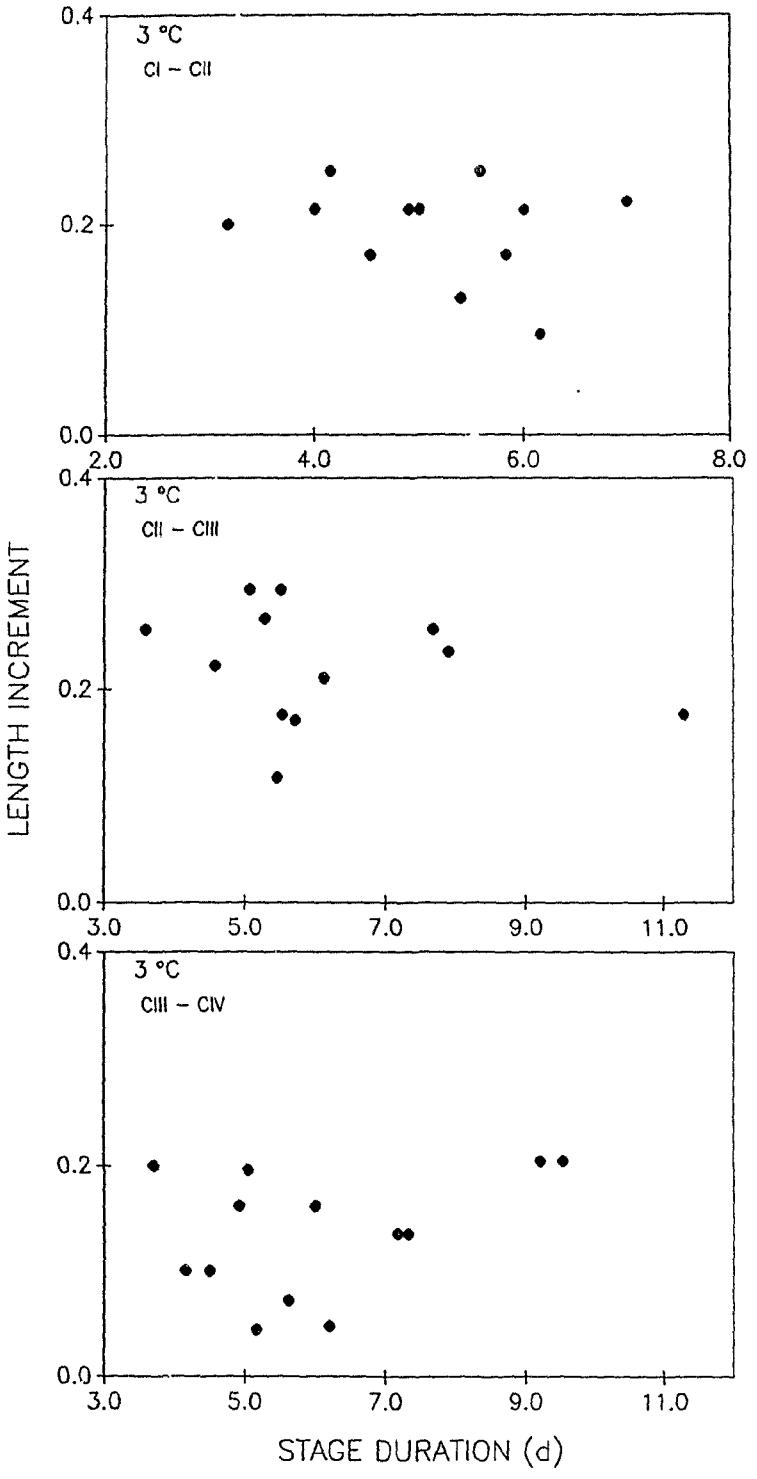

Fig.4.15 Length increments between stages plotted as a function of corresponding stage durations in E.herdmani, reared at 3 °C under excess conditions of food.

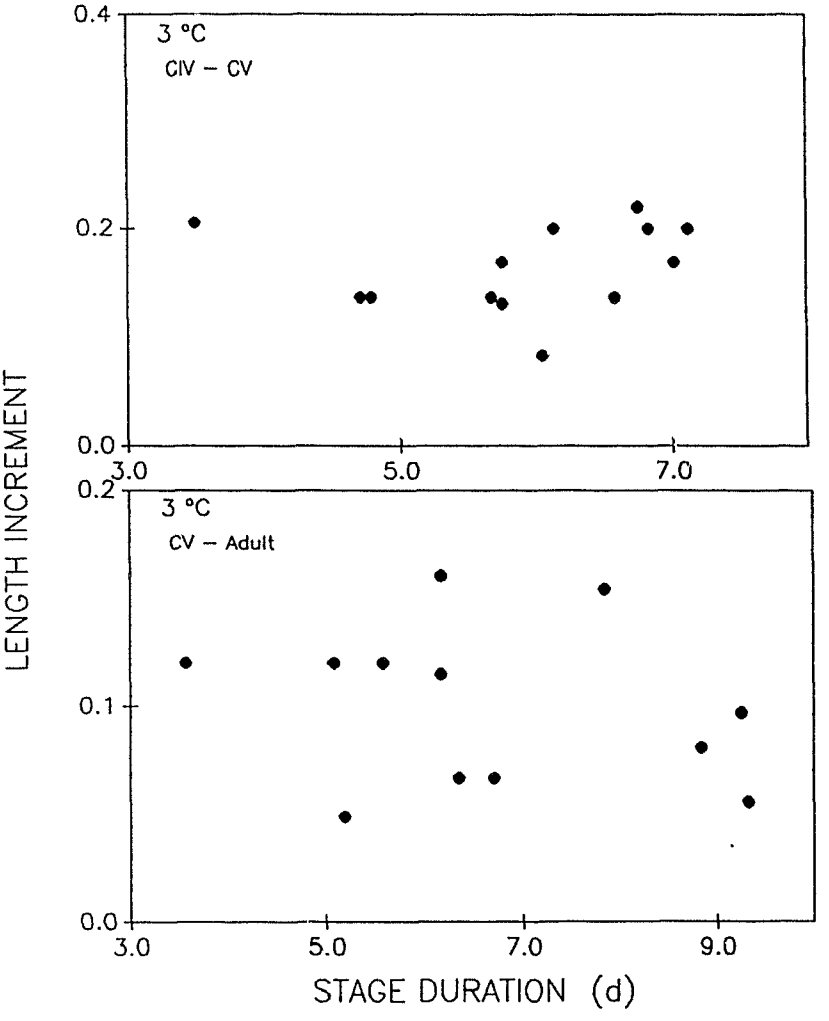

Fig. 4.15 (continued)

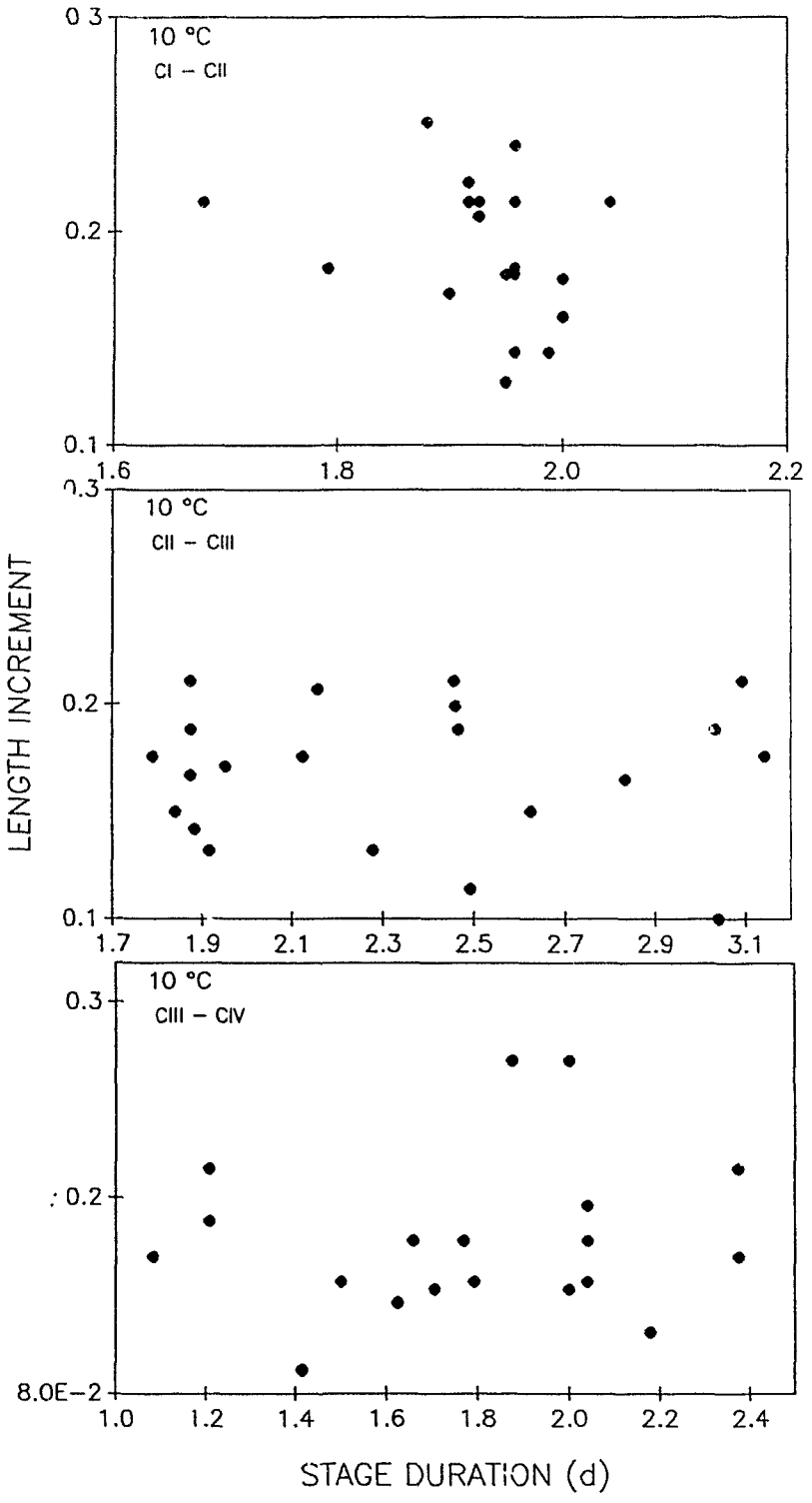

Fig.4.16 Length increments between stages plotted as a function of corresponding stage durations in E.herdmani, reared at 10 °C under excess conditions of food.

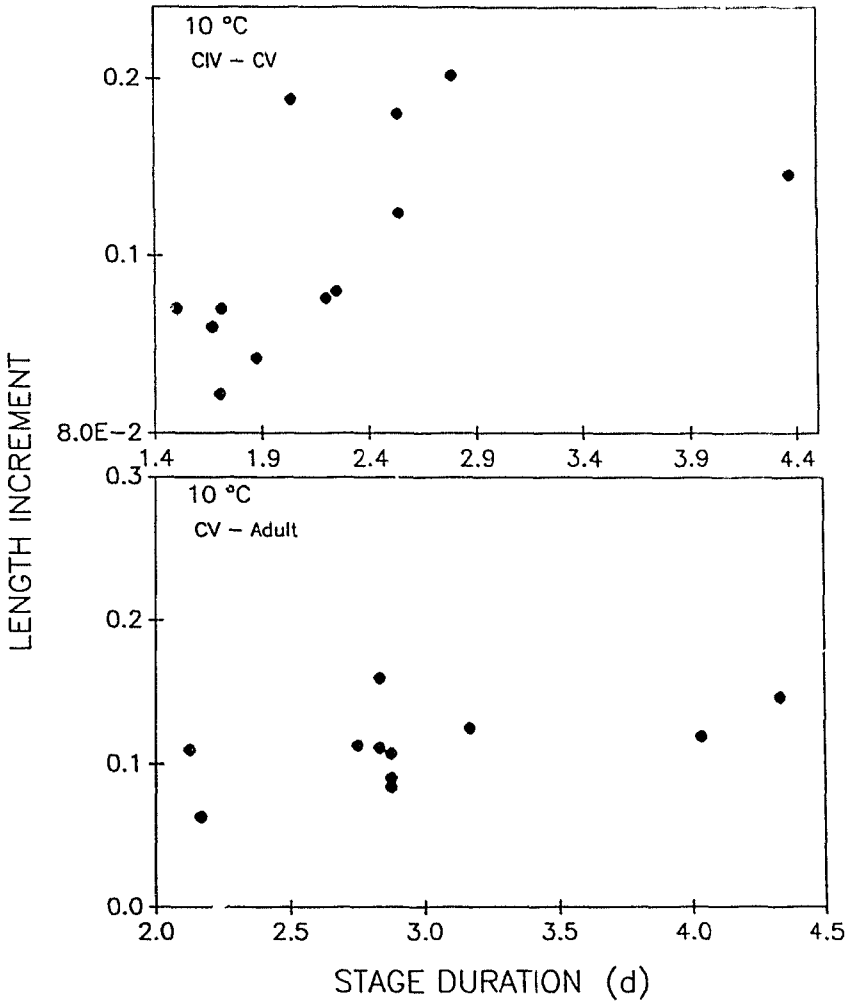

Fig.4.16 (continued)

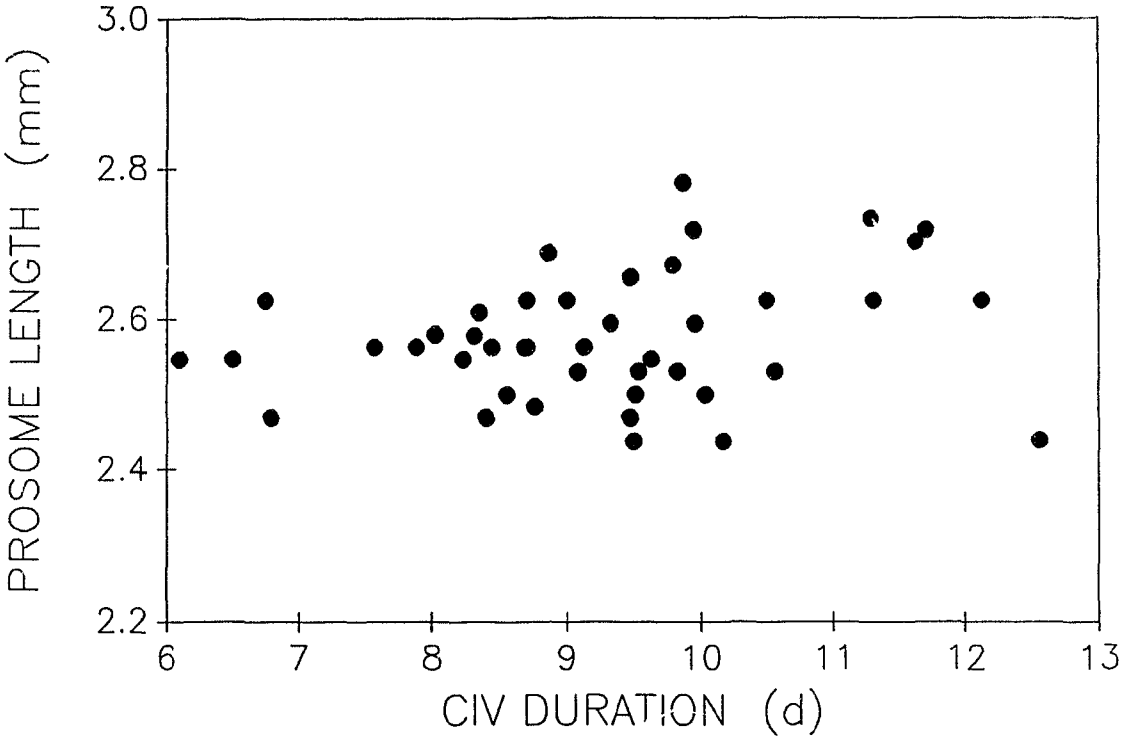

Fig.4.17 Prosome length of stage CV of C.glacialis plotted as a function of stage CIV duration after rearing at 10 ^{0}C under excess conditions of food.

In another experiment with C.glacialis a small number of individuals, reared at 3 ^{0}C and 10 ^{0}C, was selected to obtain precise estimations of stage durations along with changes in prosome length. Thus, the influence of CIV and CV duration on prosome length at stages CV and adult was examined. Duration of CIII was not considered because the starting point of this stage was unknown. It is clear that stage durations are not equal in this species, for CV lasts ca. 3 times longer than CIV (**Table 4.6**). According to McLaren and Corkett (1986) this species enters a resting stage at CIV in nature, but in this experiment, individuals apparently began resting at stage CV, given the long time to reach maturity at both temperatures, despite excess conditions of food.

In order to examine the influence of development rate on prosome length, a matrix of partial correlations was obtained for each temperature, including prosome lengths at CIII, CIV, CV and adult, and CIV and CV durations (**Table 4.7**). Since prosome length at any stage might be influenced by size at previous ones, relative increments in length between stages were also included in this matrix. At 10 ^{0}C, adult body length was correlated to body length at CV and to relative increments in length between stages CIV-CV and CV-adult (**Table 4.7**). Body lengths of neither adult, nor those of CVs were correlated with durations of the previous stage. Nor do relative increments in length between stage show any significant correlation with stage durations. These correlations, or lack

Table 4.6 Mean duration of stages CIV and CV of C.glacialis reared at 10 °C and 3 °C under excess conditions of food. Mean values were obtained from individual observation of molting times.

Stage	Mean Duration ± S.D. (d)	
	10 °C (n=15)	3 °C (n=16)
CIV	7.08 ± 1.36	14.66 ± 2.19
CV	21.62 ± 4.13	37.02 ± 3.06

Table 4.7. Correlation matrix among prosome lengths of C.glacialis at stages CIII(LCIII), CIV(LIV), CV(LCV) and adult(LAd.), duration of stages CIV(DCIV) and CV(DCV), and length increments between stage CIV-CV(LICIV) and CV-adult(LICV), obtained at 10 °C and 3 °C, after rearing from CIII to adult under excess of food.

10 °C

	LCIII	LCIV	LCV	LAd.	DCIV	DCV	LICIV	LICV
LCIII	-							
LCIV	.007 (.76)	-						
LCV	-.079 (.78)	.504 (.06)	-					
LAd.	-.050 (.86)	.105 (.71)	.700** (.004)	-				
DCIV	.420 (.16)	.134 (.64)	.205 (.46)	-.035 (.90)	-			
DCV	-.013 (.99)	-.132 (.64)	-.147 (.60)	-.265 (.34)	-.06 (.83)	-		
LICIV	-.162 (.56)	-.591 (.02)	.399 (.14)	.542** (.04)	.05 (.86)	.005 (.99)	-	
LICV	-.003 (.99)	-.325 (.24)	.058 (.84)	.750** (.001)	-.23 (.41)	-.231 (.41)	.399 (.14)	-

Table 4.7. (continued)

3 °C

	LCIII	LCIV	LCV	LAd.	DCIV	DCV	LICIV	LICV
LCIII	-							
LCIV	.550** (.03)	-						
LCV	.420 (.10)	.640** (.01)	-					
LAd.	.531** (.03)	.385 (.14)	.550** (.03)	-				
DCIV	.490 (.06)	.590** (.02)	.630** (.01)	.160 (.56)	-			
DCV	.020 (.90)	-.130 (.62)	.240 (.37)	.580** (.02)	-.26 (.32)	-		
LICIV	-.270 (.32)	-.650** (.01)	.160 (.55)	.056 (.84)	-.110 (.68)	.409 (.11)	-	
LICV	.068 (.80)	-.320 (.22)	-.530** (.04)	.420 (.11)	-.520** (.04)	.340 (.19)	-.097 (.72)	-

of them, are also illustrated in **Fig.4.18.** These results suggest that length increases and hence length variations, under these conditions, are not influenced by rate of development.

Results at the low temperature (3 ^{0}C) were somewhat different from those at 10 ^{0}C. Adult body length was also positively correlated to length of the previous stage, CV, and to that of CIII (**Table 4.7**). However, at this temperature, body lengths of adults and CVs were significantly correlated to duration of previous stages. A slight positive correlation between length increments between CV and adult and CV duration was observed (**Fig.4.19**), whereas increments in length between CIV and CV were not correlated with CIV duration (**Table 4.7**, also **Fig.4.19**).

4.4.2. Stage duration and body mass

In continuously growing animals a slow development rate would allow more tissue to accumulate and hence greater body weights at maturity. This, of course, assumes that growth rate is constant or at least does not slow down to the same extent as development rate. According to earlier evidence (e.g. McLaren 1986) and data presented here, growth of copepods can be constant through development. If true, body mass at the end of a given stage should be positively related to duration of that stage, especially, in experiments at constant temperatures and excess of food.

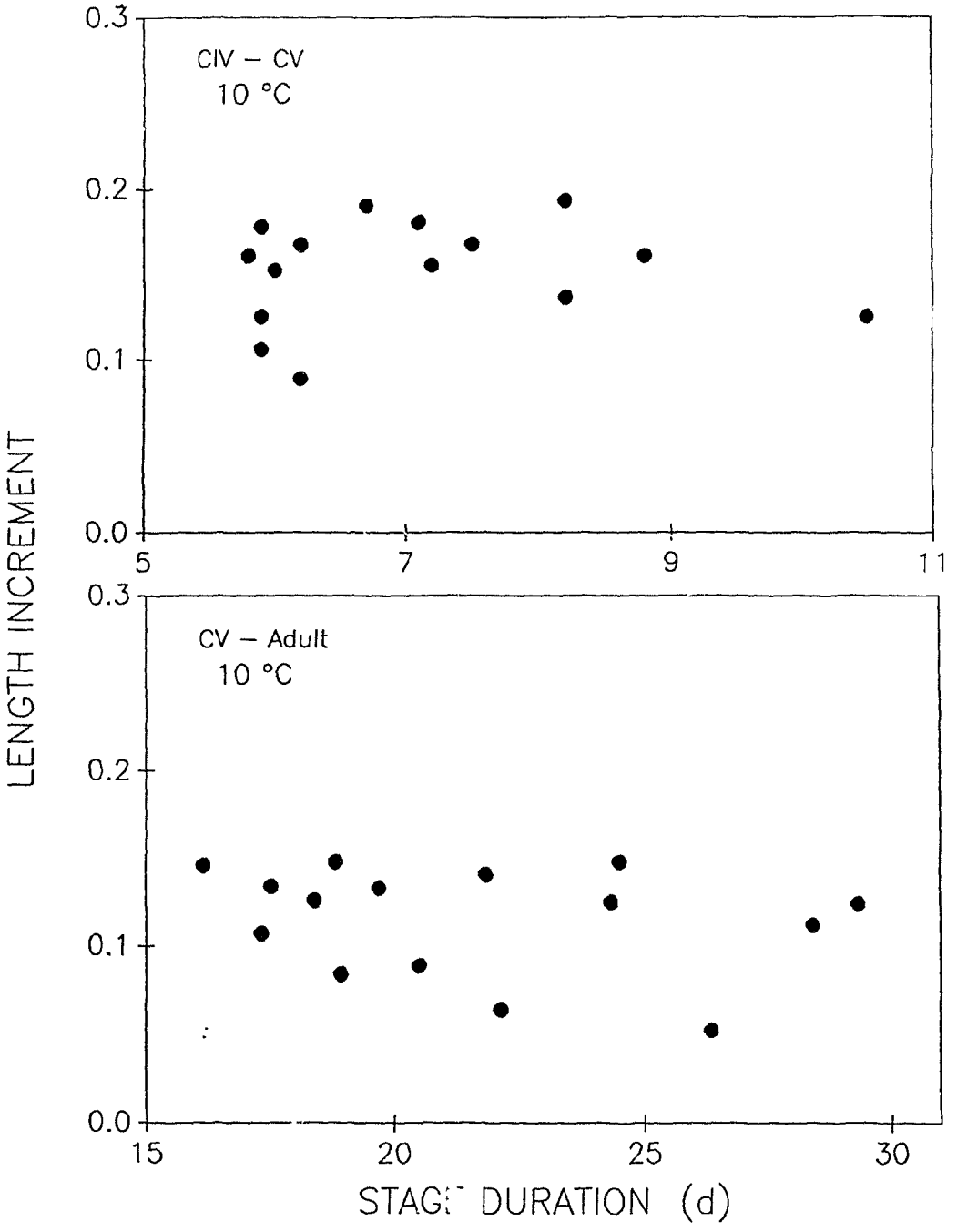

Fig.4.18 Length increments in C.glacialis plotted as a function of corresponding stage durations after rearing from stage CIII to adult at 10 °C, under excess conditions of food.

