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Effects of food-level acclimation on digestive enzyme activities and feeding behavior of *Calanus pacificus**

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Abstract

A recent hypothesis in the zooplankton literature states that zooplankton acclimate to ambient food concentrations such that higher digestive enzyme activities and, consequently, higher maximum ingestion rates are achieved at higher food levels. To test this hypothesis, adult female *Calanus pacificus*, collected from the main basin of Puget Sound, Washington, USA, in August 1979 and May 1982, were conditioned for 2 wk at different concentrations of the diatom *Thalassiosira weissflogii* (=*fluviatilis*). Ingestion rates and the activity of the digestive enzymes laminarinase, maltase, and cellobiase were measured periodically during acclimation and in a block-designed feeding experiment at the end of acclimation. Consistent with the hypothesis, maximum ingestion rate and digestive enzyme activity were positively correlated. However, in contrast to the hypothesized mechanism, this result arose because both maximum ingestion rate and digestive enzyme activity were negatively correlated with food concentration during acclimation. The enhanced ingestion of copepods following long-term (12 to 14 d) acclimation to low food is similar to that previously described for short-term (e.g. 1 d) starvation. It might be energetically optimal for copepods experiencing a patchy food environment to maintain higher levels of digestive enzymes at low food concentrations in order to exploit high concentrations of food when encountered.

Introduction

Although the feeding of marine copepods has been a focus of considerable research for the past two decades, feeding rates, relationships, and behavior under natural conditions are, even for the best-studied species, largely speculative. As a case in point, there is a remarkable diversity of opinion regarding the most basic of feeding interactions: namely, response to food concentration. Various investigators have implied that copepods are either never (e.g. McLaren, 1978), sometimes (e.g. Frost, 1974), or always (e.g. Poulet, 1974; Huntley, 1981) food-limited in nature. In the present paper, we examine one of the hypotheses generated by these divergent viewpoints – that copepods acclimate behaviorally and biochemically to food concentration.

Evidence from laboratory studies generally supports a relationship between copepod ingestion rate (biomass consumed copepod^{-1} d^{-1}) and food concentration characterized by a critical or incipient food concentration above which ingestion rate is constant and maximum (e.g. Mullin, 1963; Frost, 1972, 1977): this is termed a saturation response. Below the critical concentration, there is a range of food concentration over which ingestion rate increases approximately linearly with increasing food, i.e., where volume clearance rate per individual is constant. Above the critical concentration, the food handling or processing capabilities of the copepod appear to be saturated, as clearance rate (volume cleared copepod^{-1} d^{-1}) decreases monotonically with increasing food.

A saturation response for copepods in nature was brought into question by the results of studies of the seasonal feeding and biochemistry of field populations. Poulet (1974), for instance, observed that ingestion rate was linearly related to food abundance when copepods were fed natural particulates at ambient concentrations (see also Huntley, 1981). The range of ambient particulate carbon concentrations covered the range of phytoplankton carbon used in the laboratory studies. In addition, Mayzaud and Conover (1976) found that seasonal changes in the activities of digestive enzymes of mixed zooplankton were positively correlated with changes in the abundance of particulate matter. These observations form the basis for an acclimation hypothesis of copepod feeding behavior

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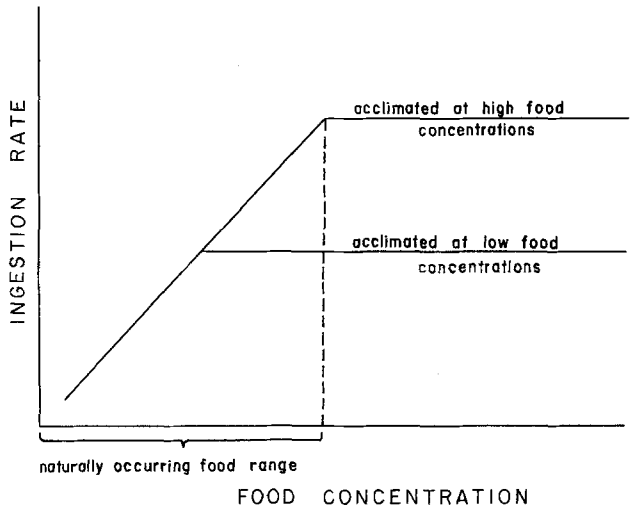

Fig. 1. Expected relationships between ingestion rate and food concentration for copepods acclimated to high and low food levels according to the acclimation hypothesis of Mayzaud and Poulet (1978)

– that digestive enzymes of copepods acclimate to the level of available food such that ingestion is directly proportional to the ambient concentration of food (Mayzaud and Conover, 1976). According to the acclimation hypothesis, the saturation response observed in the laboratory can be an artifact of short-term experiments when copepods acclimated to relatively low food concentrations in nature are exposed to high food levels exceeding their digestive capability (Conover, 1978; Mayzaud and Poulet, 1978). More significantly, the acclimation hypothesis implies that the recent food environment of a copepod will be reflected in the activity of the copepod's digestive enzymes and, thus, that enzyme analyses of field-collected individuals might provide useful insights into the quantity and quality of food in the marine environment and the temporal and spatial scales of feeding interactions (e.g. Cox, 1981).

