

Shifts in food quality for herbivorous consumer growth: multiple golden means in the life history

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Abstract. Consumer growth can be affected by imbalances between the nutrient content of the consumer and its food resource. Although ontogenetic-driven changes in animal composition are well documented, their potential consequences for the organism's sensitivity to food quality constraints have remained elusive. Here we show that the potential growth response of the copepod *Mixodiaptomus laciniatus* (as %RNA and RNA:DNA ratio) to the natural gradient of seston carbon (C):nutrient ratio is unimodal and stage specific. Solution of the equation given by the first derivative function provided the optimum C:nutrient ratio for maximum stage-specific growth, which increased during ontogeny. The peakedness of the function indicated that animal vulnerability to suboptimal food quality decreased as juveniles reached adulthood. Consistent with these results, a field experiment demonstrated that potential consumer growth responded to variations in seston C:phosphorus ratio, and that early life stages were particularly vulnerable to suboptimal food quality.

Key words: copepods; ecological stoichiometry; food quality; herbivorous consumer; Lake La Caldera, Spain; life history; *Mixodiaptomus laciniatus*; nutrient ratio; RNA:DNA ratio; unimodal response.

INTRODUCTION

Life history, shaped by natural selection to maximize organism fitness, is one of the most important concepts in evolutionary biology. Evolutionary optimization theory posits that both the wide diversity of life histories and the nature of the trade-offs among their traits are determined by the availability of resources, especially those that affect growth, survivorship, and reproduction (Stearns 1992, Soler 2002). Ecological stoichiometry (the study of the balance of energy and multiple chemical elements in ecological interactions; Sterner and Elser 2002) has made a major contribution to knowledge on this issue, elucidating the role of limiting nutrients in constraining consumer performance. Ecological stoichiometry relies on two basic principles: (1) autotrophs exhibit great flexibility in their elemental composition in response to nutrient availability, whereas heterotrophs have physiological mechanisms to strictly regulate their elemental composition around taxon- or stage-specific values, defined as stoichiometric homeostasis (Sterner and Elser 2002); and (2) differences in elemental composition underlie the nutritional imbalance between autotrophs and their herbivorous consumers, which can

have a major effect on consumer fitness and dynamics (Sterner et al. 1993, Boersma 2000) and therefore on the amount of energy and materials transferred up the food web (Urabe and Sterner 1996, Urabe et al. 2002) and the strength of trophic cascades (Elser et al. 1998).

A key insight that emerges from this approach is that the elemental mismatch between resources and consumers determines the nutritional demands for animal growth. The threshold elemental ratio (TER) is a central concept for understanding nutrient deficiency in animals (Sterner and Elser 2002). TER is the carbon (C):nutrient ratio at which growth limitation switches from energy (C) to a mineral nutrient (Sterner and Hessen 1994, Frost et al. 2006). It is calculated as the product of physiological nutrient efficiencies and somatic elemental composition (Frost et al. 2006), and may change as a function of ambient food quantity (Sterner 1997). By definition, food nutrient content would not be expected to influence consumer performance below a given threshold. However, Plath and Boersma (2001) found a unimodal growth response of *Daphnia* to a gradient of C:phosphorus (P) food, with maximal growth rates at intermediate C:P food levels and a pronounced growth impairment at both extremes (P-enriched and P-poor algae). This observation was later corroborated by Elser et al. (2005) for the interaction between snails and stromatolitic microbial communities, and these authors formulated the “knife-edge” hypothesis to argue that there is an optimum resource C:nutrient ratio (“trophic

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or stoichiometric knife-edge”) close to the TER, which satisfies the consumer’s requirements, and that growth penalties are incurred at ratios above and below this value.

A common assumption in ecological stoichiometry theory, including quantitative estimates of TER, is strict homeostasis and no variability in the chemical content of the consumer (Sterner and Elser 2002). However, while the distinctive elemental composition of consumers is not in dispute, with wide interspecific variations, several studies have reported marked intraspecific variations in somatic composition across the life cycle of a consumer. For example, DeMott et al. (1998) and Kyle et al. (2006) showed strong variations in the P content of *Daphnia* in response to the quality of food, and more recently Lukas et al. (2011) found that colimiting nutrients and biochemicals can have a great impact on consumer homeostasis. Likewise, Villar-Argaiz et al. (2002) described large intraspecific changes in somatic C:nitrogen (N):P ratios throughout the ontogenetic development of a copepod species. In a coetaneous study, Villar-Argaiz and Sterner (2002) found that P limitation prevented transition between certain copepodite stages, creating demographic bottlenecks. These data demonstrated that certain life stages may be more sensitive than others to variations in the stoichiometric content of their food resource, i.e., a given resource C:P ratio that supplies inadequate P for one developmental stage may provide surplus P for another. Hence, the degree of mismatch between the composition of the animal and food resource may vary according to the nutrient requirements at each developmental stage. Further complexity has been added to the consumer–resource interaction by reports that organism C:N:P ratios can also vary within developmental stages (Villar-Argaiz et al. 2002). For example, Carrillo et al. (2001) found that the intrastage-P content of the copepod *Mixodiaptomus laciniatus* varies by up to threefold.

According to such evidence, neither hard cutoffs nor species-specific unimodal responses should be established for the role of nutrients in limiting food quality in organisms with complex life histories, because the intrinsic ontogenetic variability in nutrient requirements must be taken into account. We therefore hypothesized that the ontogenetic-driven nutrient requirements of a consumer would yield different optimal food quality levels at which stage-specific potential growth is maximal. We also hypothesized that sensitivity to suboptimal food quality would decrease as larvae and juveniles mature into adults with lower mineral nutrient demands for growth. In order to test these propositions, we first analyzed the relationship between the seston C:nutrient ratio and the stage-specific potential growth of the calanoid copepod *Mixodiaptomus laciniatus* during three years of intensive monitoring in Lake La Caldera, Spain.

Seston C:nutrient ratios were proposed to vary according to the intensity of light and nutrient conditions (“light:nutrient hypothesis;” Sterner et al. 1997). Subsequent research reported that wavelengths within the ultraviolet radiation (UVR) range are powerful drivers of seston food quality for herbivores by reducing C:nutrient ratios (Xenopoulos et al. 2002), a finding later verified under laboratory (Hessen et al. 2008) and natural conditions (Carrillo et al. 2008). Based on this evidence and the significant relationships of seston C:P ratio with total P (TP) and UVR in Lake La Caldera, we second tested whether the in situ manipulation of these two factors affected the stage-specific growth of *Mixodiaptomus laciniatus* via food quality. RNA-derived indices were selected as proxies of growth rate for this purpose (e.g., Elser et al. 2003), because they have proven useful for both in situ studies and short-term experiments (e.g., Wagner et al. 2001, Vrede et al. 2002, Malzahn and Boersma 2012).

METHODS

Study system and organism

Our study was carried out in Lake La Caldera (36°55′–37°15′ N, 2°31′–3°40′ W), a natural permanent water body at 3050 m above sea level in the National Park of Sierra Nevada, Spain (Appendix A: Fig. A1). The elevated UVR doses at this altitude (see Fig. 1 in Carrillo et al. 2002), the lake transparency (Secchi’s disk visibility reaching maximum depth), and low dissolved organic C values (<1 mg/L) result in a deep UVR penetration in the water column during the ice-free season (from June to November) (Appendix A: Table A1; see Fig. 1 in Carrillo et al. 2008). There is a strong P limitation on lake production (TP, 1.38–11.30 µg P/L; dissolved inorganic N:TP (DIN:TP) molar ratio > 45) that is partially alleviated by transient atmospheric Saharan nutrient inputs (Villar-Argaiz et al. 2001, Morales-Baquero et al. 2006). The biological community is relatively simple, with no fish and only a few planktonic species. Of particular interest for our study is the absolute dominance of the herbivorous zooplankter *Mixodiaptomus laciniatus*, which represents >90% of the total zooplankton biomass. This species was chosen to test our hypotheses because it strictly grazes on seston and has a univoltine life cycle, allowing the study of its ontogenetic development from nauplii to adult stages in its natural habitat (Carrillo et al. 2001, Villar-Argaiz et al. 2002).

