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Medium term time acclimation of feeding and digestive enzyme activity in marine copepods: influence of food concentration and copepod species

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ABSTRACT: Inter-species differences in the acclimation strategy of the nutritional processes (ingestion, digestive enzyme activity) of female and copepodite stage V copepods were established experimentally for 3 different species: *Acartia clausi*, *Eurytemora herdmani*, and *Drepanopus pectinatus*. In all experiments food was supplied over 4 to 7 d at 2 limiting concentrations of the cultured diatom *Skeletonema costatum*. The first 3 experiments confirmed earlier results suggesting that under food limiting conditions, *Acartia*-type copepods (low level of energy reserve, high turnover of its biomass) displayed positive acclimation of both ingestion rate and digestive system. Under the experimental conditions used, both *A. clausi* and *E. herdmani* required 48 h to significantly acclimate their digestive activity to changes in food ration exceeding 1 µg C copepod^{-1}. Smaller but sustained differences in food ration could result in similar behaviour but seem to require a longer time period to show significant acclimation. Despite larger food rations, experiments with the lipid-rich species *D. pectinatus* failed to show any acclimation of digestive enzyme activities, except for individuals collected before a summer bloom which displayed partial positive acclimation of their trypsin activity. These inter-species differences agree with the hypothesis that internal control of the acclimation process is governed by the metabolic requirement of the animals. In addition, the differential response of *D. pectinatus* collected before and after a phytoplankton bloom suggests an important role for past feeding conditions (physiological memory), thus explaining the variability in responses between natural field conditions and laboratory conditions. Physiological adaptation is proposed as a mechanism which minimizes the effect of stressful trophic conditions.

INTRODUCTION

Since the early reports by Mayzaud & Poulet (1978) and Donaghay & Small (1979) on time acclimation of digestive and feeding responses, subsequent studies have reinforced the hypotheses that these responses may be more influenced by past conditions than by immediate changes in environmental conditions (Donaghay 1988, Huntley 1988). The concept of feeding history is usually understood in terms of overall history of past feeding conditions and little emphasis has been given to the metabolic characteristics of the organism and their role in the general acclimation process of the nutritional system.

The results of time acclimation of feeding and digestion have been reviewed by Donaghay (1988) who suggested that major species differences existed in feeding behaviour which could not be related directly to food concentration and size. He suggested that different species should react in different ways to temporal and spatial variation in the quantity and/or the quality of their food resources and adapt their behaviourial and physiological responses. Such differential responses raise the question of the role of the metabolic requirements which are known to differ with species and environments (Båmstedt 1986).

Most recent work suggests that the obvious inter-species differences in acclimation strategy could be extended to the time acclimation of a given species subjected to variable trophic conditions. Seasonal adaptation of the maximum feeding rates, first postulated by Mayzaud & Poulet (1978) for various neritic species, was later described by Runge (1980) and Hassett & Landry (1990a) for species as different as *Acartia clausi* and *Calanus pacificus* with or without concurrent changes in digestive enzyme activity. The

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relationship between changes in maximum ingestion rates and digestive enzyme activities was tested experimentally by Hassett & Landry (1983) using *Calanus pacificus* and Roche-Mayzaud et al. (1991) with *Acartia clausi*. Both studies concluded that the 2 systems operated on different time scales. Ingestion acclimated within a few hours while digestive enzymes dampened short-term food variations and required at least 48 h under the experimental conditions used by Roche-Mayzaud et al. (1991).

The controversy over the pattern of digestive acclimation response has been largely clarified by Roche-Mayzaud et al. (1991) who showed that positive acclimation of small neritic copepod digestive enzymes (i.e. increase in enzyme activity in agreement with the food ingested) occurred in response to food limitation (suboptimal growth) while food saturation resulted in a constant or decreasing digestive potential as reported by Harris et al. (1986). Aside from feeding conditions, they suggested also that nutrition was probably controlled by feed-back phenomena which operate to meet the organism's metabolic requirements. Thus acclimation was linked to species-specific metabolic characteristics and life history.

Our objective in this study was to confirm the existence of feeding and digestive acclimation for different species and to address 2 questions which emerge from the above considerations: First, are different copepod species with different metabolic characteristics (size specific energy expenditure or respiration rate) displaying similar patterns of acclimation? Second, does past trophic history modify the acclimation response?

MATERIAL AND METHODS

Three copepod species from 2 different sites were used in the present study: *Acartia clausi* and *Eurytemora herdmani* from the St. Lawrence estuary (Canada), and *Drepanopus pectinatus* from the subantarctic Kerguelen archipelago (South Indian Ocean). These 3 coastal species differ in their respective size and ability to store large amounts of lipid reserves. Late copepodites and the female *A. clausi* and *E. herdmani* weighed 5 to 8 µg each, and did not store high levels of neutral lipids (10 to 15 % dry weight). Similar stages *D. pectinatus* weighed 35 to 40 µg each and stored high concentrations of wax-esters (40 to 65 % dry weight). Specimens were collected with a 333 µm mesh-size net towed obliquely from 15 m depth to the surface. After capture, they were transferred to a plastic cooler, diluted with water collected with a 10 l Niskin sampler at the mean tow depth and then returned to the laboratory within 2 h. The copepods were then sorted to developmental stages (females with a few copepodite stage V) under a binocular microscope and acclimated to the laboratory conditions in natural sea water and at *in situ* temperature. Samples of adult females were deep-frozen (–70 °C) immediately after capture to determine the field levels of digestive enzymes (Day 0 condition). Known volumes of sea water collected at 5 m were filtered through prebaked Whatman GF/C filters to measure the carbon and nitrogen content of the particulate material.

The time dependency of feeding rates and digestive enzyme levels in relation to food variation was investigated in 5 experiments conducted during the summer (June, July) of 1986 for *Acartia clausi* and *Eurytemora herdmani* and during the austral summer (December, January) of 1987 for *Drepanopus prectinatus*. The experimental protocol was similar to that described by Roche-Mayzaud et al. (1991) except that the different food rations were maintained more or less constant and that the experimental densities of animals were lowered.

The diatom *Skeletonema costatum* was used as food. In Rimouski (Quebec, Canada; *Acartia clausi* and *Eurytemora herdmani* experiments), the culture was grown in batch under a 14:10 light dark cycle in f/2 nutrient medium (Guillard & Ryther 1962). In Kerguelen (*Drepanopus prectinatus* experiments), the *S. costatum* cells were isolated from local waters and cultured in the same nutrient medium but with a 12:12 light:dark cycle using a field lighting system. In all 5 experiments, batch cultures were initiated every other day and each batch was used for 2 consecutive days to provide the animals with similar age algae. The experimental food media were prepared by adding known concentrations of phytoplankton culture to GF/C filtered sea water.

In the first 3 experiments the copepods (*Acartia clausi* or *Eurythemora herdmani*) were divided immediately after collection into 3 groups and acclimated 24 to 48 h in natural sea water to laboratory conditions (dim light, 12 °C). At the end of this acclimation period, 1 group was starved throughout the experiment while the other 2 groups were maintained at 2 different concentrations of *Skeletonema costatum* food for 4 to 10 d. A similar experimental protocol was followed with *Drepanopus pectinatus* (Expts 4 and 5) except that acclimation took place in the dark at 7 °C with the duration of experiment limited to 5 d.

In Expts 1, 4 and 5 the experimental copepod mean density ranged from 80 to 150 ind. l^{-1} while in Expts 2 and 3, it varied between 300 and 350 ind. l^{-1}. In all cases, the copepods were placed in 1 l jars and placed on a rotating wheel at constant temperature. Darkness and natural temperature conditions (4 to 7 °C) were maintained at all times. Concentrations of algal food in each jar were monitored and adjusted daily using

either the ratio between microscope cell counts and chlorophyll *a* (chl *a*) concentrations (Expts 1, 2 and 3) or a TA2 Coulter counter (Expts 4 and 5).

Copepod feeding rates were calculated from changes in food concentrations between sampling times (usually 22 to 26 h). Algal growth rates (i.e. grazing control) were determined from the changes in chl *a* in 1 l samples of food media without copepods, incubated under the same conditions. Calculations of ingestion rates were made according to the equation of Frost (1972). Except in Expt 1, the food limitation imposed on the animals resulted in the consumption of 70 to 95 % of the phytoplankton present making food availability strongly time and density-dependent. Despite such drop in food level between daily adjustments, the food concentration remained at all times above the limit corresponding to starvation (i.e. the concentration of carbon needed to fulfil the respiratory needs). Indeed, the maximum respiration requirements of *Acartia clausi* or *Eurytemora herdmani* at 10 °C never exceeded 0.065 µl O_2 ind.$^{-1}$ h^{-1}, equivalent to 8.4 µg C population^{-1} h^{-1}, for an experimental population of 300 copepods. Similar computation for *Drepanopus pectinatus* indicated a respiratory requirement of 3.7 µg C population^{-1} h^{-1} for an experimental population of 80 individuals. Thus, the ingestion rates recorded should be viewed as minimum values and the food ration per copepod rather than the food concentrations should be considered in the interpretation of the trophic conditions. Copepod mortality was also monitored daily and remained below 5 % d^{-1} in all feeding experiments, confirming the absence of adverse influence of low food rations. On each sampling day, 1 (Expts 2 and 3) or 2 (Expts 1, 4 and 5) jars at each food level were randomly picked and after removal of dead and moribund specimens, the remainder were counted, and then deep-frozen (−70 °C) for subsequent enzyme analyses. The remaining jars were checked for dead copepods and their food medium adjusted to the desired concentration. Aliquot samples of the incubation media and of the new food media were filtered on precombusted Whatman GF/C glass-fiber filters for subsequent analyses (in triplicate) of proteins, total carbohydrates and/or carbon and nitrogen concentrations.