The acclimation hypothesis yields three predictions regarding the behavior or biochemistry of copepods acclimated to different food levels. The first prediction is that the feeding capacity (i.e., maximum ingestion rate at high food concentration) should be positively related to food concentration during acclimation (Fig. 1). The second prediction is that digestive enzyme activities of copepods should be positively correlated with food abundance during acclimation. The third prediction is that, at a given high concentration of food, ingestion rates should be positively correlated with digestive enzyme activity. In this paper, we describe results of experiments designed to test the predictions of the acclimation hypothesis for the copepod *Calanus pacificus*.

Materials and methods

Adult female specimens of *Calanus pacificus* were collected from the main basin of Puget Sound, Washington, USA, in late August, 1979, and subjected to an acclimation experiment in two phases. In the first phase, groups of copepods were acclimated to different food concentrations for 12 d. This was followed by a short-term, block-designed grazing experiment. Both phases of the experiment were conducted at 12 °C and in constant dim light. Cultures of the diatom *Thalassiosira weissflogii* (=*fluviatilis*) were used as food.

During the acclimation phase of the experiment, groups of 160 copepods were maintained at 4 concentrations of food particles. Initially, cell densities of 500, 1 000, 2 000, and 4 000 *Thalassiosira weissflogii* ml^{-1} were selected (1 000 cells ml^{-1} = approximately 100 μg C l^{-1}), but the food levels were increased to 1 000, 2 000, 4 200, and 6 600 cells ml^{-1} when *Calanus pacificus* showed much reduced clearance rates at 500 cells ml^{-1} (see Frost, 1975). The culture containers, 26-liter glass jars, were continuously stirred with a rotating plunger (as in Frost, 1972; Vidal, 1980) to keep cells in suspension. Settled material, fecal pellets and copepod eggs were siphoned from the bottom of the containers once a day. The discarded water, about 15% of container volume, was replaced with filtered sea water and fresh food.

Concentrations of algal cells were maintained within about ± 15% of desired levels by monitoring particle densities twice daily using a Coulter Counter (Model TAII). Copepod feeding rates were calculated from changes in cell densities between sampling times. Algal growth rates (i.e., grazing controls) were determined from changes in cell densities in samples of water (without copepods) drawn from the acclimation containers and incubated in replicated 1 000 ml beakers under similar conditions. Calculations were according to the equations of Frost (1972).

Subsamples of the copepod populations in the acclimation containers were taken on Days 0, 5, 8, and 12 for determination of dry weights and digestive enzyme activities. The copepods were quick-frozen in liquid nitrogen, freeze-dried, and stored at – 40 °C for later analysis.

The second phase of the experiment, performed on Day 13, involved constructing a functional response relationship for each acclimation condition by exposing copepods acclimated to given food levels to all four concentrations of food used during acclimation. Fifteen 500 ml jars were prepared for each food concentration (1 000, 2 000, 4 200, 6 600 cells of *Thalassiosira weissflogii* ml^{-1}). Six copepods from each acclimation condition were added to each of 3 jars, and the remaining 3 jars served as grazing controls. The jars were attached to a rotating wheel and the copepods were allowed to feed for 10 h. Ingestion and clearance rates were determined from the difference in initial and final particle counts corrected for algal growth rates (Frost, 1972). The copepods in each jar were killed in liquid nitrogen and saved for later analyses as described above.

A second acclimation experiment with some modifications was conducted with *Calanus pacificus* females collected in May, 1982. Groups of copepods were acclimated to concentrations of 500, 1 000, 2 000, 4 000, and 8 000 cells

of *Thalassiosira weissflogii* ml^{-1} for 2 wk by continuous, controlled injection of algal cultures into 16-liter holding containers with a peristaltic pump. No sampling of the copepod population was done during this phase. Grazing experiments were then run with copepods from each acclimation condition feeding at 1 000, 2 000, 5 200, and 6 000 cells ml^{-1}. Five replicates for each condition and control were prepared; otherwise, the experiment was identical to that described above. Following the grazing experiments, the remaining acclimated copepods were starved in filtered water for 36 h to test the effect of the acclimation conditions on post-starvation ingestion rates. Previous experiments (e.g. Frost, 1972; Runge, 1980) have indicated that *C. pacificus* ingests at higher rates following short-term (on the order of 1 d) starvation. The ingestion rates of these individuals were measured at a food concentration of 5 200 cells ml^{-1}.

