

# BODY SIZE REGULATION IN COPEPOD CRUSTACEANS

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## Abstract

Body size affects survival probabilities, reproductive output and individual fitness in many organisms. In freshwater zooplankton, traits ranging from demographic rates to community composition depend on body size, and predation is most often identified as the selection pressure determining body size. We examined the extent to which stage-specific growth trajectories and body sizes of copepod crustaceans are constrained, independent of selection. We used exuviae shed at each molt to quantify the relationship between size at molting and growth during the subsequent instar for two common, herbivorous calanoid copepods, *Boeckella triarticulata* and *Diatomus leptopus*. Individuals of both species were raised under diets of different food quality or quantity, and at different temperatures. Size at molting varied little among individuals of both species, as a consequence of a persistent negative relationship between size at molting and subsequent (absolute) growth increment. Individuals that were small when they molted grew more during the subsequent instar than individuals that were large. This relationship was statistically significant for nearly all instars of both species raised in different food or temperature conditions, and not affected by food quality, food quantity or temperature. Our results indicate that body size is constrained or regulated over much of the copepod life cycle, independent of the effects of environmental conditions (food, temperature, predation).

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## Abstract

Body size affects survival probabilities, reproductive output and individual fitness in many organisms. In freshwater zooplankton, traits ranging from demographic rates to community composition depend on body size, and predation is most often identified as the selection pressure determining body size. We examined the extent to which stage-specific growth trajectories and body sizes of copepod crustaceans are constrained, independent of selection. We used exuviae shed at each molt to quantify the relationship between size at molting and growth during the subsequent instar for two common, herbivorous calanoid copepods, *Boeckella triarticulata* and *Diaptomus leptopus*. Individuals of both species were raised under diets of different food quality or quantity, and at different temperatures. Size at molting varied little among individuals of both species, as a consequence of a persistent negative relationship between size at molting and subsequent (absolute) growth increment. Individuals that were small when they molted grew more during the subsequent instar than individuals that were large. This relationship was statistically significant for nearly all instars of both species raised in different food or temperature conditions, and not affected by food quality, food quantity or temperature. Our results indicate that body size is constrained or regulated over much of the copepod life cycle, independent of the effects of environmental conditions (food, temperature, predation).

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## Introduction

Body size affects survival probabilities, reproductive output and individual fitnesses of many organisms. Environmental factors including food quality and quantity, temperature, interspecific interactions and disturbance regimes influence body size, and many studies have focused on identifying the selection (environmental) pressures responsible for the size patterns observed. Ectotherms often are larger when food is abundant or high in quality (Atkinson and Sibly 1997), when temperature is lower (Sibly and Atkinson 1994; Atkinson 1994; Berrigan and Charnov 1994), or when size-selective predators prefer smaller individuals (e.g., Paine 1965; Bertness 1981; Berven and Gill 1983). Size at metamorphosis or maturity also can be affected by both food availability and predation (e.g., Collins 1979; van der Veer and Bergman 1987; Bradshaw and Johnson 1995; Smith and van Buskirk 1995). Selection acting on body size is recognized as the primary factor generating and maintaining observed patterns of variation in body size.

An implicit assumption of these life-history studies is that selection acts on completely flexible growth trajectories, and it is the consequences of body size that have attracted most attention (e.g., Peters 1983; Roff 1992; Stearns 1992). However, additional processes may affect body size. Physiological, developmental or structural constraints may limit maximum, optimum or minimum body sizes, along with variation in size at any particular point during the life cycle (e.g., Wilbur and Collins 1973; Nijhout 1975; Hartnoll and Dalley 1981). For a variety of organisms exhibiting complex life cycles, body size at metamorphosis or maturity depends on both external (environmental) and internal (physiological, structural) constraints, and models predicting either

flexible (e.g., Wilbur and Collins 1973) or fixed (Leips and Travis 1994) body sizes are supported empirically (Reznick 1990; Hensley 1993; Leips and Travis 1994). For example, the timing of amphibian and insect metamorphosis may be fixed by the onset of the endocrine cascade that effects morphological change (e.g., Gilbert et al. 1996). Given this constraint, Hensley (1993) argued for the amphibian *Pseudacris crucifer* that the body size achieved at metamorphosis cannot be optimized by selection because it depends on food encountered once metamorphosis has been triggered. Arthropod body sizes are determined by the growth achieved during discrete developmental stages; and growth achieved per molt, the size at molting, or instar duration reflect structural or physiological constraints as well as direct responses to selection (Higgins and Rankin 1996). With this evidence for body size constraints, full understanding of the ecological or the evolutionary significance of body size in any particular species or population requires examination of both the selection pressures acting on body size and the constraints that limit body size.