Field sampling and in situ experiment

The data in this paper derive from two sources: a 3-year (2005–2007) field monitoring program (hereafter, interannual study) and an in situ light × nutrient experiment carried out in July–August 2007 (hereafter, experimental study).

The interannual data were obtained by monthly monitoring during the ice-free seasons in 2005, 2006, and 2007. For each sampling day, we measured light

(UVR at 305, 320, and 380 nm and photosynthetic active radiation, PAR) and temperature profiles using a Biospherical Instruments Compact (BIC) radiometer (Biospherical Instruments, San Diego, California, USA). For each UVR wavelength and PAR, the diffuse attenuation coefficient for downward irradiance, K_d (Appendix A: Table A1), and the mean light in the mixed layer, I_m , were determined. Expressed as a fraction of surface irradiance, I_m was calculated as:

$$I_m = \frac{1 - e^{-K_d Z_m}}{K_d Z_m}$$

(Sterner et al. 1997), where Z_m is the mixed-layer depth. We defined I_{mUVR} as the mean UVR in the mixed layer, calculated as the mean I_m at three UVR wavelengths (305, 320, and 380 nm). Water samples for chemical (total N (TN) and TP) and biological (chlorophyll a [Chl a], seston C, N, and P) variables were taken in triplicate at two depths in 2005 (0.5 and 1 m) and four depths in 2006 and 2007 (0.5, 3, 5, and 8 m). Detailed collection protocols are described in Villar-Argaiz et al. (2001). Samples of *Mixodiatomus laciniatus* for analyses of nucleic acids (NAs) were collected by vertical hauls with a 64- μ m mesh net and were brought to the laboratory in lake water under dark and cold conditions. Between 10 and 20 individuals were isolated and transferred to 1.5-mL Eppendorf tubes containing 300 μ L of RNA $later$ (Ambion, Austin, Texas, USA), which were stored at -80°C until analysis (see recommendations in Gorokhova 2005). Primary production in terms of total organic carbon (TOC) and particulate organic carbon >1.0 μ m (POC $_1$) was determined following the procedure described in Carrillo et al. (2002) (see Appendix B for a detailed description of the method).

The experimental study consisted of two phases. First, water collected at 3-m depth was filtered through 64 μ m to remove zooplankton and was incubated in six UVR-transparent polyethylene mesocosms (height 5 m, diameter 0.7 m, volume 2 m 3). Three enclosures were covered and surrounded with Plexiglass UF3 sheets to prevent UVR (PAR treatment). The other three enclosures received the full spectrum of solar radiation (UVR treatment), although they were covered with polyethylene to avoid atmospheric nutrient inputs during incubation. Second, after a 1-month interval (from 19 July to 19 August), water from each mesocosm was used to fill two 20-L polyethylene microcosms (height 0.2 m, diameter 0.4 m), yielding a total of 12 microcosms. *Mixodiatomus laciniatus* individuals collected from the lake were added to each microcosm until the doubling of lake population density was reached. For each light treatment, three microcosms received P (as Na $_2$ HPO $_4$) and N (as NH $_4$ NO $_3$) to double the TP concentration observed in the lake and maintain a molar N:P ratio of 30, mimicking the mean value of the molar TN:TP ratio found in the total atmospheric deposition (Bullejos et al. 2010). The experiment had a split-plot design, with light applied at plot level and nutrients manipulated at subplot

level. This yielded a 2 (UVR vs. PAR) \times 2 (no nutrient addition vs. nutrient addition) factorial design with three replicates per treatment: UVR, UVR+NP, PAR, and PAR+NP. Microcosms were incubated for one week (from 19 August to 26 August) at the depth where the UVR was 75% of the surface value for the UVR treatments (0.1 m) or under Plexiglass UF3 sheets for the PAR treatments (Appendix C: Fig. C1). After incubation, subsamples were taken in triplicate for analysis of Chl a , seston C, N, P, and zooplankton NAs and were treated as reported previously. For further details of the experimental setup and procedures, see Souza et al. (2010).

Laboratory analyses

TN was analyzed by using the UV spectrophotometric screening method. TP was measured colorimetrically via the acid molybdate technique (APHA 1992). Seston samples, obtained by filtration of 300 mL of water per replicate using GF/B filters (Sigma-Aldrich, St. Louis, Missouri, USA), were analyzed for P following the acid-molybdate technique (APHA 1992) or were dried (24 h at 60 $^\circ\text{C}$) and analyzed for C and N using a Perkin-Elmer model 2400 CHN elemental analyzer (Perkin-Elmer Corporation, Waltham, Massachusetts, USA). Seston C:P and C:N ratios (hereafter, C: nutrient ratios) were calculated on a molar basis. Chl a was measured fluorimetrically after grinding the pigment-loaded filters (concentrated by filtration of 300 mL of water per replicate using GF/F filters; Sigma-Aldrich) and extracting the pigments in 90% acetone (24 h under dark conditions at 4 $^\circ\text{C}$).

In total, >450 and >120 individuals of *Mixodiatomus laciniatus*, distinguishing among ontogenetic stages and between adult genders, were measured and analyzed for NA content in the interannual and experimental studies, respectively. RNA and DNA contents were expressed as percentages relative to dry mass (%RNA, %DNA) after length-mass conversions (Carrillo et al. 2001) and as RNA:DNA ratio. Hereafter, %RNA and RNA:DNA ratio will be referred as NA indices (NAIs). NAs were measured using a microplate fluorometric high-range RiboGreen assay (Invitrogen, Carlsbad, California, USA) after N-laurylsarcosine extraction and RNase digestion, as described in Gorokhova and Kyle (2002). This highly sensitive method allowed individual zooplankton NA measurements to be carried out. Fluorescence measurements were converted into RNA and DNA concentrations by using standard curves for RNA (16S and 23S from *Escherichia coli*; component C of the RiboGreen Kit) and DNA (calf thymus), and were expressed as NAIs (see Appendix B for a detailed description of NA analysis).