Copepods from the acclimation experiments were weighed dry on a Cahn Model 25 Electrobalance. They were then analyzed for activities of three digestive enzymes, laminarinase (β-1,3 glucanase), cellobiase (β-glucosidase), and maltase (α-glucosidase). Laminarinase was selected because of the importance of β-1,3 glucan as a storage product in phytoplankton (Handa and Tominaga, 1969), while the latter two enzymes vary seasonally in activity in field populations (Mayzaud and Conover, 1976). The enzymes were assayed according to the procedures of Hassett and Landry (1982). Briefly, individual copepods or groups of copepods were ground in Tris buffer, pH 8.1, and centrifuged. Aliquots of the crude extract were removed, added to the substrates (2 mg ml^{-1} laminarin, 1 mg ml^{-1} cellobiose, and 5 mg ml^{-1} maltose, all in pH 5.7 acetate buffer), and incubated at 20 °C for either 45 min (laminarinase) or 90 min (cellobiase and maltase). The reaction was stopped by heating to 95° for 2 min. The glucose produced was measured fluorometrically on a Turner model 110 fluorometer by an enzymatic reaction with NADP+ to produce the fluorescent product NADPH (Lowry and Passonneau, 1972). Activities were expressed as μg glucose released copepod^{-1} h^{-1}.

Results

Long-term changes in the feeding behavior of *Calanus pacificus* were not observed during 12 d of acclimation to constant food conditions. Consequently, all of the feeding estimates at each food level were grouped to compute mean ingestion and clearance rates (Fig. 2). These rates are almost identical to those reported by Frost (1972) for *C. pacificus* feeding on various concentrations of *Thalassiosira weissflogii* and are consistent with a saturation response to increasing food concentration. The relatively low clearance rates at 500 cells ml^{-1} represents a reduced feeding effort at very low food concentrations (Frost, 1975) which is thought to be an energy optimizing strategy (e.g. Lam and Frost, 1976).

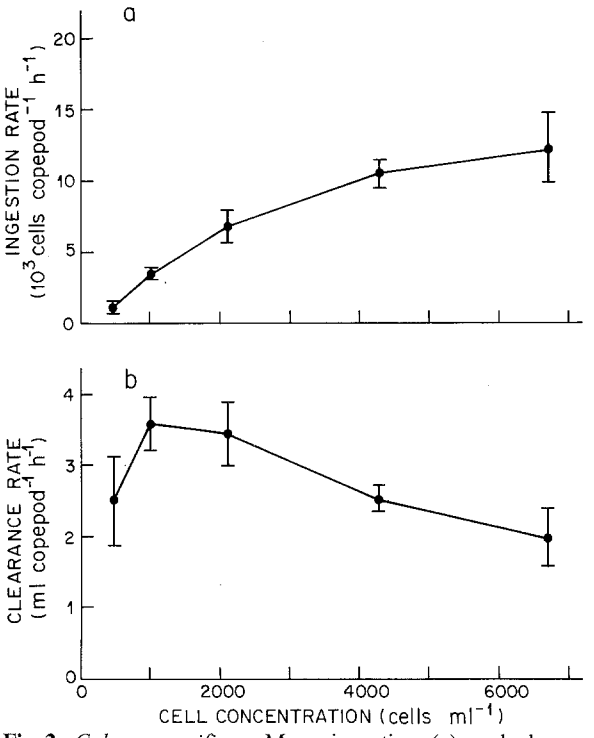

Fig. 2. *Calanus pacificus.* Mean ingestion (a) and clearance (b) rates measured during 12 d of acclimation to different cell densities of the diatom *Thalassiosira weissflogii.* Vertical lines denote 95% confidence intervals

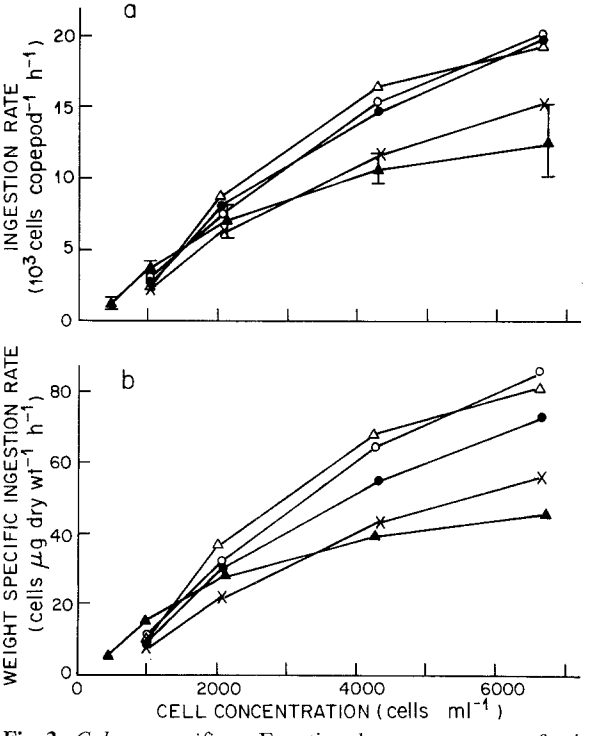

Fig. 3. *Calanus pacificus.* Functional response curves for individuals previously acclimated to constant cell densities of *Thalassiosira weissflogii* for 12 d (Experiment 1); (a) = ingestion rate per copepod, (b) weight-specific ingestion rate. ▲: mean rates during 12 d acclimation period (i.e., Fig. 2 a data). Acclimation conditions were: 1 000 (○); 2 000 (△); 4 200 (●); and 6 600 (×) cells ml^{-1}. Each point is mean of three replicates. For clarity, confidence intervals are not shown; statistically significant differences are discussed in text