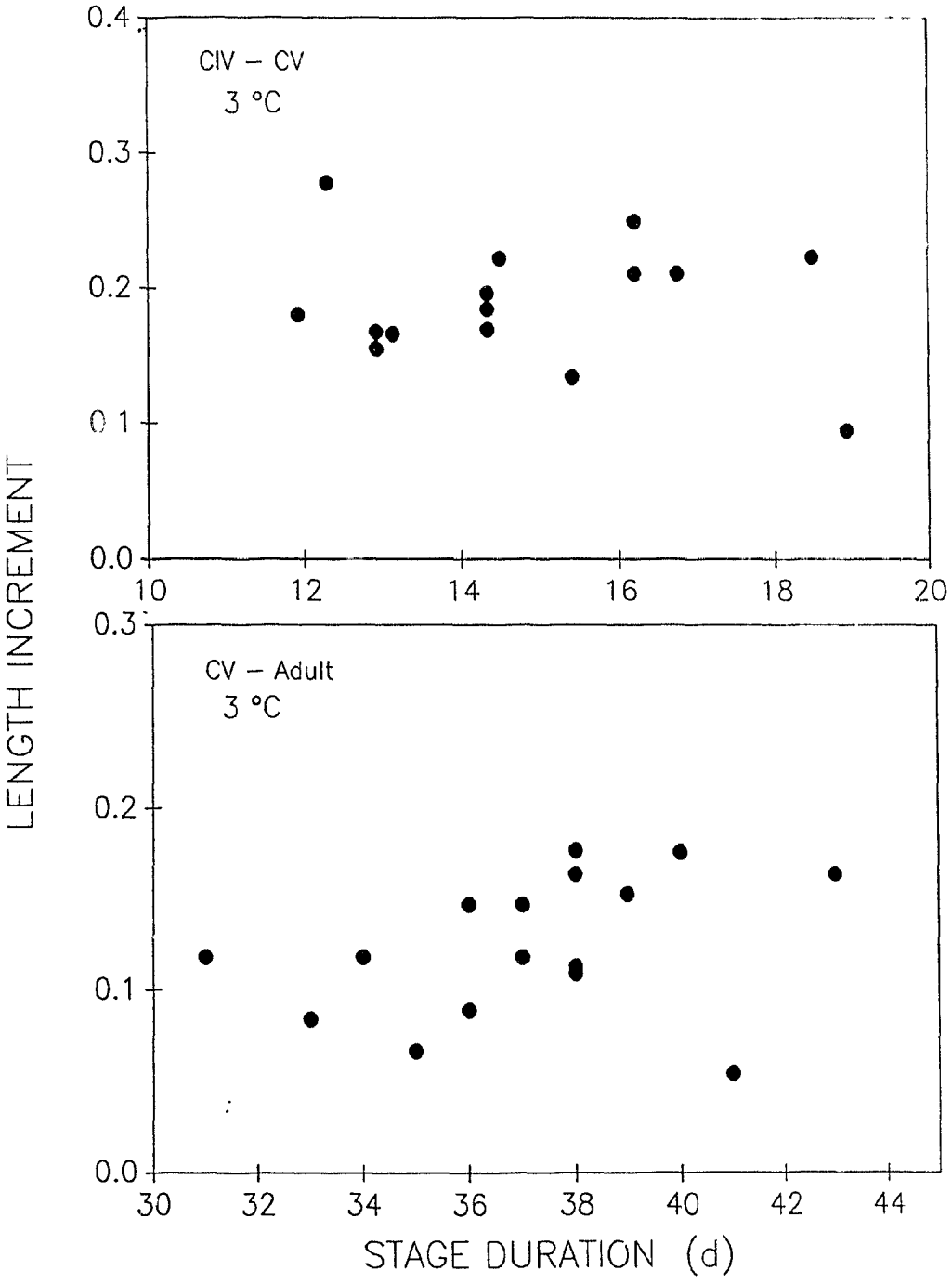

Fig.4.19 Length increments in C.glacialis plotted as a function of corresponding stage durations after rearing from stage CIIi to adult at 3 °C, under excess conditions of food.

From a first experiment performed with E.herdmani individual stage CIVs were reared to adult at 3 °C and 10 °C. The time taken between CIV and adult was recorded for each individual, and dry weights were obtained as soon as they matured. The lack of correlation at both temperatures (**Fig.4.20**) suggests that increases in weight are independent of stage durations. Unfortunately, no information is available for initial dry weights (at CIV), and this certainly limits this conclusion, because variability in adult weights might depend on weights of earlier stages.

In a second experiment, dry weights of stages CI to adult of E.herdmani reared at 10 °C and 3 °C were obtained from recently molted individuals. Since animals have to be killed, changes in dry weight cannot be followed in the same individuals through development. Moreover, weight is accumulated from one stage to another. Therefore, to analyze the influence of stage duration on body weight, increments in weight between stages were estimated from mean values of dry weight at the beginning of each stage and from mean values of duration of the previous stage. Increment in weight was estimated as,

$$G = (\ln DW_2 - \ln DW_1)/t_1 \qquad (4.5)$$

where G is the instantaneous growth rate ($\mu g.d^{-1}$), DW_2 and DW_1 are mean dry weight of two succesive stages, and t_i is mean stage duration.

It can be observed that growth rates do not relate directly to stage duration (**Table 4.8**). For example, the largest growth rates were observed between stage CII and CIII at 3 ^{0}C and between CIII and CIV at 10 ^{0}C. This increase in rates took place associated with the appearance of morphological differences between sexes. However, corresponding stage durations were not considerably shorter at these stages. Within temperatures, among stages, durations are significantly different (one-way ANOVA, $F_{4,146} = 9.312$, $P < 0.001$). Duration of stage CV was the longest one at both temperatures, but there was no notable decrease in growth rate at this stage. Therefore, a lack of association means that weight increments during discrete time intervals (in this case stages of development) do not depend on the rate of development.

The influence of stage duration on body weight was also studied in C.glacialis. An initial experiment tested the hypothesis that individuals under constant conditions of food and temperature would have similar growth rates, so that development rate would determine body mass at the end of a given stage. About 60 individuals of CIII were reared in excess of food up to stage CV, at which stage they were weighed and the time taken between CIV and CV recorded for

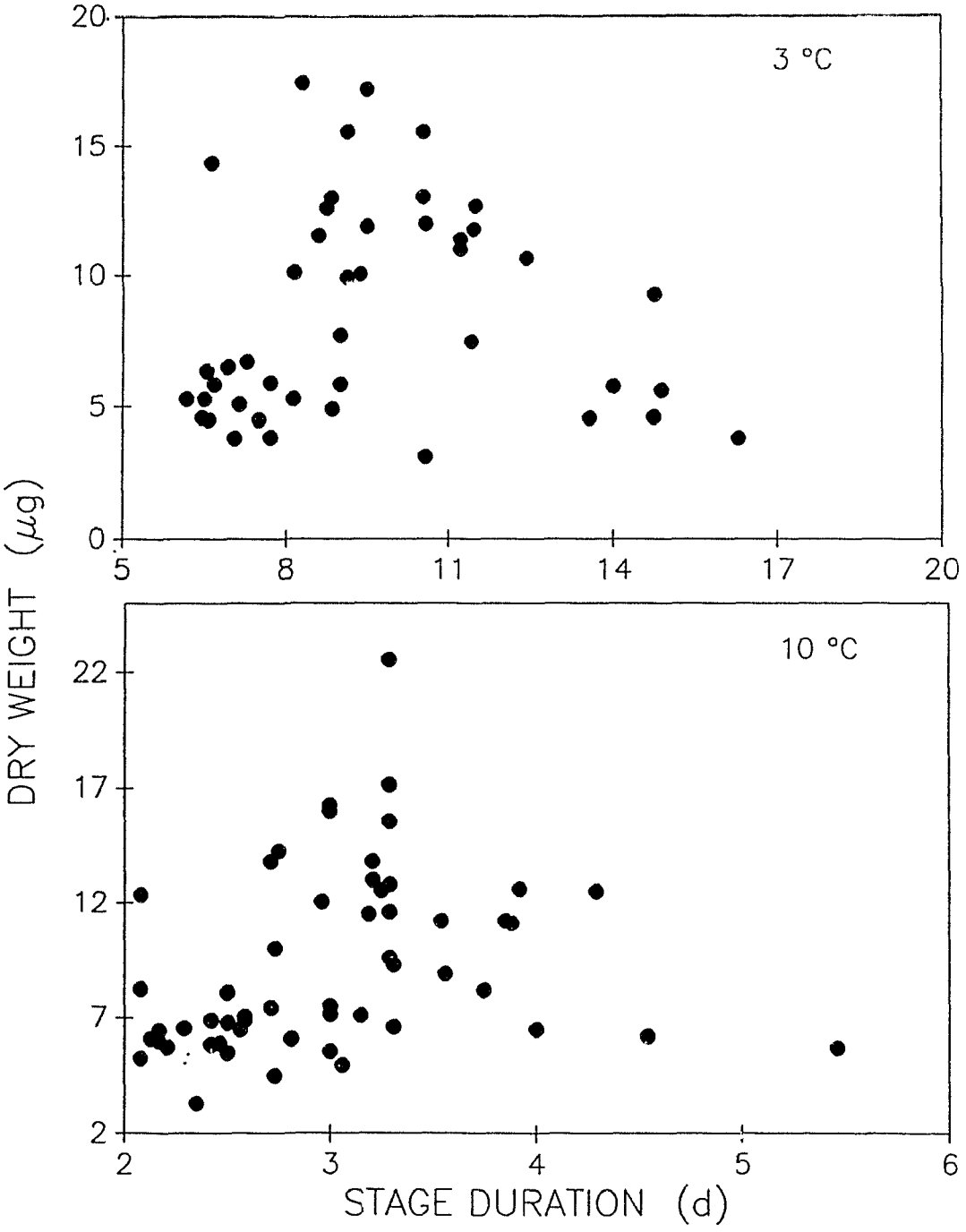

Fig.4.20 Dry weight of adult males and females E.herdmani as a function of stages CIV and CV durations after rearing at two temperatures, under excess conditions of food.

Table 4.8. Instantaneous growth rate of copepodid stages of E.herdmani reared at two temperature and under excess of food.

Temp.	Stage	Stage duration (d) + S.D.	Instantaneous growth rate ($\mu g \cdot d^{-1}$)
10 °C	CI	1.936 + 0.070	0.221
	CII	2.344 + 0.469	0.057
	CIII	1.784 + 0.378	0.395
	CIV	2.266 + 0.778	0.176
	CV	2.988 + 0.670	0.155
	Adult		
3 °C	CI	5.209 + 1.050	0.024
	CII	6.083 + 1.933	0.144
	CIII	6.045 + 1.821	0.065
	CIV	5.895 + 1.056	0.040
	CV	6.672 + 1.805	0.072
	Adult		

each individual. An absence of correlation between CIV duration and CV weight seems evident (**Fig.4.21**), although as in E.herdmani, the lack of initial weights limits conclusions.

In another experiment, CIIIs were reared until they reached adult at 3 ^{0}C and 10 ^{0}C. In this case, however, dry weights were obtained at irregular time intervals, regardless of developmental stage. Thus, body weights at the beginning and at the end of each stage are unknown. Nevertheless, assuming that mean dry weight of each stage represents body weight at the middle of the stages and using mean stage durations, the increments in weight between stages can be approximated using equation (5). **Table 4.9** shows estimated instantaneuos growth rates for each stages. At both temperatures there is a notable decline in growth rate from CIII to adult.

4.5. Discussion

In order to examine the effect of temperature or other factors on growth and development, it is necessary to analyze first the basic patterns of growth and development and then how these patterns might be affected by these factors. In this context, development has been much more studied than growth. The influence of temperature on development rate has also been well studied in copepods and some empirical relationships have been established. It is known that development time decreases logarithmically with increasing temperature. From studies of

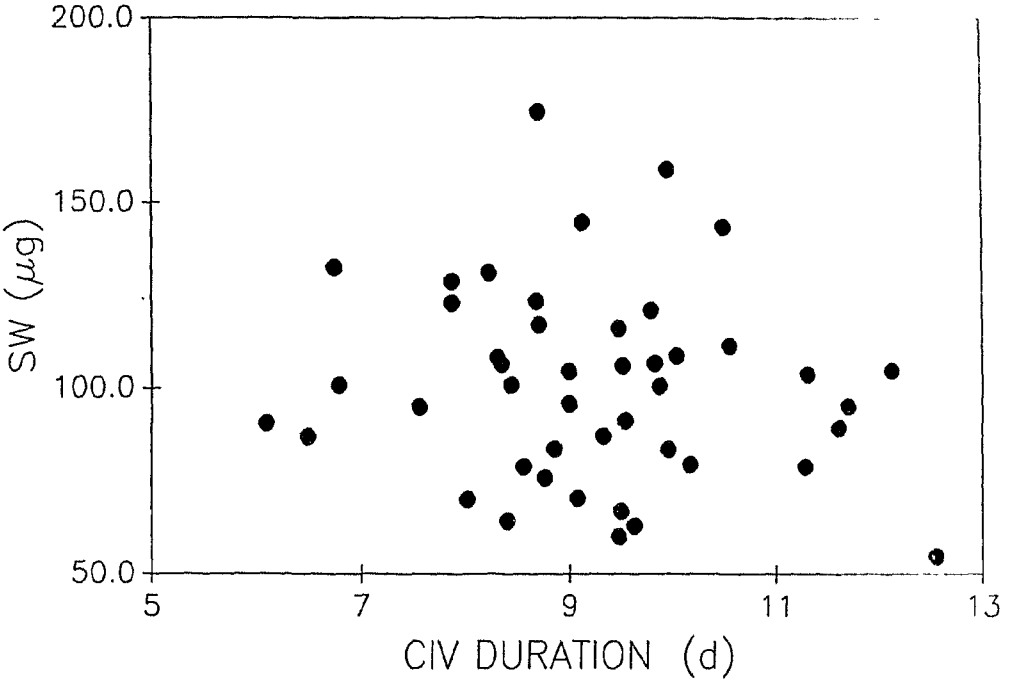

Fig.4.21 Lipid-discounted dry weight (SW) of stage CV C.glacialis as a function of stage CIV duration, after rearing from CIII to CV at 10 °C under excess conditions of food.

Table 4.9 Mean stage durations and estimated instantaneous growth rates of structural weight (SW) between stages of C.glacialis reared from CIII to adult at two temperatures under excess conditions of food.

Temp.	Stage	Stage duration (d) ± S.D.	Instantaneous growth rate (ug . d^{-1})
10 °C	CIII	1.460 ± 0.050	0.390
	CIV	8.290 ± 1.431	0.078
	CV	17.610 ± 1.220	0.013
	Adult		
3 °C	CIII	2.920 ± 1.201	0.230
	CIV	14.570 ± 2.330	0.039
	CV	38.080 ± 3.640	0.005

embryonic development rates, this relationship has been found to be well described by Béhlerădek's temperature function (Mclaren 1966), $D = a(x - T)^{-b}$, D is development time, T is temperature and a, x and b are empirical constants. This model has been found suitable and with ample application to study temperature-dependent development of copepods (e.g. McLaren et al. 1969, McLaren 1978, McLaren et al. 1988, Uye 1988).

In the present experiments development rates clearly responded to temperature. Stage durations of E.herdmani and C.glacialis are extended by factors of ca. 2.6 and 1.7 respectively at low temperature (**Tables 4.6, 4.7**). Duration of all stages changed in about the same proportion in response to temperature, suggesting that development rate may be equiproportional (Corkett 1984). However, it was clear that stage durations are not isochronal.

The course of growth in copepods, on the other hand, is not well understood, even under controlled conditions. Although patterns of growth in body mass have been documented for several species, there is no agreement on what models best describe such patterns. Experimental evidence suggests that body mass expressed as carbon or dry weight increases sigmoidally with age, such that weight-specific growth rates tend to decrease towards older stages (e.g. Mullin and Brooks 1970, Paffenhöfer 1976, Vidal 1980a, Peterson 1986, Uye 1988). In spite of this evidence, an alternative view proposes that increases in body mass are potentially exponential throughout

stages, so that weight-specific growth rates are constant (e.g. Harris 1983, McLaren 1986). Exponential growth has been found in Acartia (Landry 1975, Sekiguchi et al. 1980), in E.herdmani (McLaren and Corkett 1981), and in C.finmarchicus (McLaren 1986). To reconciliate this disagreement, Harris (1983) proposed a model for growth of Calanus in which growth rate of structural weight (lipid discounted body mass) would be only limited by size of the lipid store. According to McLaren (1986), exponential growth of "structural weight" of Calanus can occur under conditions of sufficient food to allow storage, while species that do not accumulate substantial amounts of lipid, such as Acartia and Eurytemora, tend to show an exponential pattern of growth of total weight.

C.glacialis and C.finmarchicus are lipid-storing species, and analyses of patterns of growth in total weight (TW) and structural weight (SW) obtained in the present study may give some insight into this controversy. As shown above (**Fig.4.4, 4.10**), growth of Calanus species follows a pattern in both TW and SW that may fit to an exponential one. The residual variation after non-linear fitting (**Fig.4.6, 4.11**) has to be taken into account, however. This variability is more pronounced in C.glacialis, and as shown in **Table 4.9**, the growth rate did not remain constant, but it tended to decrease through older stages. Therefore, it appears that growth in SW and TW of C.glacialis is not exponential. Even though exponential equations are significant in this species they

cannot describe or predict growth of older stages. In fact it can be easily demonstrated that they overestimate body weight of adult and CV stages.

C.finmarchicus, on the other hand, seems to show a nearly exponential growth. Although there is still much variation of TWs not explained by an exponential model of growth, some of this variation is reduced by using lipid discounted weight (SW). **Fig.4.10** is self-evident, supporting the conclusion of McLaren (1986) that this species may indeed show exponential growth if lipids are discounted and if conditions of food are non-limiting.

Growth in dry weight of E.herdmani is clearly exponential and a greatly reduced variation obtained after fits of exponential models is due, not only to the absence of highly variable lipid contents as observed in Calanus species, but also to the continuous growth of this species through stages. Even although late stages take slightly longer to develop, calculated growth rates for each stage did not show any clear pattern of decrease through older stages (**Table 4.8**).

Differences in patterns of growth among species should thus be taken into account. In this study the three species were subjected to similar conditions. However, they responded differently, and although food requirements for each species might be different, it is more likely that in the field the three species indeed show different growth patterns. For instance, it seems that the gradual decrease of growth rates

through development results from a general reduction of metabolic rates, and this is more pronounced in species that tend to have resting stages. C.glacialis was the slowest developing species in this study. This is clearly shown in reduced rates of development and growth at CV. E.herdmani, on the other hand, seems to develop more continuously and shows a clear exponential growth. These two species represent very different organisms in several aspects. The former is a high latitude species with a long overwintering period and basically annual life cycle (McLaren et al. 1989d). E.herdmani, on the other hand, appears to be a more continuously growing species of estuarine environments (Katona 1970), where there may be short-term environmental fluctuations, thus favouring a rapid and more continuous life cycle than in the strongly seasonal open ocean. In this context, C.finmarchicus seems to fall in an intermediate category with a life cycle more dependent on food supply (Diel and Klein Breteler 1986). Thus, under non-limiting conditions of food, its growth and development might be more continuous and temperature-dependent. In this study, under excess of food, it tends to show an exponential pattern of growth, especially growth in SW.

Much caution should be taken, however, when extrapolating results from laboratory experiments to field conditions. Most conclusions on growth patterns and growth rates of copepods have been deduced from rearing experiments. It is obvious that

laboratory experiments cannot entirely simulate natural conditions, and it might be that many results obtained in the laboratory represent suboptimal conditions. The evidence obtained in this study supports the hypothesis of potential exponential growth of copepods, with the exception of overwintering species such as C.glacialis, which may reduce metabolic rates in older stages despite excess conditions of food.

A different situation was found when describing growth in body length. Even though prosome lengths increase in a linear pattern through time, length increments between stages are not constant (**Table 4.2**), so that linear models, though significant are unrealistic. In addition, body length increases discretely through instars, such that growth cannot be described by a continuous function. Alternatively, length increases between stages can be estimated and then used to predict adult size (equation 4.3). Although food and temperature might influence such length increments, and this should be explored in more detail.

The advantage of using body length to analyze copepod growth is that animals do not have to be sacrificed, so that growth can be followed in the same individuals. This certainly constitutes a more direct measure of growth than using body mass. However, evidence from this study strongly suggests that both types of growth are clearly distinct processes and inferences from one to the other are hardly valid. Moreover,

measurements of body length tell little about growth of body mass. Although, the use of length-weight regressions has become a common method of estimating copepod growth and production, it seems clear that individual variation of body density (see Chapter 3) may introduce large bias in estimates of body mass.

The influence of temperature on growth rate may be deduced from results at the two temperatures considered in this study. Length increments between stages were slightly increased at low temperature in C.glacialis, by similar proportions in the stages considered. However, as concluded above, length increments within temperatures are unrelated to stage durations, and although between-temperature differences in development rates are accompanied by changes in length increments, it might be that size and development rate respond to temperature through different and perhaps independent mechanisms. This suggestion is also supported by results in the other species. Despite very extended stage durations at the low temperature in C.finmarchicus and E.herdmani, length increments between temperatures do not differ considerably (**Table 4.1**). Nevertheless, many observations from field studies have shown a correlation between temperature and body length (e.g. Deevey 1960, 1964), such that large individuals are observed at low temperature and this is also accompanied by reduced development rates. It might be that temperature exerts its effects on prosome length at earlier stages than

those considered in these experiments. However, it is not possible to rule out the possibility that prosome lengths in the present experiments were affected by rearing conditions other than temperature.

Growth in terms of dry weight, on the other hand, is clearly different between temperatures, in both C.glacialis and E.herdmani. Because growth in body weight may be continuous, extended stage durations will greatly reduce growth rates. Reduced development rates, however, do not necessarily imply greater weight increments because rates of tissue accumulation may also be reduced under the effect of low temperature. The extent to which temperature affects body weight will be analysed further in Chapter 5.

Thus far, the influence of stage durations, i.e. development rate, on body size has been under consideration. This problem may also be approached in terms of size-dependence of development rates. Size-dependence of development and hence growth, has been the basis for construction of size-related functions to estimate growth and production of copepods. Molting rates might take place at fixed body sizes, as demonstrated in some insects (Nijhout 1975, Blakley and Goodner 1978), and assumed in Harris' (1983) model of copepod growth. Information presented here shows little support for this assumption in copepods. Although development and growth rates decrease in later stages, particularly stage CV, there is a notable lack of correlation

of either body length or body mass with stage duration. Extended duration of stage CV may be related to investment of energy in sex differentiation and elaboration of reproductive material, and also to the presence of resting stages.