Data and statistical analyses

In the interannual study, ontogenetic and gender differences in NAIs were tested by using nonparametric Kruskal-Wallis ANOVAs. Polynomial regression mod-

els were adjusted to determine the response of consumer growth, measured as NAIs, to the effects of (1) temperature, (2) algal standing stock (Chl *a*, seston C, TOC, and POC₁), and (3) food quality (seston C:nutrient ratios). Quadratic functions were expressed as:

$$f(x) = cx^2 + bx + a \quad (1)$$

where x is the seston C:nutrient ratio and $f(x)$ is the NAI. Because c was always negative, these were inverted parabolic functions. The maximum point of the curve corresponds to the “optimum resource (seston) C:nutrient ratio” ($x_{\text{optC:nut}}$), at which potential consumer growth is maximal [$f(x_{\text{optC:nut}})$]. Because $f(x)$ was continuous and differentiable throughout the seston C:nutrient ratio interval, the $x_{\text{optC:nut}}$ value that provided maximum potential growth, $f(x_{\text{optC:nut}})$, was determined by the first derivative function $f'(x)$:

$$f'(x) = 2cx + b = 0 \quad (2)$$

Therefore,

$$x_{\text{optC:nut}} = -\frac{b}{2c} \quad (3)$$

By rearranging Eq. 1, maximal potential consumer growth (measured as either %RNA or RNA:DNA ratio) can be determined as (see Fig. 1A):

$$f(x_{\text{optC:nut}}) = c \left(-\frac{b}{2c} \right)^2 + b \left(-\frac{b}{2c} \right) + a = -\frac{(b^2 - 4ca)}{4c} \quad (4)$$

The absolute value of c is a measure of the degree of peakedness and yields information on the sensitivity of organism growth to the food quality (hereafter, “growth sensitivity index,” GSI). A low GSI corresponded to a flat-topped curve and was interpreted as low growth sensitivity to food quality variations around $x_{\text{optC:nut}}$, whereas a high GSI corresponded to a highly peaked curve and indicated high sensitivity to food quality variations around $x_{\text{optC:nut}}$ (Fig. 1B). In comparison to the RNA:DNA ratio %RNA has been found to be more strongly correlated with feeding and growth in copepods (Holmborn et al. 2009), due to confounding effects on individual DNA content, such as endopolyploidy (Gorokhova and Kyle 2002) or ovary development (Wagner et al. 2001, Ikeda et al. 2007). For this reason, we calculated the GSI from the mother functions in which %RNA was used as a proxy of growth.

Simple linear regression was used to test the effects of (1) $x_{\text{optC:nut}}$ and GSI on mean stage-specific size; and (2) I_{mUVR} and $I_{\text{mUVR}}:\text{TP}$ ratio on the seston C:P ratio. A homogeneity of slopes model (analysis of covariance, ANCOVA) was used to test the effect of NAI (categorical factor) on mean stage-specific size across the continuous predictor variables (covariates, $x_{\text{optC:P}}$ and $x_{\text{optC:N}}$). Nonsignificant results for the categorical factor \times covariate interaction (term “slope”) indicated

no differences between regression slopes (Quinn and Keough 2002).

In the experimental study, the effects of light (UVR vs. PAR) on Chl *a* and seston C:nutrient ratios in the mesocosms were compared by one-way ANOVA. Experimental data obtained from the split-plot design were analyzed by repeated-measures ANOVA (RM-ANOVA; Quinn and Keough 2002). The effects of light (UVR vs. PAR) and nutrients (no nutrient addition vs. nutrient addition) on seston C and C:nutrient ratios, and the effects of light, nutrients, and adult gender (male vs. female) on NAIs and %DNA in the microcosms were obtained from the results of the univariate tests for RM-ANOVA. Because the subplot factor ontogeny had more than two levels (copepodite stages II–V and adults), effects of ontogeny and its interactions with light and nutrients on NAIs and %DNA were obtained from the results of the multivariate tests of Wilks, Pillai, Hotelling, and Roy for RM-ANOVA, as recommended by O’Brien and Kaiser (1985), and Scheiner and Gurevitch (2001). When interactive effects were significant, Tukey’s HSD post hoc tests were used to determine the effect of each main factor (Dunne 2010). Normality was tested by Kolmogorov-Smirnov and Shapiro-Wilk W tests and homoscedasticity by Cochran’s and Levene’s tests. STATISTICA 10 for Windows software (StatSoft 2011) was used for data analyses.

RESULTS

Interannual study

The herbivore *Mixodiaptomus laciniatus* showed a wide intraspecific variability in %RNA, with up to >12-fold higher values in nauplii than in adults. There was a strong and significant effect of ontogeny on both NAIs (Kruskal-Wallis ANOVA tests: %RNA, $H_{5,34} = 23.43$, $P < 0.001$; RNA:DNA ratio, $H_{5,34} = 11.54$, $P = 0.042$). In particular, the mean %RNA decreased from 8.25% in nauplii to 0.64% in adults. In contrast, no gender differences in NAI values were found (Kruskal-Wallis tests: %RNA, $H_{1,11} = 1.2$, $P = 0.27$; RNA:DNA ratio, $H_{1,11} = 3.33$, $P = 0.07$) (Appendix D: Fig. D1). Much of the intraspecific variability in NAIs was due to differences within a given stage (i.e., intrastage variability; Appendix D: Fig. D1). The stage-specific coefficients of variation (CVs) ranged from 21% to 37% for %RNA and from 18% to 50% for RNA:DNA ratio. The mechanisms behind this high variability were explored by analyzing NAIs as a function of factors potentially affecting animal growth (temperature, food quantity, and food quality variables). Neither temperature nor food quantity explained the NAI values (for all correlations, $P > 0.05$; data not shown). Only food quality variables (seston C:P and C:N ratios) proved significant, and the relationship was unimodal in most cases (Fig. 2, Table 1). NAIs also showed a marked variability in nauplii and copepodite stage I, but the low number of samples precluded unimodal fits.