Amylase, laminarinase and trypsin activities were measured in 3 subsamples of 50 to 80 individuals each after homogenization in 0.7 ml of phosphate buffer (pH: 7.0, 0.05 M) and centrifugation at 12 000 × *g* and 2 °C. The methodology described by Roche-Mayzaud et al. (1991) was followed. Amylase values are given in mg amylose degraded min^{-1}; laminarinase in µg glucose released min^{-1}; and trypsin in µmol *p*-nitroaniline produced min^{-1}. The protein contents of the copepod extracts were estimated by the method of Lowry et al. (1951) using bovine albumin as a standard.

Carbon and nitrogen contents of the experimental phytoplankton media were measured with a Perkin-Elmer 240 CHN analyzer. Total protein and total carbohydrate concentrations were estimated by the methods of Lowry et al. (1951) and Dubois et al. (1956) respectively.

Statistical analyses were performed using the SYSTAT and SPSS statistical packages. Variation in concentration or activity over time was tested using a Kruskal-Wallis 1-way analysis of variance. Differences between treatments were compared using a Tukey multiple comparison test.

RESULTS

Expt 1

Acartia clausi were subjected to 2 concentrations of the exponentially growing diatom *Skeletonema costatum* for 5 d. The lower food concentration was adjusted to a mean value of 5.4 ± 0.9 µg chl *a* l^{-1} or 405 µg C l^{-1} (food ration 2.7 to 3.5 µg C copepod^{-1}) while the high food level corresponded to a mean value of 8.5 ± 1.2 µg l^{-1} chl *a* or 690 µg C l^{-1} (food ration 4.6 to 5.8 µg C copepod^{-1}). The time-changes in chlorophyll concentrations illustrated in Fig. 1A were only partially representative of the variations in biochemical composition at either food level (Fig. 1B). Proteins dominated over carbohydrates and represented 2 different nutrient levels. Except on Day 4, differences in carbohydrate concentrations were significant (Tukey multiple comparisons test; $p < 0.05$), though of smaller magnitude than the other chemical constituents (Fig. 1B).

The experimental copepods were subjected to the different food concentrations 2 d after capture. They fed at very low rates (Fig. 2) with filtration rates ranging from 0.5 to 1.5 ml ind.$^{-1}$ d^{-1} and with a total consumption of less than 30 % of the available chlorophyll standing stock per day. Over time, although the changes were significant (Kruskal-Wallis test; $p < 0.05$), the 2 food levels resulted in different ingestion rates only on Days 2 and 3 ($p < 0.01$). On Days 4 to 6, the copepods seemed to feed at increasing rates irrespective of the food concentrations, suggesting the existence of some compensation phenomenon.

The changes in amylase, laminarinase and trypsin activities were negligible during the first 24 h acclimation period but then indicated a strong relationship with food availability (Fig. 3). All activities displayed a significant increase over time at high food concentration and a significant decrease under starvation ($p < 0.05$). At the lower food level only laminarinase showed a significant decrease in activity ($p < 0.02$) while the other 2 activities remained unchanged at an

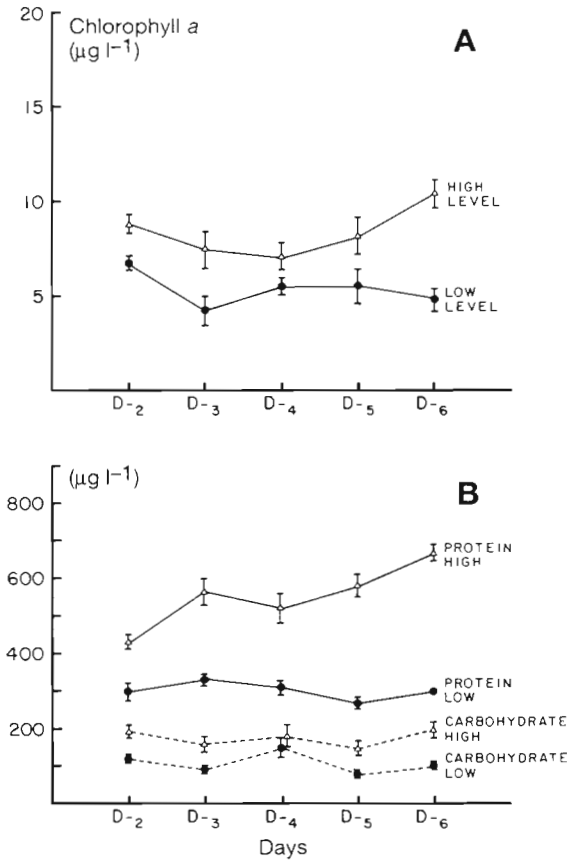

Fig. 1 *Skeletonema costatum*. Changes (± SD) in food concentration for 2 different levels of the diatom expressed as chlorophyll levels (A) and biochemical composition (B) in Expt 1. $D-_n$: 24 h period between food adjustment and ingestion measurement on Day *n*

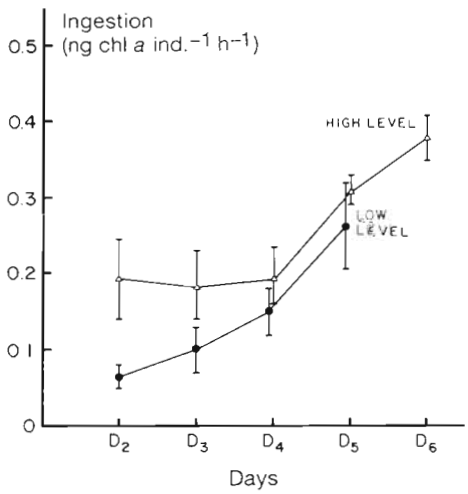

Fig. 2. *Acartia clausi*. Variations (± SD) in chlorophyll ingestion rates for the copepod feeding at 2 levels of the diatom food *Skeletonema costatum* in Expt 1

intermediate value. A Tukey multiple comparison test indicated that positive acclimation between the 2 food levels became significant only on Day 4 (i.e. 48 h after exposure to the experimental diet). Laminarinase and trypsin activities from starved individuals did not show any significant difference from the low food acclimated animals ($p < 0.05$). The significant discrimination (Tukey multiple comparison test; $p < 0.03$) of all 3 levels of amylase activity after Day 3 reinforces the hypothesis of a positive acclimation even at low food level.

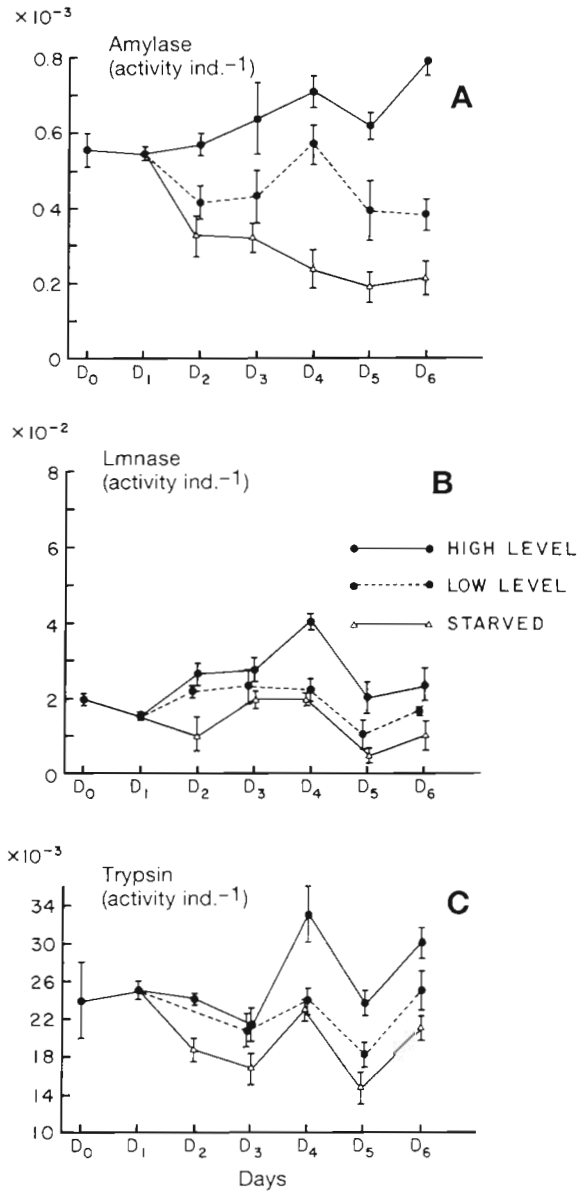

Fig. 3. *Acartia clausi*. Medium term acclimation (± SD) of the digestive enzymes amylase (A), laminarinase (Lmnase; B) and trypsin (C) activities subjected to the food changes defined in Expt 1