CHAPTER 5

TEMPERATURE AND FOOD QUANTITY AS INFLUENCES ON BODY SIZE.

5.1. Introduction

Numerous field observations and laboratory experiments have documented influences of temperature and food quantity or quality, or both, on copepod size. A negative relationship between body size of copepods and temperature has been well documented from field obsevations (e.g. Deevey 1960, 1964, McLaren 1965, Corkett and McLaren 1978). In rearing experiments similar observations have been obtained (e.g. Lock and McLaren 1970, Mullin and Brooks 1970, Klein-Breteler and Gonzalez 1982, 1988).

Food supply is also recognized as a critical factor influencing size variations. Field observations indicate that food quantity is a potential cause for small sized individuals during seasons of food shortage (e.g. Deevey 1960). This has also been observed in rearing experiments (e.g. Klein Breteler and Gonzalez 1982, 1988). A few studies have dealt with effects of food quality on size. They suggest that food requirements are species-dependent and that perhaps developmental stages also have particular nutritional needs (e.g. Huntley et al. 1987, Diel and Klein Breteler 1986, Klein Breteler et al. 1990).

In natural conditions, evaluation of relative effects of temperature and food supply on body size has proven to be difficult, mainly because the two factors covary, such that high concentrations of food coincide with low temperatures, usually at the beginning of the spring season in temperate environments. At this time of year large individuals are observed. At the end of the summer, when temperature is high and food levels reach lowest values, smaller individuals are usually found. This difficulty of separating the two factors has been the major problem creating controversy about food or temperature dependence of growth and development (Huntley and Boyd 1984, Frost 1985 for discussions), which are the processes that ultimately regulate body size.

According to Klein-Breteler et al. (1990), laboratory experiments offer the opportunity to control variables and hence to test the effects of various factors separately. However, interactions between temperature and food still have to be taken into account. Furthermore, in rearing experiments, other variables may be involved, such as mortality during molting (Mullin and Brooks 1970). For example, if larger individuals are more resistant to stress conditions of food, temperature, or other conditions, this may result in apparent influence of temperature or food on body size when the real cause might be size-dependent mortality. Therefore, whenever possible differential mortalities rate among treatments of food and temperature should also be considered.

It is evident that eventual food-temperature effects take place during ontogeny, i.e. during processes of development and growth. Thus, the longer the exposure to certain conditions, the greater the expected effects on body size. In this context, experiments with controlled conditions for entire generations are most valuable. However, it is often extremely difficult to rear copepods through the whole of development, and even when possible it is likely that individuals respond differently when compared to natural conditions (see Davis 1983 for discussion). Thus, information obtained from short-term experiments may also be valuable to examine effects of food and temperature on size. In general, adult size is of most interest, since it reflects accumulated effects during development.

As discussed in previous chapters, body size may be viewed in such terms as total body mass, structural body mass, body length or size of the lipid store. It is not clear that these components respond differently to temperature-food effects and, if they do, which components are most or least affected. Analysis of effects of food and temperature on body size of copepods presented here is based on data on body length, body mass and size of the lipid-store of adult females C.glacialis reared from stage CIII to adult under four combinations of food quantity and temperature, and on data on prosome length of adult females of P.acuspes and P.elongatus, reared from the egg to adult at four combinations of food

quantity and temperature. Additional information comes from rearing of E.herdmani from CI to adult at two temperatures. All this information will be mainly analyzed through analysis of variance and covariance. Therefore, these techniques, their assumptions and validity for this particular study, will first be briefly discussed.

5.2. Statistical procedures and assumptions.

Analysis of variance (ANOVA) is commonly applied in physiological and ecological studies. However, there are many situations when basic assumptions are hardly met, leading to biased conclusions. Unfortunately, criteria for choosing procedures and tests of basic assumptions, are seldom given in the literature. It is known by statisticians that violation of ANOVA assumptions may lead to two type of errors (as stated recently, e.g. Day and Quinn 1989). In Type I, means between treatments are declared as significantly different, when the population means for the treatments are equal. In Type II, means for treatments are not declared as significantly different when the population means do differ. Given multifactorial effects on organisms' responses in most ecological experiments, clear-cut results are hardly expected, so that conclusions have to rely on robustness of statistical tests. This robustness, however, can only be properly assumed when basic assumptions are satifastorily met. Therefore,

adequate tests for assumptions should be the first stage in application of ANOVA.

A fundamental assumption of ANOVA is randomness of sampling. Violation of this assumption may be lead to lack of independence of experimental units or heteregeneity of variances or nonnormal distribution (Sokal and Rohlf 1981). Fortunately, there are ways to detect lack of randomness by testing normality, homogeneity of variances and independence. Alternatively, transformation of data to different scales may correct some of these violations.

Another very common violation of ANOVA occurs when there are interactions among variates, i.e. there are multiplicative effects of treatments rather than additive ones. Multiplicative factors are often found in physiological responses. This effect, however, may often be solved by logarithmic transformation.

5.2.1. Testing assumptions of ANOVA

A critical assumption of ANOVA is homogeneity of variance among treatments. Heterogeneity of variance may reflect lack of randomness of sampling and non-normality distribution of variables within treatments (Sokal and Rohlf 1981). A suitable method for testing homogeneity of variances is Barlett test. This test was thus applied to all the variables for which food-temperature effects were to be tested (**Table 5.1**).

Table 5.1 Barlett test for homogeneity of variance among food-temperature treatments of prosome lengths of adult females P.acuspes and P.elongatus, and prosome lengths, TW, SW and OSW of adult females C.glacialis. Critical values are for 3 and n-4 degrees of freedom.

Species	n	Prosome length	TW	SW	OSW
C.glacialis	37	5.66	5.96	2.32	31.31**
P.acuspes	64	1.57	-	-	-
P.elongatus	60	1.024	-	-	-

5.3. Combined effect of food and temperature on body size

In all the experiments usually only adult females were obtained, so that throughout the text adult size refers to female adult size. Food-temperature effects will be first analyzed for separate components of body size, i.e. prosome length, TW, SW and OSW, and then eventual effects on the length-weight relationship will be examined. Additional information will be obtained from analysis of food-temperature effects on the condition factor (CF, as defined in Chapter 3, p.54) of C.glacialis and E.herdmani.

5.3.1. Food-temperature effects on body length

Mean adult prosome lengths obtained from the four combinations of food and temperature are shown in **Table 5.2.** One-way ANOVA of food-temperature treatments is shown in **Table 5.3.** However, this does not distinguish between food and temperature effects, and it does not test for interactions between the two treatments. Thus, two-way ANOVA was applied to the data (**Table 5.4**).

In C.glacialis and P.elongatus there were clear effects of food and temperature, as expected from previous studies (e.g. Klein Breteler and Gonzalez 1982, 1988). In P.acuspes, on the other hand, food also exerted a similar effect. However, temperature effects on body length of this species were found not significant, although the estimated probability to reject the hypothesis that temperature indeed affected

Table 5.2 Prosome lengths (mm ± S.D) of adult females C.glacialis, P.acuspes and P.elongatus after rearing at four combinations of food and temperature. High temperature was 12 °C for C.g., 10 °C for P.a. and 15 °C for P.e., low temperature was 3 °C for C.g., 5 °C for P.a. and 8 °C for P.e. More details on food levels and temperatures are given in Materials and Methods.

Temperature		Food level 1	n	1/16	n
High	C.g.	2.84 ± 0.125	15	2.74 ± 0.060	9
	P.a.	0.95 ± 0.071	17	0.85 ± 0.050	9
	P.e.	0.91 ± 0.061	13	0.77 ± 0.055	17
Low	C.g.	3.04 ± 0.103	8	2.97 ± 0.153	5
	P.a.	0.94 ± 0.060	26	0.80 ± 0.054	12
	P.e.	1.02 ± 0.046	15	0.83 ± 0.053	15

Table 5.3 One-way ANOVA of food-temperature treatment effects on prosome length of C.glacialis (C.g.), P.acuspes (P.a.) and P.elongatus (P.a.), reared in the laboratory under four combinations of food and temperature.

Source of variation		d.f.	SS	F-ratio	P
Among Treatments	C.g.	3	0.435	11.575	<0.001***
	P.a.	3	0.212	19.046	<0.001***
	P.e.	3	0.542	62.202	<0.001***
Within treatments	C.g.	33	0.413		
	P.a.	60	0.223		
	P.e.	56	0.163		

Table 5.4 Two-way ANOVA of food-temperature effects on prosome length of adult females C.glacialis, P.acuspes and P.elongatus reared in the laboratory under four combinations of food and temperature.

Source of variation		d.f.	SS	F-ratio	P
Food	C.g.	1	0.059	4.686	<0.05**
	P.a.	1	0.238	54.508	<0.001***
	P.e.	1	0.404	138.864	<0.001***
Temperature	C.g.	1	0.356	28.442	<0.001***
	P.a.	1	0.016	3.681	>0.05 n.s.
	P.e.	1	0.104	35.705	<0.001***
Food x Temperature	C.g.	1	0.001	0.110	>0.05 n.s.
	P.a.	1	0.009	2.046	>0.05 n.s.
	P.e.	1	0.008	2.754	>0.05 n.s.
Error	C.g.	33	0.413		
	P.a.	60	0.262		
	P.e.	56	0.163		

prosome length was 0.06, which is very close to the conventional level of 5%. Even if significant, this effect was positive (**Table 5.2**). This is an unexpected result given the well-documented negative correlation between temperature and body length.

Food and temperature effects on prosome length are more notable in P.elongatus than in the other two species. Individuals of C.glacialis were only reared from CIII to adult, so that they experienced the treatments only during a small part of their development. Moreover, variation of stage CIII from the field may have influenced sizes of adults, because as shown in Chapter 4, lengths of late stages are usually influenced by lengths of earlier ones.

P.acuspes, on the other hand, although cultivated throughout development, also showed a greater residual variation not explained by treatment effects compared with P.elongatus (**Table 5.4**). Mean size of parental females P.acuspes obtained from the field in June 1989 was 1.010 ± 0.062 (mean ± S.D., n = 50). The grand mean for the four combinations of food and temperature was 0.902 ± 0.083 (mean ± S.D.), which is significantly different from the parental mean (t-test = 7.66, $P < 0.01$). Although the grand variance of the rearing experiment is significantly greater than that of the parental females ($F = 1.792$, $P < 0.05$), some of the parental variation may have influenced size of the progeny.

Moreover, this species is highly variable in body size throughout the year in Bedford Basin (McLaren et al. 1989a).

P.elongatus has been reared for more than 50 generations in the laboratory, and the animals for this study were obtained from one of these stocks. There is much inbreeding in these stocks (Klein-Breteler et al. 1990). Thus, it is not surprising that treatments effects are more clearly expressed since the genetic factor has been somehow controlled.

The lack of interaction between food and temperature in all cases studied is interesting (**Table 5.4**). However, this may be expected since the media containing the food was frequently changed, avoiding effects of temperature on the quantity and quality of food. This may have caused the lack of dependence between the two factors.

5.3.2. Food-temperature effects on body weight

Measurements of dry weight as TW, SW and estimations of OSW, were obtained for adult females C.glacialis subjected to food-temperature treatments from stage CIII to adult (**Table 5.5**).

Barlett tests showed significant heterogeneity of variances among treatments for estimated weight of the oil sac (OSW) (**Table 5.1**). Log-transformed OSW's, however, showed homogeneous variance among treatments (Barlett test, $F_{3,33}$ = 0.15, P >.05), thus ANOVA could be applied to test food-temperature effects on OSW's. An initial one-way ANOVA test

Table 5.5 Dry weight (mean ± S.D.) measured as total weight (TW), lipid-discounted dry weight (SW) and weight of the lipid store (OSW) in C.glacialis reared under four combinations of food and temperature.

Temperature		Food level 1	n	1/16	n
12 °C	TW	327.729 ± 115.221	15	172.966 ± 59.808	9
	SW	163.893 ± 39.627	15	165.033 ± 60.642	9
	OSW	163.835 ± 115.725	15	7.932 ± 12.761	9
3 °C	TW	355.024 ± 115.035	8	227.644 ± 55.278	5
	SW	162.346 ± 50.783	8	188.986 ± 38.506	5
	OSW	192.678 ± 117.555	8	38.678 ± 32.376	5

indicated that both TW and OSW are significantly affected by food-temperature treatments ($F_{3,33}$ = 6.74 and 8.21 for TW and OSW respectively, P <0.001). No significant treatment effect on SW was detected ($F_{3,33}$ = 0.40, P >0.05).

In order to test independent effects of food and temperature and their interaction on TW, SW and OSW, two-way ANOVA's were applied (**Table 5.6**). It seems that food and temperature affect mainly OSW, which in turn leads to effects on TW. This is implied from the absence of food-temperature effects on SW.

It is relevant that lipid contents, but not the structural weight (SW) are influenced by food and temperature. This would certainly alter the relationships among TW, SW, OSW and body length. Moreover, if variation in individual dry weight induced by environmental factors is determined only by lipid content, this would certainly mislead about the effects of food and temperature on body size.

Significant effects of food and temperature on body weight have been documented from rearing experiments (e.g. Vidal 1980a, Foran 1986), but relative influence on lipid contents and body weight has not hitherto been explored. Field samples reveal high variability in body weight, but this has not been related to lipid content, and, as stressed above, because food and temperature covary in the field it is difficult to evaluate their separate roles. The lack of interaction between food and temperature on body weight in the

Table 5.6 Two-way ANOVA of food-temperature treatment effects on dry weight of C.glacialis reared under four combinations of food and temperature. Dry weight has been measured as total dry weight (TW), lipid-discounted dry weight (SW) and dry weight of the lipid store (OSW).

Source of variation		d.f.	SS	F-ratio	P
Food	TW	1	158307.133	16.359	<0.001***
	SW	1	1534.902	0.672	>0.05 n.s.
	OSW	1	55.625	24.852	<0.001***
Temperature	TW	1	13371.591	1.382	>0.05 n.s.
	SW	1	998.473	0.437	>0.05 n.s.
	OSW	1	10.030	4.662	<0.05**
Food x temperature	TW	1	1493.591	0.154	>0.05 n.s.
	SW	1	1293.290	0.566	>0.05 n.s.
	OSW	1	8.350	3.881	>0.05 n.s.
Error	TW	33	319333.566		
	SW	33	75386.424		
	OSW	33	71.005		

present study (**Table 5.6**), indicates that the independent effects of the two factors have been evaluated to some extent.

5.4. The influence of food and temperature on the length-weight relationship.

As shown above, prosome length and TW, but not SW are influenced by food and temperature. This differential effect on body length and structural weight should be reflected in the length-weight relationship, such that body density would be altered by differences in food and temperature.

Estimations of TW and SW of C.glacialis were obtained at variable time intervals from stages CIV to adult, during the rearing experiment for the four combinations of food and temperature. These data, when plotted as a function of prosome length (**Fig.5.1**), and fitted by linear, exponential and power models, were in all cases best described by power functions (**Table 5.7**). Covariance analysis on log-transformed variables indicated that food affected significantly the regression lines ($F_{3,33} = 4.45$, $P < .05$), whereas temperature effects and interactions between treatments and variables were not significant ($F_{3,33} < 1.00$, $P > .05$). Covariance analysis of the relationship between prosome length and SW suggested that neither food nor temperature had significant effects on the regression lines ($F_{3,33} < 1.00$, $P > .05$).

Differential effects of food and temperature on length and weight can also be viewed (**Table 5.8**) in terms of

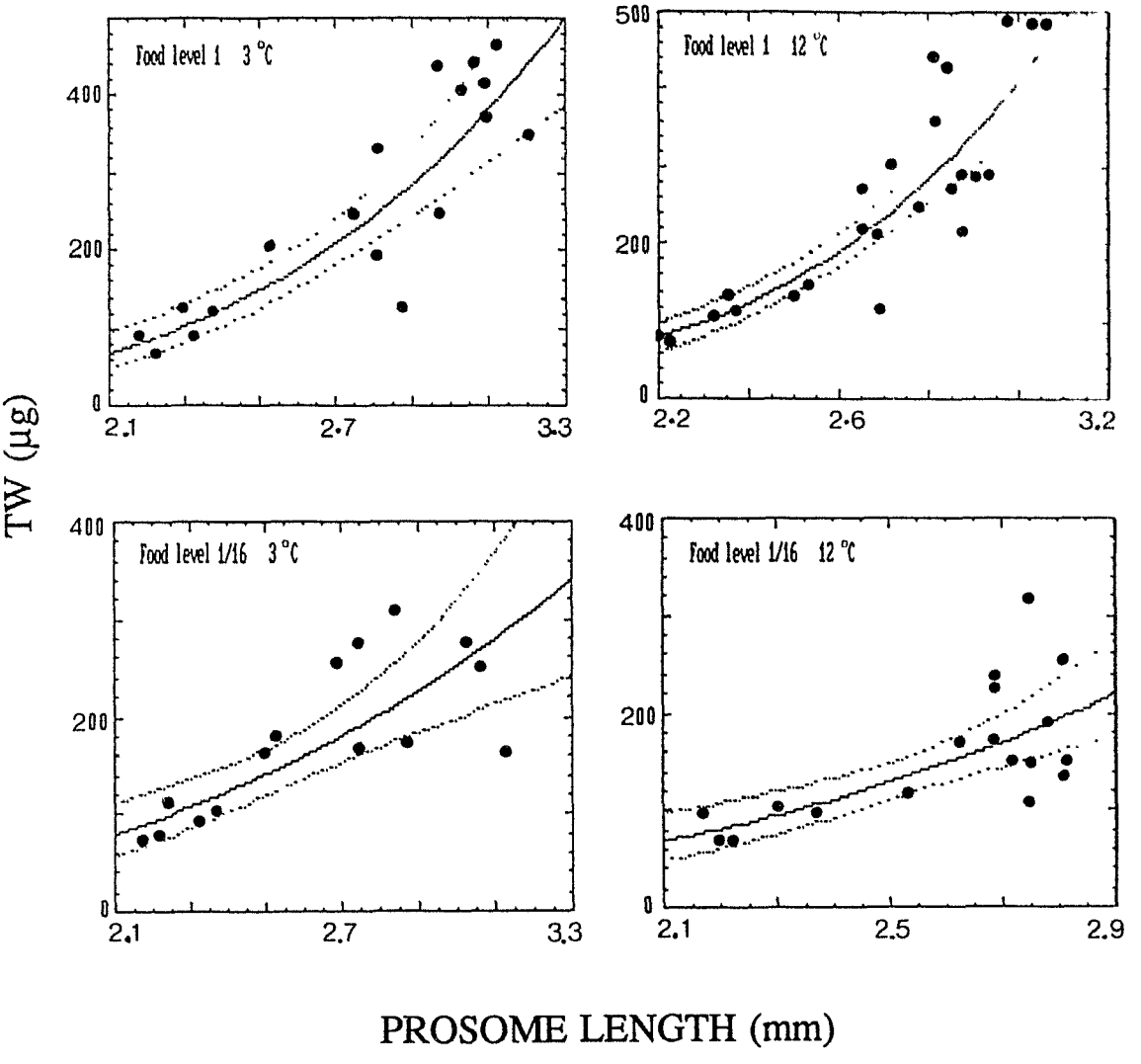

Fig.5.1. The length-weight relationship in C.glacialis reared under four combinations of food and temperature. Stage CIV to adult females are included. Dry weight is expressed as total weight (TW). Dotted lines are 95% confidence limits.

Table 5.7 Power equations for the length-weight relationship in C.glacialis reared under four combinations of food and temperature. Stage CIV to adult females are included. Dry weigth is expressed as total weight (TW). S.E. is standard error for estimated slopes, FL is food level (1 and 1/16) and n is the number of cases.

Best fitted model	r^2	n	S.E.	Treatment
$TW = 2.671\ Length^{4.386}$	0.81	18	0.473	FL 1 3 °C
$TW = 1.148\ Length^{5.348}$	0.83	24	0.521	FL 1 12 °C
$TW = 7.507\ Length^{3.195}$	0.68	15	0.609	FL 1/16 3 °C
$TW = 4.694\ Length^{3.609}$	0.60	18	0.738	FL 1/16 12 °C

Table 5.8 Condition factor for C.glacialis stage CIV to adult reared in the laboratory under four combinations of food and temperature. ** =significantly different from the other (t-test, P >.05).

	Food level	
	1	1/16
3 °C	1.131 S.D. = 0.340 n = 18	0.937** S.D. = 0.251 n = 15
12 °C	1.226 S.D. = 0.401 n = 24	0.870** S.D. = 0.260 n = 18

condition factors (CF, as defined in Chapter 3). Within treatments condition factors were positively correlated to OSW (**Fig.5.2**), thus suggesting that body mass per unit of volume is altered by lipid contents.

By influencing lipid content both temperature and food alter the total weight (TW), and since these two factors also affect body length, animals are heavier per unit of length. However, since structural weight is not affected, animals at low temperature should also be lighter per unit of length if lipids are discounted. This interesting effect of temperature was looked for in E.herdmani, reared from stage CI to adult under excess of food at 3 ^{0}C and 10 ^{0}C. About 15 individuals females were obtained from each temperature (see Appendix B1). One-way ANOVA showed that dry weight of adult females was not influenced by temperature ($F_{1,29}$ = 1.635, P >.05), but prosome length was significantly affected ($F_{1,29}$ = 5.037, P<.05). This indicates that although animals were large at the low temperature, they were also lighter per unit of length.

