Vol. 44: 15–24, 1988 | MARINE ECOLOGY – PROGRESS SERIES
Mar. Ecol. Prog. Ser. | Published April 21

Interspecific, seasonal and diel variations in zooplankton trypsin and amylase activities in Kosterfjorden, western Sweden

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ABSTRACT: High interspecific variability in trypsin and amylase activities of boreal macrozooplankton species, prevailing during an 8 mo investigation, could not be explained by differences in trophic position of the species. A pronounced but delayed response to the spring phytoplankton bloom was recorded for herbivorous but not for carnivorous species. The 2 copepods *Calanus finmarchicus* and *Metridia longa*, having different life strategies, showed significantly different enzyme activity during most of the investigation. The non-feeding adult males of 2 carnivorous copepods showed a depressed trypsin activity but normal amylase activity compared with feeding developmental stages. The copepod *Chiridius armatus* from Kosterfjorden, as well as from the Norwegian west coast, always displayed exceptionally high trypsin activity. A diurnal rhythm in enzyme activity, significantly correlated with the feeding activity and respiration rate, was recorded for Stage V copepodites *Calanus finmarchicus* studied in late September. Results suggest that data on enzyme activities of mixed zooplankton samples cannot be used as a general indicator of zooplankton feeding levels. But for a given species the activities of digestive enzymes may reflect both long-term (seasonal) and short-term (diurnal) changes in feeding activities. However, the causal relationships between feeding and enzyme activities appear very complex, and measurements of digestive enzymes cannot be used as a direct quantitative method to estimate ingestion rates of zooplankton.

INTRODUCTION

For a general understanding of the dynamics of a pelagic ecosystem, knowledge of zooplankton feeding processes has been considered very important. Quantification of such processes, using laboratory experiments and in situ measurements, has therefore attracted many ecologists. Because direct determination of feeding activity requires that the animals be subject to unnatural conditions, results from such experiments will always be debatable. An alternative approach where grazing rates of herbivorous zooplankters are indirectly estimated from the content of algal pigments in the guts of freshly caught animals has proved useful (e.g. Mackas & Bohrer 1976, Dagg & Wyman 1983, Nicolajsen et al. 1983, Tande & Båmstedt 1985, Harris & Malej 1986). However, this method has the limitation of only including grazing of chlorophyll-containing particles. Moreover the mathematical calculation requires that ingestion and gut evacuation are simultaneous processes for unbiased estimation of the contemporaneous feeding rate, a condition which is probably not always fulfilled. Another indirect method was suggested by Boucher & Samain (1974) and is based on an empirically determined relationship between digestive enzyme activity and ingestion rate. Later investigations, both on mixed zooplankton and single species, refuted the relationship postulated by Boucher & Samain (1974). The suggested models of environmental regulation of zooplankton enzyme activities, developed from such studies, were partly contradictory (cf. Mayzaud & Conover 1976, Mayzaud & Poulet 1978, Cox 1981, Hasset & Landry 1983, Head & Conover 1983, Baars & Oosterhuis 1984). In a recent review Mayzaud (1986) emphasized the importance of food quality and the complexity of the regulating mechanisms involved in the digestive system of copepods. Trophic acclimation, in which slowly occurring changes in the nutritional environment will affect the enzyme system differently to rapidly occurring ones, has been suggested as such a regulating factor

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0171-8630/88/0044/0015/$ 03.00

for digestive enzymes (Mayzaud & Poulet 1978, Mayzaud et al. 1984, Harris et al. 1986).

Inconsistencies in apparent relationships between activities of digestive enzymes and food ingestion have been taken into account in a model of 'constant assimilation' (Samain et al. 1981, 1985, Harris et al. 1986). At present this is the only model intended to describe the functional relationship between food intake and enzyme activity. However, this model, although attractive for explaining variable enzyme responses in different food environments, does not permit an estimation of the in situ rate of grazing from activities of digestive enzymes. Results for a few species have been fitted to this model but we still need more information in order to evaluate its generality. Interspecific, seasonal, and short-term variations are particularly poorly covered in previous studies. These aspects are considered here by using alkaline protease (trypsin) and one carbohydrase (amylase) in a study on the enzyme/environment relationships within a macrozooplankton community over an 8 mo period.