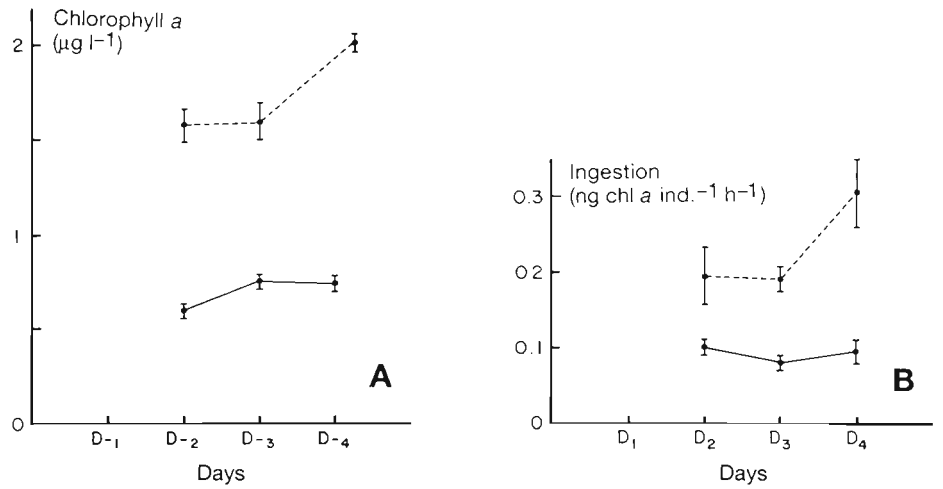

Fig. 4. *Skeletonema costatum* and *Eurytemora herdmani*. Changes (± SD) in chlorophyll concentrations for 2 different levels of the diatom food (A) and the corresponding ingestion rates (B) for the copepod in Expt 2. $D-_n$: as in Fig. 1

Expt 2

Eurytemora herdmani were acclimated to laboratory conditions in natural sea water for 24 h, and then placed for 4 d at 2 low levels of the diatom food *Skeletonema costatum* harvested during the stationary growth phase. One set of individuals was kept under starvation in filtered sea water. The lower food concentration corresponded to 0.8 µg chl $a\ l^{-1}$ or 220 µg C l^{-1} (food ration 0.7 to 0.9 µg C copepod^{-1}) while the higher food concentration was adjusted to 1.8 µg chl $a\ l^{-1}$ or 495 µg C l^{-1} (food ration 1.6 to 2.1 µg C copepod^{-1}). Despite an accidental loss of the chlorophyll data for the first experimental period (Day 1), the 2 food levels are well separated (Fig. 4A).

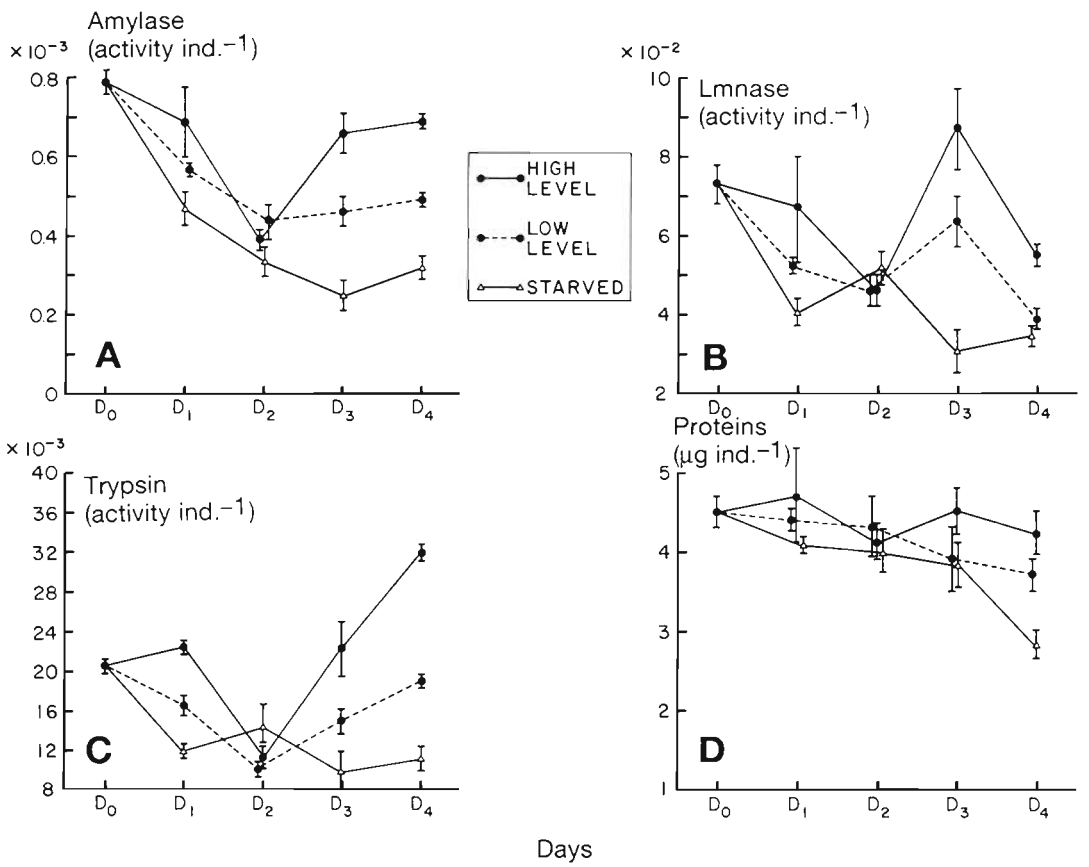

Fig. 5. *Eurytemora herdmani*. Medium term acclimation (± SD) of the digestive enzymes amylase (A), laminarinase (Lmnase; B) and trypsin (C) activities, and changes in body protein concentrations (D) for the different trophic experimental conditions used in Expt 2

Ingestion rates followed the food availability (Fig. 4B) but should be considered as minimum estimates since close to 95 % of the initial chlorophyll standing stock was consumed during the 24 h experimental periods. As expected, severe food limitations resulted in food shortage and time- and density-dependent ingestion rates.

As anticipated in a situation of food shortage, all 3 digestive enzyme activities showed an initial decrease followed, after Day 2, by a significant recovery at the higher food level ($0.015 < p < 0.05$; Fig. 5A to C). Significant positive acclimation to the food concentration had set in by Day 3 ($p < 0.05$) with slightly different patterns of changes depending on the enzyme considered. Amylase activities appeared to stabilize at 3 different levels while trypsin activities continued to increase with increasing ingestion. In contrast laminarinase activity showed a transient increase and maintained significant acclimation only for the higher food level ($p < 0.02$).

Copepod protein content (Fig. 5D) showed little change with varying feeding conditions but a significant decreasing trend occurred under starvation ($p < 0.05$). Although the same trend was apparent at the lower food level, a multiple comparison test indicated that neither of the 2 food levels induced significantly different body protein content ($0.3 < p < 0.9$) which in turn were different from the starved individuals only on Day 4 ($p < 0.05$).

Expt 3

Once it was established that *Eurytemora herdmani* displayed positive acclimation under food limitation, the next experiment was designed to evaluate the influence of small differences in food ration on the occurrence of nutritional acclimation. By increasing duration of the experiment, we also examined the possibility that the concentration threshold below which acclimation does not take place could be related to the duration of the experimental exposure to different food rations.

In an attempt to eliminate the influence of the natural trophic history, the duration of acclimation to laboratory conditions was increased to 72 h in natural sea water The copepods were then provided with 2 concentrations of *Skeletonema costatum* harvested during the early stationary growth phase. The differences between food levels were kept minimal and the diatoms were supplied to the animals over a total period of 7 d. As previously, 1 group of copepods was starved. The lower food concentration was set at a mean value of 1.9 ± 0.3 µg chl *a* l^{-1} or 486 ± 60 µg C l^{-1} (food ration 1.1 to 1.8 µg C copepod^{-1}) whereas the higher food level was adjusted to a mean value of 2.7 ± 0.6 µg chl *a* l^{-1} or 663 ± 130 µg C l^{-1} (food ration 1.5 to 2.6 µg C copepod^{-1}). The difference between the 2 food levels was maintained below 1 µg C copepod^{-1} (range 0.2 to 0.9 µg C copepod^{-1}). Changes in chlorophyll concentration in either food medium (Fig. 6A) showed unexpected maxima on Days 7 and 9. Both levels of chlorophyll were, significantly different ($p < 0.02$). The biochemical composition was dominated by protein, though on Day 7 carbohydrates reached levels similar to those of protein (Fig. 6B) introducing a shift in food quality. Protein concentrations at both food levels were also different at all times ($p < 0.03$), in contrast to the total carbohydrate levels which were significantly separated only on Days 7 and 9 ($p < 0.008$) (Fig. 6B).