Covariance analysis of the length-weight relationship of E.herdmani at the two temperature (**Fig.5.3**) showed that the regression lines for log-transformed variables were significantly different ($F_{1,29}$ = 6.022, P <.05), resulting from a differential effect of temperature on body weight and length. Mean values of conditions factors (± S.D.) were 0.95 ± 0.194 and 1.06 ± 0.206 for 3 ^{0}C and 10 ^{0}C respectively. These values are significantly different (t-test = 2.27, P <.05),

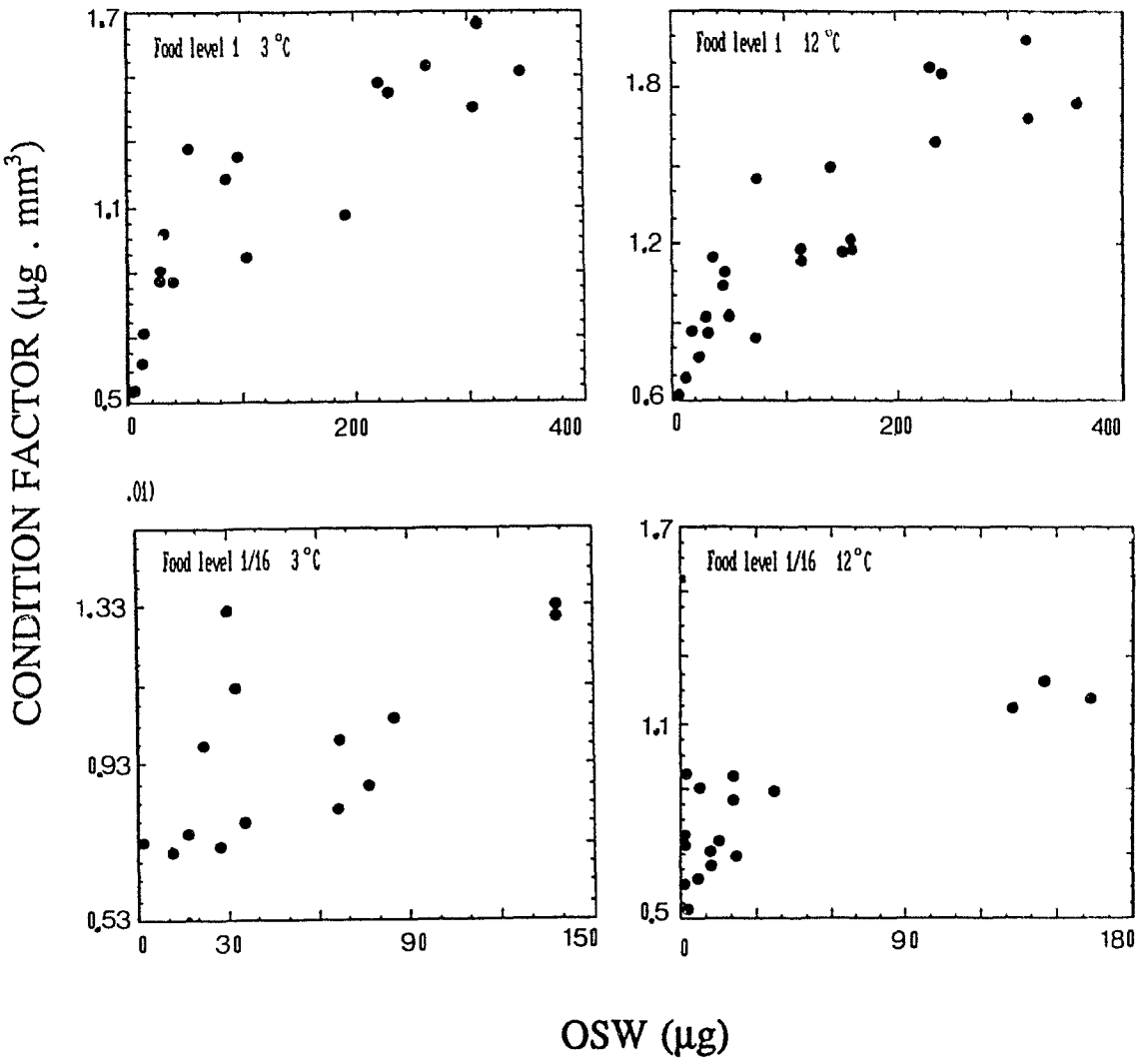

Fig.5.2. The relationship between the condition factors (CF) and size of the lipid store (OSW) in C.glacialis reared under four combinations of food and temperature.

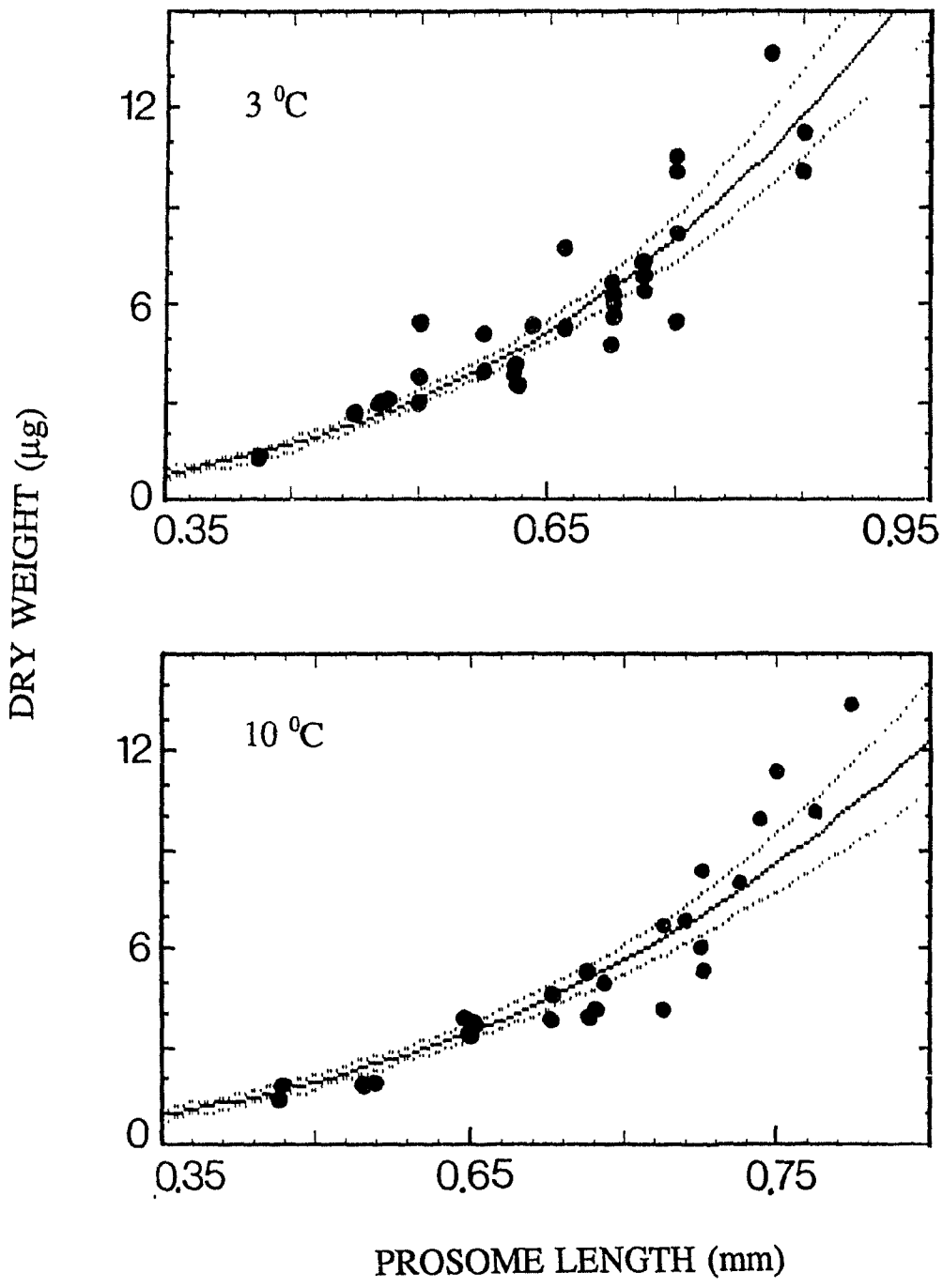

Fig.5.3. The length-weigth relationship in E.herdmani stages CI to adult males and females, reared at two temperatures under excess conditions of food. Dotted lines are 95% confidence limits.

thus confirming that temperature altered body density.

5.5. Mortality during rearing experiments

Great mortality rates are a major problem in long-term experiments when rearing copepods (Davis 1983). Mullin and Brooks (1970) obtained up to 30% survivors when rearing Rhincalanus nasutus through seven generations, after which mortality reached 100%. Peterson (1986) obtained between 10 and 68% of survivors in rearing of Calanus marshallae, only through one generation. Obviously survivorship will largely depend on rearing techniques and on the species in question. However, variability in mortality rates during experimentation might affect conclusions about relative importance of treatment effects. This would be critical if responses being studied are subject to differential mortality. For instance, Peterson (1986) argued that variability in development rates of C.marshallae reared in the laboratory, may result from differential mortality rates among developmental stages.

In the present study information on the number of survivors for each food-temperature treatment was obtained for C.glacialis and for P.elongatus. In P.acuspes a high mortality was observed in naupliar stages in every treatment, so that the experiment was repeated several times until a sufficient number of individuals reached adulthood for each treatment, but no information on survivorship was obtained.

Observations at irregular time intervals allowed counting of dead animals for each food-temperature treatment, although some individuals were also sampled at those times (**Table 5.9**). Accumulated number of dead and live individuals for each treatment were compared and no differential mortality among treatments was found (Contingency Chi-square = 7.175, d.f=3, P >.05).

In experiments with P.elongatus, population density was recorded at irregular time intervals until more than 30% of the individuals in each treatment had reached adulthood (**Table 5.10**). Density at this last sampling time was compared among treatments as a proportion of the density at the beginning of the experiment. Mortality was found to be dependent on treatment (Contingency Chi-square = 13.345, d.f.=3, P <.05). Mortality was significantly higher at low food and high temperature (**Table 5.10**). However, this treatment also produced the smallest individuals (**Table 5.2**), and thus it is unlikely that size differences among treatments arose from differential mortality rates, since at high mortality rates large individuals might be expected to survive. This is because large individuals, with probably more lipid, since size of the lipid store is positively correlated with body length (see Chapter 1), should be able to cope better with food-shortage.

Table 5.9 Mortality of copepodids C.glacialis stage CIII to adult when reared under four combinations of food and temperature. Time is from the beginning of the experiment until they reached adulthood. Numbers of dead animals are indicated at each observation, and in parentheses the number of remaining individuals after sampling.

	Treatment			
	Food level 1		Food level 1/16	
Time (d)	3 °C	12 °C	3 °C	12 °C
0	(50)	(70)	(50)	(70)
13	4 (46)	1(59)*	0 (50)	5 (55)*
21	2 (34)*	0 (59)	2 (43)*	6 (49)
28	1 (30)	3 (56)*	1 (37)	3 (46)*
41	5 (25)	n.a.	n.a.	n.a.
48	2 (23)*	8 (45)	4 (33)*	11 (16)*
52	0 (23)	10 (35)	2 (31)	10 (0)
55	1 (17)	2 (33)	0 (31)	-
63	0 (15)	6 (25)*	2 (29)	-
72	3 (12)*	7 (16)	3 (26)	-
76	0 (10)	4 (12)	3 (19) *	-
85	4 (4)	4 (8)	4 (15)	-
92	1 (3)	4 (0)	6 (9)	-
99	0 (0)	-	3 (6)	-
109	-	-	0 (0)	-

* = approximate time when individuals were changing stage
n.a. = not available

Table 5.10 Mortality (decline of specimens per liter) during rearing of P.elongatus from naupliar stages to adult under four combinations of food and temperature. Time is indicated from the beginning of the experiment until they reached adulthood. High and low food levels are described in Chapter 2 (**Table 2.1**). At the last observation more than 30% of the population was adult.

	Food level			
	High		Low	
Time (d)	8 °C	15 °C	8 °C	15 °C
0	63	63	63	63
7	62	53	72	58
14	62*	52*	62	34*
17	n.a.	36	n.a.	n.a.
20	43	-	32	20
22	38	-	33*	15
27	42	-	35	9
28	21	-	n.a.	-
31	-	-	22	-
35	-	-	15	-
37	-	-	18	-
41	-	-	23	-

* = approximate time at stage CI , n.a. = not available

5.6. Discussion

As in numerous studies body size was clearly influenced by food and temperature. This effect was observed in body length of all species and lipid content of C.glacialis. Temperature, however, had little effect on prosome length of P.acuspes. This may have resulted from a low resolution in the low and high temperatures selected for this species (5 and 10 ^{0}C).

Although some adult males where obtained when rearing C.glacialis, they were not included in the analysis, because they were too few, and were only obtained at high food level and high temperature. In E.herdmani, sex differentiation takes place at stage CIV, and sex ratios obtained were nearly 1 : 1. The absence of adult males in the rest of the experiments cannot be easily explained. Sex ratios in copepod samples are often biased toward females (e.g. Grainger 1959, Mednikov 1961), especially in copepods from deep waters and the open ocean. In rearing experiments of Calanus and Pseudocalanus species, adult males are seldom obtained (e.g. Conover 1965, Uye 1988, Marcus and Alatalo 1989). However, there is evidence that in the field sex ratios are nearly 1 : 1 in stages CIV and CV (e.g. Fontaine 1955, McLaren 1969). In rearing experiments, sex ratios vary in according to experimental conditions. For instance, Mullin and Brooks (1970) obtained a 1 : 1 sex ratio when rearing Rhincalanus nasutus, whereas Peterson & Painting (1990) obtained different sex ratios in

C.australis depending on temperature treatments. Evidence suggests that adult males have shorter lives than females, and that there might be environmental influences determining sex ratios in nature (e.g. Corkett and McLaren 1978). In the laboratory, it is unknown if alteration of sex ratios is determined by differential mortality or by a condition effect on sex determination (Grigg et al. 1985, for discussion). The absence of adult males, however, might be an indication of sub-optimal conditions. Although, it is not possible to know which factors, or which combination of factors are involved in determining sex ratios of adults.

In addition to food quantity and temperature, there are other potential factors that should be mentioned. Light conditions may influence feeding rhythms and endocrine activity (Omori and Ikeda 1984). This obviously would affect growth and development. However, very little experimental work has been done to study light conditions effects on copepods, and a variety of light regimes has been applied when rearing copepods. Although, some authors have used semi-diurnal light-dark periods (e.g. Paffenhöfer 1970, Marcus and Alatalo 1989), in the present experiments semidark and dark conditions were used, as in other studies (e.g. Mullin and Brooks 1967, Hirota 1972). Although the possibility that light conditions may have affected development and growth in the present study cannot be ruled out, application of same conditions to all treatments leads us to assume that data among treatments are comparable.

Bacterial infections during rearing is another potential factor affecting development and growth of copepods (Davis 1983). Eventual bacterial populations may show differential rates of growth depending on treatments, so that if they affect copepod growth, such effects might be differential among treatments. Therefore, this is a potential source of error that, unfortunately, it is not possible to evaluate, and that might have introduced bias in conclusions about food-temperature effects on copepod size. There are other factors, such as handling of individuals, size of containers, and water quality that might have influenced growth and development of individuals. However, for comparisons, they may be assumed as affecting equally all treatments, although this shortcoming obviously limits conclusions about growth of copepods in natural conditions.

CHAPTER 6

GENOME SIZE IN CALANOID COPEPODS AND ITS FUNCTIONAL ROLE

6.1. Introduction

The term C-value (Swift 1950) was originally proposed to refer to the supposedly constant amount of DNA in the unreplicated haploid genome of a species. This C-value, also known as genome size (Hinegardner 1976), has proven to vary over a million-fold among organisms (Cavalier-Smith 1985). This variation has constituted a riddle for biologists. Once it was established that genes consist of DNA, it was thought that organisms with similar levels of complexity would also have similar genetic requirements, so that genome size could be associated to such complexity. However, the genome size is highly variable even among closely related species with same chromosome numbers (e.g. Raina and Rees 1983, Bennett and Smith 1976, Laird 1973), and without conection to organism complexity (Mirsky and Ris 1951, Cavalier-Smith 1978, John and Miklos 1988 for reviews). Thomas (1971) proposed the term "C-value paradox" to refer to this unexplained variability.

According to Cavalier-Smith (1985) six kinds of solutions have been suggested, some of which have been definitely discarded with the advance of molecular biology. Three remaining explanations deserve consideration, but are still

controversial: 1) much DNA may be genetically or physiologically inert (Darlington 1937), also termed "junk DNA" (Ohno 1972); 2) much DNA is "selfish DNA" (Doolitle and Sapienza 1980), functionless parasite (Ostergren 1945); and 3) in addition to its genetic function, DNA has a quantitative non-genic, or "nucleotypic" (Bennett 1971) function (Bennett 1971, 1972, Rees 1972, Cavalier-Smith 1978, 1985).

The idea of a functionless or useless DNA implies that genome size of organisms is non-adaptive. Nevertheless, as stressed by Bennett (1971), many authors have overlooked the extensive evidence for strong correlations between genome sizes and various quantitative characters of clear adaptive significance to organisms. In plants genome size is associated with climate and temperature (Bennett 1971, Grime 1983) and also with rates of development and life-history (Bennett 1972, Smith and Bennett 1975). Furthermore, since developmental rates correlate negatively to cell sizes, genome size also shows a negative correlation to cell size (e.g. Darlington 1965, Olmo 1983).

Similar correlations have also been documented in animals. Cell division rates are associated with nuclear DNA contents in six species of insects (Bennett 1977), and rate of embryonic development is also related to genome size in anurans (Larson 1984 for review), although the relationship is not a simple one (Oeldorf et al. 1978). Sessions and Larson (1987) failed to find any correlation between nuclear DNA

content and cell division rate of salamander species, but through a phylogenetic analysis they concluded that limb regeneration rate is an evolutionary correlate of changes in genome size.

Although significant correlations between genome size and life-history characteristics do not explain causal relationships, they constitute strong evidence against the functionless role of genome size. This evidence was the basis for the "nucleotypic" hypothesis (Bennett 1971), which argues that genome size is a consequence of selection for cell size, the rate of cell division, the rate of development and hence development time. The hypothesis has been modified by Cavalier-Smith (1985), who views genome size as an adaptive feature which acts as the "nucleoskeleton", maintaining a metabolically favorable ratio between cell volume and nuclear volume.

The nucletypic hypothesis has been strongly criticized (e.g. John and Miklos 1988), mostly because it does not offer specific mechanisms and also because the correlation between genome size and cell metabolism is far from being clear (Szarski 1976, John and Miklos 1988 for reviews).

In spite of the controversy regarding the function of genome size, the correlation between cell size and nuclear DNA content is fairly strong and well documented in protists, plants and animals (reviewed by Cavalier-Smith 1985). This

unexplained and controversial correlation certainly deserves more exploration in animals (Cavalier-Smith 1985).

As in other organisms, crustaceans show a high interspecific variability in nuclear DNA contents (Rheinsmith et al. 1974, McLaren et al. 1988). Among species of calanoid copepods genome size follows a significant non-random series and this might be an indication of abrupt speciation (McLaren et al. 1989c). This variation is also associated with copepod size (Robins and McLaren 1982) and developmental rates (McLaren et al. 1988). Among closely related species, copepods may have similar nucleus number at a given stage (McLaren and Marcogliese 1983, McLaren et al. 1988). Robins and McLaren (1982) had earlier suggested that if cell number were fixed, body size could be directly related to cell size and consequently to genome size.

Although, the hypothesis of nucletypic control of cell size and associated properties of organisms is based mainly on interspecific comparisons, intraspecific variation in nuclear DNA contents associated with life-history characters has been documented in plants (e.g. Durrant 1971, Bennett 1972, Mowforth and Grime 1989). In animals this has not yet been explored. Although Robins and Mclaren (1982) found a significant correlation between body size and nuclear DNA content of Pseudocalanus, the species was unidentified and probably more than one species was present (McLaren et al. 1989c).

Intraspecific variation of copepod size, under the influence of environmental factors, might result from variations in cell size. This in turn could reflect nucleotypic effects of genome size. This hypothesis is explored in the present study through the analysis of nuclear DNA content and its relationships with body size in copepods subjected to different conditions of food and temperature. Although the specific mechanisms by which nuclear DNA content may regulate cell size and hence body size, cannot be revealed from this work, it might be used as another test of current hypotheses about the functional role of genome size.

6.2. Some methodological considerations

Although there is evidence for at least roughly determinate cell number of copepods (McLaren and Marcogliese 1983, McLaren et al. 1988), there is no information on how cell number might be affected by environmental factors. This is obviously a crucial point for studying the relationships between genome size, cell size and body size under the effect of the environment. Nuclei counts from one species will permit some exploration of this subject.

Estimations of nuclear DNA contents in this study have been made through measurements of optical density of Feulgen-stained somatic cells of copepod. Schiff's reagent has been widely applied in qualitative and quantitative studies of

aldehydes (Shriner et al. 1964 for review). Feulgen and Rossenbeck (1924) first used Schiff's reagent to identify nuclear DNA, which after hydrolyzation and treatment with this reagent, develops an intense violet colour. Since then this technique has been known as the Feulgen reaction and it has been principally applied to quantify DNA. Although for quantitative studies the resolution obtained with the Feulgen reaction has been questioned, a large body of evidence supports a proportionality between amounts of DNA and stain uptake (Robins 1978 for review and discussion). However, standarization of the technique is difficult to achieve, especially during hydrolysis with HCl (see Robins 1978). Frequent agitation of samples during hydrolysis was performed to obtain a slightly more intense staining, as recommended by McLaren et al. (1988). Moreover the use of chick blood standards for each batch of stained slides allowed means of detecting variations of conditions among batches.

In the process of choosing nuclei for measuring nuclear DNA through microspectrophotometry, some somatic nuclei were found to have twice the amount of DNA observed in other nuclei from the same individual or within the same species. These nuclei were assumed to be 4C and were not used in the analysis. Other large nuclei with enormous amounts of DNA were recognized as oocytes (Robins and McLaren 1982) and they were also discarded from the analysis. It was also observed that in areas where nuclei were more aggregated, nuclei usually showed

somewhat more intense staining. Whenever possible nuclei were chosen from these areas, as recommended by McLaren et al. (1988). This variation in the degree of staining was also observed in the chick blood standards, although to much less extent.

Variation among different batches can be inferred from values of the chick-blood standards obtained for all the experiments (Appendix C1).

Nuclear DNA content as influenced by food and temperature was estimated for 3 species, P.acuspes, P.elongatus and C.glacialis. However, experimental designs differed among species; thus results will be to some extent presented and discussed separately for each species.

6.2.1. Statistical analyses

Analysis of variance (ANOVA) was used to test food-temperature effects on DNA content. To test validity of basic assumptions of ANOVA, homogeneity of variances among treatments was tested through Barlett tests. Values of Barlett tests were: 4.91 (d.f.= 3) for P.acuspes, 0.968 (d.f. = 3) for P.elongatus, and 0.988 (d.f.= 3) for C.glacialis respectively. These values are not significant (P >.05), indicating that in the three species variances among treatments can be assumed to be homogeneous.

6.3. Pseudocalanus acuspes

An initial experiment was performed with mature females obtained from the field in October 1987. About 30 females were randomly sorted at 3 ^{0}C and 10 ^{0}C and kept under excess of food for 12 days. Since adults do not molt and do not appear to have somatic cell mitoses, this experiment was performed to test stability of DNA contents after all development had ceased. One-way ANOVA indicated non-significant differences in mean DNA content/nucleus of individuals between temperatures ($F_{1,21}$ = 1.75, P >.05). Pooled data from both temperatures showed no significant correlation ($F_{1,21}$ = 0.016, P >.05) between nuclear DNA contents and prosome length (**Fig.6.1**).

Newly hatched nauplii of P.acuspes were reared at 5 ^{0}C or 10 ^{0}C at high or low food levels (see Chapter 2 for details on food levels). When individuals reached adulthood, they were fixed and their nuclear DNA contents estimated (**Table 6.1**). From measurements of single nuclei (10 per individual), it was found that variation of DNA content among females within food-temperature treatments is highly significant (nested ANOVA, $F_{16,180}$ = 35.401, P <.01). Despite this variation there were also significant effects of food temperature and their interaction on DNA contents (**Table 6.2**).