MATERIAL AND METHODS

Oblique hauls from ca 200 m to the surface were taken with a conical zooplankton net with 400 µm mesh size in Kosterfjorden, western Sweden (58°52'N, 11°06'E). The zooplankton captured in the non-filtering cod end was transferred to a large carboy containing seawater at 5 to 7 °C, transported to the laboratory, and sorted by using a small sieve. The animals were homogenized in distilled water within a few hours after capture. From this homogenate amylase activity was measured by the method of Street & Close (1956), trypsin activity by the method of Erlanger et al. (1961), and protein by the method of Dorsey et al. (1977). Table 1 lists the species included in the investigation, and their taxonomic status and trophic position (cf. Matthews & Bakke 1977), together with their average individual protein contents. A separate study to evaluate the diel variation in feeding and digestive enzyme activity of the copepod *Calanus finmarchicus* was carried out in late September. Oblique hauls from 70 m to the surface were take every 4 h with a 300 µm meshed WP2-net in Kosterfjorden. The material caught in the non-filtering cod end was used for enzyme and protein analyses as described above, in which 15 Stage V copepodites were used for each assay. In addition subsamples with 5 healthy individuals in each were used for measurements of ETS (electron transport system) activity as described by Båmstedt (1984), and subsamples preserved in formalin solution were used for microscopic determination of population structure and gut fullness (length of filled gut). The latter variable is suggested as an indicator of in situ feeding activity of the animal (see Båmstedt 1984). For comparative purposes results on enzyme activities are also included for zooplankton collected on the Norwegian west coast in late November. These animals were sampled by vertical hauls with a 0.5 m diameter Juday net, 180 µm mesh aperture (see Båmstedt & Ervik 1984), and analysed as described above.

RESULTS

Seasonal and interspecific variability

Information on seasonal and interspecific variations is given in Tables 2 to 4 and Fig. 1. Most species were represented by only a single or a few samples. Since the representation of species and developmental stages differed between the 3 seasons, one cannot postulate any seasonal variation in the zooplankton community as a whole from the average values of the species. This is mainly due to the highly species-specific level of enzyme activity at any given time. It does not obliterate the existence of significant seasonal variation, since the community is heavily dominated by a few species (Båmstedt in press), but suggests that at present an evaluation of seasonal variation must be kept at the species level.

Fig. 1 shows the results for 2 truly carnivorous, 1 herbivorous, and 1 omnivorous species of copepod. Both Stage V copepodites of *Calanus finmarchicus* and adult females of *Metridia longa* showed pronounced increases in trypsin and amylase activity in April, followed by decreases during May and June. The seasonal pattern in enzyme activity of the 2 carnivores was less distinct, although highest amylase activity occurred in March/April. A 1-way analysis of variance (Zar 1974) rejected the null hypothesis of equality in enzyme activity over the investigation period for the 2 non-carnivorous species ($p < 0.0005$ for both trypsin and amylase activity of the species), whereas the null hypothesis could not be rejected ($p > 0.05$) for the 2 carnivorous species.

The herbivore/omnivore species *Calanus hyperboreus* and *Meganyctiphanes norvegica* showed the same tendency of high enzyme activity in spring as *C. finmarchicus* and *M. longa*, whereas the carnivores *Parathemisto abyssorum* and *Tomopteris helgolandica* had low activities in spring (Tables 2 to 4). To further evaluate these differences between the 2 main trophic groups a non-parametric test (Mann-Whitney *U*-test; Zar 1974) was used for the total data on each sampling occasion. The null hypothesis of equal enzyme activity of the groups could not be rejected at the 5% significance level for any of the 10 sampling occasions. This was mainly an effect of high interspecific variability

Table 1. Seasonal range in average individual protein content, taxonomic status and trophic position of macrozooplankton species from Kosterfjorden, used in the study of digestive enzymes