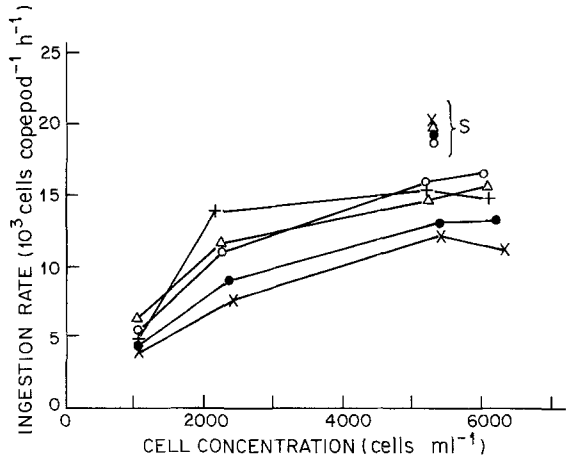

Fig. 4. *Calanus pacificus.* Functional response curves for individuals previously acclimated to constant cell densities of *Thalassiosira weissflogii* for 14 d (Experiment 2). Acclimation conditions: 500 (○); 1 000 (+); 2 000 (△); 4 000 (●); and 8 000 (×) cells ml^{-1}. Symbols denoted by "S" are ingestion rates following 36 h starvation. Each point is mean of 5 replicates

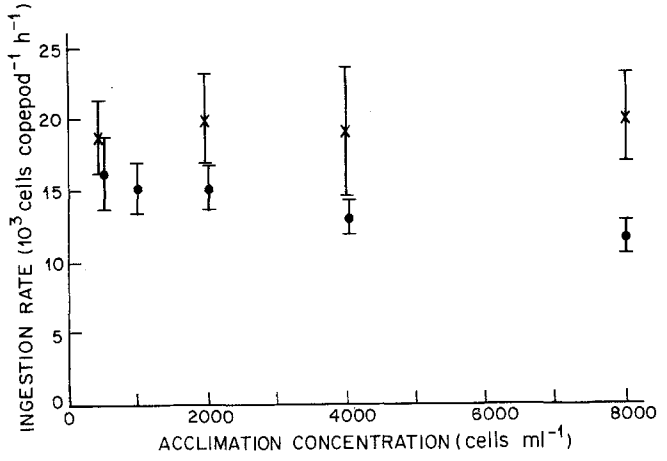

Fig. 5. *Calanus pacificus.* Ingestion capacity at high food concentration (5 200 to 6 000 cells ml^{-1}) versus previous acclimation conditions before (●) and following (×) 36 h starvation. Vertical bars denote 95% confidence intervals for 5 to 10 replicates

The functional response curves for copepods acclimated to different food concentrations are presented in Fig. 3 a. Copepods acclimated to the 3 lowest food concentrations had significantly ($p < 0.01$, Kruskal-Wallace test) higher ingestion rates at 4 200 and 6 600 cells ml^{-1} than individuals acclimated to the highest food level. Ingestion rates of copepods acclimated to the 3 lowest food levels also departed significantly from the long-term mean for copepods fed at constant food concentrations. Because the acclimation condition affected the weight of the copepods used in the second phase of the experiment (Table 1), the functional response curves were recast as weight-specific ingestion rates (Fig. 3 b). This manipulation reduced ingestion rates for individuals acclimated to 4 200 cells ml^{-1} relative to the other functional response curves, but did not alter the statistical differences found above.