As shown in Chapter 5, prosome lengths were significantly influenced by food-temperature treatments. Prosome lengths were plotted as a function of mean nuclear DNA content of

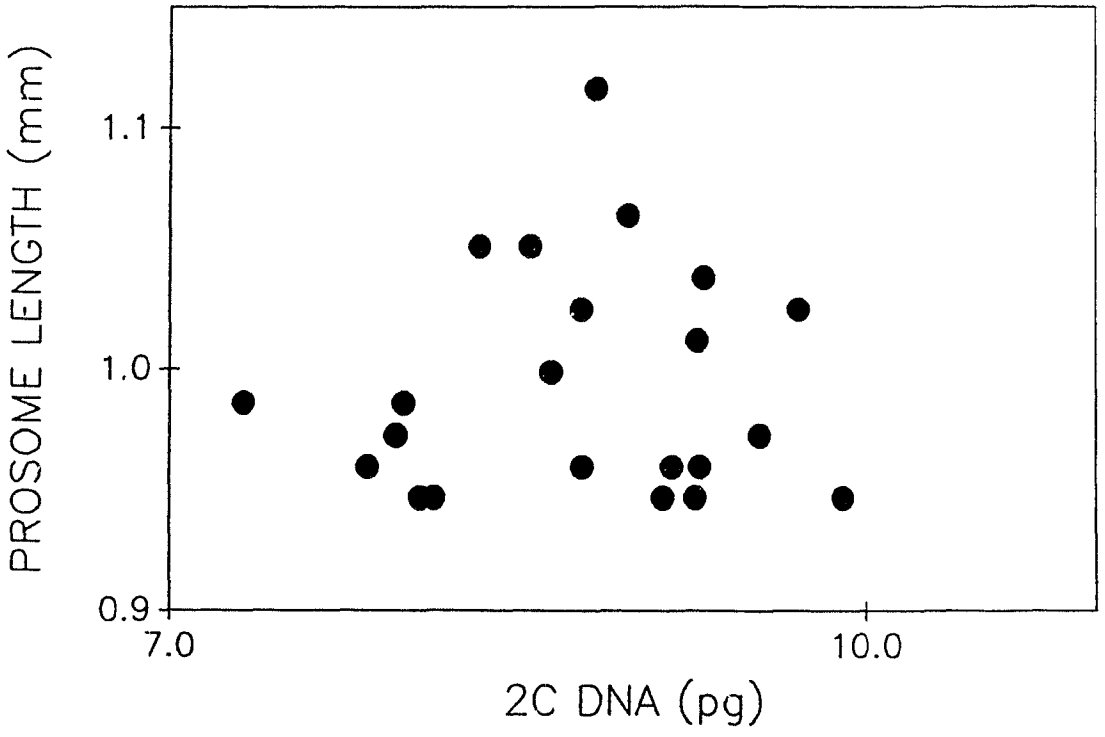

Fig.6.1 The relationship between prosome length and 2C nuclear DNA content of adult females of P.acuspes obtained from the field and kept for 12 days at 3 ^{0}C and 10 ^{0}C under excess of food.

Table 6.1. 2C nuclear DNA content (mean ± S.D.) of adult females of P.acuspes reared at four combinations of food and temperature. DNA content of individuals was estimated from the average of 10 nuclei of each individual.

Food level	Temp.(°C)	n	DNA (pg)	S.D.
1	5	4	7.45	1.320
	10	7	7.63	1.690
1/16	5	5	9.07	0.640
	10	4	7.65	0.930

Table 6.2 Nested ANOVA testing the effects of food-temperature treatments on 2C nuclear DNA content of adult female of P.acuspes reared under four combinations of food and temperature. Female (1 to 4) is variation of individuals within food-temperature treatments.

Source of Variation	SS	d.f.	F-ratio	P
Female (1)	62.428	3	38.719	<.001 ***
Female (2)	199.721	6	61.936	<.001 ***
Female (3)	16.399	4	7.628	<.001 ***
Female (4)	25.869	3	16.044	<.001 ***
Food level	31.841	1	59.244	<.001 ***
Temperature	18.293	1	34.036	<.001 ***
Food level x temperature	30.788	1	57.287	<.001 ***
Error	96.740	180		

individuals (**Fig.6.2**). There was no significant correlation between prosome length and DNA content ($F_{1,18}$ = 0.862, P >.05).

Several attempts were made to obtain estimates of nuclear DNA at earlier stages of this species under the four combinations of food and temperature. However, mature females obtained in late summer 1989 produced only small numbers of eggs and too few individuals survived treatments of low food and low temperature, such that only 10 individuals stage CI reared at high food and high temperature were obtained. Their estimated nuclear DNA content was, 4.81 ± 0.772 (mean ± S.E.). This value is significantly lower than that of the adult females (grand mean ± S.D. = 7.96 ± 1.429; t-test = 4.45, P <.05). Variability in DNA content of stages CI was also significantly greater than that of adults ($F_{9,19}$ = 2.92, p <.05).

Indications of seasonal variation in nuclear DNA of P.acuspes were observed by McLaren et al. (1989c). In the copepod, Euchaeta norvegica, total amount of DNA of individuals also shows seasonal variation (Skjoldal and Båmstedt 1976), although this could involve cell numbers, and oogenesis cycles, as well as variations in somatic cell DNA contents. Since seasonal variation in prosome length is associated with variability in conditions of food supply and temperature (see Chapter 5), nuclear DNA content of individuals might also vary according to environmental conditions. Seasonal samples of adult females (about 10

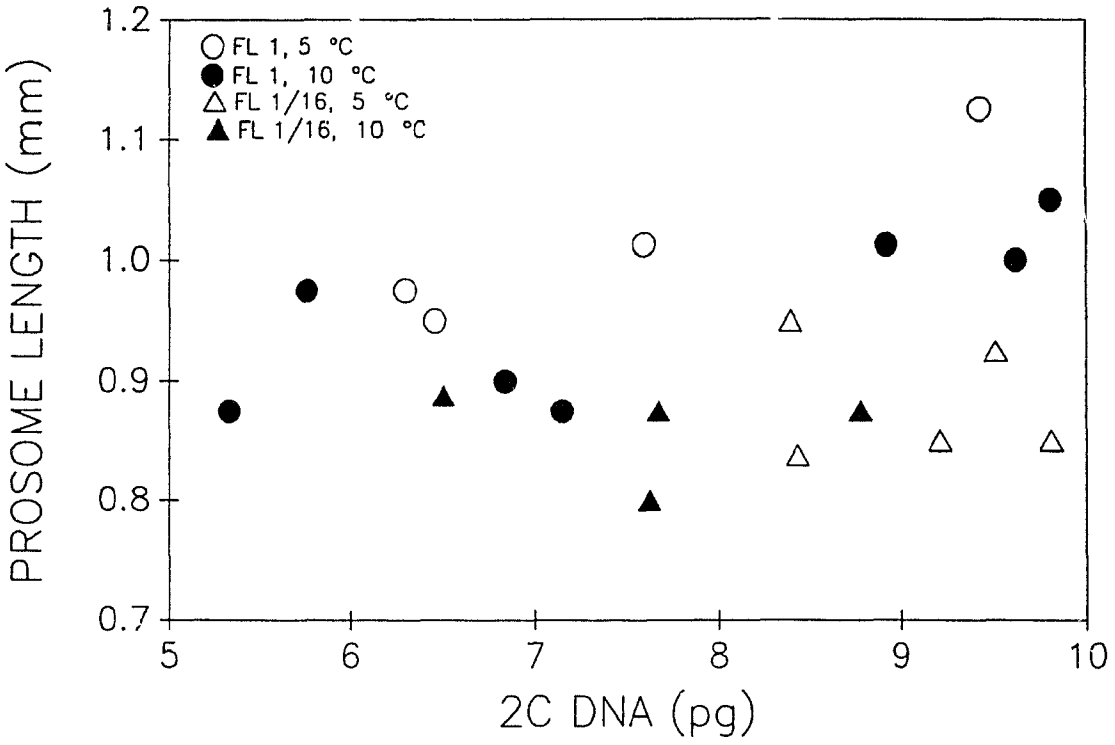

Fig.6.2 The relationship between prosome length and 2C nuclear DNA content of adult females of P.acuspes reared in the laboratory under four combinations of food and temperature.

individuals per sample) were obtained from the field and analyzed for DNA content. Prosome lengths followed the usual pattern of seasonal variation, i.e. large animals in early spring samples and small ones in late summer and fall. Seasonal variation in nuclear DNA contents, on the other hand, did not show any clear trend (**Fig.6.3**). There is much overlap in 95% confidence limits for mean nuclear DNA contents of individuals among seasons (**Fig.6.3**), due to a high variance within seasons, mean nuclear DNA contents of individuals are not significantly different (One-way ANOVA, $F_{3,32} = 1.455$, $P > .05$) among seasons. However, variances are significantly different among seasons as shown by a Barlett test for homogeneity of variances (Chi-square = 28.591, d.f.=3, $P < .001$). Although heterogeneity of variances may invalidate the result of ANOVA, it also suggests that DNA contents follow different patterns of variation according to seasons.

6.4. *Pseudocalanus elongatus*

From newly hatched nauplii, this species was reared at 8 ^{0}C or 15 ^{0}C with high food or low food (see Chapter 2 for food levels). Although more than 20 females were obtained for each food-temperature treatment, only a few of them were in suitable condition for DNA analysis. Preservation in ethanol 70% apparently affected physical conditions of the copepod tissues, so that there was a poor retention of nuclei on the

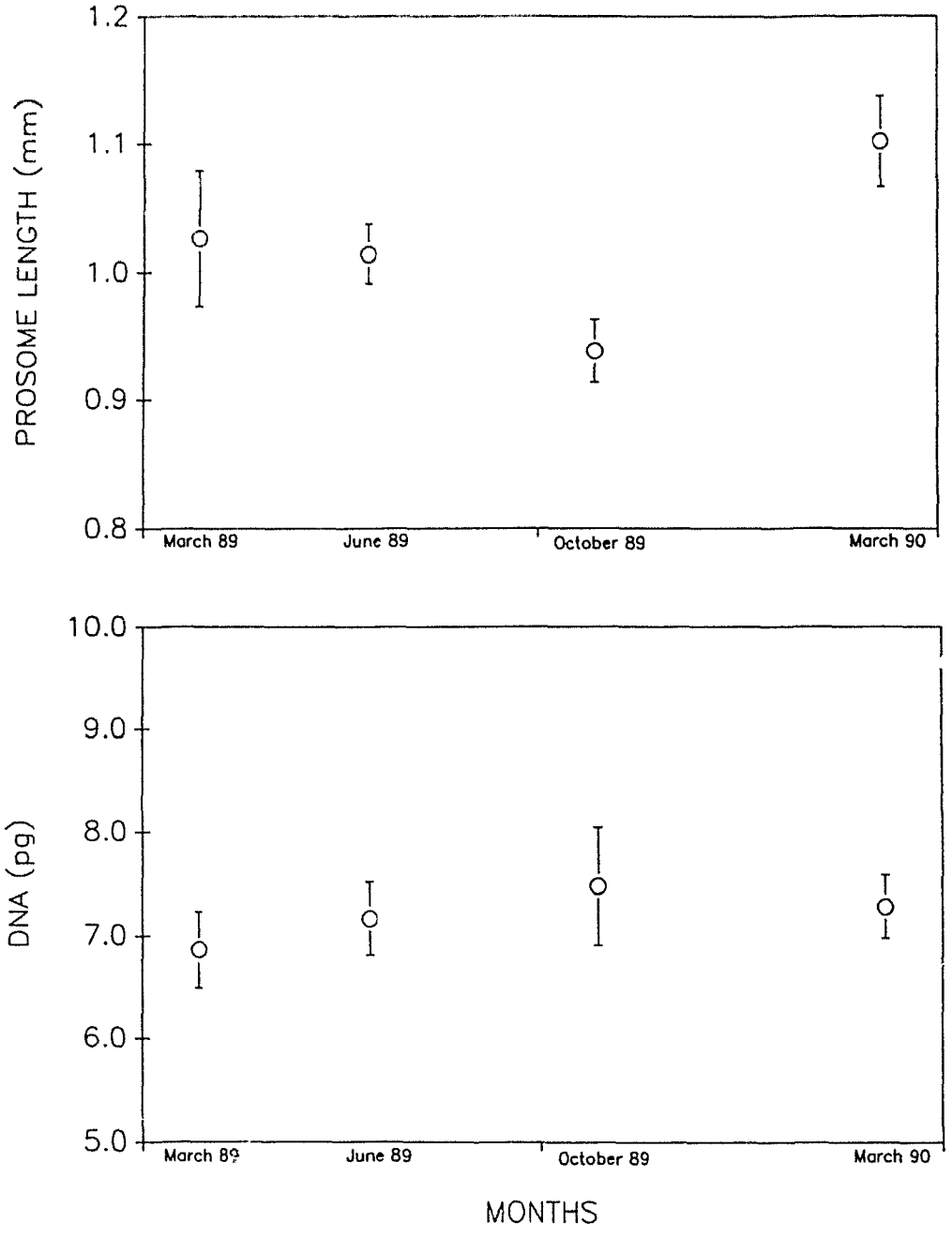

Fig.6.3 Seasonal variation in prosome length and nuclear DNA content of adult females of P.acuspes obtained from Bedford Basin during March 1989 and March 1990. Vertical lines represent 95% confidence limits for the mean.

slides for the Feulgen technique. Six females per treatment were available for DNA analysis. Mean values of nuclear DNA are shown in **Table 6.3.**

Although the variation of nuclear DNA content among females within treatment is high (nested ANOVA: $F_{20,216}$ = 10.035, P <.001), there were highly significant effects of food and temperature and their interaction on DNA contents (**Table 6.4**).

Using pooled data from the four food-temperature treatments, prosome length was plotted as a function of nuclear DNA content (**Fig.6.4**). A linear regression between body length and nuclear DNA content is highly significant ($F_{1,22}$ = 14.205, P <.001).

6.5. Calanus glacialis

This species was reared from stage CIII to adult at 3 ^{0}C or 12 ^{0}C and in high or low food levels. Five initial, stage CIII individuals, were fixed for DNA analysis and thereafter samples of usually 5 individuals were obtained from each treatment at stages CIV, CV and adult, although number of adults depended on the number surviving each treatment. Mean nuclear DNA content of initial stage CIII was (mean ± S.E., n = 5), 20.63 ± 0.394, and nuclear DNA contents of subsequent stages are summarized in **Table 6.5.**

As in the other species there was considerable variation

Table 6.3 2C nuclear DNA content of adult females of P.elongatus reared at four combinations of food and temperature. DNA content is estimated from the average of 10 nuclei of each individual.

Food level	Temp. (^{0}C)	n	DNA (pg)	S.D.
1	8	6	4.51	0.213
	15	6	3.64	0.280
1/16	8	6	3.26	0.323
	15	6	3.59	0.244

Table 6.4 Nested ANOVA testing the effects of food-temperature treatments on 2C nuclear DNA content of female of P.elongatus reared under four combinations of food and temperature. Female (1 to 4) is variation of individuals within food-temperature treatmens.

Source of Variation	SS	d.f.	F-ratio	P
Female (1)	2.237	5	6.561	<.001 ***
Female (2)	4.647	5	13.631	<.001 ***
Female (3)	3.894	5	11.422	<.001 ***
Female (4)	2.907	5	8.526	<.001 ***
Food level	24.762	1	363.159	<.001 ***
Temperature	4.546	1	66.668	<.001 ***
Food level x temperatue	20.762	1	304.499	<.001 ***
Error	14.728	216		

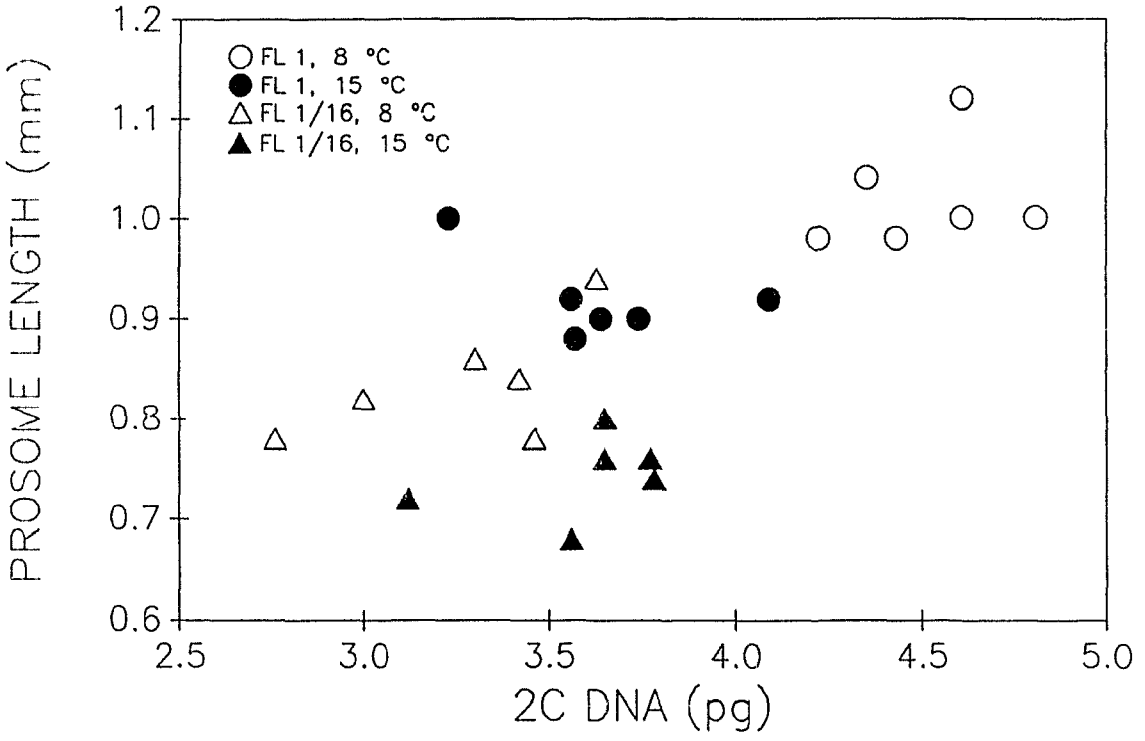

Fig.6.4 The relationship between prosome length and nuclear DNA content of females P.elongatus reared under four combinations of food and temperature. The linear regression, length = 0.346 + 0.143 DNA, is highly significant (r^2 = 0.441, P <.001).

Table 6.5 Nuclear DNA content of late developmental stages of C.glacialis reared from CIII to adult under four combinations of food and temperature. n is number of individuals.

Food level	Temp. (^{0}C)	Stage	n	DNA (pg) ± S.D.	
1	3	CIV	5	20.77	0.571
		CV	3	19.85	0.711
		Ad.	7	22.36	1.660
	12	CIV	4	20.34	0.245
		CV	5	19.797	1.382
		Ad.	11	19.80	1.240
1/16	3	CIV	3	20.05	0.422
		CV	8	19.96	0.955
		Ad.	5	19.81	1.240
	12	CIV	3	20.238	0.722
		CV	5	18.59	1.894
		Ad.	5	18.99	1.470

of DNA content among adult females within treatments (nested ANOVA, $F_{24,252}$ = 7.083, P <.001). Food-temperature effects on nuclear DNA content are also significant, but not their interaction (**Table 6.6**).

In adult females from the four treatments there was a weak, although significant, positive correlation between prosome length and nuclear DNA (**Fig.6.5**). The fitted regression line was, Prosome length = 1.915 + 0.05 DNA, ($F_{1,26}$ = 6.561, P <.05).

Changes in nuclear DNA content through stages in the four combinations of food and temperature are consistent with divergence in body length (**Fig.6.6**), i.e. small individuals are associated with lower amounts of nuclear DNA. It was also observed that DNA amounts in adults tend to decrease at low food and high temperature as compared to initial stage CIII (**Fig.6.6**). At high food and low temperature, on the other hand, larger adult females are obtained whose nuclear DNA is not significantly different from that of initial stages CIII (t-test = 2.121, P >.05). This suggests that modulation of nuclear DNA under the influence of food supply and temperature might not involve amplification, but a reduction of DNA under food shortage and high temperature. This also implies that stages CIII obtained from the field might reflect conditions of high food and temperature similar to the low value during the experiment (3 ^{0}C). Indeed, the temperature at the surface during sampling in March 1989 was ca. 3 ^{0}C.

Table 6.6 Nested ANOVA testing the effects of food-temperature treatments on 2C nuclear DNA content of female of C.glacialis reared from CIII to adult under four combinations of food and temperature. Female (1 to 4) is variation of individuals within food-temperature treatments.

Source of Variation	SS	d.f.	F-ratio	P
Female (1)	166.171	6	10.085	<.001 ***
Female (2)	152.764	10	5.563	<.001 ***
Female (3)	61.510	4	5.600	<.001 ***
Female (4)	86.390	4	7.865	<.001 ***
Food level	28.679	1	10.443	<.01 ***
Temperature	29.985	1	10.919	<.01 ***
Food level x temperature	1.052	1	0.383	>.05 n.s.
Error	698.022	252		

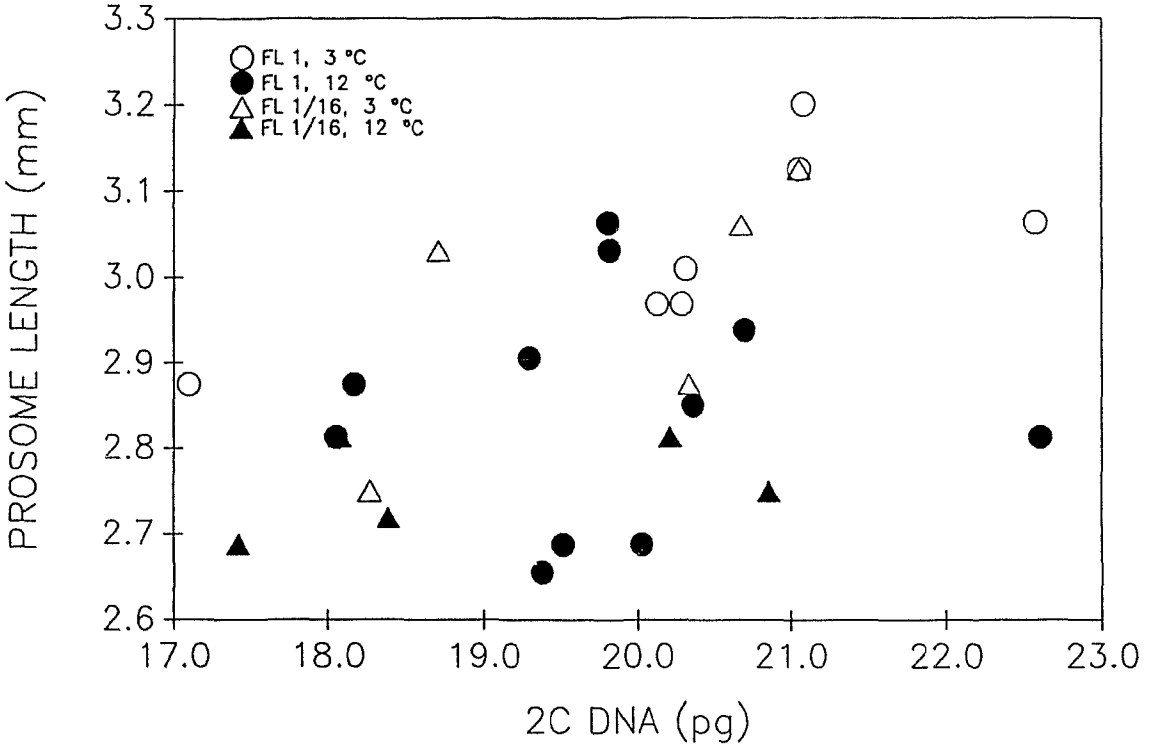

Fig.6.5 The relationship between prosome length and 2C nuclear DNA content of adult females C.glacialis reared from stage CIII to adult under four combinations of food and temperature.