The pattern of variation of ingestion rates followed the changes in food availability (Fig. 7) though the differences between the 2 food levels were only significant for Days 7 and 9 ($p < 0.03$). As indicated for

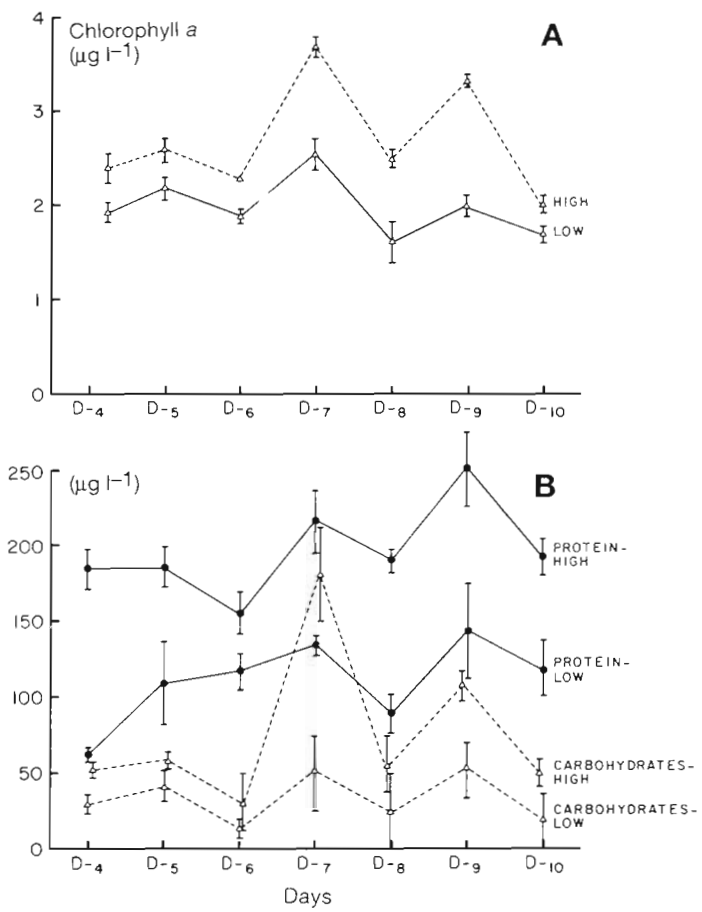

Fig. 6. *Skeletonema costatum*. Variations (± SD) in chlorophyll concentrations (A) and biochemical constituents (B) for 2 levels of the diatom food in Expt 3. D$-_n$: as in Fig. 1

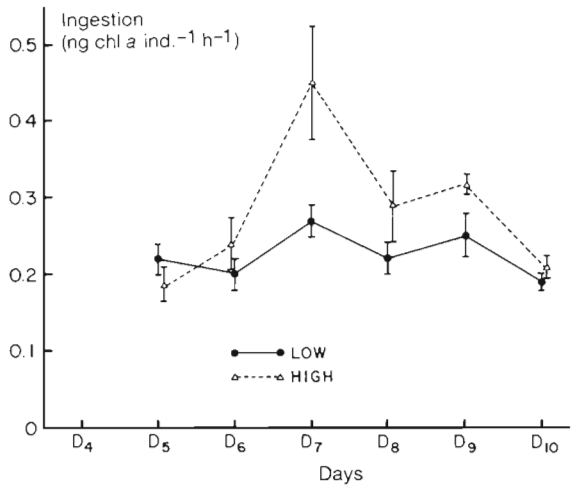

Fig. 7. *Eurytemora herdmani*. Changes (± SD) in chlorophyll ingestion rates for the copepod feeding at 2 levels of phytoplankton food in Expt 3

the previous experiment these rates should be considered as minimum values as most of the initial chlorophyll concentration was consumed by the experimental population each day (87 to 93 % for the lower food level and 75 to 85 % for the higher one). The resulting time and density dependency rates were minimized by restricting the sampling intervals to the range 22 to 24 h and by using similar numbers of copepods. Under such food-limiting conditions changes in food ration of 0.5 µg C ind.$^{-1}$ or smaller did not result in significant differences in ingestion rate.

Changes in digestive enzyme activities from Days 4 to 10 were significant for all 3 enzymes considered and all food levels ($p < 0.03$). Under starvation amylase and laminarinase activities decreased steadily in contrast to trypsin which showed an initial increase above feeding levels until Day 6 when it decreased sharply (Fig. 8A to C). When compared to the natural activity levels, the 3 d acclimation in natural sea water induced different responses for each enzyme: amylase activity decreased while laminarinase increased and trypsin remained more or less stable. At both food concentrations the increase in ingestion rate observed on Day 7 resulted in parallel changes in amylase and laminarinase activities but produced a 24 h shifted peak of activity for trypsin (Fig. 8A to C). The secondary peak of ingestion on Day 9 did not induce any carbohydrase changes but it again produced a 24 h shifted trypsin activity increase for the higher food level. When comparing the fed and the starved

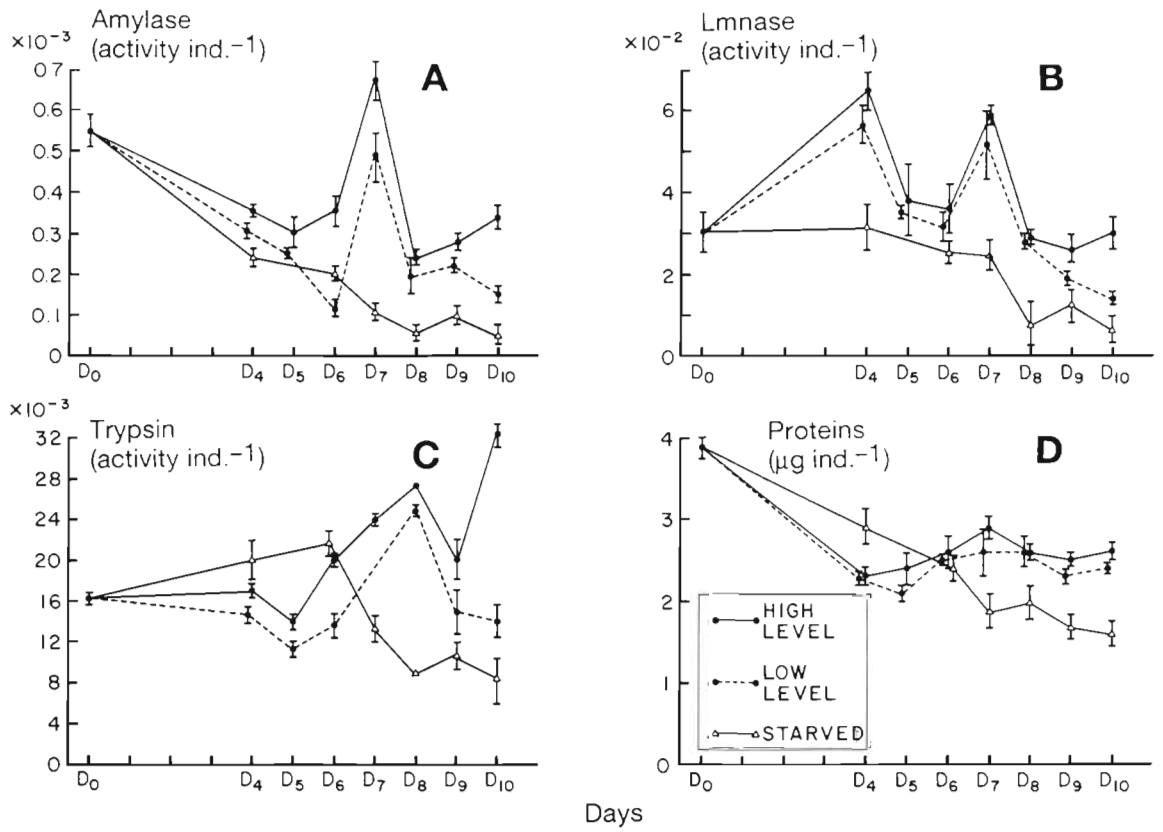

Fig. 8. *Eurytemora herdmani*. Medium term acclimation (± SD) of the digestive enzymes amylase (A), laminarinase (Lmnase; B) and trypsin (C) activities, and body protein concentrations (D) for individuals subjected to 2 food levels and under starvation in Expt 3

individuals, positive acclimation was significant only after Day 6 ($p < 0.05$). Acclimation to the 2 food levels did not occur before Day 9 for amylase and laminarinase and Day 10 for trypsin ($p < 0.05$), suggesting a delayed influence under the conditions of small differences in food rations.

As expected under strong food limitations, copepods could not maintain their natural content of body protein which dropped significantly at the end of Day 4 ($p < 0.02$). The decreasing trend continued for the starved individuals but stabilized to a common intermediate level for both feeding conditions ($0.09 < p < 0.97$; Fig. 8D).

Expt 4

Drepanopus pectinatus, a lipid-rich species, was sampled under different natural trophic conditions than specimens in the previous experiments. The copepods were sampled early in a summer bloom after a period of moderate food availability (December 22, 1986). After 48 h acclimation to laboratory conditions, they were divided into 2 groups and subjected to 2 food concentrations for 4 d. A third group was kept under starvation. The lower food concentration was set at a mean value of 3.2 ± 0.6 µg chl *a* l^{-1} or 800 µg C l^{-1} (food ration 7.8 to 12.5 µg C copepod^{-1}) while the higher food level was set at 7.7 ± 0.2 µg chl *a* l^{-1} or 1230 µg C l^{-1} (food ration 14.9 to 15.7 µg C copepod^{-1}). Over time, the variation in chlorophyll concentrations was non-significant (Fig. 9A; $p = 0.34$) and proteins dominated the biochemical composition of the diet (Fig. 9B). Though the culture of *Skeletonema costatum* was harvested in the early stationary phase, the proportion of total carbohydrates at the higher food level increased significantly ($p < 0.002$) up to Day 4 (Fig. 9C) but within a limited range.