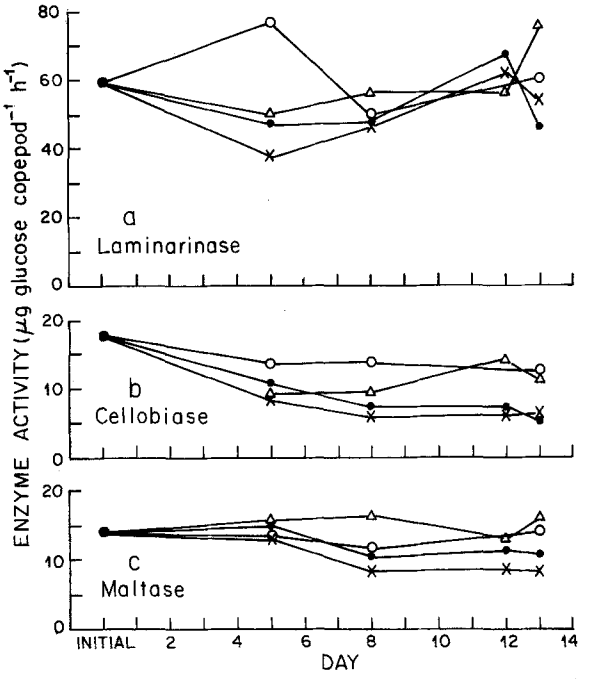

Fig. 6. *Calanus pacificus.* Changes in activities of three digestive enzymes during acclimation to different food concentrations of *Thalassiosira weissflogii.* Acclimation conditions: 1 000 (○); 2 000 (△); 4 200 (●); and 6 600 (×) cells ml^{-1}. Initial value is for mean of 48 copepods assayed in groups of 6. Activities on other sampling dates are for 12 to 18 copepods assayed individually (Days 8, 12, and 13) or as a group (Day 5)

Table 1. *Calanus pacificus.* Mean dry weights (μg copepod^{-1}) and confidence limits (CL) for adult females prior to (field-collected) and following 12 d of acclimation to different food concentrations of *Thalassiosira weissflogii* (cells ml^{-1})

Acclimation condition (cells ml^{-1})	Copepod dry wt (μg)	95% CL
Initial	211	203 – 219
1 000	234	200 – 248
2 000	240	221 – 259
4 200	270	254 – 286
6 600	273	261 – 285

A similar pattern for ingestion rates emerged for the second acclimation experiment conducted in May, 1982. Copepods acclimated to low food concentrations tended to feed at higher rates at a given food level than individuals acclimated to high food concentrations (Fig. 4). By the Kruskal-Wallace test, this trend was significant at concentrations of 2 000 ($p < 0.05$) and 6 000 cells ml^{-1} ($p < 0.025$), but not at 5 200 cells ml^{-1} although the pattern remained consistent. Post-starvation (36 h) ingestion rates were significantly greater than the rates for acclimated copepods feeding at the same concentration without a starvation period (Fig. 5), except for copepods acclimated at 500 cells ml^{-1} (Wilcoxon two-sample test, $p < 0.025$).

Table 2. *Calanus pacificus.* Effects of short-term changes in food concentration on digestive enzyme activities. Copepods acclimated to 4 concentrations of *Thalassiosira weissflogii* (cells ml^{-1}) for 12 d were exposed to each of 4 feeding concentrations for 10 h. For a given acclimation concentration, the Kruskal-Wallace tests was used to determine significant trends for copepods at different feeding concentrations. Rank orders of enzyme activities for copepods of different feeding concentrations were determined by the Wilcoxon paired test (">" denotes significance at $p < 0.05$; "," is nonsignificant)

Enzyme	Acclimation concentration (cells ml^{-1})	Trend (Kruskal-Wallace)	Rank (Wilcoxon)
Laminarinase	1 000	NS	–
	2 000	$p < 0.01$	1 000, 2 000 > 4 200, 6 600
	4 200	NS	–
	6 600	NS	–
Cellobiase	1 000	NS	–
	2 000	NS	–
	4 200	NS	–
	6 600	NS	–
Maltase	1 000	NS	–
	2 000	$p < 0.005$	2 000 > 4 200
	4 200	NS	–
	6 600	$p < 0.05$	4 200 > 1 000

Table 3. *Calanus pacificus.* Effects of long-term food acclimation on digestive enzyme activities. Copepods acclimated to 4 concentrations of *Thalassiosira weissflogii* (cells ml^{-1}) for 12 d were exposed to each of 4 "feeding concentrations" for 10 h. For a given feeding concentration, the Kruskal-Wallace test was used to determine significant trends in enzyme activities for copepods of different acclimation histories. Rank orders of enzyme activities for copepods of different histories were determined by the Wilcoxon paired test (" > " denotes significance at $p < 0.05$; "," = nonsignificant)

Enzyme	Feeding concentration (cells ml^{-1})	Trend (Kruskal-Wallace)	Rank (Wilcoxon)
Laminarinase	1 000	NS	–
	2 000	$p < 0.005$	1 000, 2 000 > 4 200, 6 600
	4 200	NS	–
	6 600	NS	–
Cellobiase	1 000	$p < 0.01$	1 000, 2 000 > 4 200, 6 600
	2 000	$p < 0.005$	2 000 > 1 000 > 4 200 > 6600
	4 200	$p < 0.005$	1 000 > 2 000 > 4 200, 6 6000
	6 600	$p < 0.005$	1 000, 2 000 > 4 200, 6 600
Maltase	1 000	$p < 0.025$	1 000, 2 000, 4 200 > 6 600
	2 000	$p < 0.01$	1 000, 2 000, 4 200 > 6 600
	4 200	$p < 0.05$	1 000 > 6 600
	6 600	NS	–

There was no effect of acclimation condition on post-starvation ingestion rates.