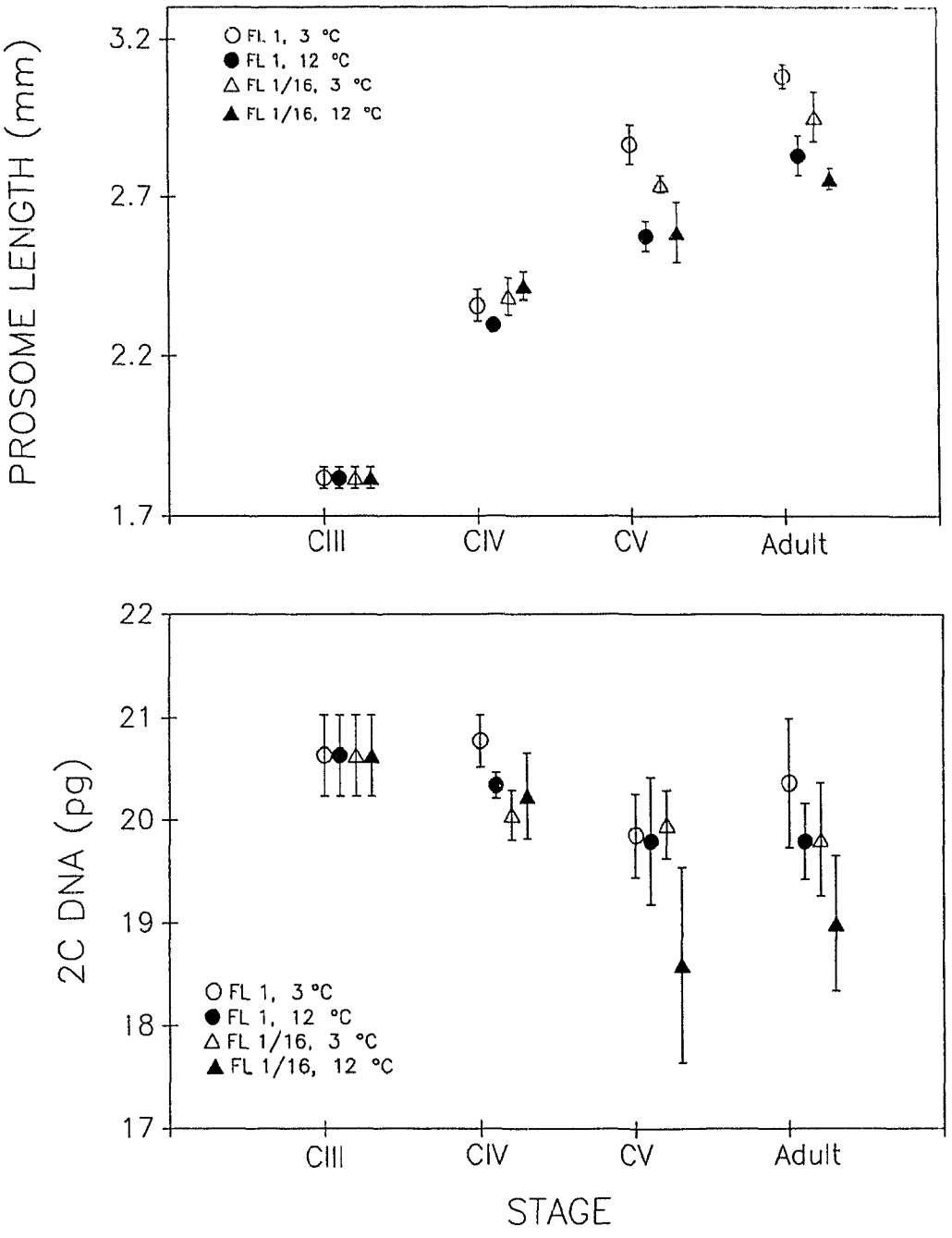

Fig.6.6 Variation in prosome length and 2C nuclear DNA content of late stages of C.glacialis reared from CIII to adult under four combinations of food and temperature. Vertical lines represent standard errors for the mean.

Although food availability at the collection site is unknown, observations from other studies in Nova Scotian waters indicate that phytoplankton concentration and primary production reach their maximal values during the period March-April (e.g. Irwin et al. 1988, 1989). The high amounts of lipids in the collected stage CIII's may also indicate good nutritional conditions (Klein-Breteler and Gonzalez 1988, also observed in the present study, Chapter 5).

6.6. Nuclear DNA content and development rates

McLaren et al. (1988) observed that among Calanus species, body size and development rate are correlated, such that large species develop slower than small ones. They also found a similar correlation between development rate and genome size, i.e. species with large genomes show slow development rates as compared to species with smaller genome sizes. In the present study, within species there was little correlation between body size and development rate among individuals reared under the same conditions (see Chapter 4). However, as shown above, even within treatments genome size was highly variable, so that this variation might be associated with development rate.

In a rearing experiment with C.glacialis from stage CIII to adult, at 10 ^{0}C and under excess conditions of food, durations of stages CIV and CV were estimated for animals kept

in individual containers. Development rate of individuals was calculated as,

$$1/D = 1/(DCIV + DCV)$$

where 1/D is development rate (d^{-1}), and DCIV and DCV are durations of stages CIV and CV respectively.

Unfortunately due to an extended duration of stage CV and consequently an increased mortality, only 12 out of 30 individuals reached adulthood. Among these survivors development rate showed a low variability [$0.040 \pm 1.282 \times 10^{-3}$ (mean ± S.E.)]. Despite the lack of correlation between development rate and prosome length ($F_{1,10} = 0.198$, $P > .05$), and between prosome length and nuclear DNA ($F_{1,10} = 2.052$, $P > .05$), the negative correlation between development rate and nuclear DNA content was highly significant ($r^2 = 0.631$, $F_{1,10} = 17.106$, $P < .01$). The relationship between development rate and nuclear DNA is shown in **Fig.6.7.**

6.7. Nucleus number in copepods

Among invertebrates, various groups show a certain constancy in cell number. Nematoda and Rotifera apparently acquire a constant number of cells during development, and then growth is achieved by increasing cell size (Hyman 1951). Embryonic stages of Artemia salina also possess a constant number of cells (Olson and Clegg 1978).

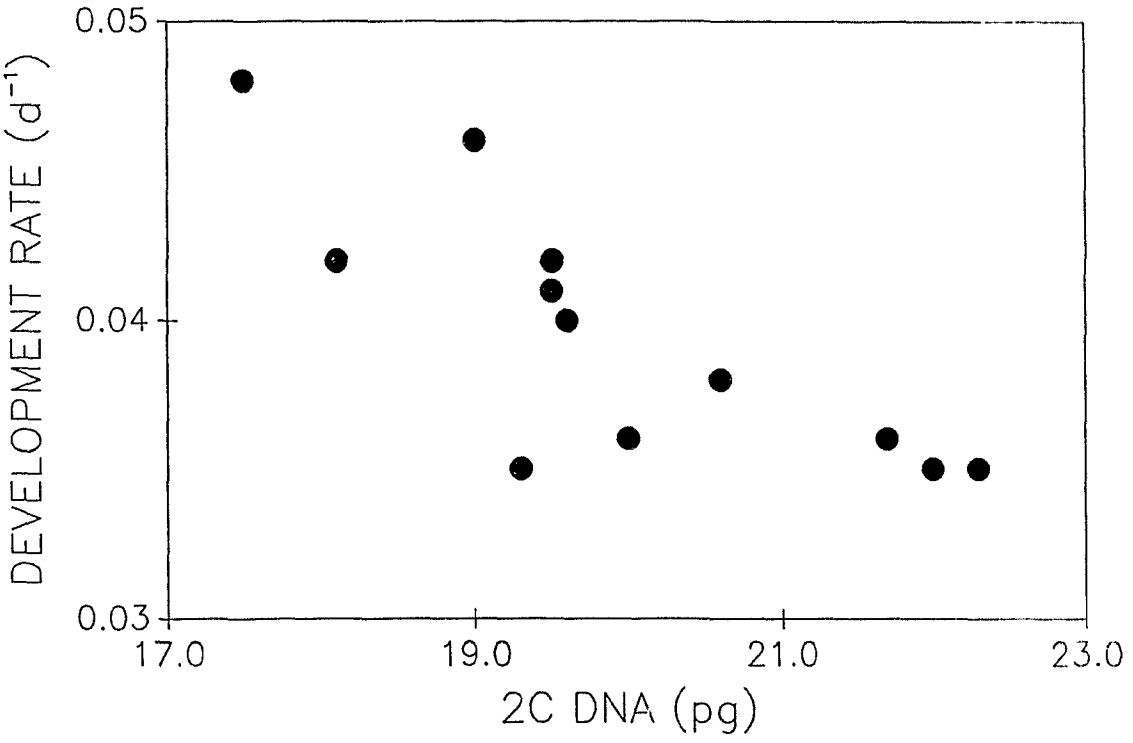

Fig.6.7 The relationships between development rate of late stages and 2C nuclear DNA content at stage adult females C.glacialis reared from stage CIII to adult at 10 ^{0}C, under excess conditions of food.

Estimating cell number is difficult, however, especially for late stages of development. Some estimates have been derived from measurements of DNA content, assuming a constant C-value (nuclear DNA) for the species (e.g. Mirsky and Ris 1949). It is now clear from this study that nuclear DNA within copepod species may vary in a great extent, so that numbers of cells estimated through this approach are far from being realistic. Furthermore, there could be large variations among individuals in numbers and DNA contents (Robins and McLaren 1982) of oocytes. Alternatively, direct counting of cells or nuclei can be performed (e.g. Olson and Clegg 1978, McLaren and Marcogliese 1983). Using this technique, in the present study the total number of nuclei were counted in individuals of P.acuspes reared at 5 ^{0}C or 10 ^{0}C and with high or low food. This experiment attempted to test the hypothesis that, despite size variations induced by food-temperature treatments, the number of cells of adults remains nearly constant, so that size variations might be explained by changes in cell size, but not in cell numbers.

Total number of nuclei was estimated for Feulgen-stained adult females (see Chapter 2 for details on the technique). A total number of 25 individuals were analyzed. However, several counts probably underestimated the total number of nuclei, when they were too aggregated. Other counts were assumed to overestimate number of nuclei, when many fragments of broken

nuclei were present. To avoid such sources of error, quality of slides were judged without reference to the counts. Therefore, those slides judged to be poor on the basis of these criteria were not considered in the analysis.

Estimates of total nuclei number still showed considerable variance (grand mean ± S.D. = 15904 ± 2621, n = 18). Among food-temperature treatments, variances were found homogeneus (Barlett test = 2.755, P <.05). Food-temperature effects on nuclei number were found not significant, when tested through two-way ANOVA (**Table 6.7**). There was no significant correlation ($F_{1,16}$ = 0.576, P >.05) between prosome length and nuclei number (see **Fig.6.8**).

6.8. Discussion

Drastic changes in nuclear DNA content of copepods have been observed through the phenomenon of chromatin diminution (Beerman 1977, Ammermann 1985 for reviews). This process takes place during early cleavage divisions of the embryo and involves excision of DNA from somatic cells. Thus, chromatin diminution possibly provides a mechanism to explain variations in genome size and hence body size (Robins and McLaren 1982). It is now clear that such variations do not result from polyploidy, i.e. increase in chromosome number, but they usually involve changes in chromosome size (e.g. Tobler 1986).

Changes in genome size under the effect of the environment have not been reported in animals. However, recent

Table 6.7 Two-way ANOVA testing food temperature effects on the number of nuclei of adult females P.acuspes reared under four combinations of food and temperature.

Source of Variation	d.f.	SS	F-ratio	P
Food	1	1.023×10^7	1.544	>.05 n.s.
Temperature	1	1.311×10^7	1.978	>.05 n.s.
Food x Temperature	1	1.462×10^6	0.221	>.05 n.s.
Error	14	9.280×10^7		

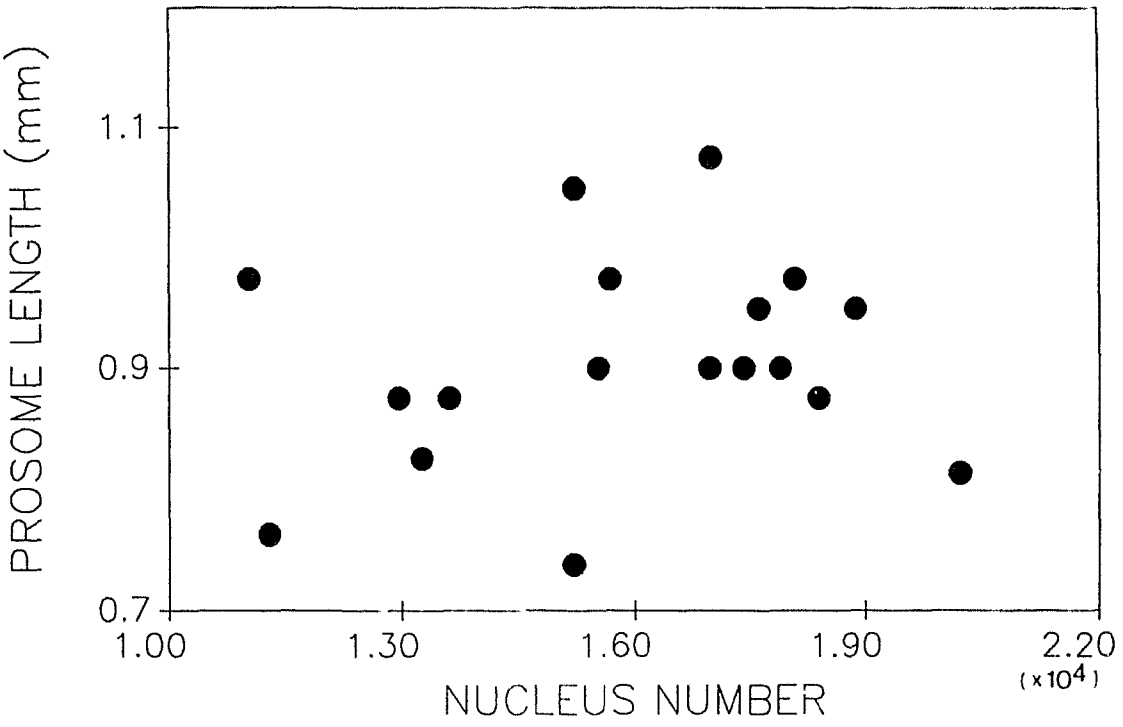

Fig.6.8 The relationship between prosome length and nuclei number in adult females P.acuspes reared under four combinations of food and temperature.

work in plants documents selective chromatin diminutions in Scilla siberica under environmental influence, possibly as a mechanism for quick adaptation to environmental stress (Deumling and Clermont 1989). Information obtained in the present study is in agreement with this hypothesis. However, food-temperature effects on DNA of late stages of C.glacialis suggest that modulation of genome size of somatic cells can take place during late development, and not necessarily through chromatin diminution in embryonic stages. It is also clear from these results that such changes in DNA would take place during ontogeny, presumably during cell cycles, since no temperature effects were detected on adult P.acuspes, indicating stability of DNA amounts after cessation of cell divisions. However, inherent variation in DNA contents associated with body sizes could also produce changes in DNA from differential, treatment-dependent mortality. Although, no differential mortality among treatments was detected in C.glacialis, mortality was treatment-dependent in P.elongatus (see Chapter 5) and no information was available for P.acuspes. Thus, the present results cannot definitively rule out the possibility of treatment-dependent selection of body size and corresponding genome sizes.

At this point, it is not possible to elucidate whether genome size is responsible for regulating cell sizes and consequently body size. Although a weak positive correlation exists in two of the three species between body length and

nuclear DNA, it is clear from their relationships (**Fig.6.2**, **Fig.6.4** and **Fig.6.5**) that DNA only partially explains length variations. Furthermore, a positive correlation between size and nuclear DNA does not necessarily imply a causal relationship. There might be body size variations independent of cell size. For instance, although no treatment effects on nucleus number were detected in P.acuspes, estimates of nucleus number are uncertain given the high variance in nucleus counts, possibly because of technical difficulties. McLaren and Marcogliese (1983) using a similar method found that numbers of nuclei of copepodids stage CI Pseudocalanus and other species vary between 9600 and 13000. This range is not very different from that of adult females obtained in this study (mean = 15904), suggesting a small increase in cell numbers from stage CI to adult. Thus body size increases would result from increases in cell sizes. This is consistent with significantly smaller amounts of nuclear DNA of stage CI as compared to adults, as shown above. However, amounts of DNA of stage CIII in C.glacialis are not significantly different from that of adult females. Thus increase in body size through late stages may also involve mechanisms other than cell size increases.

In spite of the complications in relationships of body size and genome size, a negative correlation between development rate and nuclear DNA in C.glacialis supports the thesis that genome size is an adaptive feature to confront

environmental conditions (Cavalier-Smith 1985). Although rapid development under conditions of high temperature might result from inevitable increased metabolic rates, an even more rapid development as a consequence of decreased genome size is possibly an advantage under conditions of food-shortage and high temperature, such that animals reaching maturity sooner avoid increased mortality, at cost of a smaller size. In excess conditions of food and low temperature, on the other hand, a slow development would allow a larger body size which is certainly an advantage in terms of reproductive potential (see Peters 1983). This hypothesis does not postulate any causal relationship between genome size and body size. Genome size might regulate cell developmental rates by modulating the nucleocytoplasmatic ratio (Cavalier-Smith 1978), which is a crucial component in cell metabolism. Cavalier-Smith (1985) postulated that the bulk of DNA might act as a nucleoskeleton regulating nuclear volume and hence corresponding cell volumes. This process, according to Cavalier-Smith (1985), would be subject to natural selection, which acts on organisms to determine developmental rates, and associated cell sizes. This hypothesis is in agreement with inherent variation in genome size correlated to body size and development rate. However, the extent to which genome size is heritable and hence subject to natural selection, has to be elucidated. Moreover, environmental effects on DNA during ontogeny of

individuals, as shown by the present study is a phenomenon that requires exploration at the molecular level.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

7.1. Understanding size variations

Since there are multiple selection pressures to which organisms are exposed, and which may influence body size, it has been argued that a more holistic approach should be taken to understand how size variation develops (Mayr 1956, Roff 1981). Such theoretical analyses show that sizes of species fall within optimal ranges for maximal fitness in terms of population growth (Roff 1981, 1986). These approaches may be used to obtain insights about ultimate causes of organisms' sizes. However, they might tell us little about proximate causes, and the mechanisms involved. Moreover, in order to achieve more accuracy, they necessarily require information on such proximate causes and mechanisms.

The multiplicity of factors that may influence body size has to be recognized, however. There might be situations when a large part of the variance in size can be explained by a single or a few factors, but these are probably special exceptions. Moreover, different components of the body, such length, mass or lipid store may respond differently to changing conditions, limiting extrapolations of results from one component to another. In this context, effects on "body

size" should be explicitly referred to as effects on body volume, body mass or other components.

It was the intention of this study to examine the role of those factors that are well recognized as most important in influencing size variations, namely food availability and temperature, and to obtain insights on particular mechanisms through which such factors may influence body size. Nevertheless, there are various other factors that might influence intrapopulation variations. Although their relative importance as determinants of body size is not clear, they may be considered potential proximate or ultimate causes of size variation.

7.2. Potential influences on copepod size

Proximate causes of size variations in natural populations of copepods can be summarized as genetic components (e.g. McLaren 1976, McLaren and Corkett 1978, Tepper and Bradley 1989), temperature during development (e.g. Deevey 1960, McLaren 1965), food conditions during development (e.g. Klein Breteler and Gonzalez 1982), population density (Whitehouse and Lewis 1973) and size dependent mortality through vertebrate predation (e.g. O'Brien 1979, Warren et al. 1986).

Although, in copepods some evidence indicates that parental size influences size of the progeny (e.g. McLaren 1976, Corkett and McLaren 1978), seasonal size variations need

not be attributed to genetic differences (e.g. Elgmork 1976, Klein Breteler et al. 1990). Alternatively, a significant role has been given to food supply and temperature, to explain seasonal size variations. Evidence comes from both field observations (e.g. Deevey 1960, Klein Breteler and Gonzalez 1982) and experiments (e.g. Lock and McLaren 1970, Klein Breteler et al. 1990).

Population density and vertebrate predation, as influences on size variations, have been mostly studied in lakes (e.g. Warren et al. 1986). In the ocean, some theoretical works suggest that seasonal changes in size may be related to mortality rather than to physiological factors (e.g. Myers and Runge 1983). However, this hypothesis still has to be tested in the field, and even if predation plays a significant role, it should be considered as an ultimate cause. Moreover, Roff (1981), based on theoretical considerations, showed that in most situations there is no need to invoke density-dependent effects to explain size variations in natural conditions.

7.3. Growth and development as regulating body size

Divergence in body size takes place during ontogeny. Thus, variation in size of adults and inmature individuals must result from differential growth and development rates. However, growth in body mass and growth in body volume or length have to be distinguished. This distinction is important

because changes in body mass and body length may respond differently to food and temperature, resulting in a large unexplained variation when attempting to predict body mass through measurements of body length.

Although, it was shown that growth can be exponential, stage durations were not equal. This variation in duration of stages resulted in a decrease in the growth rate from young to older stages. Since body size increases with stages, it follows that growth rate also decreases with body size. This has been viewed as body size influencing growth and development rates (Vidal 1980a, 1980b, Bottrell and Robins 1984). In the present study, however, there was a lack of correlation between body sizes and stage durations. Although as expected, size at a terminal stage is influenced by size at an initial one. However, there is no need to invoke size dependence to explain the decrease in growth rate, it rather seems that differential stage durations determines changes in growth rate, but not body size itself. In fact, in E.herdmani, where development was more isochronal compared to C.glacialis, the growth rate did not show a pattern of decrease toward older stages.

Therefore, in order to describe changes in body size through development, timing of development and initial body size have to be considered. Growth in body length, although not continuous, shows a linear pattern with time, and may be described as,

$$L = L_0 + b\ t \qquad (7.1)$$

where, L is length at a terminal stage (e.g. adult), L_0 is length at an initial stage (e.g. CI), b is the slope for a linear regression of length against time (t).

Assuming body weight (W) as related to L^3, and exponential growth in weight, the ratio between weight and length at a terminal stage may be expressed as,

$$L/L^3 = (L_0 + b\ t) \ / \ (W_0\ e^{Gt}) \qquad (7.2)$$

where W_0 is body weight at an initial stage (CI) and G is the instantaneous growth rate.

Rearranging equation (7.2) and applying logarithms, the relationship between G and development time can be expressed as,

$$G = [3\ \ln\ (L_0 + b\ t) - \ln\ W_0] \ / \ t \qquad (7.3)$$

Equation (7.3) suggests a logarithmic decrease of G with development time (Fig. 7.1), and implies that in rapidly developing individuals, small changes in development time will produce drastic changes in G. Slow developing individuals, on the other hand, would show less dependence of G on development time. Since development time is highly dependent on temperature, small species and early stages that develop more

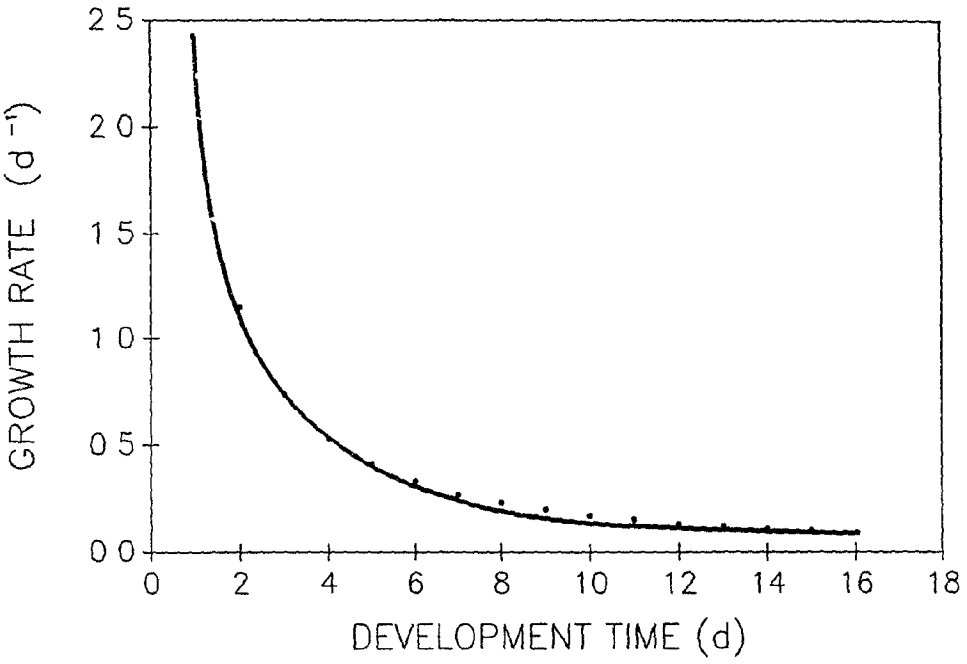

Fig.7.1. A theoretical relationship between the instantaneous growth rate and development time in copepods.

rapidly than large species and late stages, would show a high dependence of G with temperature.