Constraints on growth or body size, and the interdependence of growth and development, have received far less attention in many organisms, including the crustaceans we examine here, than the consequences of body size. Among crustaceans, larvae of the caridean shrimp *Palaemon elegans* (Hartnoll and Dalley 1981) and the barnacle *Balanus eburneus* (West and Costlow 1987) accomplish size regulation by a negative feedback between molt increment and premolt size: individuals that are large at a particular larval molt accomplish a small growth increment during the next molt and vice versa. This relationship suggests the ability to modify growth rates based on previous growth history such that body size at molting varies little among individuals in any particular stage.

Since Brooks and Dodson (1965) proposed the Size-Efficiency Hypothesis, body size has played a major role in studies of the ecology and evolution of freshwater zooplankton. However, our understanding of the factors affecting zooplankton body size has been limited almost exclusively to environmental (selective) factors, and has focused on predation as the principal of these. Food and temperature have clear immediate effects on body size, but predation pressures are usually considered the ultimate causes of size-specific life history transitions (Lynch 1980; Hairston et al. 1983; Riessen et al. 1984; Papinska 1988; Hairston 1987), behaviors (e.g., Zaret and Suffern 1976; Neill 1990), morphology (Dodson 1974, 1989), and community composition (Carpenter et al. 1985; DeMelo et al. 1992; Strong 1992). This limitation is not purely conceptual; logistically, it has been difficult if not impossible to measure individual size nondestructively, particularly of larval copepod (naupliar) stages. This difficulty has severely limited our understanding of the range of factors affecting individual growth and body size (Thompson 1982; Hart 1990; van den Bosch and Gabriel 1994), including factors that might constrain body size.

Recent use of exuviae shed by crustacean zooplankton at each molt to measure individual size at successive stages and individual growth trajectories (Twombly and Burns 1996) provides an opportunity to examine growth dynamics and the factors affecting body size in more detail. Analyses of exuviae shed over the entire life cycle of a large number of individuals of the copepod *Boeckella triarticulata* revealed limited size variation at each molt (Twombly and Burns 1996a). These results suggested that copepod body size is constrained over some or much of the life cycle (see also Twombly 1993, 1995). Our purpose in the studies reported here was to identify both the

potential mechanism of size constraint as well as the instars during which it acts for two freshwater calanoid copepod species, *Boeckella triarticulata* Thomson and *Diaptomus leptopus* Forbes. Growth dynamics throughout the life cycle, including the relationships between molt increment and size at molting or instar duration, were quantified, and the effects of diet and temperature on these relationships were measured.

## Materials and Methods

Because ovigerous females of each species were collected from different field locations at different times, and their offspring were raised under different conditions (light, temperature, food), the experimental protocol used is described separately for each species.

*Boeckella triarticulata*: Ovigerous females were collected from Saddle Hill Quarry Pond, approximately 20 km west of Dunedin, New Zealand, isolated in approx. 30 ml spring water, and kept at 15°C and 14:10 light:dark photoperiod until their eggs hatched. Eleven newborn nauplii from each of 5 sibships were reared in two different diets: 1) *Cryptomonas* sp. at a concentration of 0.4 mg dry weight (DW) L<sup>-1</sup> (henceforth referred to as CR), and a combination of *Cryptomonas* sp. and *Anabaena flos-aquae* (1:1 by dry weight) at the same concentration (i.e., 0.2 mg DW L<sup>-1</sup> of each species, referred to as C-A). Nauplii were reared individually in plastic vials containing 15 ml of a mixture (1:1) of modified MBL medium (Stemberger 1981) and spring water, at 15°C and 14:10 photoperiod. Food and medium were replaced every second day. When nauplii metamorphosed to the first copepodite stage, food concentrations were increased to 1.2 mg DW L<sup>-1</sup>; food was increased again (to 2.0 mg DW L<sup>-1</sup>) when animals reached maturity. Adult

food concentration was kept above the incipient limiting concentration for *B. triarticulata* (Burns and Xu 1990; Burns and Hegarty, unpublished).