Species	Stage	µg protein	Abbr.	Taxonomic status	Trophic status
Calanus finmarchicus (Gunnerus)	CV	62–107	*C. f.*	Copepoda, Calanoida	Herbivore
Calanus hyperboreus (Kröyer)	CV, VI	429–1885	*C. h.*	Copepoda, Calanoida	Herbivore
Chiridius armatus (Boeck)	CVI ♀ CVI ♂ CV	174–426 220–303 165	*C. a.*	Copepoda, Calanoida	Carnivore
Euchaeta norvegica Boeck	CVI ♀ CVI ♂ CV CIV CIII	763–1698 484 474 104 47	*E. n.*	Copepoda, Calanoida	Carnivore
Metridia longa (Lubbock)	CVI ♀ CV	83–143 40–68	*M. l.*	Copepoda, Calanoida	Omnivore
Meganyctiphanes norvegica (M. Sars)	Adults	4509–35895	*M. n.*	Euphausiacea	Omnivore
Boreomysis arctica (Kröyer)	Adults	2752–4059	*B. a.*	Mysidacea	Omnivore
Parathemisto abyssorum Boeck	–	247–2094	*P. a.*	Amphipoda, Hyperiidea	Carnivore
Pasiphaea multidentata Esmark	–	2259–10086	*P. m.*	Decapoda, Natantia	Carnivore
Concoecia borealis/elegans G. O. Sars	Adults	134–178	*C.* spp.	Ostracoda	Omnivore
Balanus sp. Da Costa	Nauplii	3.5–4.4	*B.* sp.	Cirripedia	Herbivore
Eukrohnia bathypelagica Alvarino	Adults	808–1445	*E. b.*	Chaetognatha	Carnivore
Eukrohnia hamata (Möbius)	Adults	1926–2996	*E. h.*	Chaetognatha	Carnivore
Sagitta elegans Verrill	Adults	458–1045	*S. e.*	Chaetognatha	Carnivore
Tomopteris helgolandica Greeff	Adults	1080–8987	*T. h.*	Polychaeta, Errantia	Carnivore
Aurelia aurita Lamarck	–	1011	*A. a.*	Scyphozoa	Carnivore
Eutonina indicans Romanes	–	673	*E. i.*	Hydrozoa	Carnivore
Pleurobrachia pileus Fabricius	–	393–922	*P. p.*	Ctenophora	Carnivore
Beroe cucumis Fabricius	–	1023–2628	*B. c.*	Ctenophora	Carnivore

within both trophic groups. The present data do not, therefore, support the theory that there is a general difference in digestive-enzyme activities between herbivores and carnivores. Exceptionally high trypsin activity was recorded for adult females of *Chiridius armatus* and *Boreomysis arctica* (Tables 2 to 4; Fig. 1), but because these species have different food habits the argument for systematic differences between trophic groups is weakened rather than strengthened.

Previous results by Båmstedt & Ervik (1984) and Hopkins et al. (1984, 1985) on the 2 copepods *Calanus finmarchicus* and *Metridia longa* indicate that they have different nutritional strategies during the overwintering period. A comparison, using the Mann-Whitney *U*-test, showed that the trypsin activity and the trypsin/amylase ratio were often significantly different for the 2 species ($p < 0.05$), mostly during spring, whereas the amylase activity was different for the species during summer/autumn (Table 5).

Variations due to developmental stage

Different developmental stages of the 2 copepods *Euchaeta norvegica* and *Chiridius armatus* were separately analysed. The adult male does not feed in either species (Båmstedt & Matthews 1975, J. Matthews pers. comm.). This fact was strongly reflected in the trypsin activity but not in the amylase activity (Fig. 2). Younger developmental stages of *E. norvegica* had higher enzyme activity than adult females, whereas this was not the case for *C. armatus*.

Geographical variations

The values for species collected on the Norwegian west coast in November agree reasonably well with the data from Kosterfjorden. *Calanus finmarchicus, Chiridius armatus, Euchaeta norvegica*, and *Metridia*

Table 2. Average activities of trypsin and amylase (mU mg^{-1} protein) and the ratio trypsin/amylase of macrozooplankton species collected on 5 occasions in spring. 1 = 3 Mar; 2 = 17 Mar; 3 = 30 Mar; 4 = 13–14 Apr; 5 = 6 May. $\bar{X}$ and standard deviation (SD) calculated from the average values of each sampling occasion. Abbreviations as given in Table 1