7.4. The role of food supply and temperature

Although body mass and body length are inevitably correlated and this was described through allometric equations, it was evident that body density does not remain constant when individuals have undergone different culture conditions. For instance, results from this study indicate that in lipid-storing species such as C.glacialis body mass is affected by food and temperature because of changes in content of lipids, but mass of the structural copepod remains unaffected. In fact, Gatten et al. (1979), found that variation in dry weight in field samples of C.helgolandicus were correlated to total content of lipids. Under excess of food, on the other hand, temperature affected body length, but not body mass. This also alters the relationship between body mass and body length, thus suggesting that under variable conditions of food and temperature the use of body length to estimate body mass is inadequate. In the context of copepod production body mass is of most interest, thus direct measurements of individual body mass should be made.

In rearing experiments, it has been shown that under conditions of high food levels size variations can be explained by temperature (e.g. Lock and McLaren 1970, McLaren 1965, Thompson 1976). In the field, although there is often a

lack of correlation between food quantity and body size (e.g. McLaren 1963, Bottrell and Robins 1984), effects of food quality on body size have not been investigated. Diel and Klein Breteler (1986) observed that development rates of C.finmarchicus in the North Sea were dependent on phytoplankton composition. Thus, food quality may be an important factor influencing body size.

In the present study, it was shown that both temperature and food influenced body length. Although only quantity of food was tested, it seemed that body size, as either body length or body mass, was more affected by food quantity than by temperature. C.glacialis was exposed to different food levels only during three stages of development, so that only slight differences in body length and body mass were observed. However, in Pseudocalanus species exposed throughout development, a drastic reduction in body length was observed at the low food level. The effect of temperature on body length, although significant, was less notable and not consistent in all cases.

The mechanisms through which food and temperature influence body size are unknown. However, there are indications that food shortage retards development rates (e.g. Vidal 1980b, Diel and Klein Breteler 1986). Retardation of development by food shortage must be accompanied with reduced growth rates in order to produce small individuals. Temperature, on the other hand, also acts on development

rates. However, reduced development rates at low temperatures should not be accompanied by highly reduced growth rates in order to produce large individuals. Thus, although both food and temperature may indirectly affect body size by influencing mainly development time, their mechanisms may be very different. Food shortage might limit metabolic reactions by a lack of substrates, whereas temperature may slow down metabolic reactions, but it also may slow down turnover rates or catabolic reactions resulting in a net increase of material. This obviously requires exploration at the biochemical level.

7.5. Genome size, development rates and body size of copepods

Although a large body size is advantageous in terms of reproductive success (Peters 1983), it is not clear that size is directly related to fitness of the species. For instance, among species of insects, Gaston (1988) found a lack of correlation between size and the intrinsic rate of increase "r". In insects, it has been argued that size is a by-product of selection acting on timing of development (e.g. Masaki 1978, Taylor 1981). Indeed, results from the present study indicate that, in copepods, timing of development might be the major determinant of variations in growth rates, which in turn determine size variations, so that as in insects, body size may be considered a by-product of selection for development times.

In understanding the importance of timing of development, heterogeneity of the environment has to be considered. As shown when comparing growth patterns of species, in some copepods, such as E.herdmani, exposed to estuarine environments with short-term fluctuations in conditions, selection will favour a rapid development rate and more continuous growth. In more stable, but strongly seasonal environments, a rapid development rate is only favoured during the spring season, when food is abundant, but for the other seasons, under scarcity of food, reduced metabolic rates and diapause when individuals have reached an advanced stage of development are clearly favoured. C.glacialis with an annual life cycle and long overwintering period represents this type of selection. Both kinds of selection are usually referred as "r" and "k" selection (MacArthur and Wilson 1967, Pianka 1970).

Development and growth of individuals are undoubtedly the expression of metabolic rates at the cellular level. Cavalier-Smith (1978) argues that individual cells may also be subject to processes of selection for development rates associated with cell volumes. In unicellular organisms there is a close association between cell division rate and cell size (Cavalier-Smith 1978 and references therein). In multicellular plants and animals the same association is observed (Cavalier-Smith 1985 for review). The argument is that cell size results from conflicting selection for a rapid development and large

cell size (Cavalier-Smith 1978). The mechanisms through which cells may modify their metabolic rates and consequently their volumes is not well understood. According to Bennett (1972), the total DNA of nuclei might be responsible for regulating nuclear volume and consequently cell volume. This nucleotypic effects of genome size will in turn determine cell metabolic rates (Cavalier-Smith 1978).

Among calanoid copepods genome size has been found related to development rate and body size (McLaren et al. 1988). Within species development rate of individuals was correlated to genome size in C.glacialis in the present study. The association between body size and genome size was less clear and not consistent in all cases in my study. Therefore, it seems that the role of genome size is more directly related to cell development rates rather than to cell size.

In order for the genome size to be considered an adaptive trait, certain conditions should be met. There should be intrapopulation variation, such that selective pressures may act on such variation. This condition is clearly met in copepods, as shown in this and previous studies (e.g. Robins and McLaren 1982). However, the most important condition should be inheritance of genome size. This is an open question that requires exploration. However, what is relevant from the present study is the finding that genome size may be modulated during ontogeny of individuals under the effect of environmental factors. Although a recent study in plants has

shown shifts in genome size during the process of chromatin diminution, associated with environmental factors (Deumling and Clermont 1989), results in the present study may be considered novel in animals.

Although the hypothesis of nucleotypic control by genome size has been strongly criticized in a recent review (John and Miklos 1988), my results support the hypothesis that genome size plays a significant role in modulating cell metabolic rates and consequently organism metabolic rates, which in turn would determine individual body sizes. This hypothesis does not postulate a direct relationship between genome size and body size, but a rather indirect correlation due to the universally known association between metabolism and body size. It was clear from my results that variation of genome size only partially explains variation of body sizes. Perhaps a closer correlation between genome size and body size of copepods would be expected if size variations are only due to changes in cell size, but not in cell number. This was a question that was not entirely answered in this study. Moreover, there might be variations of body size independently of cell size and cell number (Cavalier-Smith 1978). Furthermore, the positive correlation between nuclear DNA and cell volume is expected if nucleocytoplamatic ratios remain constant. Variations in such ratios might also obscure the correlation between genome size and body size.

In attempting to explain the adaptive value, if any, of a potentially modificable genome size during ontogeny of individuals, the physiological plasticity of poikilotherms animals should be recalled. Metabolic compensation to confront changing temperature has long been recognized in these organisms (Bullock 1955). Thus, it may be hypothesized that genome size is involved in such compensation by modifying metabolic rates. Responses to food shortage, on the other hand, might reflect substrate-limited synthesis of DNA. However, it might also reflect shifts in nuclear DNA as a mechanism of adaptation to food conditions. Under food-shortage, reduced genome size may allowed a rapid development to reach maturity and avoid increased mortality of young stages; this at cost of a smaller size.

APPENDIX A1

Table A1. Mean total dry weight (TW), lipid discounted dry weight (SW) and oil sac volume (OSV) in different stages of Calanus species. Mean values for each $(TW-SW)_i$ group were obtained from animals of the same stage reared in same conditions. n is the sample number for each $(TW-SW)_i$ group.

TW (µg)	SW (µg)	n	(TW-SW)i (µg)	OSV (mm^3 x1000)
63.55	34.22	6	29.33	8.6
83.10	56.20	4	26.90	8.7
110.40	66.25	6	44.15	20.30
200.60	118.40	5	82.20	38.00
85.80	56.70	6	29.10	13.30
111.73	70.50	4	41.23	17.10
190.95	120.15	8	70.81	35.71
247.42	138.70	6	108.70	73.80
261.97	169.50	4	92.47	128.80
83.30	48.80	5	34.50	25.00
122.10	65.00	4	57.10	31.00
111.90	61.00	6	50.90	27.60
116.50	63.10	4	53.40	34.00
143.20	126.60	8	16.60	14.10
130.00	122.00	4	8.00	8.80
379.20	298.90	6	80.30	120.00
435.30	383.70	4	51.60	70.00
330.50	275.30	5	55.20	39.80

APPENDIX B1

Table B1. Prosome length and dry weight (DW) of adult females *E.herdmani* reared from stages CI to adult at two temperatures and under excess conditions of food.

3 0C		10 0C	
P.length (mm)	DW (µg)	P.length (mm)	DW (µg)
0.700	8.45	0.738	5.30
0.675	4.10	0.750	8.20
0.688	6.85	0.763	7.65
0.638	5.00	0.725	4.15
0.700	5.38	0.685	3.70
0.675	6.70	0.725	7.10
0.750	11.35	0.700	6.30
0.725	8.00	0.725	7.00
0.775	10.15	0.825	6.30
0.750	11.33	0.700	6.60
0.700	6.00	0.850	10.00
0.625	5.30	0.850	10.00
0.800	13.40	0.825	13.70
0.738	9.90	0.750	5.30
		0.750	4.80
		0.825	7.20
		0.650	3.90

APPENDIX C1

Table C1. Chick blood standards used for estimate nuclear DNA contents of copepod. 1 or 2 standards were included in each batch. Mean values (± S.D.) are units of absorbance (integrated in log scale). These means were assumed to represent 2.5 µg of DNA (Rash et al. 1971).

Experimental species	Standards (abs.units)	n
	72.23 ± 7.260	30
	78.97 ± 7.069	30
	68.40 ± 2.440	15
P.acuspes	73.30 ± 2.831	15
	52.40 ± 5.630	20
	64.10 ± 4.201	15
	91.67 ± 3.750	30
P.elongatus	88.30 ± 2.911	30
	72.35 ± 2.470	30
	72.50 ± 2.380	30
	61.75 ± 1.962	15
C.glacialis	64.22 ± 3.211	30
	76.44 ± 5.204	30

BIBLIOGRAPHY

AMMERMAN, D. 1985. Chromatin diminution and chromosome elimination: mechanisms and adaptive significance. **In**: T.Cavalier-Smith (ed.) **The evolution of genome size**. John Wiley & Sons, NY.

ANRAKU, M. 1964. Influence of the Cape Cod Canal on the hydrography and the copepods in Buzzards Bay and Cape Cod Bay, Massachussetts. II. Respiration and feeding. Limmnol. Oceanogr. **9**:195-206.

AKSNES, D.L. and T. MAGNESEN. 1988. A population dynamics approach to the estimation of production of four calanoid copepods in Lindaspdlene, Western Norway. Mar. Ecol. Progr. Ser. **45**:57-68.

BÅMSTEDT, U. 1984. Diel variations in the nutritional physiology of Calanus glacialis from lat. 78° N in the summer. Mar.Biol. **79**:257-267.

BÅMSTEDT, U. 1988. Ecological significance of individual variability in copepod bioenergetics. Hydrobiologia **167/168**:43-59.

BÅMSTEDT, U. and K.S. TANDE. 1985. Respiration and excretion rates of Calanus glacialis in arctic waters of the Barents Sea.Mar.Biol. **87**:259-266.

BARNES, H. and M. BARNES. 1953. Biometry of the copepod Calanus finmarchicus (Gunn.) in stages V and VI. J. Mar. Biol. Ass. U.K. **32**:305-313.

BEAUCHAMP, J.J. and J.S. OLSON 1973. Correction for bias in regression estimates after logarithmic transformations. Ecology **54**:1403-1407.

BEERMAN, S. 1977. The diminution of heterochromatic chromosomal segments in Cyclops (Crustacea, Copepoda). Chromosoma **60**:297-344.

BEERS, J.R. 1976. Determination of zooplankton biomass. In H.F. Steedman (ed.). **Zooplankton fixation and preservation**. Monographs on Oceanographic Methodology. The UNESCO Press.

BENNETT, M.D. 1971. The duration of meiosis. Proc.R.Soc.Lond. **178B**:259-275.

BENNETT, M.D. 1972. Nuclear DNA content and minimum generation time in herbaceous plants. Proc.R.Soc.Lond. **181B**:109-135

BENNETT, M.D. 1977. The time and duration of meiosis. Phil.Trans.R.Soc.Lond. **277B**:201-226.

BENNETT, M.D. and J.B. SMITH. 1976. Nuclear DNA amounts in angiosperms. Phil.Trans.R.Soc.Lon. **274B**:227-274.

BERNARD, M. 1971. La forme elliptique de la relation temperature-dure de development embryonnaire chez les copepodes pelagiques et ses propiets. In D.J. Crisp (ed.). 4th Cambridge, **Symp. European Mar.Biol.**

BLAKLEY, N. and S.R. GOODNER, 1978. Size-dependent timing of metamorphosis in milkweed bugs (Oncopeltus) and its life history implications. Biol.Bull. **155**:499-510.

BOGOROV, V,G. 1934. Seasonal changes in biomass of Calanus finmarchicus in the Plymouth Area in 1930. J. Mar. Biol. Ass. U.K. **19**:585-612.

BOTTRELL, H.H. 1975. The relationships between temperature and duration of egg development in some epiphytic Cladocera and Copepoda from the river Thames, Reading, with a discussion of temperature functions. Oecologia (Berlin) **18**:63-84.

BOTTRELL, H.H. and D.B. ROBINS. 1984. Seasonal variations in length, dry weight, carbon and nitrogen of Calanus helgolandicus from Celtic Sea. Mar.Ecol.Prog.Ser. **14**:259-268.

BRODY,S. 1964. **Bioenergetics and Growth**. 2nd ed.,Hafner Pub. Corp., N.Y.

BULLOCK,T.H. 1955. Compensation for temperature in the metabolism and activity of poikilotherms. Biol.Rev.Cambridge Phil.Soc. **10**:311-342.

CAVALIER-SMITH, T. 1978. Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox. J.Cell Sci. **34**:247-248.

CAVALIER-SMITH, T. 1985. **The Evolution of Genome Size**. John Wiley & Sons, LTD. London.

CLARKE, G.L. and D.J. ZINN. 1937. Seasonal production of zooplankton off Woods Hole with special reference to Calanus finmarchicus. Biol. Bull. **73**:464-487.

COHEN, R.E. and R.G. LOUGH. 1981. Length-weight relationships for several copepods dominant in the Georges Bank-Gulf of Maine area. J.northw.Atl.Fish.Sci. **2**:47-52.

COMITA, G.W., MARSHALL, S.M. and A.P. ORR. 1966. On the biology of Calanus finmarchicus 13. Seasonal change in weight, calorific value and organic matter. J.Mar Biol.Ass. U.K. **46**:1-17.

COMMONER, B. 1964. Roles of deoxyribonucleic acid in inheritance. Nature, London, **202**:960-968.

CONDREY, R.E. 1982. Ingestion-limited growth of aquatic animals: the case for Blackman kinetics. Can.J.Fish.Aquat. Sci. **39**:1585-1595.

CONOVER, R.J. 1965. Notes on the moulting cycle, development of sexual characters and sex ratio in Calanus hyperboreus. Crustaceana **8**:308-320.

CONWAY, D.V.P. and R.C. MINTON. 1975. Identification of the copepoid stages of some common calanoid copepods. Mar.Lab. Aberdeen Internal. Rep. N.S. **7**:1-2.

CORKETT, C.J. 1984. Observations on development in copepods. Studies on Copepoda II. Proceedings of the First International Conference on Copepoda, Amsterdam, 1981. Crustaceana, Suppl., **7**:150-153.

CORKETT, C.J. and I.A. McLAREN. 1978. The biology of Pseudocalanus. Adv.mar.Biol. **15**:1-231.

CORNER, E.D.S. and S.C.M. O'HARA (eds.). 1986. **The Biological Chemestry of Marine Copepods**. Oxford Science Publications, Oxford.

CUSHING, D.H. 1975. **Marine Ecology and Fisheries**. Cambridge University Press, Cambridge.

DAGG, M.J. 1976 Complete carbon and nitrogen budgets for the carnivorous amphipod, Calliopius laeviusculus (Kroyer). Int. Rev. gesamten Hydrobiol. **61**:297-357.

DARLINGTON, C.D. 1937. **Recent Advances in Cytology**. 2nd. edn., Churchill, London.

DARLINGTON, C.D. 1965. **Cytology**. Churchill, London.

DAVIS, C.C. 1976. Overwintering strategies of common planktic copepods in some North Northway fjords and sounds. Astarte **9**:37-42.

DAVIS, C.S. 1983. Laboratory rearing of marine calanoid copepods. J.Exp.Mar.Biol.Ecol. **71**:119-133.

DAVIS, C.S. 1987. Components of the zooplankton production cycle in the temperate ocean. J.Mar.Res. **45**:947-983.

DAY, R.W. and G.P. QUINN. 1989. Comparisons of treatments after an analysis of variance in ecology. Ecol. Monographs **59**:433-463.

DEEVEY, G.B. 1960. Relative effects of temperature and food on seasonal variations in length of marine copepods in some eastern American and western European waters. Bull. Bingham Oceanogr. Coll. **17**:54-86.

DEEVEY, G.B. 1964. Annual variations in length of copepods in the Sargasso Sea off Bermuda. J.Mar.Biol.Ass.U.K. **44**:589-600.

DEUMLING, B. and L. CLERMONT. 1989. Changes in DNA and chromosomal size during cell culture and plant regeneration of Scilla siberica: selective chromatin diminution in response to environmental conditions. Chromosoma **97**:439-448.

DIEL, S. and W.C.M. KLEIN BRETELER. 1986. Growth and development of Calanus spp. (Copepoda) during spring phytoplankton succession in the North Sea. Mar. Biol. **91**:85-92.

DOOLITTLE, W.F. and C. SAPIENZA. 1980. Selfish genes, the phenotype paradigm and genome evolution. Nature, London, **284**:617-618.

DURBIN, E.G. and A.G. DURBIN. 1978. Length and weight relationships of Acartia clausi from Narragansett Bay, R.I. Limnol. Oceanogr. **23**:958-969.

DURRANT, A. 1971. Induction and growth of flax genotrophs. Heredity **27**:277-298.

EDMONDSON, W.T., COMITA, G.W. and G.C. ANDERSON 1962. Reproductive rate of copepods in nature and its relation to phytoplankton populations. Ecology **43**:625-634

ELGMORK, K. and G. HALVORSEN. 1976. Body size of free-living copepods. Oikos **27**:27-33.

EPPLEY, R.W. 1972. Temperature and phytoplankton growth in the sea. Fishery Bull. **70**:1063-1085.

FEULGEN, R. and H. ROSSENBECK. 1924. Microchemical test for nucleic acid of the thymonucleic acid type and the selective staining of cell nuclei in microscopic preparations. Z. Physiol. Chem. **135**:203-248.

FONTAINE, M. 1955. The planktonic copepods (Calanoida, Cyclopoida, Monstrilloida) of Ungava Bay, with special reference to the biology of Pseudocalanus minutus and Calanus finmarchicus. J.Fish.Res.Bd.Can. **12**:858-898.

FORAN, J.A. 1986. A comparison of the life history features of a temperate and a subtropical Daphnia species. Oikos **46**:185-193.

FRANSZ, H.G. and S. DIEL. 1985. Secondary production by Calanus finmarchicus (Gunnerus) (Copepoda:Calanoidea) in a transitional system of the Fladen Ground Area (Northern North Sea) during spring 1983. **In** Proc. 19th **Symp.European Mar.Biol.**, pp.123-133. E. Gibbs (ed.) Cambridge University Press.

FROST, B.W. 1974. Calanus marshallae, a new species of calanoid copepod closely allied to the sibling species C.finmarchicus and C.glacialis. Mar.Biol. **26**:77-99,

FROST, B.W. 1985. Food limitation of the planktonic marine copepods Calanus pacificus and Pseudocalanus sp. in a temperate fjord. Arch.Hydrobiol.Beih.Ergebn.Limnol. **21**:1-13.

FROST, B.W. 1989. A taxonomy of the marine calanoid copepod genus Pseudocalanus. Can.J.Zool. **67**:525-551.

GASTON,K.J. 1988. The intrinsic rates of increase of insects of different sizes. Ecol.Entomol. **14**:399-409.

GELLER, W. and H. MÜLLER. 1985. Seasonal variability in the relationship between body length and individual dry weight as related to food abundance and clutch size in two coexisting Daphnia species. J.Plankton. Res. **7**:1-18.

GATTEN, R.R., CORNER, E.D.S., KILVINGTON, C.C. and J.R. SARGENT. 1979. A seasonal survey of the lipids in Calnus helgolandicus (Claus) from the English Channel. **In**: E.Naylor and R.G. Hartnoll (eds.) **Cyclic phenomena in Marine Plants and Ainmals**. Pergamon Press, Oxford.

GATTEN, R.R., SARGENT, J.R., FORSBERG, T.E.V., O'HARA, S.C.M. and E.D.S. CORNER. 1980. On the nutrition and metabolism of zooplankton. XIV. Utilization of lipid by Calanus helgolandicus during maturation and reproduction. J.mar.biol.Ass. U.K. **60**:391-399.

GOULD, S.J. 1966. Allometry and size in ontogeny and phylogeny. Biol.Rev.Cambridge Phil.Soc. **41**:587-640.

GRAINGER, E.H. 1959. The annual oceanographic cycle at Igloolik in the Canadian arctic. 1. The zooplankton and physical and chemical observations. J.Fish.Res.Bd.Can. **16**:453-501.

GRAINGER, E.H. 1961. The copepods Calanus glacialis Jaschnov and Calanus finmarchicus (Gunnerus) in Canadian Arctic-Subarctic Waters. J.Fish.Res.Bd. **18**:663-678

GREEZE, V.N. 1978. Production in animal populations. pp.89-114. In: O.Kinne (ed.). **Marine Ecology**. vol 4. John Wiley & Sons.

GRIGG, H., HOLMES, L.J. and S.J. BARDWELL. 1985. Seasonal observations on the biometry and development in copepodite stage CV of Calanus finmarchicus from the Firth of Clyde. Mar.Biol. **88**:73-83.

GRIME, J.P. 1983. Prediction of weed and crop response to climate based upon measurements of DNA content. **In: Aspects of Applied Biology**, Volume 4. Influence of environmental factors on herbicide performance and crop and weed biology. The Association of Applied Biologist, National Vegetable Research Station, Wellesbourne, Warwick.

GRIME, J.P. and M.A. MOWFORTH. 1982. Variation in genome size and ecological interpretation. Nature, London **299**:151-153.

GUILLARD, R.R.L. and J.H. RYTHER. 1962. Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt and Denotula confervacea (Cleve) Gran. Can J. Microbiol. **8**:229-239.