Individuals were examined daily for shed exuviae using a stereomicroscope with darkfield illumination at 15-40X. Exuviae were measured at 100X (N1-CI) or 40X (CII-CV) under a compound microscope using a calibrated ocular micrometer. The lengths of entire naupliar exuviae were measured from the anterior mid-dorsal margin to the base of the caudal spines; copepodite exuviae were measured from the anterior to mid-dorsal posterior margins of the prosome. The developmental stage of each exuvium was verified at measurement.

*Cryptomonas* sp. stock cultures were maintained in modified MBL medium (Stemberger 1981) at 20°C in dim light at 12:12 light:dark cycle; *A. flos-aquae* cultures were grown in WC medium (Guillard and Lorenzen 1972) and kept at ambient temperature and photoperiod. Algal stock concentrations were determined turbidimetrically and translated to DW/L using a previously-determined dry weight regression. Appropriate volumes of each algal stock culture were added to culture medium (modified MBL medium and spring water) to obtain the final food concentration required, depending upon individual developmental stage.

*Diaptomus leptopus*: Ovigerous females were collected from Little Bullhead Pond in Perryville, Rhode Island, in February and May 1998, and kept individually in the laboratory in 12 ml modified MBL medium at near ambient field conditions (for February females, 8°C and 10:14 photoperiod; for June females, 16°C and 14:10 photoperiod) until their eggs hatched. Nauplii from the February females were raised individually in small petrie dishes in 12 ml modified MBL medium at 8°C and 10:14 Photoperiod. Fifteen offspring from each of 5 sibships were reared on a

combined diet (1:1 by carbon content) of *Chlamydomonas reinhardtii* and *Cryptomonas erosa* at two different concentrations,  $0.6 \mu\text{g C ml}^{-1}$  and  $1.2 \mu\text{g C ml}^{-1}$ . Nauplii hatched from field-collected females in May were pooled and 45 individuals were reared individually, on the same combined diet and at the same food concentrations as February offspring, at  $16^\circ\text{C}$  or  $19^\circ\text{C}$  and at 14:10 photoperiod. All individuals were observed daily ( $16^\circ\text{C}$ ,  $19^\circ\text{C}$ ) or every second or third day ( $8^\circ\text{C}$ ) for the presence of exuviae, and both food and medium were changed every second or third day. Exuviae were measured at 50X under a stereomicroscope using a calibrated ocular micrometer. Lengths of naupliar exoskeletons were measured from the anterior mid-dorsal margin to the base of the caudal spines, and entire copepodite exuviae were measured from the anterior mid-dorsal margin to the base of the caudal rami.

Stock cultures of both *C. reinhardtii* and *C. erosa* were maintained in modified MBL medium at  $16\text{-}18^\circ\text{C}$  in dim light at 12:12 light:dark cycle. Algal stock concentrations were determined using a hemacytometer and translated to  $\mu\text{g C ml}^{-1}$  using equations reported by Strathmann (1967). Appropriate volumes of each algal stock culture were added to modified MBL medium to obtain the final food concentrations required.

*Statistical Analyses:* Means, variances, coefficients of variation and other statistical analyses described below were calculated or carried out for 7 - 11 of the possible 13 developmental stages. In both species, the second naupliar (N2) molt was the first one reliably recorded in the experimental chambers (because the first, non-feeding larval instar is completed quickly), with the result that the first molt increment data that could be related to size at molting were for the third naupliar (N3) stage. We measured different exuvium dimensions for *B. triarticulata* nauplii and

copepodites (see above) and therefore were unable to calculate an accurate molt increment for the first copepodite stage (CI). Growth statistics are reported for CII-CVI stages only. This problem was eliminated for *D. leptopus* by measuring the entire length of copepodite exuviae.