Chlorophyll ingestion rates followed the variation in food availability quite closely (Fig. 10A) although time changes were only significant for the higher food level ($p < 0.01$). In contrast to the previous experiment, the depletion of the initial chlorophyll concentrations did not exceed a range of 70 to 80 % and yielded normal values of individual filtration rates (i.e. 10.2 ± 0.9 ml d^{-1} for the lower food level and 9.9 ± 1.2 ml d^{-1} for the higher food level). Ingestion in terms of proteins and carbohydrates showed more complex patterns which are probably related to the inclusion of the faecal material in such types of measurement. Time changes in protein ingestion were significant for both food concentrations ($p < 0.002$) and indicated a maximum on Day 3 which exceeded the variations in protein standing stocks (Fig. 10B). Carbohydrate ingestion did not exhibit significant changes at the lower food level ($p = 0.08$) but increased steadily at the higher food level ($p = 0.005$), though at a lower rate than the changes in carbohydrate food concentrations (Fig. 10C).

Digestive enzyme activities showed 3 different patterns of variations (Fig. 11A to C): (1) amylase activity of the starved individuals decreased over time ($p < 0.02$), but remained more or less constant at either food level ($0.14 < p < 0.66$); (2) laminarinase activities at all 3 experimental regimes did not vary significantly ($0.36 < p < 0.44$); and (3) trypsin activities showed significant changes under starvation ($p = 0.04$) and under the higher food level conditions ($p = 0.03$) but not at the lower level ($p = 0.06$). Both amylase and laminarinase failed to acclimate to any of the food situations except on Day 5 when amylase activity of the starved animals became significantly lower than those of the fed animals ($p < 0.02$). Positive acclimation had set in on Day 3 for the 3 levels ($p < 0.02$) but was evident as early as Day 1 if we consider the difference between the activities of the starved and higher food level groups ($p < 0.005$).

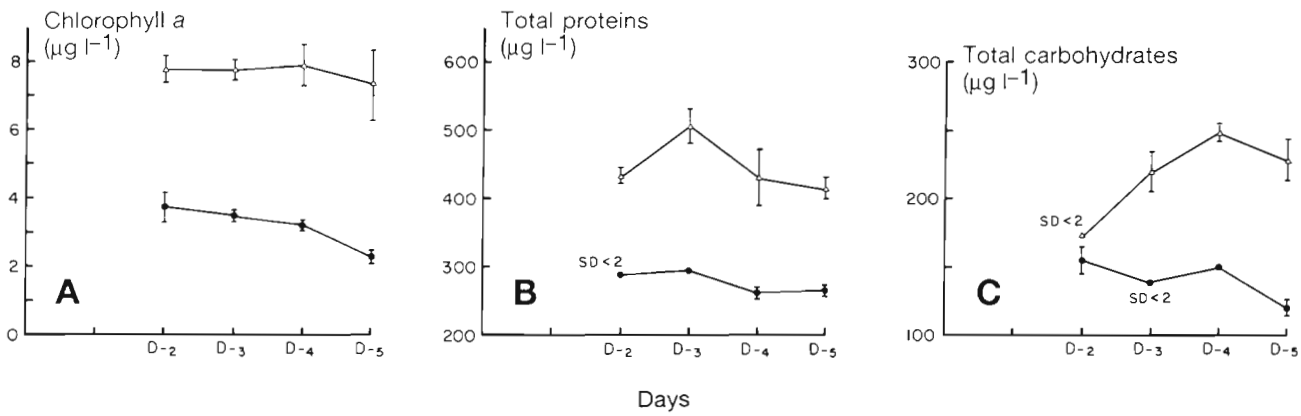

Fig. 9. *Skeletonema costatum*. Variations (± SD) in phytoplankton chlorophyll (A), protein (B) and carbohydrate (C) concentrations for the 2 experimental food levels used in Expt 4. $D-_n$: as in Fig. 1

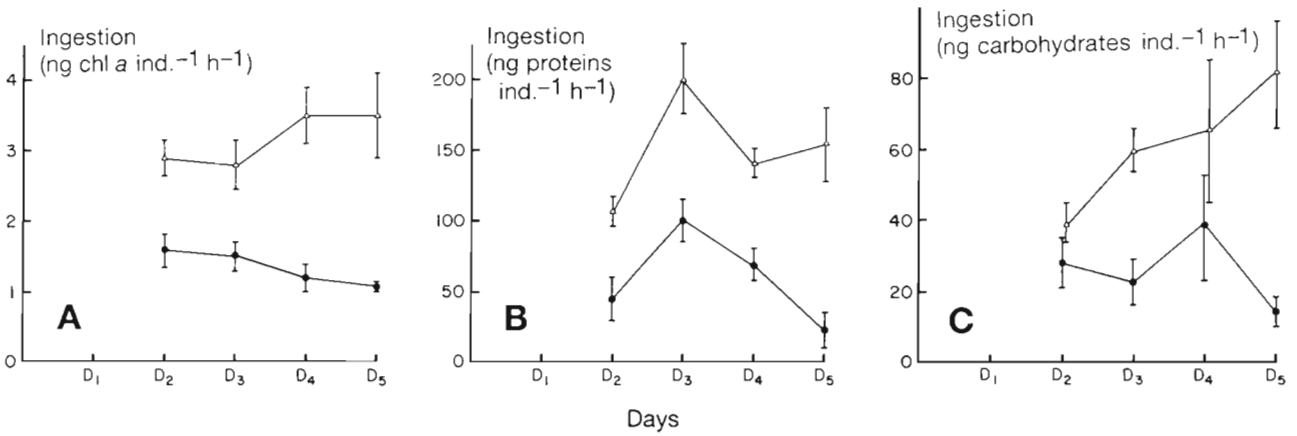

Fig. 10. *Drepanopus pectinatus*. Changes (± SD) in ingestion rates for chlorophyll (A), protein (B) and carbohydrate (C) ingestion for copepods fed 2 levels of diatom food during Expt 4

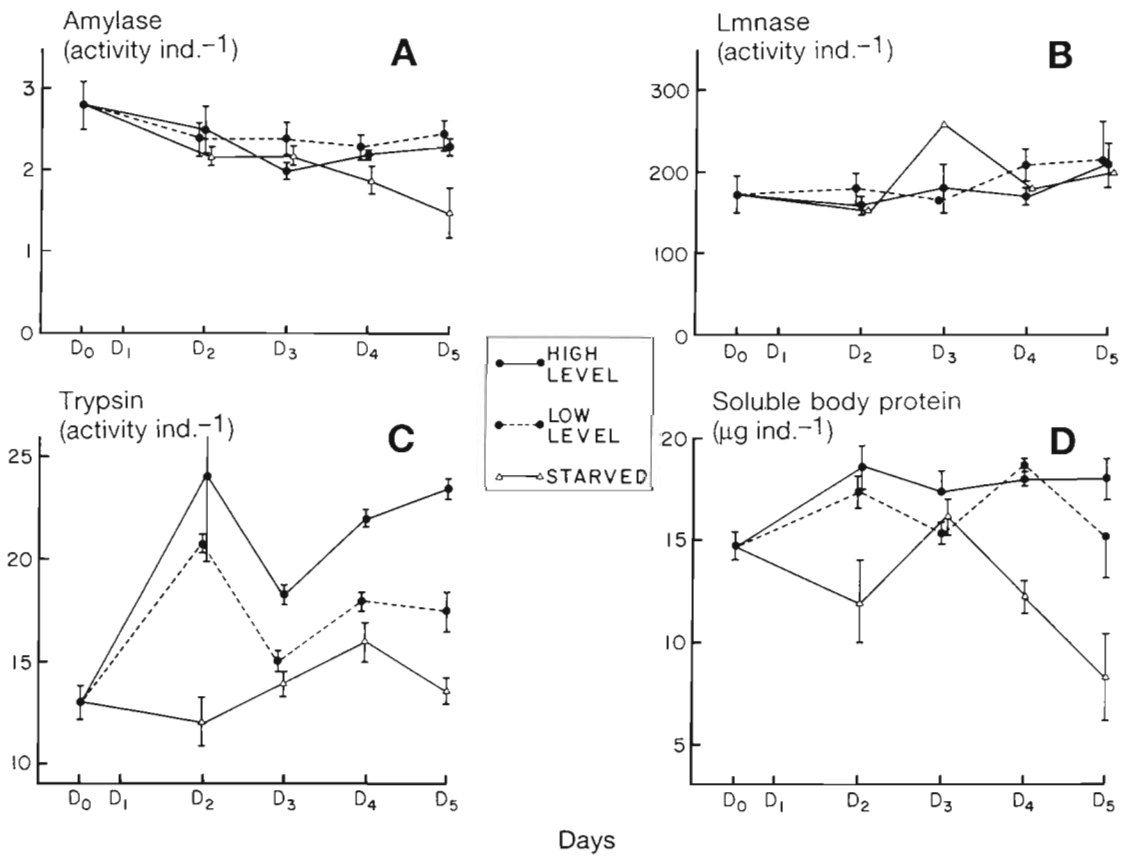

Fig. 11. *Drepanopus pectinatus*. Medium term acclimation (± SD) of the digestive enzymes amylase (A), laminarinase (Lmnase; B) and trypsin (C) activities and body protein concentration (D) for copepods fed 2 levels of diatom-food and starved in Expt 4

Protein content per copepod at either food level remained more or less constant and decreased significantly under starvation (Kruskal-Wallis test; $p < 0.02$; Fig. 11D). As expected, differences between starved and fed individuals were significant ($p < 0.02$), with the exception of Day 3. In contrast, no real influence of the food concentration could be established ($0.12 < p < 0.76$) despite a trend suggesting that experimental conditions were more favourable (i.e. less limiting) than those of the natural environment.