HALDANE,J.B.S. 1928. On being the right size. In: **Possible Worlds**. Harper, N.Y.

HARRIS, J.R.W. 1983. The development and growth of Calanus copepodites. Limnol.Oceanogr. **28**:142-147.

HART, R.C. and I.A. McLAREN. 1978. Temperature acclimation and other influeneces on embryonic duration in the copepod Pseudocalanus sp. Mar. Biol. **45**:23-30.

HEIP, C. 1974. A comparison between models describing the influence of temperature on the development rate of copepods. Biol. Jaarb. **42**:121-125.

HEINLE, D.R. 1969. Temperature and zooplankton. Chesapeake Sci. **10**:186-209.

HEINLE, D.R. and D.A. FLEMER. 1975. Carbon requirements of a population of the estuarine copepod Eurytemora affinis. Mar.Biol. **31**:235-247.

HERON, G.A. 1964. Seven species of Eurytemora (Copepoda) from Northwestern North America. Crustaceana **7**:199-211.

KINEGARDNER, R. 1976. Evolution in viruses. **In**: Ayala, F.J. (ed). **Molecular evolution**, Sinauer, Sunderland.

HIRCHE, H.J. and R.N. BOHRER. 1987. Reproduction of the arctic copepod Calanus glacialis in the Fram Strait. Mar.Biol. **94**:11-17.

HIROTA, J. 1972. Laboratory culture and metabolism of the planktonic ctenophore, Pleurobrachia bachei A. Agassiz. **In**: A. Takenouchi et al. (eds.), **Biological Oceanography of the Northern North Pacific Ocean**. Idemitsu-Shoten, Tokyo, Japan.

HUMPHREYS,F.W. 1979. Production and respiration in animal populations. J.Animal.Ecol. **48**:427-453.

HUNTLEY, M. and C. BOYD. 1984. Food-limited growth of marine zooplankton. Am.Nat. **124**:455-478

HUNTLEY, M., CIMINIELLO, P. and M.D.G. LOPEZ. 1987. The importance of food quality in determining development and survival of Calanus pacificus (Copepoda:Calanoida). Mar.Biol. **95**:103-113.

HUXLEY,J.S. 1932. **Problems of relative growth**. London: Methuen.

HYMAN, L.H. 1951. The invertebrates: Acanthocephala, Aschelminthes, and Entoprocta. The pseudocoelomate bilateria. New York; McGraw-Hill Book Co.

IKEDA, T. 1970. Relationship between respiration rate and body size in marine plankton animals as a function of the temperature of habitat. Bull.Fac.Fish., Hokkaido Univ., **21**:91-112.

IRWIN, B., ANNING, J., CAVERHILL, C., ESCRIBANO, R. and T. PLATT. 1988. Carbon and oxygen primary production in Bedford Basin from January to April 1986. Can.Data Rep.Fish.Aquat.Sci.No. **719**

IRWIN, B., ANNING, J., CAVERHILL, C., ESCRIBANO, R. and T. PLATT. 1989. Carbon and oxygen primary production in Bedford Basin from July to December 1986. Can.Data Rep.Fish.Aquat.Sci. No. **720**.

JASCHNOV, V.A. 1955. Morphology, distribution and systematics of Calanus finmarchicus. Zool. Zhurnal. **34**:1210-1223.

JOHN, B. and G. MIKLOS. 1988. **The Eukaryote Genome in Development and Evolution**. Allen & Unwin Inc. Winchester, MA. USA.

JOHNSON, J.K. 1981. Population dynamics and cohort persistence of Acartia californiensis (Copepoda:Calanoidea) in Yaquiner Bay, Oregon. Ph.D. Thesis. Oregon State University.

JOHNSON, M.W. 1966. The nauplius larvae of Eurytemora herdmani Thompson & Scott 1897 (Copepoda, Calanoidea). Crustaceana **11**:307-313.

KANE, J. 1982. Effects of season and location on the relationship between zooplankton displacement volume and dry weight in the Northwest Atlantic. Fish.Bull. **80**:631-642.

KANKAALA, P. and S. JOHANSSON. 1986. The influence of individual variation on length-biomass regressions in three crustacean zooplankton species. J.Plankton.Res. **8**:1027-1038.

KATTNER, G. and M. KRAUSE. 1987. Changes in lipids during the development of Calanus finmarchicus s.L. from copepodid I to adult. Mar.Biol. **96**:511-518.

KATONA, S.K. 1970. Growth characteristics of the copepods Eurytemora affinis and E. herdmani in laboratory cultures. Helgoländer wiss. Meeresunters. **20**:373-384.

KERMACK, K.A. and J.B.S. HALDANE. 1950. Organic correlation and allometry. Biometrika 37:30-41.

KIMMERER, W.J. 1983. Direct measurements of the production: biomass ratio of the subtropical Calanoid Copepod Aerocalanus inermis. J. Plankton Res. **5**:1-14.

KIMMERER. W.J. 1987. The theory of secondary production calculations for continuously reproducing populations. Limnol.Oceanogr. **32**:1-13.

KLEIN BRETELER, W.C.M. and S.R. GONZALEZ. 1982. Influence of cultivation and food concentration on body length of calanoid copepods. Mar.Biol. **71**:157-161.

KLEIN BRETELER, W.C.M. and S.R. GONZALEZ. 1986. Culture and development of Temora longicornis (Copepoda:Calanoida) at different conditions of food and temperature. Syllogeus **58**:71-84.

KLEIN BRETELER, W.C.M. and S.R. GONZALEZ. 1988. Influence of temperature and food concentration on body size, weight and lipid content of two calanoid copepod species. Hydrobiologia **167/168**:201-210.

KLEIN BRETELER, W.C.M., SCHOGT, N. AND S.R. GONZALEZ. 1990. On the role of food quality in grazing and development of life stages and genetic change of body size during cultivation of pelagic copepods. J.Exp.Mar.Biol.Ecol. **136**:177-189.

LaBERGE, E. 1980. Life cycle and seasonal accumulation of lipids in a population of the copepod Pseudocalanus. PhD. thesis, Dalhousie University, Halifax, N.S.

LAIRD, C.D. 1973. DNA of Drosophila chromosomes. Ann.Rev.Genet. **7**:177-204.

LAMPERT, W. 1977. Studies on the carbon balance of Daphnia pulex as related to environmental contitions. I, II. Arch. Hydrobiol. Suppl. **48**:287-335.

LANDRY, M.R. 1975. Seasonal temperature effects and predicting development rates of marine copepod eggs. Limnol.Oceanogr. **20**:434-440.

LANDRY, M.R. 1983. The development of marine calanoid copepods with comments on the isochronal rule. Limnol.Oceanogr. **28**:614-624.

LARSON, A. 1984. Neontological inferences of evolutionary pattern and process in the salamander family Plethodontidae. Evol.Biol. **17**:119-217.

LECREN, E.D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (Perca fluviatis). J.Anim.Ecol. **20**:201-219.

LEE, R.F., NEVENZEL, J.C. and G.-A. PAFFENHÖFER. 1971. Importance of wax esters and other lipids in the marine food chain.: phytoplankton and copepods. Mar.Biol. **9**:99-108.

LEE, R.F., NEVENZEL, J.C. and G.-A. PAFFENHÖFER. 1972. The presence of wax esters in marine planktonic copepods. Naturwissenschaften **59**:406-411.

LEE, R.F., NEVENZEL, J.C. and A.G. LEWIS. 1974. Lipid changes during life cycle of marine copepods, Euchaeta japonica Marukawa. Lipids **9**:891-898.

LEVINS, R. 1966. The strategy of model building in population biology. Amer. Scient. **54**:421-431.

LOCK, A.R. and I.A. McLAREN. 1970. The effect of varying and constant temperatures on size of a marine copepod. Limnol.Oceanogr. **15**:638-640.

LONGHURST, A.R. 1986. Instar increments in copepod growth. Can.J.Fish.Aquat.Sci. **43**:1671-1674.

MacARTHUR, R.H. and E.O. WILSON. 1967. **The theory of island biogeography**. Princeton University Press, Princeton, N.J.

MARCUS, N.H. and P. ALATALO. 1989. Conditions for rearing Calanus finmarchicus (Gunnerus, 1770) (Copepoda, Calanoida) through multiple generations in the laboratory. Crustaceana **57**:101-103.

MARSHALL, S.M. and A.P. ORR. 1955. **The Biology of a Marine Copepod Calanus finmarchicus (Gunnerus)**. Oliver & Boyd, Edinburgh.

MARSHALL, S.M., NICHOLLS, A.G. and A.P. ORR. 1934. On the biology of Calanus finmarchicus. V: seasonal distribution, size, weight and chemical composition in Loch Striven in 1933 and their relation to the phytoplankton. J.mar.biol.Ass. U.K. **19**:793-828.

MASAKI, S. 1978. Seasonal and latitudinal adaptations in the life cycles of crickets, pp. 72-100. In **Evolution of insect migration and diapause**, E.Dingle (ed.). Springer-Verlag, N.Y.

MAYR, E. 1956. Geographical character gradients and climatic adaptation. Evolution **10**:105-108.

McCLATCHIE, S. 1987. Experimental test of an allometric method for estimating potential copepod production. Mar.Biol. **94**:597-603.

McLAREN, I.A. 1963. Effects of temperature on growth of zooplankton and the adaptive value of vertical migration. J.Fish.Res.Bd.Can. **20**:685-727.

McLAREN, I.A. 1965. Some relationships between temperature and body size, development rate and fecundity, of the copepod Pseudocalanus. Limnol.Oceanogr. **10**:528-538.

McLAREN, I.A. 1966. Predicting development rates of copepod eggs. Biol.Bull. **131**:457-469.

McLAREN, I.A. 1969. Population and production ecology of zooplankton in Ogac lake, a landlocked fjord in Baffin Island. J.Fish.Res.Bd.Can. **26**:1485-1559.

McLAREN, I.A. 1976. Inheritance of demographic and production parameters in the marine copepod Eurytemora herdmani. Biol. Bull. **151**:200-213.

McLAREN, I.A. 1978. Generation lengths of some temperate marine copepods: estimation, prediction, and implications. J.Fish.Res.Bd.Can. **35**:1330-1342.

McLAREN, I.A. 1986. Is "structural" growth of Calanus potentially exponential?. Limnol.Oceanogr. **31**:1342-1346.

McLAREN, I.A. and C.J. CORKETT. 1978. Unusual genetic variation in body size, development times, oil storage and survivorship in the marine copepod Pseudocalanus. Biol.Bull. **155**:347-359.

McLAREN, I.A. and C.J. CORKETT. 1981. Temperature-dependent growth and production by a marine copepod. Can.J.Fish. Aquat.Sci. **38**:77-83.

McLAREN, I.A. and C.J. CORKETT. 1986. Life cycles and production of two copepods on the Scotian Shelf, eastern Canada. Syllogeus No.58 362-368.

McLAREN, I.A. and D.J. MARCOGLIESE. 1983. Similar nucleus numbers among cepods. Can.J.Zool. **61**:721-724.

McLAREN, I.A., CORKETT, C.J. and E.J. ZILLIOUX. 1969. Temperature adaptations of copepod eggs from the arctic to the tropics. Biol.Bull. **137**:486-493.

MCLAREN, I.A., SEVIGNY, J.-M. and C.J. CORKETT. 1988. Body sizes, development rates, and genome sizes among Calanus species. Hydrobiologia **167/168**:275-284.

McLAREN, I.A., LABERGE, E., CORKETT, C.J. and J.-M. SEVIGNY. 1989a. Life cycles of four species of Pseudocalanus in Nova Scotia. Can.J.Zool. **67**:552-558.

McLAREN, I.A., SEVIGNY, J.-M. and C.J. CORKETT. 1989b. Temperature-dependent development in Pseudocalanus species. Can.J.Zool. **67**:559-564.

McLAREN, I.A., SEVIGNY, J.-M. and B.W. FROST. 1989c. Evolutionary significance of genome sizes in the copepod genus Pseudocalanus. Can.J.Zool. **67**:565-569.

McLAREN, I.A., TREMBLAY, M.J., CORKETT, C.J. and J.C. ROFF. 1989d. Copepod production on the Scotian Shelf based on life-history analyses and laboratory rearings. Can.J.Fish.Aquat.Sci. **46**:560-583.

MEDNIKOV, B.M. 1961. On the sex ratio in deep water Calanoida. Crustaceana **3**:105-109.

MIDDEBROOK, K. and J.C. ROFF. 1986. Comparison of methods for estimating annual productivity of the copepods Acardia hudsonica and Eurytemora herdmani in Passnaquoddy Bay, New Brunswick. Can.J.Fish.Aquat.Sci. **43**:656-664.

MILLER, C.B., JOHNSON, J.K. and D.R. HEINLE. 1977. Growth rules in the marine copepod genus Acartia. Limnol.Oceanogr. **22**:326-335.

MILLS, E.L. and R.O. FOURNIER. 1979. Fish production and the marine ecosystems of the Scotian Shelf, eastern Canada. Mar.Biol. **54**:101-108.

MIRSKY, A.E. and H. RIS. 1949. Variable and constant components of chromosomes. Nature, London **163**:666-667.

MIRSKY, A.E. and H. RIS. 1951. The DNA content of animal cells and its evolutionary significance. J.Gen.Physiol. **34**:451-462.

MOWFORTH, M.A. and J.P GRIME. 1989. Intra-population variation in nuclear DNA amount, cell size and growth rate in Poa annua L. Funct.Ecol. **3**:289-295.

MULLIN, M.M. and E.R. BROOKS. 1967. Laboratory culture, growth rate, and feeding behavior of a planktonic marine copepod. Limnol.Oceanogr. **12**:657-666.

MULLIN, M.M. and E.R. BROOKS. 1970. Growth and metabolism of two planktonic, marine copepods as influenced by temperature and type of food. In. **Marine Food Chains**, J.H. Steele (ed.), University of California Press, Berkeley.

MYERS, R.A. and J.A. RUNGE. 1983. predictions of seasonal natural mortality rates in a copepod population using life-history theory. Mar.Ecol.Prog.Ser. **11**:189-194.

NEVENZEL, J.C. 1970. Occurrence, function and biosynthesis of wax esters in marine organisms. Lipids **5**:308-319.

NIJHOUT,H.F. 1975. A threshold size for metamorphosis in the tobacco hornworm, Manduca sexta (L.). Biol.Bull.Mar.Bio.Lab., Woods Hole **149**:214-225.

O'BRIEN, W.J. 1979. The predator-prey interaction of planktivorous fish and zooplankton. Am.Sci. **67**:572-581.

OELDORF, E., NISHIOKA, M. and K. BACHMANN. 1978. Nuclear DNA amounts and developmental rate in holarctic anura. Z.Zool.Syst.Evolut.-forsch **16**:216-224.

OHNO, S. 1972. So much 'junk' DNA in our genome. **In**: Smith, H.H. (ed). **Evolution of Genetic Systems**, Brookhaven Symposium in Biology. 23:366-370.

OLMO, E. 1983. Nucleotype and cell size in vertebrates: a review. Basic and Applied Histochemistry **27**:227-255.

OLSON, C.S. and J.S. CLEGG. 1978. Cell division during the development of Artemia salina. Wilhem Roux's Arch. **184**:1-13.

OMORI, M. and T. IKEDA. 1984. **Methods in Marine Zooplankton Ecology**. John Wiley & Sons, Inc.,USA.

OSTERGREN, G. 1945. Parasitic nature of extra fragment chromosomes. Bot.Notiser **2**:157-163.

PAFFENHOFER, G.A. 1970. Cultivation of Calanus helgolandicus under controlled conditions. Helgol.Wiss.Meeresunters. **20**:346-359.

PAFFENHOFER, G.A. 1976. Feeding, growth, and food conversion of the marine planktonic copepod Calanus helgolandicus. Limnol.Oceanogr. **21**:39-50.

PAINE, R. 1964. Ash and caloric determinations of sponge and opisthobranch tissues. Ecology **45**:384-387.

PETERS, R.H. 1983. Ecological implications of body size. Cambridge University Press, Cambridge.

PETERSON, W.T. 1986. Development, growth and survivorship of the copepod Calanus marshallae in the laboratory. Mar.Ecol.Prog.Ser. **29**:61-72.

PETERSON, W.T. and S.J. PAINTING. 1990. Developmental rates of the copepod Calanus australis and Calanoides carinatus in the laboratory, with discussion of methods used for calculation of development time. J.Plankton Res. **12**:283-293.

PIANKA, E.R. 1970. On r- and K-selection. Amer. Nat. **104**:592-597.

RAINA, S.N. and H. REES. 1983. DNA variation between and within chromosome complements of Vicia species. Heredity **51**:335-346.

RASH, E.M.; H.J. BURR and R.W. RASH. 1971. The DNA content of sperm of Drosophila melanogaster. Chromosoma (Berlin) **33**:1-18.

REES, H. 1972. DNA in higher plants. **In**: Smith, H.H. (ed.). **Evolution of Genetic Systems**, Brook haven Symposium in Biology **23**:394-418.

RHEINSMITH, E.L , HINEGARDNER, R. and K. BACHMANN. 1974. Nuclear DNA amounts in Crustacea. Comp.Biochm.Physiol. **48B**:343-348.

RILEY, G.A. 1947. A theoretical analysis of the zooplankton population on Georges Bank. J.Mar.Res. **6**:104-113.

ROBINS, J.H. 1978. An investigation of the Schiff reaction and variations in the DNA content of the marine copepod Pseudocalanus. M.Sc. thesis, Dalhousie University, Halifax, N.S.

ROBINS, J.H and I.A. McLAREN. 1982. Unusual variations in nuclear DNA contents in the marine copepod Pseudocalanus. Can.J.Genet.Cytol. **24**:529-540.

ROFF, D.A. 1981. On being the right size. Amer. Nat. **118**:405-422.

ROFF, D.A. 1986. Predicting body size with life history models. BioScience **36**:316-323.

RUTHMANN, A. 1970. **Methods in cell research**. G. Bell, London.

SARGENT, J.R., GATTEN, R.R., CORNER, E.D.S and C.C. KILVINGTON. 1977. On the nutrition and metabolism of zooplankton. J.mar.biol.U.K. **57**:525-533.

SEKIGUCHI, H., McLAREN, I.A. and C.J. CORKETT. 1980. Relationship between growth rate and egg production in the copepod Acartia clausi hudsonica. Mar. Biol. **58**: 133-138.

SESSIONS, S.K. and A. LARSON. 1987. Developmental correlates of genome size in plethodontid salamanders and their implications for genome evolution. Evolution **4**:1239-1251.

SEVIGNY, J.-M. and I.A. McLAREN. 1988. Protein polymorphism in six species of the genus Calanus. Hydrobiologia **167/168**:267-274.

SHRINER, R.L., FUSON, R.C. and D.Y. CURTIN. 1964. The systematic identification of organic compounds. John Wiley & Sons. NY.

SHUTER, B.J., THOMAS, J.E., TAYLOR, W.D. and A.M. ZIMMERMAN. 1983. Phenotypic correlates of genomic DNA content in unicellular eukariotes and other cells. Amer. Nat. **122**:26-44.

SKJOLDAL, H.R. and U. BÅMSTEDT. 1976. Studies on the deep-water pelagic community of Korsfjorden, Western Norway. Adenoside phosphates and nucleic acids in Meganyctiphanes norvegica (Euphasiacea) in relation to the life cycle. Sarsia **61**:1-14.

SMITH, R.J. 1980 Rethinking allometry. J.Theor.Biol. **87**:97-111.

SMITH, J.B. and M.D. BENNETT. 1975. DNA variation in the genus Ranunculus. Heredity **35**:231-239.

SOKAL, R.R. and F.J. ROHLF. 1981. **Biometry**. Second edition, W.H. Freeman, San Francisco, Cal. USA.

STEELE, J.H. 1974. **Structure of Marine Ecosystems**. Harvard University Press, Cambridge, MA.

STEELE, J.H. and E.W. HENDERSON. 1985. Modeling long-term fluctuations in fish stocks. Science **224**:985-986.

SWIFT, H. 1950. The constancy of deoxyribose nucleic acid in plant nuclei. Proc.Natl.Acad.Sci.USA. **36**:643-654.

SZARSKI, H. 1976. Cell size and nuclear DNA content in vertebrates. Int.Rev.Cytol. **44**:93-111.

TAYLOR, F. 1981. Ecology and evolution of physiological time in insects. Amer.Nat. **117**:1-23.

TEPPER, B.W. and B.P. BRADLEY. 1989. Temporal changes in a natural population of copepods. Biol.Bull.(Woods Hole, Mass.) **176**:32-40.

TESSIER, A.J., HENRY, L.L., GOULDEN, C.E and M.W. DURAND. 1983. Starvation in Daphnia: energy reserves and reproductive allocations. Limnol.Oceanogr. **28**:667-676.

THOMAS, C.A. 1971. The genetic organization of chromosomes. Ann.Rev.Genet. **5**:237-256.

THOMPSON, B.M. 1976. The biology of Pseudocalanus elongatus (Boeck). PhD.Thesis, University of East Anglia, Norwich.

TOBLER, H. 1986.The differentiation of germ and somatic cell lines in nematodes. **In**: W. Hennig (ed.) **Results and Problems in Cell Differentiation**, Vol.13. Springler, Berlin Heidelberg, NY.

TRANTER, D.J. 1976. Herbivore production. pp.186-224. In: D.H. Cushing and J.J. Walsh (ed.). **The Ecology of the seas**. Blackwell Scientific Publications, Oxford.

TREMBLAY, M.J. and J.C. ROFF. 1983. Production estimates for Scotian Shelf copepods based on mass specific P/B ratios. Can. J. Fish Aquat. Sci. **40**:749-753.

UYE, S. 1982. Length-weight relationships of important zooplankton from the inland sea of Japan. J.Oceanogr.Soc.Japan. **38**:149-158.

UYE, S. 1988. Temperature-dependent development and growth of Calanus sinicus (Copepoda:Calanoida) in the laboratory. Hydrobiologia **167/168**: 285-293.

VIDAL, J. 1980a. Physioecology of zooplankton. I. Effects of phytoplankton concentration, temperature, and body size on the growth rate of Calanus pacificus and Pseudocalanus sp. Mar.Biol. **56**:111-134.

VIDAL, J. 1980b. Physioecology of zooplankton. II. Effects of phytoplankton concentration, temperature, and body size on the development and molting rates of Calanus pacificus and Pseudocalanus sp. Mar.Biol. **56**:135-146.

WARREN, G.J., EVANS, M.S., JUDE, D.J. and J.C. AYERS. 1986. Seasonal variations in copepod size: effects of temperature, food abundance, and vertebrate predation. J.Plankton Res. **8**:841-853.

WATERS, T.F. 1977. Secondary production in inland waters. Adv. Ecol. Res. **10**:91-165.

WHITEHOUSE, J.W. and B.G. LEWIS. 1973. The effect of diet and density on development, size and egg production in Cyclops abyssorum Sars, 1863. (Copepoda, Cyclopoida). Crustaceana **25**:225-236.

WILLIAMS, R. and J.A. LINDLEY. 1980. Plankton of the Fladen Ground during FLEX 76. III. Vertical distribution,

population dynamics and production of Calanus finmarchicus (Crustacea:Copepoda). Mar.Biol. **60**:47-56.

WILSON, C.B. 1932. The copepods of the Woods Hole region, Massachussetts. Bull. U.S. Nat.Mus. **158**:635 p.

WINBERG,G.G. (Ed.). 1971. Methods for the estimation of production of aquatic animals. Academic Press, London.

YENTSCH, C.S. and D.W. MENZEL. 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep-Sea Research **10**:221-234.