Reproductive maturity is achieved at the molt from the CV to the CVI stage, when molting ceases, and a CVI molt increment was determined by measuring length of all adults at death.

We used parametric correlation analysis to examine the relationship between molt increment<sub>x+1</sub> and size at molt<sub>x</sub> (where x=developmental stage or instar) because both variables were measured with error. Analysis of covariance (ANCOVA) was performed to detect differences in this relationship due to diet (food quality or quantity) or temperature (*D. leptopus* only). Spearman rank correlations were used to investigate the relationship between molt increment and instar duration because instar durations were not normally distributed. Females and males were analyzed separately in the penultimate and ultimate instars, as growth trajectories diverged in later copepodite stages and males were usually smaller than females by the fifth copepodite stage. Despite this sexual dimorphism, sexes did not differ statistically in the strength of the correlation between molt increment and size at molting, or in the effects of environmental conditions on this relationship. As a result, sexes are pooled in the results presented below. All statistical tests were run using SAS version 6.08 or 6.12 (SAS Institute 1985).

## Results

**Growth dynamics:** Size at each molt varied little among individuals raised on a given diet; coefficients of variation (C.V.) ranged from 3.5-6% (Figure 1). Absolute molt increments (the

change in size from one molt to the next) increased from N3 to CIV stages in both species. Stage-specific variation in molt increments was much higher (C.V. 20-35%) than that for size at molting and was equal across all stages (Figure 1).

These highly variable molt or growth increments are not due to large differences among individuals in the amount of time spent in any particular stage (instar duration). Instar duration was not correlated with molt increment for either *B. triarticulata* or *D. leptopus* (only one of 34 correlations was significant at  $p < 0.05$ ), so that larger molt increments were not the result of prolonged development. In fact, growth within a stage was often negatively correlated with stage duration.

Correlation analyses revealed highly significant negative relationships between molt increment and size at molting for 60-80% of the developmental stages quantified (Figures 2 and 3). This relationship was negative but not significant ( $p > 0.05$ ) among most older juvenile stages. As a result of individual variation in molt increment, size at each molt varied little. When individuals in successive instars overlapped in size at molting, they achieved different absolute molt increments (see Figure 2, stages N3 and N4; Stages CIII and CIV), suggesting that the relationship between molt increment and size at molting is 'reset' at each instar (West and Costlow 1987). Individuals in the younger stage were large at molting and exhibited relatively small molt increments, while equal-sized individuals in the next instar were relatively small and exhibited large molt increments.

Food quality had small but statistically significant ( $p < 0.05$ ) effects on body sizes achieved in later instars of *B. triarticulata* (Figure 1). Similarly, temperature affected body size of the last

naupliar and all copepodite stages of *D. leptopus* ( $p < 0.05$ ), even though the absolute changes were small and the trends exhibited were opposite those recorded for many ectotherms. In all later copepodite stages of *D. leptopus*, individuals raised at 16°C were larger than those raised at 8°C.

**Effects of diet and temperature on growth dynamics:** Although food quality had a significant effect on body size and absolute molt increment in *B. triarticulata* (Figure 1A; Twombly and Burns 1996), and food quantity caused significant differences in body size of adult *D. leptopus* (Figure 1B, C), neither food quantity (for *D. leptopus*) or quality (for *B. triarticulata*) affected the relationship between molt increment and premolt size in either species, with the exception of the CV size-CVI increment relationship in male *B. triarticulata* (Table 1A, B nonsignificant size\*diet interaction terms). Similarly, temperature did not alter the molt increment-size at molting relationship in *D. leptopus* (Table 1C), even though temperature had a statistically significant effect on overall body size. Constancy of the stage-specific relationship between molt increment and size at molting, regardless of these experimental conditions, lends further support to a general mechanism for size regulation. Both *B. triarticulata* and *D. leptopus* retain the ability to adjust growth rates based on body size, and thus to minimize size variation, over most developmental stages.