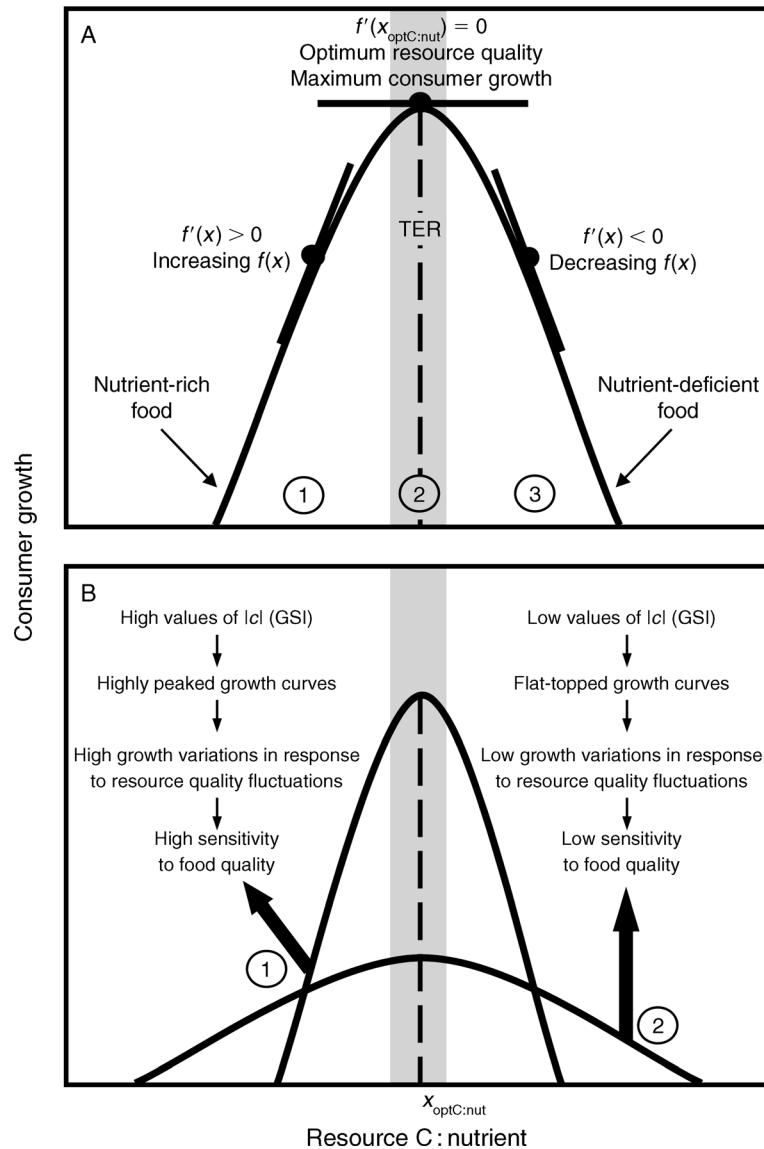


FIG. 1. Conceptual diagram illustrating: (A) the unimodal response of consumer growth, $f(x)$, to the resource C : nutrient ratio, x , where $f(x) = cx^2 + bx + a$. Three regions characterize this curve: (1) a resource C : nutrient ratio that yields increasing $f(x)$ with $f'(x) > 0$, indicating impaired growth due to nutrient-rich food; (2) an optimum resource C : nutrient ratio ($x_{\text{optC:nut}}$, dashed line) that yields maximum consumer growth $f(x_{\text{optC:nut}})$, close to the likely threshold elemental ratio (TER, gray-shaded region), with $f'(x_{\text{optC:nut}}) = 0$; (3) a resource C : nutrient ratio that yields decreasing $f(x)$ with $f'(x) < 0$, indicating impaired growth due to nutrient-deficient food. (B) Two hypothetical scenarios of the unimodal response of consumer growth to the resource C : nutrient ratio: (1) highly peaked growth curves [high values of $|c|$ (growth sensitivity index, GSI)] representing high consumer sensitivity to food quality fluctuations around $x_{\text{optC:nut}}$; and (2) flat-topped growth curves (low values of $|c|$) representing low consumer sensitivity to food quality fluctuations.

The seston food quality that supported maximum potential growth varied throughout ontogeny, as indicated by the significant correlations between $x_{\text{optC:nut}}$ and mean stage-specific size (Fig. 3, Table 2). The slopes of the relationships were positive and were similar when the covariate $x_{\text{optC:nut}}$, obtained from %RNA or RNA:DNA ratio growth functions, was $x_{\text{optC:P}}$ (ANCOVA: intercept, $F_{1,8} = 10.14$, $P = 0.012$; slope, $F_{1,8} = 2.01$, $P = 0.194$) to when it was $x_{\text{optC:N}}$ (ANCOVA: intercept, $F_{1,8} = 2.35$, $P = 0.163$; slope, $F_{1,8} = 1.34$, $P =$

0.280). Furthermore, the sensitivity of consumers to food quality varied throughout ontogeny and decreased in more advanced stages, as indicated by the negative linear relationships between GSI and organismal size (Fig. 3, Table 2).

We measured I_{mUVR} and calculated $I_{\text{mUVR}}:\text{TP}$ in the study lake, as described by Sterner et al. (1997). Regression analyses were performed to evaluate the underlying mechanisms controlling the seston C:P ratio as a proxy for food quality. Results showed this ratio to

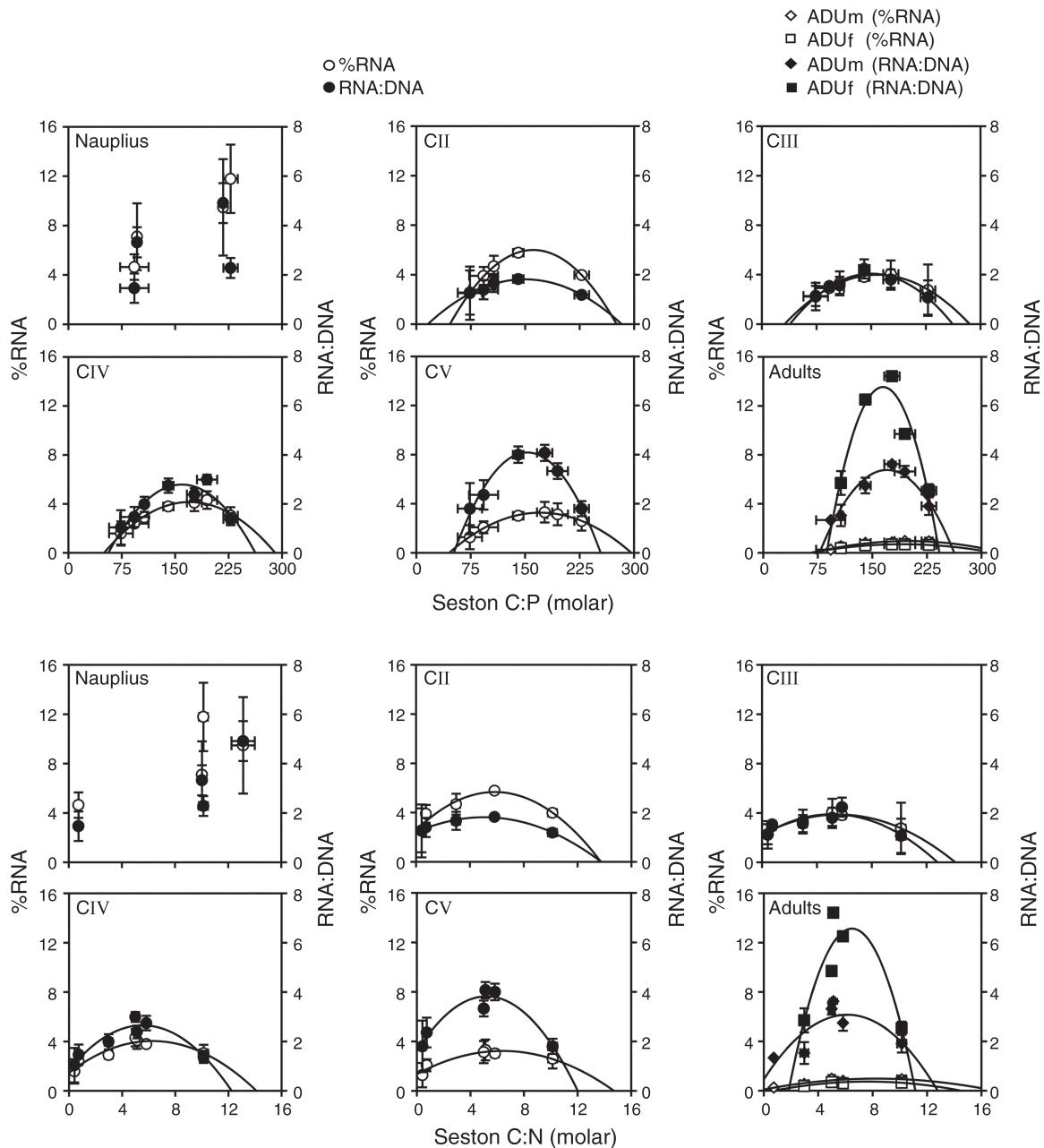


FIG. 2. Relationships between the seston C:P and C:N ratios and individual RNA content (percentage of dry mass, %RNA) and RNA:DNA ratio for each ontogenetic stage (nauplius; CII–V, copepodite stages II–V) and adults (ADUm, adult male; ADUf, adult female) of the copepod *Mixodiaptomus laciniatus*. Circles and error bars represent mean values \pm SD for each sampling day in the ice-free seasons of 2005, 2006, and 2007. Lines represent parabolic regression fits. See Table 1 for regression parameters and statistics.

be negatively correlated with both I_{mUVR} (seston C:P ratio = $-231.13I_{mUVR} + 244.62$; $P = 0.005$, $R^2 = 0.56$) and I_{mUVR} :TP ratio (seston C:P ratio = $-15.90I_{mUVR}$:TP ratio + 205.32; $P = 0.004$, $R^2 = 0.58$) (Appendix D: Fig. D2).