Expt 5

This was designed as a repetition of Expt 4 except that the copepods used were collected after the summer bloom of phytoplankton (January 4, 1987). The lower food level corresponded to 3.8 ± 0.3 µg chl *a* l^{-1} or 756 ± 14 µg C l^{-1} (food ration 7.2 to 9.0 µg C $ind.^{-1}$) while the higher food level was set at 6.2 ± 0.3 µg chl *a* l^{-1} or 1021 ± 56 µg C^{-1} (food ration = 10.0 to 12.7 µg C $ind.^{-1}$). The changes in chlorophyll concentrations throughout the 4 days were minimal though significant ($p < 0.04$) with slightly higher values being observed on Day 2 (Fig. 12A) The biochemical composition of the diatom culture harvested in the early stationary phase was again dominated by proteins but because 2 different batches of diatom culture had to be used to prepare the food media the carbohydrate/protein ratios were different for the 2 levels (i.e. lower = 0.69 ± 0.09; higher = 0.51 ± 0.04). As a consequence, though the 2 food media showed different levels of proteins ($p < 0.002$), they displayed similar concentrations of total carbohydrates ($p = 0.24$; Fig. 12B, D). The changes in particulate carbon and nitrogen (Fig. 12C) confirmed the previous observation with C/N ratios of 7.8 ± 0.3 and 6.2 ± 0.6 for the lower and upper food levels respectively.

Ingestion rates in term of chlorophyll (Fig. 13A) followed the changes in chlorophyll concentrations and, except on Day 2, ranked accordingly ($p < 0.04$). The daily depletions of the initial chlorophyll standing stock were lower than in the previous experiment and ranged between 45 and 75 %. The corresponding filtration rates were somewhat lower (i.e. 7.8 ± 0.6 ml d^{-1} for the lower food level and 6.1 ± 0.4 ml d^{-1} for the upper one) which suggested that feeding took place above the critical level of concentration below which the filtration rate is constant. Time changes in protein ingestion (Fig. 13B) were significant ($p < 0.02$) with a maximum for both food levels on Day 5 which largely exceeded the variations in protein standing stock. Differences between the 2 food levels were significant at all times ($p < 0.035$) but smaller than in the previous experiment despite similar food availability. As expected, variation in carbohydrate ingestion rates was more or less random, and differences, when significant (Days 3 and 5), showed the higher rates for the lower food levels (Fig. 13C).

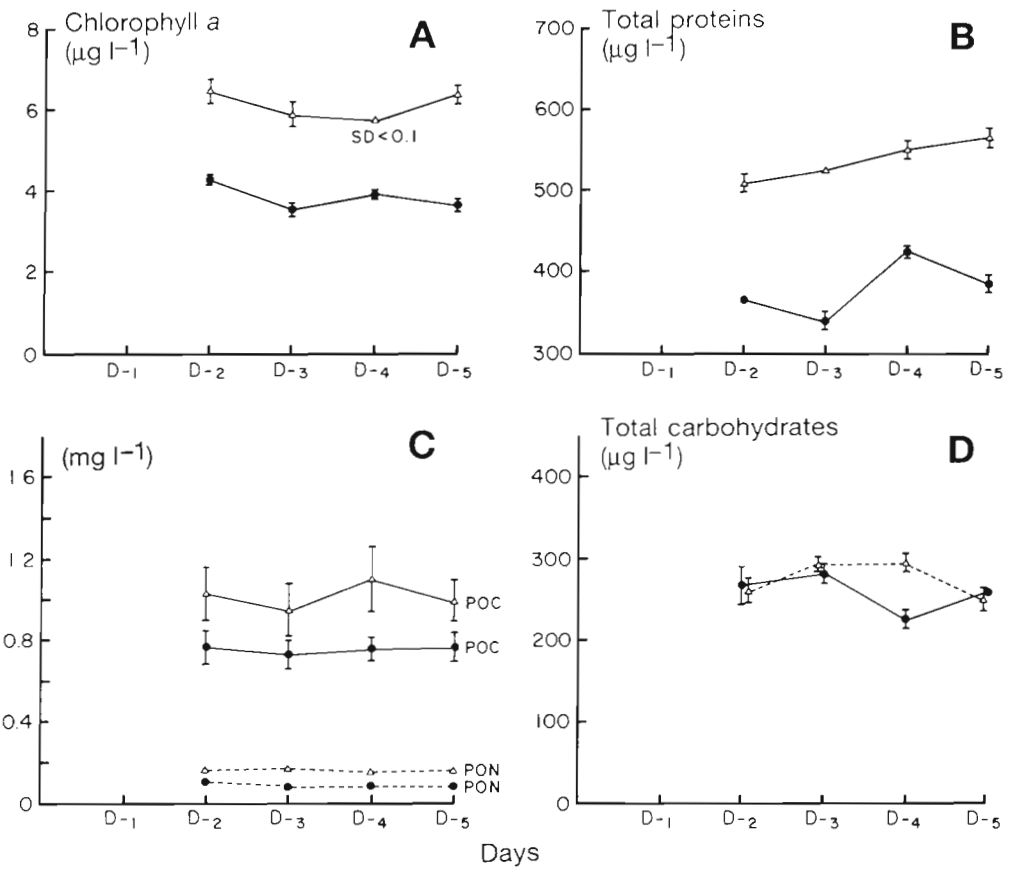

Fig. 12. *Skeletonema costatum*. Variations (± SD) in chlorophyll (A), protein (B), carbon and nitrogen (C) and carbohydrate (D) concentrations for 2 levels of the diatom food in Expt 5. $D-_n$: as in Fig. 1

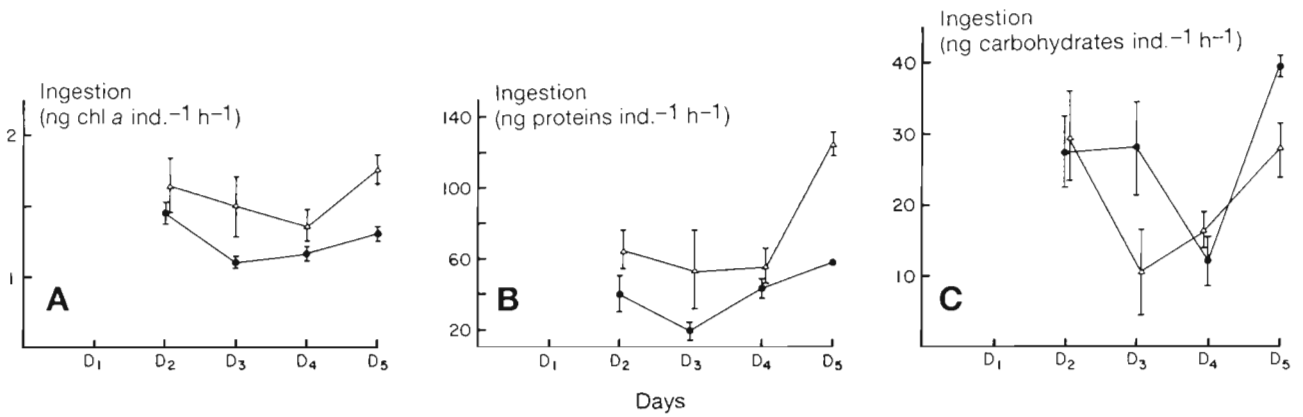

Fig. 13. *Drepanopus pectinatus*. Changes (± SD) in ingestion rates for chlorophyll (A), protein (B) and carbohydrate (C) ingestion for copepods fed 2 levels of diatom food in Expt 5