## **Discussion**

### **Body size regulation**

Body size achieved at each of several successive molts in two common calanoid copepods varied little among individuals raised in controlled laboratory conditions. Age at molting and molt

increment were more variable than body size among these individuals, and intra-instar variation in molt increment was significantly related to size at molting over larval and the first three juvenile stages. Growth achieved within a particular instar (the molt increment) varied negatively with body size of individuals entering that instar; these data demonstrate clear constraints on body size in two copepod crustaceans. Less complete data also show the existence of the same negative relationship for naupliar and copepodite stages of two other copepods, *Diaptomus sanguineus* and *Acartia tonsa* (Twombly, unpublished). The negative relationship was weaker in older stages, perhaps because size regulation weakens when morphogenesis ceases in copepods.

Size regulation within an instar via a negative feedback mechanism has been reported in two other crustaceans, the barnacle *Balanus eburneus* (West and Costlow 1987) and the decapod *Palaemon elegans* (Hartnoll and Dalley 1981). Barnacle molt increments were negatively related to size at molting over all larval stages up to - but not including - the cyprid stage, at which point size regulation ceased. In contrast, size appeared regulated only at specific stages (metamorphosis and maturity) during the life cycle in *P. elegans*, and varied considerably in other instars (Hartnoll and Dalley 1981). West and Costlow (1987) concluded that the negative feedback between molt increment and size at molting in *B. eburneus* was reset at each molt, and the same appears to be true for both copepod species described here. For example, when successive stages of *B. triarticulata* overlapped in size at molting, the earlier instar exhibited a smaller molt increment than the later one did (see Figure 2).

Our experimental conditions - changes in food quality, food quantity, and temperature - effected only small changes in copepod body sizes over the life cycle, and these changes were

statistically significant only in older copepodite stages (including the adult). While surprising, these results are corroborated by other studies showing only small effects of food or temperature on zooplankton body size (measured as length or dry weight) and significant effects primarily on the oldest stages (e.g., Vidal 1980; Tessier and Consolatti 1991; Ban 1994), and they provide further evidence for constraints on body size. Similarly, our experimental conditions had no detectable effect on the negative relationship between molt increment and size at molting. The potential feedback exhibited appears to be independent of immediate environmental conditions. It also appears to be unrelated to at least one major exoskeletal constraint: if maximum growth per ecdysis is limited by how tightly new cuticle can be packed within the existing exoskeleton (Cheng and Chang 1994; Higgins and Rankin 1996), then larger individuals within an instar should exhibit larger molt increments.

The constraints on growth and body size that we document here suggest that body size may not be as important a fitness trait in copepods as it is in other organisms. Although larger body size results in increased reproductive output in many invertebrates (including other crustaceans), the effect of adult body size on reproductive output in several copepods is unclear (Twombly et al. 1998, unpublished data; see also Peterson et al. 1991; Hart et al. 1995). Individual lifetime fitness depends on body size in *B. triarticulata*, but not in *D. leptopus* (unpublished data). Reproductive output or effort may relate more directly to individual energy storage than to body length for invertebrates that broadcast their gametes or carry their eggs externally (e.g., Thompson 1982). In several copepods, size at specific life cycle transitions (metamorphosis, maturity) varies less than age at these transitions (Twombly 1995, 1996), and

there is no apparent relationship between instar duration and size at molting (this study).

Individuals cannot grow larger by remaining within a particular stage for a longer period of time.

Finally, lower temperatures did not result in larger body sizes (at maturity, for example) in *D.*

*leptopus*, in contrast to the response documented in many other ectotherms (Berrigan and

Charnov 1994). These observations indicate that body size has different life-history consequences

in copepods than those typically assumed.

### **Interdependence of growth and development (morphogenesis)**

Growth and development are often assumed to be interdependent processes, largely because age and size at particular life-history transitions (such as metamorphosis or maturity) are positively correlated (e.g., Collins 1979; Smith-Gill and Berven 1979; Chambers et al. 1988; Mitchell-Olds 1996). Alternatively, these correlations may represent similar but independent responses of growth and development to the same ecological conditions (Leips and Travis 1994; Blanckenhorn 1998), with the result that statistical correlations between age and size at any particular life cycle transition say little about the degree to which growth and development are genetically coupled. Copepod age and size at metamorphosis or maturity are often negatively correlated (Twombly 1996; Twombly and Burns 1996); and in both *B. triarticulata* and *D. leptopus* (this study), molt increment (or growth achieved during an instar) was not correlated (significantly) with instar duration.