Experimental study

Based on the results of the interannual study, we experimentally tested the effect on consumer NAIs of

food quality differences due to UVR and nutrient manipulation. In the mesocosms, the presence and absence of UVR affected seston food quality in terms of the C:P (one-way ANOVA: $F_{1,4} = 11.48$, $P = 0.028$) and C:N (one-way ANOVA: $F_{1,4} = 10.10$, $P = 0.034$) ratios, with seston C:P and C:N ratios being significantly lower under UVR vs. PAR treatment (mean \pm SD; $C:P_{UVR} = 97.39 \pm 31.23$ vs. $C:P_{PAR} = 409.77 \pm 156.77$; $C:N_{UVR} = 3.01 \pm 2.42$ vs. $C:N_{PAR} = 9.81 \pm 2.81$). In

TABLE 1. Regression analyses of food quality effects for each ontogenetic stage and adult gender of the copepod *Mixodiaptomus laciniatus*, measured as the independent variables, x (seston C:P and C:N molar ratios), on two dependent variables, $f(x)$: (A) individual RNA content (percentage of dry mass, %RNA) and (B) RNA:DNA ratio.

Dependent and independent variables	Stage-gender	n	c	b	a	P_c	P_b	P_a	R^2
A) %RNA									
Seston C:P	CII	5	-0.00045 ± 0.00000	0.15 ± 0.00	-5.78 ± 0.17	<0.001	<0.001	<0.001	1.00
	CIII	6	-0.00025 ± 0.00002	0.08 ± 0.01	-2.20 ± 0.40	0.001	0.001	0.012	0.98
	CIV	7	-0.00029 ± 0.00004	0.10 ± 0.01	-4.19 ± 0.73	0.001	0.001	0.004	0.97
	CV	6	-0.00021 ± 0.00001	0.07 ± 0.00	-2.80 ± 0.24	<0.001	<0.001	0.001	1.00
	ADU	6	-0.00005 ± 0.00000	0.02 ± 0.00	-1.12 ± 0.21	<0.001	<0.001	<0.001	0.98
	ADUm	6	-0.00006 ± 0.00002	0.02 ± 0.01	-1.28 ± 0.39	0.043	0.024	0.047	0.96
	ADUf	5	-0.00004 ± 0.00000	0.02 ± 0.00	-0.90 ± 0.09	<0.001	<0.001	<0.001	1.00
Seston C:N	CII	5	-0.09102 ± 0.02620	1.06 ± 0.28	2.55 ± 0.50	0.074	0.063	0.037	0.88
	CIII	6	-0.05320 ± 0.01004	0.59 ± 0.11	2.23 ± 0.22	0.013	0.012	0.002	0.91
	CIV	7	-0.06571 ± 0.01365	0.82 ± 0.14	1.46 ± 0.32	0.009	0.005	0.011	0.90
	CV	6	-0.04635 ± 0.01091	0.59 ± 0.11	1.34 ± 0.25	0.024	0.014	0.013	0.91
	ADU	6	-0.01109 ± 0.00376	0.18 ± 0.04	0.11 ± 0.11	0.060	0.026	0.393	0.90
	ADUm	6	-0.01299 ± 0.00424	0.21 ± 0.05	0.09 ± 0.13	0.055	0.022	0.514	0.92
	ADUf	5	-0.01570 ± 0.00708	0.24 ± 0.10	-0.17 ± 0.30	0.157	0.135	0.627	0.78
B) RNA:DNA									
Seston C:P	CII	5	-0.00010 ± 0.00002	0.03 ± 0.00	-0.45 ± 0.33	0.023	0.025	0.304	0.96
	CIII	6	-0.00016 ± 0.00003	0.05 ± 0.01	-1.64 ± 0.62	0.012	0.013	0.078	0.91
	CIV	7	-0.00026 ± 0.00006	0.08 ± 0.02	-3.86 ± 1.24	0.012	0.010	0.036	0.85
	CV	6	-0.00040 ± 0.00004	0.12 ± 0.01	-5.24 ± 0.86	0.003	0.002	0.009	0.97
	ADU	6	-0.00067 ± 0.00009	0.22 ± 0.03	-13.87 ± 2.05	0.005	0.004	0.007	0.96
	ADUm	6	-0.00039 ± 0.00008	0.13 ± 0.03	-7.98 ± 1.90	0.017	0.014	0.025	0.92
	ADUf	5	-0.00113 ± 0.00023	0.37 ± 0.08	-23.87 ± 6.14	0.039	0.040	0.060	0.92
Seston C:N	CII	5	-0.02394 ± 0.00200	0.24 ± 0.02	1.20 ± 0.04	0.007	0.008	0.001	0.99
	CIII	6	-0.03307 ± 0.01067	0.34 ± 0.11	1.08 ± 0.23	0.053	0.058	0.019	0.76
	CIV	7	-0.05906 ± 0.01009	0.65 ± 0.11	0.84 ± 0.24	0.004	0.004	0.024	0.90
	CV	6	-0.08222 ± 0.01340	0.85 ± 0.14	1.60 ± 0.31	0.009	0.009	0.014	0.93
	ADU	6	-0.11639 ± 0.03680	1.41 ± 0.43	-0.03 ± 1.09	0.051	0.045	0.983	0.79
	ADUm	6	-0.06962 ± 0.02846	0.85 ± 0.33	0.46 ± 0.84	0.092	0.081	0.625	0.69
	ADUf	5	-0.30282 ± 0.09540	3.93 ± 1.32	-6.17 ± 4.06	0.087	0.097	0.268	0.85

Notes: Stages are copepodites (CII–CV, stages II–V) and adults, analyzed with (ADUm, adult males; ADUf, adult females) and without (ADU) gender distinction. The regression model was $f(x) = cx^2 + bx + a$. Parameter estimates ± SE are reported. When c and b were significant, regressions are shown in boldface.

contrast, UVR had no significant effect on Chl a (one-way ANOVA: $F_{1,4} = 4.24$, $P = 0.11$), which was low (mean ± SD; Chl $a_{\text{UVR}} = 1.44 \pm 0.18 \mu\text{g/L}$ mean ± SD; Chl $a_{\text{PAR}} = 2.52 \pm 0.89 \mu\text{g/L}$) and similar to that found in the lake (mean Chl $a < 2 \mu\text{g/L}$, and 69% of observations $< 0.7 \mu\text{g/L}$). In the microcosms, the combined manipulations of light and nutrients had no effect on either the quantity (seston C) or the quality of food for zooplankton with the exception of the seston C:P ratio, which was higher in the absence vs. presence of UVR when no nutrients were added (Fig. 4, Table 3) (Tukey's HSD post hoc tests: $P = 0.003$ for PAR vs. UVR; $P = 0.008$ for PAR vs. UVR+NP; $P = 0.029$ for PAR vs. PAR+NP).