Species/ stage	Number of samples 1	2	3	4	5	Trypsin $\bar{X}$	Trypsin SD	Amylase $\bar{X}$	Amylase SD	Trypsin/Amylase $\bar{X}$	Trypsin/Amylase SD
C. f. V	3	5	6	9	8	57.84	64.75	9.88	3.85	4.95	4.93
C. h. V, VI	2	4	2	–	–	98.27	100.69	2.68	1.73	35.95	32.86
C. a. ♀	1	4	5	8	–	214.63	76.13	3.68	2.82	63.08	24.06
C. a. ♂	–	–	1	6	–	2.81	1.46	2.32	0.71	1.14	0.32
C. a. juv.	–	–	–	1	–	256.42	–	2.62	–	97.87	–
E. n. ♀	3	3	4	1	–	14.16	3.57	2.50	2.11	22.13	34.36
E. n. ♂	–	1	–	–	–	1.65	–	6.77	–	0.24	–
E. n. V	–	–	2	2	–	38.84	16.38	5.40	2.53	7.28	0.38
E. n. IV	–	–	3	2	–	39.44	3.44	6.71	0.01	5.89	0.57
E. n. III	–	–	–	1	–	33.70	–	15.06	–	2.24	–
M. l. ♀	2	5	7	8	11	23.71	34.70	9.98	5.02	3.92	4.00
M. n.	–	1	–	–	–	85.14	–	3.37	–	25.26	–
P. a.	1	3	–	–	3	5.74	4.01	5.83	7.54	3.67	4.82
P. m.	–	1	–	–	–	1.00	–	0.55	–	1.82	–
B. sp.	4	–	–	–	–	5.68	–	21.27	–	0.28	–
C. spp.	1	1	–	–	–	1.11	0.16	2.78	0.28	0.90	0.60
E. b.	–	1	1	–	–	38.58	52.60	2.69	0.95	11.62	15.46
E. h.	3	2	1	1	–	2.24	1.67	2.25	1.20	1.88	2.46
S. e.	–	1	1	1	2	2.51	1.11	5.38	3.09	0.73	0.76
T. h.	1	–	1	1	–	2.55	0.79	4.44	1.85	0.63	0.31
A. a.	–	1	–	–	–	3.34	–	5.14	–	0.65	–
P. p.	2	1	1	2	–	4.18	2.81	13.47	5.58	0.51	0.47
B. c.	1	1	–	–	–	1.02	0.69	2.14	3.03	0.35	–

Table 3. Average activities of trypsin and amylase (mU mg^{-1} protein) and trypsin/amylase ratio of macrozooplankton species collected on 3 occasions in summer. 1 = 4 Jun; 2 = 26 Jun; 3 = 21 Jul. $\bar{X}$ and SD calculated from the average values of each sampling occasion. Abbreviations as given in Table 1

Species/ stage	Number of samples 1	2	3	Trypsin $\bar{X}$	Trypsin SD	Amylase $\bar{X}$	Amylase SD	Trypsin/Amylase $\bar{X}$	Trypsin/Amylase SD
C. f. V	–	6	5	32.31	35.75	2.73	0.23	11.71	12.66
C. h. V, VI	–	1	3	12.96	13.19	1.23	0.23	9.19	8.20
C. a. ♀	6	–	2	428.11	326.59	2.99	0.09	141.21	101.49
C. a. ♂	1	–	–	2.06	–	1.58	–	1.30	–
E. n. ♀	1	–	3	28.88	11.94	2.58	0.08	11.07	4.18
M. l. ♀	8	6	5	23.08	24.17	5.29	0.51	4.78	5.39
P. a.	–	–	2	29.96	–	2.81	–	10.68	–
M. n.	–	–	4	7.70	–	2.41	–	2.91	–
B. a.	2	–	2	343.22	290.33	3.05	0.88	95.24	76.95
C. spp.	–	–	2	59.30	–	5.29	–	10.72	–
T. h.	1	–	–	2.31	–	5.50	–	0.42	–
E. i.	–	–	3	44.93	–	10.28	–	4.17	–
B. c.	–	–	2	17.56	–	5.68	–	2.91	–

longa usually had low enzyme activity, corresponding to the lower part of the ranges found in Kosterfjorden (Table 6), where minima usually were recorded in September/October. Low enzyme activity during winter seems therefore to be a general trend.

Diel variations

Stage V copepodites of *Calanus finmarchicus* studied in late September 1981 showed a pronounced diurnal cycle, both in trypsin activity and gut fullness (Fig. 3).

Table 4. Average activities of trypsin and amylase (mU mg^{-1} protein) and trypsin/amylase ratio of macrozooplankton species sampled on 2 occasions in autumn. 1 = 18 Sep; 2 = 15 Oct. $\bar{X}$ and SD calculated from the average values at each sampling occasion. Abbreviations as given in Table 1

Species/ stage	Number of samples 1	2	Trypsin $\bar{X}$	SD	Amylase $\bar{X}$	SD	Trypsin/Amylase $\bar{X}$	SD
C. f. V	7	3	12.02	6.06	1.57	0.74	10.39	9.23
C. h. V, VI	–	1	14.13	–	1.01	–	9.71	–
C. a. ♀	–	4	214.96	–	1.36	–	157.04	–
E. n. ♀	–	1	28.68	–	0.73	–	39.29	–
M. l. ♀	3	5	35.88	9.42	3.38	1.14	11.56	0.11
P. a.	–	2	15.96	–	1.38	–	11.99	–
S. e.	3	3	28.10	11.84	3.53	0.32	7.77	2.73
T. h.	–	2	19.11	–	2.70	–	7.79	–