Copepod crustaceans have a fixed number of instars (Elgmork and Langeland 1970; Czaika 1982), and morphogenesis (the addition of segments, appendages and setae) must be

closely coupled with molting although the relationship between these two processes has not yet been thoroughly examined. West and Costlow (1988) proposed that growth and morphogenesis were coupled in *Balanus eburneus* in order to maximize the probability that morphogenesis, once initiated, would be completed by the end of a particular instar. They also considered that the coupling of growth and development resulted from an equal allocation of energy to each process. In contrast, the uncoupling of growth and morphogenesis or morphogenesis and molting in decapod crustaceans has been well documented (Gore 1985). Supernumerary instars are often associated with delayed or extended development in particular environmental conditions and this flexible number of instars indicates that variable morphogenesis can be achieved with each molt (Knowlton 1975; Gore 1985; McConaugha 1985). Decapods appear to allocate energy first to maintenance, second to molting and finally to morphogenesis (McConaugha 1985).

The constraints on growth and body size described here for two copepod crustaceans may limit their ability to respond phenotypically (via plasticity) to temporal and spatial variation in habitat quality and may therefore limit their ecological success (Higgins and Rankin 1996, Blanckenhorn 1998). Other aspects of copepod life-histories are similarly constrained: Hairston and Bohonak (1998) characterized freshwater, free-living copepods as iteroparous annuals with resting stages. Despite these constraints, however, copepods are successful in spatially and temporally variable inland habitats. A more complete understanding of the evolution of copepod life-histories, and of the life-history variation maintained within the constraints mentioned above, requires quantification of the genetic bases for the various constraints that exist, their phenotypic manifestation, and both the short- (ecological) and long-term (evolutionary) consequences of life-

history constraints in this highly successful group.

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Table 1. Analysis of covariance for the effect of A. diet on the molt increment-size at molting relationship for *Boeckella triarticulata*, n=94 for all relationships; B. diet (food concentration); and C. temperature on the relationship between molt increment and size at molting for *Diaptomus leptopus*. F value, level of statistical significance (p) and sample size (n) given for each stage

A.	Source	F value	p
N2 size-N3 increment	Size	25.35	0.0001
	Diet	0.48	0.4888
	Diet*Size	0.42	0.517
N3 size-N4 increment	Size	21.08	0.0001
	Diet	1.07	0.303
	Size*Diet	1.16	0.284
N4 size-N5 increment	Size	88.98	0.0001
	Diet	0.67	0.416
	Size*Diet	0.59	0.445
N5 size-N6 increment	Size	39.51	0.0001
	Diet	0.03	0.866
	Size*Diet	0.01	0.929
N6 size-CI increment	Size	56.51	0.0001
	Diet	0.07	0.794
	Size*Diet	0.04	0.851
CI size-CII increment	Size	24.26	0.0001

	Diet	0.11	0.735
	Size*Diet	0.05	0.820
CII size-CIII increment	Size	2.62	0.108
	Diet	0.18	0.670
	Size*Diet	0.10	0.752
CIII size-CIV increment	Size	1.04	0.311
	Diet	0.01	0.926
	Size*Diet	0.01	0.913
CIV size-CV increment			
females	Size	26.10	0.0001
	Diet	1.70	0.199
	Size*Diet	1.37	0.248
males	Size	14.23	0.0005
	Diet	0.66	0.421
	Size*Diet	0.65	0.421
CV size-CVI increment			
females	Size	24.78	0.0001
	Diet	1.53	0.223
	Size*Diet	1.18	0.284
males	Size	4.90	0.032
	Diet	7.19	0.01