In the experimental study, NAI values were within the range observed for the individuals collected from the lake. Similar to the interannual study, a clear ontogenetic pattern was observed, with a decrease in %RNA values and an increase in RNA:DNA ratios from early copepodite stages to adulthood. The ontogeny explained most of the variance in %RNA (80%) and RNA:DNA ratio (22.6%), although light and nutrients contributed 1.7% and 5.2% of the variance in %RNA and 11% and

10.5% of that in the RNA:DNA ratio, respectively (Fig. 5, Table 4). In contrast, no effects of light and nutrients on %DNA were observed (RM-ANOVA: light, $F_{1,4} = 7.20$, $P = 0.055$; nutrients, $F_{1,4} = 1.00$, $P = 0.373$; ontogeny, $F_{4,1} = 3132.61$, $P = 0.013$). The highest seston C:P ratio and lowest %RNA were found in early copepodite stages under PAR treatment (Tukey's HSD post hoc tests: $P < 0.05$ for most inter-treatment comparisons for copepodites; $P > 0.05$ for adults), consistent with the higher sensitivity to food quality found for these stages vs. adults in the interannual field study. In adults, inter-treatment differences in %RNA and RNA:DNA ratio were only due to gender (RM-ANOVA: for %RNA, light, $F_{1,4} = 2.37$, $P = 0.199$; nutrients, $F_{1,4} = 0.11$, $P = 0.752$; gender, $F_{1,4} = 21.15$, $P = 0.010$; for RNA:DNA ratio, light, $F_{1,4} = 1.35$, $P = 0.310$; nutrients, $F_{1,4} = 0.19$, $P = 0.682$; gender, $F_{1,4} = 13.02$, $P = 0.023$) (see insets in Fig. 5).

DISCUSSION

The results of this study support our hypothesis that the potential consumer growth response to food quality would be stage-dependent and unimodal. Optimal food

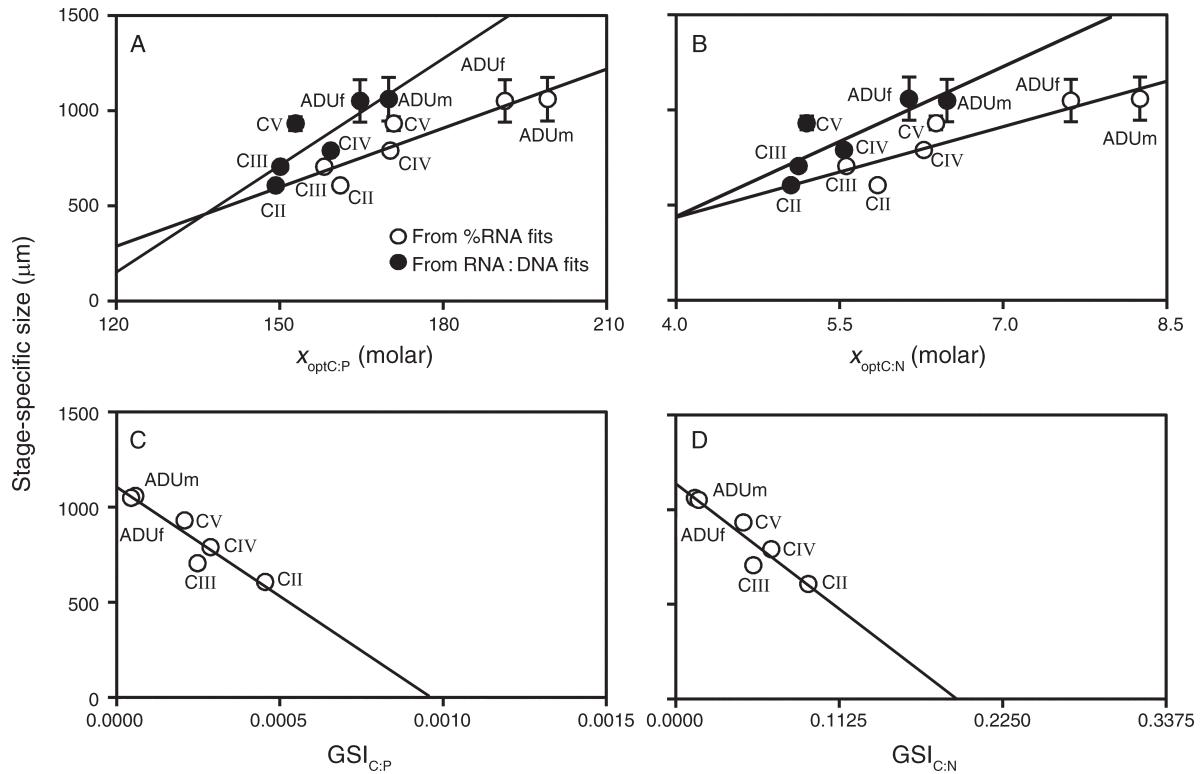


FIG. 3. Relationships of (A) $x_{optC:P}$, (B) $x_{optC:N}$, (C) $GSI_{C:P}$, and (D) $GSI_{C:N}$ with stage-specific size of the copepod *Mixodiaptomus laciniatus* in the interannual study. Circles are mean stage-specific sizes and vertical error bars indicate SD. Solid lines represent linear regression fits. Stages are copepodites (CII–CV, stages II–V) and adults (ADUm, adult male; ADUf, adult female). See Table 2 for regression parameters (slope, intercept) and statistical results.

quality produced maximal potential growth, and sub-optimal food qualities were found in situations of mineral nutrient excess or deficiency. Although this is consistent with predictions of the “knife-edge hypothesis” (Elser et al. 2005), we further show that this response is not only species-specific (Boersma and Elser 2006), but also stage-specific in consumers with complex life cycles (“multiple ontogenetic golden means or stoichiometric knife-edges”). Analysis of stage-specific unimodal curves showed differences in optimum C : nutrient ratios and function peakedness during ontogeny, indicating that consumer sensitivity to mineral nutrient constraints characteristically decreased as ontogeny

progressed, possibly attributable to differential energy and nutrient demands during the life history of the consumer.

Before making inferences about the importance of the unimodal responses observed in this study, it is necessary to highlight the sources of intraspecific variability in NAIs. First, both %RNA and RNA:DNA ratios showed strong and significant changes during the life history of the animal from nauplii to late copepodite and adult stages, i.e., there was pronounced variation in NA content during ontogeny. Similar ontogenetic patterns in NAIs have been observed for other crustacean species in both freshwater and marine

TABLE 2. Simple linear regressions of $x_{optC:P}$, $x_{optC:N}$, $GSI_{C:P}$, and $GSI_{C:N}$ (independent variables, x) against mean stage-specific size of the copepod *Mixodiaptomus laciniatus* (dependent variable, y).

Independent variable (x)	NAI	b	a	P	R^2
$x_{optC:P}$	%RNA	10.32 ± 2.29	-950.77 ± 402.50	0.011	0.84
$x_{optC:P}$	RNA:DNA	18.69 ± 5.93	-2090.96 ± 936.57	0.034	0.71
$x_{optC:N}$	%RNA	158.88 ± 38.85	-200.24 ± 261.26	0.015	0.81
$x_{optC:N}$	RNA:DNA	262.63 ± 86.21	-610.57 ± 484.11	0.038	0.70
$GSI_{C:P}$	%RNA	-1 147 184.05 ± 198 757.41	1105.41 ± 51.23	0.004	0.89
$GSI_{C:N}$	%RNA	-5870.76 ± 1061.61	1136.15 ± 58.14	0.005	0.88

Notes: NAI is the nucleic acid index (%RNA or RNA:DNA ratio) used to obtain $x_{optC:P}$, $x_{optC:N}$, $GSI_{C:P}$, and $GSI_{C:N}$. The linear regression model was $y = bx + a$. Parameter estimates ± SE are reported; $n = 6$ in all cases. Significant regressions are shown in boldface.