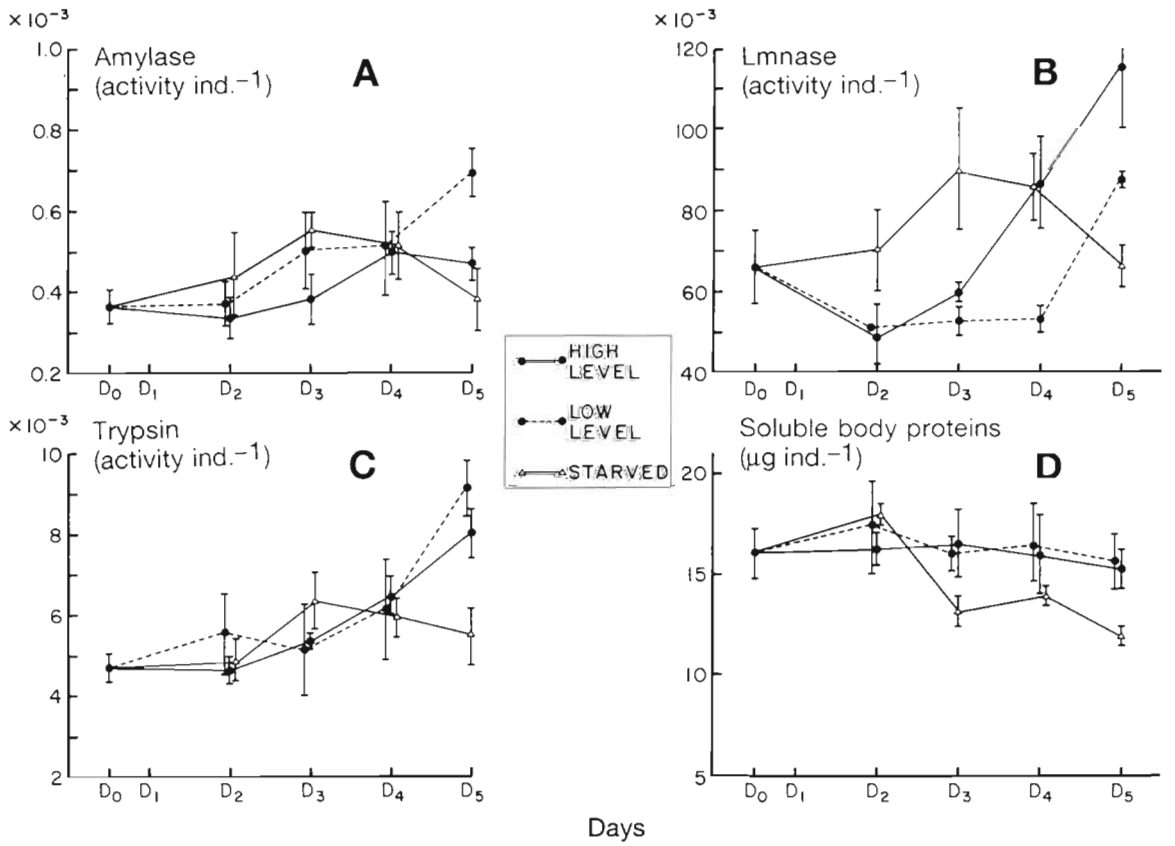

Fig. 14. *Drepanopus pectinatus*. Medium term acclimation (± SD) of the digestive enzymes amylase (A), laminarinase (Lmnase; B) and trypsin (C) activities, and body protein concentration (D) for copepods fed 2 levels of diatom food and starved in Expt 5

Compared to the previous experiment, the digestive enzyme activities were generally lower and their variations showed little changes over time and little evidence of acclimation to food supply (Fig. 14A to C). Regardless of the food status, amylase activities did not vary significantly over time ($0.06 < p < 0.22$) and, up to Day 5, failed to react to the different food concentrations (Fig. 14A). Laminarinase (Fig. 14B) increased substantially after Day 4 at both food levels ($p < 0.05$), when the activity of the starved individuals started to decrease. Thus, full positive acclimation was achieved only on Day 5 ($p < 0.03$). Trypsin activities of feeding individuals increased over time but did not show any acclimation to food concentrations ($0.18 < p < 0.98$; Fig. 14C).

Body protein showed maximum values (Fig. 14D) and remained more or less constant for both feeding levels ($0.83 < p < 0.88$). It decreased under starvation after Day 2 but showed significant differences with fed animals only on Days 3 and 5 ($p < 0.045$).

Assimilation efficiency: Expts 4 and 5

Assimilation was not measured directly but could be derived from the different estimates of chlorophyll and protein or carbohydrate ingestion. Chlorophyll is usually entirely transformed into phaeopigments during gut transit (Schuman & Lorenzen 1975) and can be considered as a true measurement of ingestion. Conversely, ingestion measured in terms of protein or carbohydrates includes a proportion of faecal material and reflects both ingestion and digestion. If the assimilation efficiency was 100 %, the phytoplankton protein (or carbohydrate)/chlorophyll ratios in the initial food (initial or control flask) and in the remaining phytoplankton in the experimental vessels at the end of the incubation period, should be equal. As expected, the ratio was always lower and a rough estimate of assimilation could thus be computed using:

(Ratio from experiment/Ratio from control) × 100.

The results from each experiment are presented on the same graph (Fig. 15) to show the influence of food availability and trophic history. The efficiency (%) of protein assimilation was positively correlated with the phytoplankton-protein concentrations in both experiments (Expt 4: $r = 0.70$, $df = 37$; Expt 5: $r = 0.576$, $df = 38$) but the degree of efficiency was significantly higher in Expt 4 than in Expt 5. Similar conclusions could be drawn from the carbohydrate assimilation data except that no significant relationship could be established between % assimilation and carbohydrate availability in Expt 5 (Expt 4: $r = 0.537$, $df = 37$; Expt 5: $r = 0.257$, $df = 38$). The respective mean protein and carbohydrate % assimilations were 68 ± 18 and 49 ± 13 in Expt 4 and dropped to 38 ± 13 and 22 ± 10 in Expt 5.

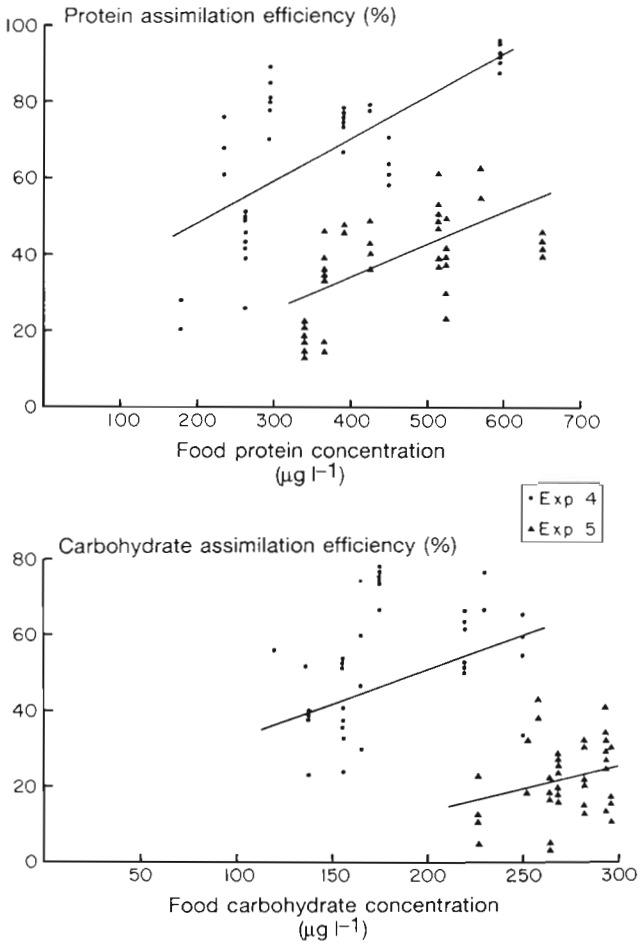

Fig. 15. *Drepanopus pectinatus*. Linear regressions between protein assimilation rates and diatom protein concentrations (top panel) and carbohydrate assimilation rates and diatom-carbohydrate concentrations (bottom panel) in Expts 4 and 5

DISCUSSION

As indicated earlier, the 3 species differ in dry weight, biochemical composition, and thus in energy requirement. Using the general relationship between respiration and dry weight for boreal species of zooplankton reported by Ikeda (1974, Table 3; $\log R = 0.783 \log W + 0.057$), weight specific respiration rate for *Acartia clausi* and *Eurytemora herdmani* can be estimated within the range of 80 to 90 µl O_2 mg^{-1} dw d^{-1}, and for *Drepanopus pectinatus* to a mean value of 55 µl O_2 mg^{-1} dw d^{-1}. Actual measurements on adult stages, at natural temperatures (5 to 7 °C), revealed a difference larger than anticipated from computation. Indeed, if the mean value of oxygen uptake in *D. pectinatus* was close to the predicted value (57 µl O_2 mg^{-1} dw d^{-1}; Razouls 1985), the respiration rates of North-Atlantic *A. clausi* and *E. herdmani*, were found to vary between 90 and 200 µl O_2 mg^{-1} dw d^{-1} (Gauld & Raymont 1953, Raymont 1959, Conover 1960, Anraku 1964, Conover & Mayzaud 1975). In a comparative study on herbivorous copepods, Conover & Corner (1968) showed that metabolic rates were strongly affected by the amount of 'nitrogen-containing tissue' (proteins) and depressed by the concentration of fat reserves suggesting some internal control in addition to the size-respiration relationship.

Since the early study by Mayzaud & Poulet (1978), the experimental evidence on the acclimation of copepod nutritional systems has yielded conflicting views (cf.