Changes in the activities of digestive enzymes during the acclimation experiment were assessed in terms of (1) potential short-term and long-term effects during the 12 d acclimation to constant food level, and (2) potential short-term effects induced over the course of the 10 h grazing phase (i.e., when individuals acclimated to given food levels were fed at different food concentrations). For the enzymes cellobiase and maltase, there is little trend in the response of acclimated copepods to short-term food conditions (Table 2). Although not highly significant, the data for laminarinase suggest a more consistent short-term response to food; namely, that laminarinase activity tends to be inversely related to food concentration (Table 2). Over the long-term, acclimation to food concentration had a pronounced effect on activity levels of cellobiase and maltase; in both cases copepods had higher enzyme activities when acclimated to lower food concentrations (Table 3; Fig. 6). For given acclimation conditions, no significant differences were found for cellobiase and maltase activities measured on Days 8, 12, and 13, indicating that the observed acclimation response of the digestive enzymes occurred within a week. A clear, long-term acclimation of laminarinase activity was not apparent. However, in the one instance where a significant effect was observed

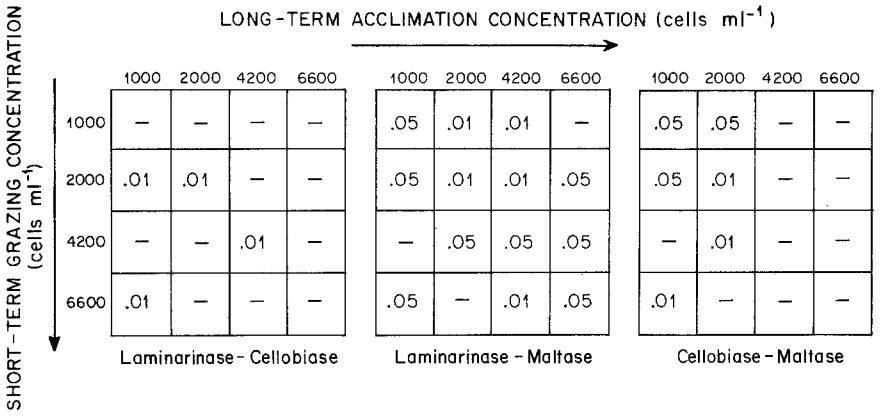

Fig. 7. *Calanus pacificus.* Cross-correlations for pairs of digestive enzymes from copepods acclimated to 4 food concentrations for 12 d and fed at 4 concentrations for 10 h. Numbers in boxes are level of significance for Spearman rank correlation test

(i.e., at 2 000 cells ml^{-1}; Table 3), the trend toward an inverse relationship between enzyme activity and food concentration was consistent with the short-term observations for laminarinase and the long-term results for cellobiase and maltase. The significance of the high activity of laminarinase at the 1 000 cell ml^{-1} condition on Day 5 (Fig. 6) is unknown since it was based on a single, group analysis of 12 individuals. The data point (1) could simply be aberrant, although cellobiase and maltase activities measured on the same group of copepods are not, or (2) could reflect a real response to low food concentration that occurs on the order of a few days, possibly the result of initial feeding (until Day 3) at 500 cells ml^{-1}.

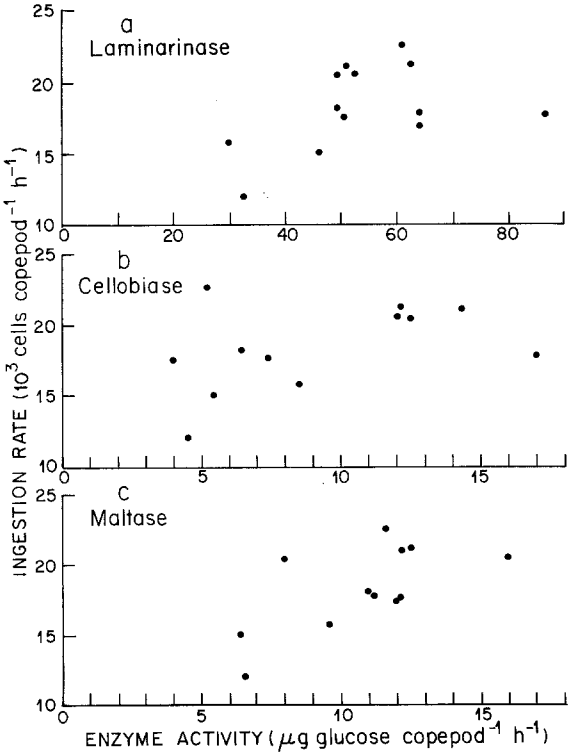

Fig. 8. *Calanus pacificus.* Relationship between ingestion capacity at high food concentration (6 600 cells of *Thalassiosira weissflogii* ml^{-1}) and activities of three digestive enzymes: (a) laminarinase, r_s=0.57, NS – Spearman rank correlation; (b) cellobiase, r_s=0.43, NS; (c) maltase, r_s=0.66, significant at $p < 0.05$ level

There was some indication of a positive correlation among the enzyme activities in individual copepods (Fig. 7). Laminarinase and maltase activities were significantly correlated in 13 of the 16 samples. However, significant correlations of cellobiase activity with the activity of either of the other two enzymes were less frequent (although r was almost always positive) and were probably attributable to the low activity of cellobiase for individuals acclimated at high food concentrations.