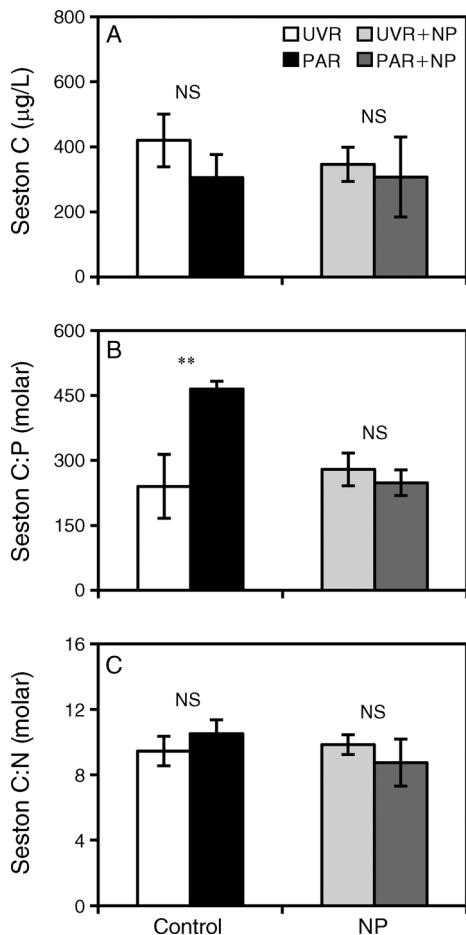


FIG. 4. Seston (A) C, (B) C:P, and (C) C:N ratios in the experimental treatments after 1-week incubation in Lake La Caldera. Treatments are: UVR, full sunlight; UVR+NP, full sunlight + nutrient addition; PAR, screened sunlight (>380 nm); PAR+NP, screened sunlight (>380 nm) + nutrient addition. Columns and error bars represent mean values ± SD. See Table 3 for statistical results for the effects of light and nutrients on seston C, C:P, and C:N ratios.

** P < 0.01; NS, not significant, for Tukey's HSD post hoc test comparisons between light treatments for each nutrient treatment.

systems (e.g., Wagner et al. 2001, Gorokhova and Kyle 2002) and are consistent with ontogenetic variations described for other biochemical and mineral constituents (Carrillo et al. 2001, Villar-Argaiz et al. 2002, Ventura and Catalan 2010). The overall higher RNA content of nauplii compared to that of juveniles and adults in *Mixodiptomus laciniatus* is consistent with the "growth rate hypothesis" that organisms lacking major P storage capacity have elevated demands for increased P allocation to P-rich ribosomal RNA under rapid growth (Elser et al. 2000). Second, a given developmental copepod stage exhibited marked differences in NA content, supporting previous observations that other factors besides ontogeny might contribute to NA variation in consumers (e.g., Wagner et al. 2001, Van Geest et al. 2010). The stage-specific relationship between NAIs and seston C:nutrient ratio strongly supports the proposition that mineral availability is a key condition for consumer growth (e.g., Villar-Argaiz and Sterner 2002, Vrede et al. 2002, Elser et al. 2005).

The response of consumers to food quality was not linear but parabolic, with a maximal growth performance at an intermediate C:nutrient ratio. Similar responses to various ecological factors (e.g., temperature, salinity, and so forth) have been observed, and they have important implications for defining the niche of a species. Because suboptimal food quality occurs at both low and high concentrations of a potentially limiting nutrient, it is reasonable to suggest that previously established C:nutrient ratio thresholds should not be considered as hard cutoffs below which consumers do not respond to the nutrient content of food. Thresholds should rather contemplate shifts associated with the varying demands of nutrients throughout the consumer's life history. In their review, Frost et al. (2006) suggested that the TER_{C:P} was, on average, 2.4-fold the body C:P ratio. It could therefore be expected that when food is below the TER, the organism would be limited by C and P would not affect its growth. Villar-Argaiz et al. (2002) found that the molar C:N:P ratio for the copepod *Mixodiptomus laciniatus* varied ontogenetically from 99:3:1 in nauplii to 165:13:1 in immature copepodites and 234:25:1 in adults. In the present study, seston C:P and C:N ratios were always below Frost's hypothetical TER (C:P < 228, C:N < 18); therefore,

TABLE 3. Results of the repeated-measures ANOVAs examining the effects of light (plot factor) and nutrients (subplot factor) on seston C (µg C/L), C:P, and C:N molar ratios.

Factor	Seston C				Seston C:P				Seston C:N			
	df	F	P	PV	df	F	P	PV	df	F	P	PV
Main plot effects												
Light	1, 4	1.55	0.281	20.86	1, 4	30.39	0.005	24.20	1, 4	0.00	0.967	0.01
Subplot effects												
Nutrients	1, 4	1.17	0.341	4.60	1, 4	7.68	0.050	20.21	1, 4	1.24	0.328	10.92
Light × nutrients	1, 4	1.27	0.322	5.01	1, 4	15.92	0.016	41.88	1, 4	3.09	0.154	27.18

Notes: Sample size was 2 × 2 × 3 (light × nutrients × replicates) = 12. The percentage of variance (PV) is calculated as (sum of squares of treatment/total sum of squares) × 100. Significant effects are shown in boldface.

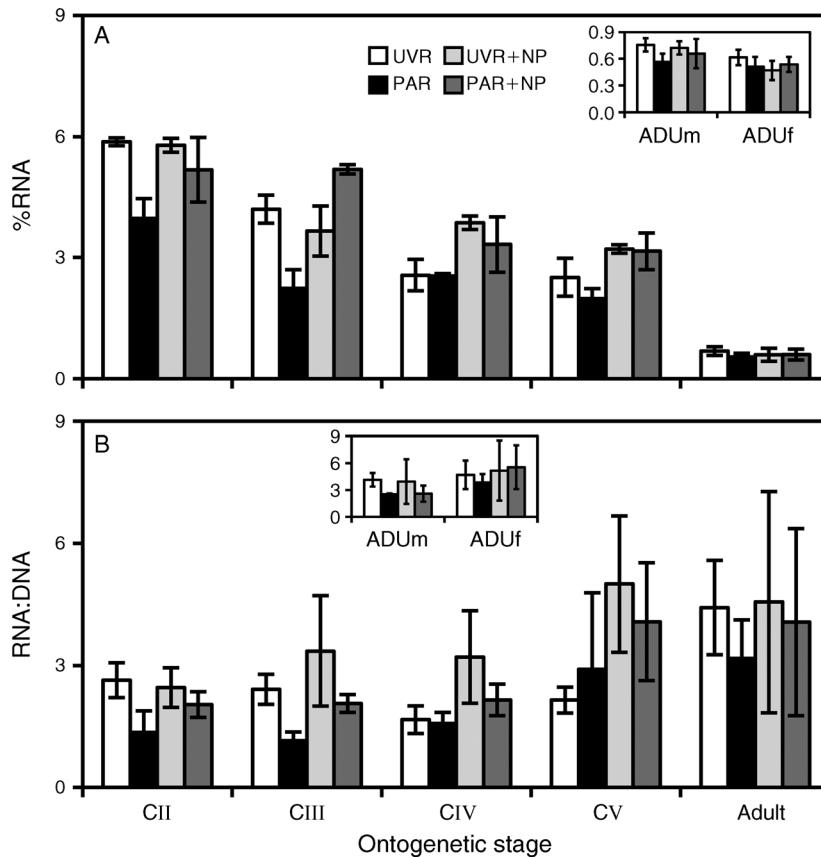


FIG. 5. (A) Individual RNA content (percentage of dry mass, %RNA) and (B) RNA:DNA ratio for each ontogenetic stage of the copepod *Mixodiaptomus laciniatus* in the field light \times nutrient experiment after 1-week incubation. Insets represent adult gender-specific (A) %RNA and (B) RNA:DNA ratio. Treatments are: UVR, full sunlight; UVR+NP, full sunlight + nutrient addition; PAR, screened sunlight (>380 nm); PAR+NP, screened sunlight (>380 nm) + nutrient addition. Columns and error bars represent mean values \pm SD. Stages are copepodites (CII–CV, stages II–V) and adults (ADUm, adult male; ADUf, adult female). See Table 4 and *Results* section for statistical results.

copepods would not be expected to respond to the P and N content of the seston. However, copepods showed strong growth response to food P content at low food quantity, consistent with recent observations of a strong

relationship between *Mixodiaptomus laciniatus* growth and seston P content in this ultraoligotrophic lake (Villar-Argaiz et al. 2012). If copepods with complex life cycles and high C:N:P ratios suffer growth penalties due

TABLE 4. Results of the repeated-measures ANOVAs examining the effects of light (plot factor) and nutrients and ontogeny (subplot factors) on RNA content (percentage of dry mass, %RNA) and RNA:DNA ratio of the copepod *Mixodiaptomus laciniatus*.