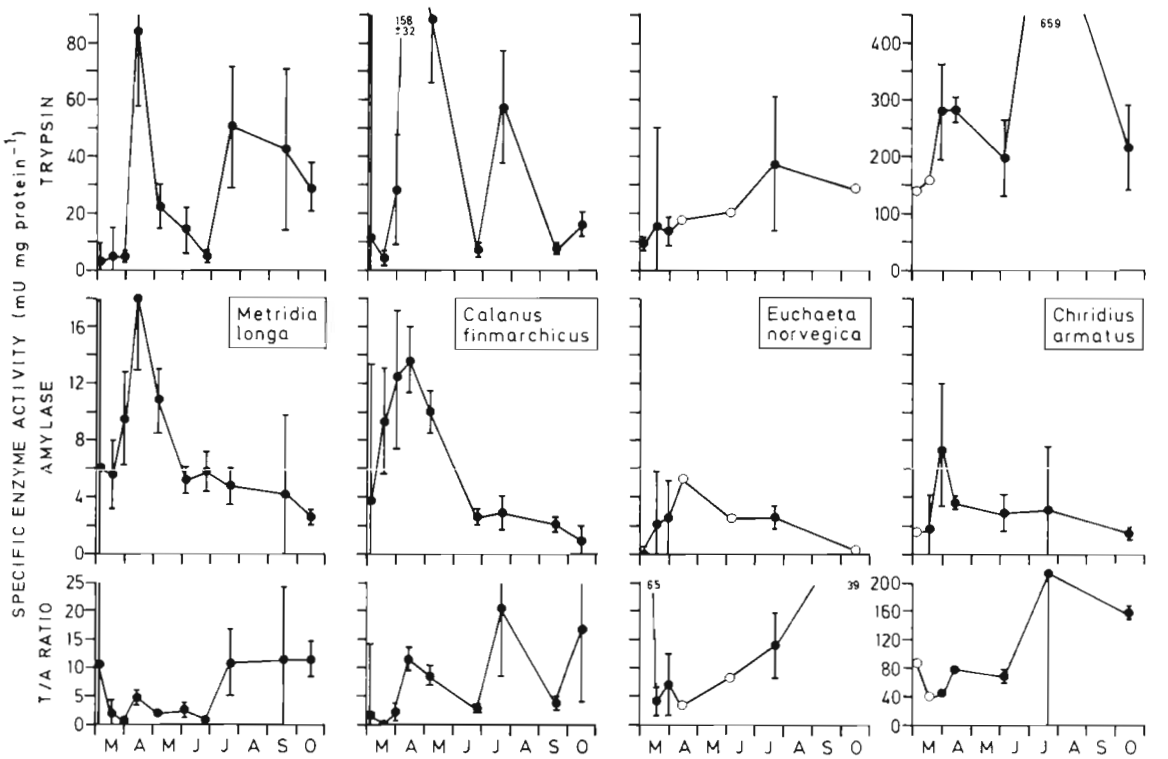

Fig. 1. Seasonal variation in the specific activities of trypsin and amylase and in the trypsin/amylase (T/A) ratio of adult females of *Metridia longa*, *Euchaeta norvegica* and *Chiridius armatus* and of Stage V copepodites of *Calanus finmarchicus*, in Kosterfjorden 1981. Vertical bars: 95 % confidence intervals. (o) Single measurements

This cycle was more weakly reflected in amylase activity and respiration rate (as determined by the ETS activity). The proportion of animals in the population which were actually feeding (determined by the proportion of animals with food in their guts) varied synchronously with the other parameters, but never exceeded 70 % (Fig. 3). The relatively stable population structure and individual body mass (protein content) of Stage V copepodites in the samples (Fig. 3E) indicate that the same population was sampled all the time and therefore that the displayed cycles reflect a physiological rhythm at the individual level.