Size\*Diet 6.72 0.013

B. 8° C	Source	F value	p	n
N6 size-CI increment	Food	4.66	0.033	82
	Size	57.26	0.0001	
	Size*food	4.20	0.044	
CI size- CII increment	Food	2.64	0.109	78
	Size	9.63	0.003	
	Food*Size	2.60	0.111	
CII size-CIII increment	Food	1.20	0.277	68
	Size	70.21	0.0001	
	Food*Size	0.98	0.326	
CIII size-CIV increment	Food	1.78	0.187	64
	Size	11.71	0.0011	
	Food*Size	1.71	0.196	
CIV size-CV increment	Food	0.15	0.695	64
	Size	0.10	0.756	
	Food*Size	0.08	0.782	
CV size-CVI increment	Food	1.26	0.263	64
	Size	0.69	0.406	

	Food*Size	1.22	0.271	
16° C				
N2 size-N3 increment	Food	1.84	0.182	52
	Size	28.73	0.0001	
	Food*Size	1.64	0.206	
N3 size-N4 increment	Food	0.97	0.328	58
	Size	30.99	0.0001	
	Food*Size	0.95	0.334	
N4 size-N5 increment	Food	0.09	0.765	57
	Size	21.53	0.0001	
	Food*Size	0.09	0.763	
N5 size-N6 increment	Food	2.14	0.149	57
	Size	22.87	0.0001	
	Food*Size	1.96	0.168	
N6 size-CI increment	Food	0.63	0.433	47
	Size	54.53	0.0001	
	Food*Size	0.66	0.423	
CI size-CII increment	Food	0.75	0.391	47
	Size	9.44	0.004	
	Food*Size	0.72	0.400	
CII size-CIII increment	Food	0.51	0.481	48

	Size	8.71	0.005	
	Food*Size	0.44	0.511	
CII size-CIV increment	Food	0.31	0.582	48
	Size	4.69	0.04	
	Food*Size	0.37	0.548	
CIV size-CV increment	Food	0.00	0.952	43
	Size	0.01	0.931	
	Food*Size	0.00	0.957	
CV size-CVI increment	Food	0.04	0.848	43
	Size	0.80	0.376	
	Food*Size	0.04	0.836	

<b>C.</b>	<b>Source</b>	<b>F value</b>	<b>p</b>	<b>n</b>
N6 size-CI increment	Size	78.60	0.0001	129
	Temp	3.41	0.067	
	Size*Temp	5.33	0.022	
CI size-CII increment	Size	29.84	0.0001	125
	Temp	0.11	0.737	
	Size*Temp	0.04	0.842	
CII size-CIII increment	Size	44.75	0.0001	116
	Temp	1.50	0.223	
	Size*Temp	1.67	0.198	

CIII size-CIV increment	Size	16.80	0.0001	111
	Temp	0.59	0.445	
	Size*Temp	0.79	0.375	
CIV size-CV increment	Size	0.12	0.724	112
	Temp	0.02	0.901	
	Size*Temp	0.03	0.871	
CV size-CVI increment	Size	8.25	0.005	117
	Temp	0.58	0.449	
	Size*Temp	1.14	0.287	

## Figure Captions

Figure 1. Stage-specific body size and molt increment for A. *Boeckella triarticulata*, B. *Diaptomus leptopus* raised at 8°C, and C. *D. leptopus* raised at 16°C. Right-hand Y-axis shows coefficients of variation for both traits. Asterisks indicate significant ( $p < 0.05$ ) differences between individuals raised on different diets (food quality or quantity).

Figure 2. Stage-specific correlations of size at molting and molt increment for *Boeckella triarticulata* raised on *Cryptomonas* diet. Stage, Pearson product-moment correlation coefficients ( $r^2$ ) and level of statistical significance ( $p$ ) are shown for each cluster of points. Sample size = 47 for all correlations.

Figure 3. As for Figure 2, for *Diaptomus leptopus* raised at 8°C in A. low and B. high food concentrations. Sample sizes for each correlation: Low Food N6-CI=41, CI-CII=37, CII-CIII=32, CIII-CIV=32, CIV-CV=36, CV-CVI=37; High Food N6-CI=41, CI-CII=41; CII-CIII=36, CIII-CIV=32, CIV-CV=33, CV-CVI=37.

A

*Boeckella triarticulata*