Factor	%RNA				RNA:DNA			
	df	F	P	PV	df	F	P	PV
Main plot effects								
Light	1, 4	21.25	0.010	1.70	1, 4	8.90	0.041	10.94
Subplot effects								
Nutrients	1, 4	16.35	0.016	5.15	1, 4	6.68	0.061	10.50
Light \times nutrients	1, 4	6.84	0.059	2.15	1, 4	0.22	0.664	0.35
Ontogeny†	4, 1	23 543.21	0.005	80.00	4, 1	5 256.24	0.010	22.63
Light \times ontogeny†	4, 1	453.85	0.035	1.58	4, 1	1 092.02	0.023	3.75
Nutrients \times ontogeny†	4, 1	141.71	0.063	1.59	4, 1	8.93	0.245	7.70
Light \times nutrients \times ontogeny†	4, 1	1 588.28	0.019	4.38	4, 1	6.28	0.290	3.09

Notes: Sample size was $2 \times 2 \times 5 \times 3$ (light \times nutrients \times developmental stages \times replicates) = 60. The percentage of variance (PV) was calculated as (sum of squares of treatment/total sum of squares) \times 100. Significant effects are shown in boldface.

† Results of the multivariate tests of Wilks, Pillai, Hotelling, and Roy examining the subplot effects of ontogeny and its interactions with light and nutrients on %RNA and RNA:DNA ratio. Significant effects are shown in boldface.

to P deficiency, a much stronger impact could be expected on P-rich species such as *Daphnia*. The considerable research on herbivore nutrition to date has focused on establishing the boundary at which the mineral content of food can slow or limit the growth of consumers (Frost et al. 2006). The present results not only show that thresholds may vary ontogenetically but also indicate that consumer growth is sensitive to an excess of nutrient content in the diet.

Are unimodal responses common in nature? Accumulating evidence indicates that this may be the case. For example, Marañón et al. (2013) showed that the unimodal size scaling of phytoplankton is associated with size-related nutrient constraints. Similar nonlinear responses of plankton to global change-related abiotic stressors were also recently reported (Bullejos et al. 2010, Medina-Sánchez et al. 2013). The unimodal fits documented here are consistent with trends reported by Plath and Boersma (2001), Elser et al. (2005), and Boersma and Elser (2006), who found that consumer growth or overall performance was maximal at intermediate nutrient contents, but was reduced toward the lower and higher ends of the nutrient gradient. Therefore, a nonlinear response of consumers to nutrient limitation may be the rule rather than merely a common phenomenon. In addition, our results provide the first evidence of differences in the unimodal consumer response to food quality in natural conditions within a given species and within a developmental stage.

We further argue that exploring derivatives of the quadratic function has a high potential for analyzing the nutritional ecology of consumers. The first derivative is a useful tool to estimate optimum consumer nutrient demands. In addition, the direct relationship between $x_{\text{optC:nut}}$ and organismal size in this study reflects changes in the specific element requirements throughout the life history of the copepod. Thus, the increasing ontogenetic trend in its optimum $x_{\text{optC:nut}}$ is consistent with previous findings of a higher overall demand for C in adults that can divert their C sources to maintenance and reproduction (Villar-Argaiz et al. 2002). The peakedness of the function provides a powerful indication of consumer sensitivity to deviations in food quality. Thus, our observation of a decrease in growth sensitivity to food quality from early stages to late copepodite and adult stages is consistent with reports of a marked ontogenetic variation in copepod sensitivity to P food quality, from a higher sensitivity in nauplii and immature copepodites to a lower sensitivity at more mature stages (Villar-Argaiz and Sterner 2002).

Natural selection operates on life history to maximize individual fitness. Hence, the selective pressure imposed by limiting nutrients in nature might be expected to determine the organism's adaptive strategy, coupling the life history with nutrient availability in order to minimize the elemental mismatch in autotroph-herbivore interactions. Conceivably, the ontogenetic variations described here might be sustained by fluctuating

selection (Reznick et al. 2000), consistent with the fairly predictable seasonal variation in resource quality for herbivorous consumers. Thus, nutrient-rich algae (low seston C:N:P ratios) regularly grow early in the season (ice-out in high-mountain lakes), coinciding with the development of high-growth nauplii with elevated nutrient requirements. Nutrient limitation in resources is not likely to occur toward the end of the growing season, because populations consist of copepodites and adults with lower growth rates and nutrient demands (Villar-Argaiz et al. 2001). Søreide et al. (2010) observed a predictable variation in consumer life history with the biochemical quality of its resource in Arctic marine ecosystems and suggested that these types of selection constraints may extend to other food quality parameters and environments.

UVR also contributes to the qualitative yield of primary producers, consistent with reports of UVR-induced changes in food quality (e.g., Xenopoulos et al. 2002, Carrillo et al. 2008, Hessen et al. 2008). The present findings demonstrate that these effects transfer to consumers in nature, especially at early stages in their life history when nutrient demands are high. The fact that experimental suppression of UVR increased seston C:P ratios and dampened copepod growth suggests that UVR may exert more complex effects than previously thought, by reducing the nutritional imbalance at the primary producer-consumer interface. In contrast to the widely documented detrimental effects of UVR (e.g., Helbling and Zagarese 2003, Häder et al. 2007), our study shows that UVR may indirectly enhance copepod growth, possibly by driving seston C:nutrient ratios toward values close to $x_{\text{optC:nut}}$.

Our study shows that optimum C:nutrient ratios vary across the life history of *Mixodiaptomus laciniatus*, a herbivorous consumer that represents the most abundant metazoan group on Earth (Mauchline 1998). To paraphrase Aristotle's statement of the central dogma of the Golden Mean Philosophy, "Virtue is a mean between two vices, that which depends on excess and that which depends on defect" (Aristotle, IV century BC). We here show that this "Golden Mean" is not only species-specific but also a dynamic property of the homeostatic consumer that may vary ontogenetically, decreasing the organism's susceptibility to mineral nutrient deficiency.

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SUPPLEMENTAL MATERIAL

Appendix A

Supplementary study site information, including photographs and information relative to the optical properties of Lake La Caldera in the National Park of Sierra Nevada (Spain) ([Ecological Archives E095-109-A1](#)).

Appendix B

Detailed description of methods for estimating primary production and measuring nucleic acids in zooplankton ([Ecological Archives E095-109-A2](#)).

Appendix C

Schematic diagram illustrating the field split-plot experimental design ([Ecological Archives E095-109-A3](#)).

Appendix D

Supporting figures for the results described in the main text for the interannual study ([Ecological Archives E095-109-A4](#)).