For *Calanus pacificus* acclimated to different food concentrations then fed at a high food density (6 600 cells ml^{-1}), ingestion rates were positively correlated to digestive enzyme activities (Fig. 8). The relationship was statistically significant (i.e., $p < 0.05$; Spearman rank test) for maltase, but marginally significant ($0.05 < p < 0.10$) for laminarinase.

Discussion

The acclimation hypothesis suggests that copepods acclimate behaviorally and biochemically to ambient food concentration such that individuals exposed to more food have increased digestive enzyme activity and, therefore, enhanced feeding capacity. Our experiments confirm that feeding behavior and digestive enzyme activities can change in response to different food concentrations; however, the results contradict the acclimation hypothesis in two important ways. First, copepods acclimated to lower, not higher, food concentrations exhibit higher maximum ingestion rates. Second, lower, not higher, activities of digestive enzymes are associated with individuals acclimated to high food conditions. This second result is consistent with that of Fong and Parsons (unpublished data) on the marine amphipod *Eogammarus confervicolus.* They found lower activities of the same three carbohydrases that we studied, as well as trypsin and cathepsin-like proteases, when *E. confervicolus* was exposed to high food concentrations.

The results of the present study agree with past laboratory investigations (e.g. Frost, 1972; Runge, 1980) in that short-term starvation of *Calanus pacificus* results in an

elevated ingestion rate. Frost (1972) found that the functional response curve for starved copepods departed from that for fed copepods only at food levels above the critical concentration where ingestion rates for well fed copepods are saturated (about 4 000 cells ml^{-1} of *Thalassiosira weissflogii*). Runge (1980), however, found that prior starvation also led to increased ingestion rates at lower food concentration. Our results suggest that enhanced feeding of copepods following short-term starvation is merely one aspect of the potential continuum of acclimation responses to different food concentrations. As in Runge (1980), ingestion rates at food concentrations both above and below the critical concentration are affected by acclimation condition. Short-term starvation (i.e., 36 h) apparently overrides the effects of long-term feeding acclimation (Fig. 5).

The acclimation hypothesis as proposed by Mayzaud and Poulet (1978) implicitly assumes a significant energetic cost of maintaining high enzyme activity when food is scarce. The tendency for digestive enzyme activities of low-food acclimated *Calanus pacificus* to be higher than (cellobiase and maltase) or equal to (laminarinase) those for individuals acclimated to high food is clearly contrary to this assumption. We suspect that, as demonstrated for the energetic cost of swimming during vertical migration (e.g. Vlymen, 1970), the cost of enzyme production, maintenance, or storage may be trivial relative to basal metabolism or may be offset by other energetic considerations. McAllister (1970) and Enright (1977), for instance, have argued that starvation-enhanced ingestion rates may be energetically important to zooplankton migrating into and out of high food concentrations on a daily cycle. Our results suggest an underlying relationship between higher enzyme activity and higher potential ingestion rate for *C. pacificus:* maintenance of high levels of digestive enzyme activity during periods of low food availability could be in anticipation of future feeding opportunity. Thus, if the potential for enhanced-ingestion is greater than the cost of enzyme maintenance, our results would be consistent with an energy-optimizing strategy for zooplankton living in a patchy food environment.

Differences in the response of digestive enzymes to food conditions might be expected for copepod species with differing life histories (migratory vs non-migratory) or inhabiting environments with differing scales of food variability. Dadd (1956), for example, found that proteases of a predatory aquatic beetle, *Dytiscus marginalis,* accumulated in midgut cells during starvation, to be released within an hour after feeding. In contrast, another beetle, *Tenebrio molitor,* evidently synthesized and secreted proteases continuously, accelerating secretion during feeding, but with no accumulation during starvation. The former beetle encounters food on a periodic basis, while the latter, a flour beetle, lives continuously in the substrate upon which it feeds.

Differences in the activities of digestive enzymes among copepods acclimated to different food levels may be due to storage of enzymes at low food concentrations, decreased production of enzymes at high food concentrations, or a combination of both. It is not known whether storage of digestive enzymes occurs in copepods. Arnaud *et al.* (1978) and Hallberg and Hirche (1980) identify vacuolated cells in mid-gut epithelium of several calanoid species which they associate with enzyme production. If a feeding stimulus is required for the expulsion of the vacuolar contents, then these vacuoles may allow a temporary storage. Activities of cellobiase and maltase, but not laminarinase, declined substantially at higher acclimation conditions in the present study. Similarly, Mayzaud and Poulet (1978) found that, among 6 digestive enzymes tested, laminarinase had the weakest correlation with substrate concentration in natural particulate matter. The reason for differences in the response of the different enzymes is unclear.