Mayzaud 1986, Donaghay 1988). According to the recent study by Roche-Mayzaud et al. (1991), a large part of this controversy could be attributed to experimental conditions which failed to establish food-limiting conditions (i.e. suboptimal growth). They suggested that digestive acclimation was not so much controlled by food changes but by feedback mechanisms which operate to meet the metabolic requirements of individuals. Under such experimental conditions, they showed that female *Acartia clausi* from the Mediterranean Sea acclimated positively to quantitative changes in food supply and characterized some of the key features of the process. *A. clausi* appeared to require: (1) 48 h to demonstrate significant acclimation and (2) food changes exceeding a threshold of 1.5 to 2 µg C ind.$^{-1}$ d^{-1}. Positive responses of the enzyme system were recorded over a range of food ration from 2 to 12 µg C ind.$^{-1}$ and were sustainable under discontinuous food stimuli. Larger saturating food rations resulted in a stabilization or a small decrease of the enzyme response.

To what extent such results can be applied to other environments and other species can be assessed from the present study. Experiments with *Acartia clausi* from North Atlantic waters confirmed the existence of a positive acclimation of the digestive system and of a time threshold of 48 h to reach significant response between food levels. The results obtained with *Eurytemora herdmani*, extend to another species the previously observed pattern of acclimation and confirm that differences in food ration lower than 0.9 µg C copepod^{-1} do not produce significant enzyme activity changes over the time span usually considered. Copepods with large energy reserves such as *Drepanopus pectinatus*, displayed a different pattern of acclimation which affected the digestion of proteins. In this species carbohydrases did not acclimate despite differences in food ration of 3 to 7 µg C copepod^{-1} or acclimated negatively depending on the enzyme considered.

Various studies on the influence of food quality on copepod feeding have reached the conclusion that the animals are maximizing protein ingestion (Libourel-Houde & Roman 1987, Cowles et al. 1988). The results on *Drepanopus pectinatus* support in a large part this conclusion with higher efficiencies of assimilation for proteins than for carbohydrates and positive acclimation solely for trypsin. The assimilation efficiency computed from our experimental data should be considered as minimum estimates because of the uncertainties in chloropigment stability during gut transit (Conover et al. 1986, Penry & Frost 1991). The few data available, using chlorophyll as a tracer (Landry et al. 1984, Hassett & Landry 1988, 1990a) support the present observations in that the assimilation efficiency is generally higher or equal for nitrogen related compounds than for carbon related compounds such as carbohydrates.

Interspecies comparison of digestive acclimation patterns bring support to the hypothesis developed by Roche-Mayzaud et al. (1991). They proposed that the intensity of digestive acclimation is related to the difference between the metabolic requirements of the animals and the ability of the trophic environment to meet them. The larger the difference, the larger the need to acclimate. One would expect that species with high metabolic requirements, i.e. low energy reserves, high turnover of their biomass (e.g. *Acartia* spp., *Temora* spp., *Eurytemora* spp.), would acclimate more readily under given food-limiting situations than species with low metabolic requirements, i.e. high level of lipid reserves, low turnover of their biomass (e.g. *Calanus* spp., *Pseudocalanus* spp., *Drepanopus* spp.). When we consider the present results and those of Roche-Mayzaud et al. (1991), it is clear that *A. clausi* and/or *E. herdmani* enzyme activities acclimated for food concentrations ranging from 220 µg C l^{-1} to 5.6 mg C l^{-1} or food rations ranging from 0.7 to 18 µg C copepod^{-1} while *D. pectinatus* carbohydrases failed to acclimate for food concentrations ranging from 800 to 1200 µg C l^{-1} or food rations ranging from 7 to 15 µg C ind.$^{-1}$. Interestingly, other lipid rich species such as *C. helgolandicus* or *C. pacificus* seemed to reach experimental food saturation for similar phytoplankton concentrations of 970 and 925 µg C l^{-1} respectively (Hassett & Landry 1983, Harris et al. 1986). Similar differential behaviour between these 2 groups of copepods have been reported from field enzyme activity data between 2 co-occurring species: *A. clausi* and *Pseudocalanus* sp. (Mayzaud & Mayzaud 1985), as well as for other time dependent physiological responses such as egg production. In the latter case, increases in food supply resulted in an immediate and continuous response for *A. tonsa* (Donaghay 1985), a delayed response for *C. pacificus* (Runge 1984), and an absence of response for *Pseudocalanus minutus* (Dagg 1977). Such behaviour is probably linked to previous feeding history and degree of metabolic requirements.

The apparent contradiction between experimental studies supporting (Mayzaud & Poulet 1978, Cox 1981, Cox & Willason 1981) or not (Hassett & Landry 1983, Landry & Hassett 1985) the so-called 'acclimation hypothesis', can also be attributed to interspecies differences. Such a possibility was raised by Hassett & Landry (1983) but not really considered in their interpretation. The present results with *Drepanopus pectinatus* clearly illustrate that under limiting conditions (Expt. 4), lipid-rich species display different patterns of changes depending on the class of digestive enzyme considered. Lack of acclimation or reverse acclimation of glycosidase activities was recorded in agreement with earlier findings by Hassett & Landry (1983, 1990a, b) and Landry & Hassett (1985) on *Calanus pacificus*

Table 1. *Drepanopus pectinatus*. Particulate carbon (POC) concentration, ingestion, and digestive enzyme activity at the time of capture and averaged over different periods in December 1986 and January 1987 prior to capture. Δ: differential changes between periods (POC) or between mean values of ingestion averaged over periods [1] and [3] and sampling time; –: not measured

	Expt 4			Expt 5		
	12–16 Dec [1]	17–21 Dec [2]	21 Dec	24–31 Dec [3]	31 Dec – 4 Jan [4]	5 Jan
POC (μg C l^{-1})	179 ± 15	350 ± 53	293 ± 5	530 ± 65	250 ± 45	200 ± 8
ΔPOC [2] – [1]	–	–	171	–	–	–
ΔPOC [4] – [3]	–	–	–	–	–	−280
Ingestion (ng chl *a* $ind.^{-1}$)	10.1 ± 7.1	–	17.5 ± 2.0	2.3 ± 1.4	–	0.9 ± 1.4
ΔIngestion	–	–	7.4	–	–	−1.4
Amylase (activity × 10^{-3} $ind.^{-1}$)	–	–	2.8 ± 0.2	–	–	0.4 ± 0.4
Laminarinase (activity × 10^{-3} $ind.^{-1}$)	–	–	170 ± 6	–	–	66 ± 13
Trypsin (activity × 10^{-3} $ind.^{-1}$)	–	–	13 ± 2	–	–	5.3 ± 1.5

but differs from the positive acclimation observed for the trypsin activity, an enzyme not considered by these authors. This does not mean that under the experimental conditions used, *C. pacificus* trypsin would have acclimated but illustrates the potential error involved with the use of an incomplete set of enzymes in the study of digestion metabolism.

The influence of trophic history on the physiological responses of zooplankton organisms in the laboratory has been of growing concern and has been shown to affect a wide range of functions such as feeding selectivity, efficiency of particle capture, feeding rate and reproduction (see reviews by Donaghay 1988, Huntley 1988). Comparison of the digestive response of *Drepanopus pectinatus* with the same experimental conditions confirmed that major differences occurred for individuals captured either 4 d after the initiation of a summer bloom or 4 d after its end. The natural trophic conditions for both groups are summarized in Table 1 and show that on the day of capture, the specimens in Expt 4 faced higher food concentrations than those used in Expt 5. Averaged over 4 d before capture, the difference in food availability was similar, though the absolute levels were slightly higher. The corresponding ingestion rates and mean levels of digestive enzymes acclimated positively to the changes in particulate carbon in agreement with the immediate and recent past conditions. If acclimation was related only to the immediate or recent food limitation, one would have expected a positive or partially positive response from the individuals used in Expt 5 rather than in Expt 4. The difference between the *D. pectinatus* natural and experimental courses of acclimation could indicate that under laboratory conditions, the enzymatic response of the copepods could have been influenced by the food conditions recorded before and during the bloom, i.e. 8 d before collection (Table 1). If so, *D. pectinatus* experimental nutritional adaptability could be controlled by longer term trophic history corresponding to periods ranging from 4 to 10 d. It would be worth considering whether such memory effect could result in shifted periodicities between changes in food supply and ingestion or digestive activity, as was observed by Tande & Slagstad (1982) for female and copepodite stage V *Calanus finmarchicus*, by Mayzaud, Razouls & Roche-Mayzaud (unpubl.) for female and copepodite stage V *Drepanopus* and to a lesser extent by Hassett & Landry (1990a) for *C. pacificus*. Integration over such long periods of time is certainly not typical of all species and environments (Donaghay 1988), but confirms that to various degrees the nutritional response (ingestion, digestion and assimilation) we measure under laboratory conditions is the product of the current environment and some integral of the past history (Huntley 1988).

Physiological adaptations have this far been considered in relation to optimum foraging theory to predict whether a given food will be assimilated by the organism at reasonable energetic costs. Although, it is tempting to believe that organisms always optimize their energy budget in a variable trophic environment, the significance of metabolic acclimation should be more readily considered as a means to minimize the disturbing effects of these changes. In other words, if an animal in equilibrium with its food supply is subjected to nutritional stress, its metabolism should react in such a way as to minimize or to undo entirely the effects of the stress, if the animal is to benefit from the adaptation. As food limitation (quality and/or quantity) is a more common event than the exception, it becomes very important not only to specify the constraints imposed by the digestive processes on the feeding activity (Penry & Jumars 1987) but also to define the degree of adaptability of the organism

in relation to its metabolic characteristics and its contemporary as well as evolutionary environment (Donaghay 1988).

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