A possible mechanism by which enzyme production could be reduced with increasing ingestion rate, as would occur during acclimation to high food concentration, is suggested by the relative simplicity of the copepod digestive system. Both enzyme production (B-cells) and digestive product absorption (R-cells) occur in epithelial cells lining the copepod gut. Arnaud *et al.* (1978) noted a third cell type (D-cell) in the gut lining of *Centropages typicus* which they interpreted as an R-cell suppressed by the development of B-cells. Similarly, Hallberg and Hirche (1980) reported a positive correlation between the number of B-cells and level of enzyme activity for *Calanus finmarchicus* and *C. helgolandicus,* suggesting that increased enzyme activity occurs when B-cells increase and R-cells are suppressed. Thus, a balance may be required between enzyme production and assimilation capabilities. Under high-food conditions, i.e., saturated capacity of absorptive cells, reduction of enzyme production might increase net assimilation (perhaps with more important enzymes, e.g. laminarinase, being favored). Under low-food conditions fewer absorptive cells would be needed, and more B-cells could be produced, either to allow more complete digestion of consumed material (greater diversity in enzymes) or for storage of enzymes in anticipation of future increases in food. An important question is the time scale on which such changes in the distribution of gut cells might occur. Our data are consistent with a time scale of greater than a half day but less than a week for changes in enzyme activity, approximately the same time scale suggested by Mayzaud and Poulet (1978) for acclimation of digestive enzymes. A response time greater than a half day would be necessary for vertical migrators to adapt to the long-term mean since they experience considerable variation on a diel basis.

We can think of several explanations for the negative correlations of food concentration and enzyme activity found in the present study and the positive relationships suggested by others. One possibility, of course, is that the discrepancy reflects differences among zooplankton species analyzed. For instance, Mayzaud and Conover (1976) and Mayzaud and Poulet (1978) investigated the digestive enzymes of small, nonmigrating species while the experi-

ments reported here deal with a large, migrating species. As pointed out previously, differences in the life history strategies of various species or the variability of the food environment in which they live may determine the optimal response of their digestive enzymes to changes in food quantity or quality.

Another possibility is that enzyme studies of natural, mixed populations of zooplankton, rather than single species, might be biased by seasonal shifts in dominance among species. Mayzaud (1980) demonstrated considerable variability in weight-specific enzyme activities among 4 dominant small copepods in Bedford Basin, Canada, *Acartia clausii*, for example, having enzyme activities one-half to 3 times greater than *Pseudocalanus minutus* for most enzymes studied. The peak in weight-specific enzyme activity observed for the mixed field population by Mayzaud and Conover (1976) corresponded to a late July–early August shift in species dominance from *P. minutus* to *A. clausii* (Conover and Mayzaud, 1976). Carbohydrate concentration in particulate matter was also at the summer maximum during this period. Conceivably, then, the observed correlations between weight-specific enzyme activities and various components of particulate matter could be a secondary result of a correlation between species composition and the quantity or quality of available food.

Cox and Willason (1981) reported that laminarinase activity of *Calanus pacificus* declined significantly when the copepods were starved for 1 to 4 d. Although their experimental design is not directly comparable to ours, their result is contrary to our expectations regarding the short-term starvation response. Since their starved copepods suffered increasing levels of mortality after 4 d and all died by Day 6, we think that their specimens may have been in an unusually poor physiological state, perhaps due to conditions in the field prior to capture. In our experience, *C. pacificus* is highly tolerant of starvation, females surviving 2 to 3 wk without food. We would not expect the digestive enzyme levels of dying individuals to behave normally.

Finally, we suggest that data supporting the negative relationship between food concentration and digestive enzyme activity reported here may have, in the past, been ignored or misinterpreted. Cox (1981), for instance, found a significant negative correlation between laminarinase activity of net zooplankton and chlorophyll *a* in the Santa Barbara Channel, USA. However, he also noted a negative correlation between enzyme activity and zooplankton biomass and raised the possibility that the low chlorophyll *a* concentrations may have indicated recent heavy grazing pressure, and hence high enzyme levels.

In summary, in agreement with the predictions of the acclimation hypothesis, the maximum ingestion rate of *Calanus pacificus* shows a positive (though not always significant) correlation with digestive enzyme activity. However, this feature is not the result of positive correlations of maximum ingestion rate and digestive enzyme activity with food concentration during acclimation, as predicted by the hypothesis. Rather, it is the result of two negative correlations: between maximum ingestion rate and acclimation food level on the one hand, and between digestive enzyme activity and acclimation food level on the other. Therefore, while digestive enzyme activity of *C. pacificus* might indicate the feeding potential of an individual, the enzyme activity is not a good measure of the food environment experienced by the copepod at the time of sampling. Whether our results are applicable to other species may in part depend on life history strategies of the species involved, specifically the degree to which vertical migration or food patchiness present a copepod with sudden extremes in food concentration.

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