DISCUSSION

From an ecological point of view the most interesting aspect of animal digestive-enzyme activity is its relationships with nutritional variation and environmental events. Boucher & Samain (1974) attempted to estimate

Table 5. Results of Mann-Whitney *U*-test on the significance of differences in digestive enzyme activities between the copepods *Calanus finmarchicus* and *Metridia longa*. ns: not significant ($p > 0.05$)

Date	Trypsin		Amylase		Trypsin/Amylase	
	U	*p*	*U*	*p*	*U*	*p*
3 Mar	4	ns	5	ns	4	ns
17 Mar	13	ns	22	ns	14	ns
30 Mar	41	<0.005	21	ns	40	<0.005
13, 14 Apr	67	<0.002	49	ns	63	<0.01
6 May	88	<0.001	51	ns	88	<0.001
26 Jun	28	ns	36	<0.005	36	<0.005
21 Jul	17	ns	23	<0.05	22	ns
18 Sep	21	<0.02	20	<0.05	21	<0.02
15 Oct	15	<0.05	15	<0.05	12	ns

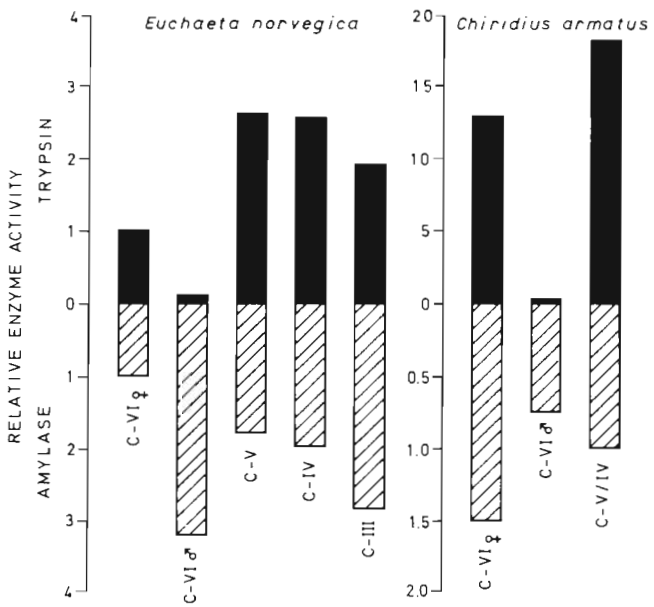

Fig. 2. *Euchaeta norvegica* and *Chiridius armatus*. Trypsin and amylase activities of different developmental stages. Most groups were inconsistently and unpredictably available. Therefore, to eliminate the possible effects of seasonal variability in comparing activities between groups, results are expressed in terms relative to the enzyme activities in females of *E. norvegica*, which were always available

ingestion rates from data on phytoplankton biomass (chlorophyll concentration) and zooplankton amylase activity, and gave a mathematical relation on the basis of results for mixed zooplankton samples from the Mauritanian upwelling area. Mayzaud & Conover (1976) showed that different digestive enzymes responded differently to changes in the nutritional environment and questioned the simple relationship suggested by Boucher & Samain (1974). Mayzaud & Conover (1976) could not give a simple mathematical expression for the relationship between food and enzyme activity, but suggested that a multiple linear regression might be calculated, estimating feeding

Table 6. Trypsin and amylase activities (mU mg^{-1} protein) of zooplankton species sampled in late November on the Norwegian west coast

Species/stage	Trypsin	Amylase
Calanus finmarchicus V	1.7–4.8*	0.2–3.5*
Chiridius armatus ♀	109.1	1.9
Euchaeta norvegica ♀	5.8	0.1
Euchaeta norvegica ♂	2.1	0.9
Euchaeta norvegica CV	3.9	0.3
Metridia longa ♀	4.1–82.1*	0.9–4.5*
Sagitta elegans	3.4	1.5–36.5
Aglantha digitale	3.1	1.8
Dimophyes arctica	2.0	2.3

* Range of average values from 13 localities

intensity from digestive-enzyme activities. Both investigations quoted dealt with mixed zooplankton samples and later studies have shown that there is considerable interspecific variation (cf. Mayzaud 1979; this study), suggesting that enzyme activities from multi-species samples may be hard to interpret uniformly. However, dominance of one or a few species in a mixed sample will certainly reduce the effects of interspecific variability due to trophic events, and increase the possibility of explaining variations in enzyme activities in the zooplankton community as a whole.

Phytoplankton abundance in Kosterfjorden, as reflected by total ($a+b+c$) chlorophyll concentration, was high from the end of February to mid April, in June, and in the first part of October (Fig. 4, from B. Rex unpubl.). The high phytoplankton occurrence in spring was thus reflected by high activities of trypsin and amylase among herbivorous zooplankton, but later 'blooms' did not affect the enzyme activity significantly (Figs. 1 and 4; Tables 2 to 4). It is noteworthy, however, that both *Calanus finmarchicus* and *Metridia longa* showed low activities in the beginning of March, when the chlorophyll data indicated a surplus of food.

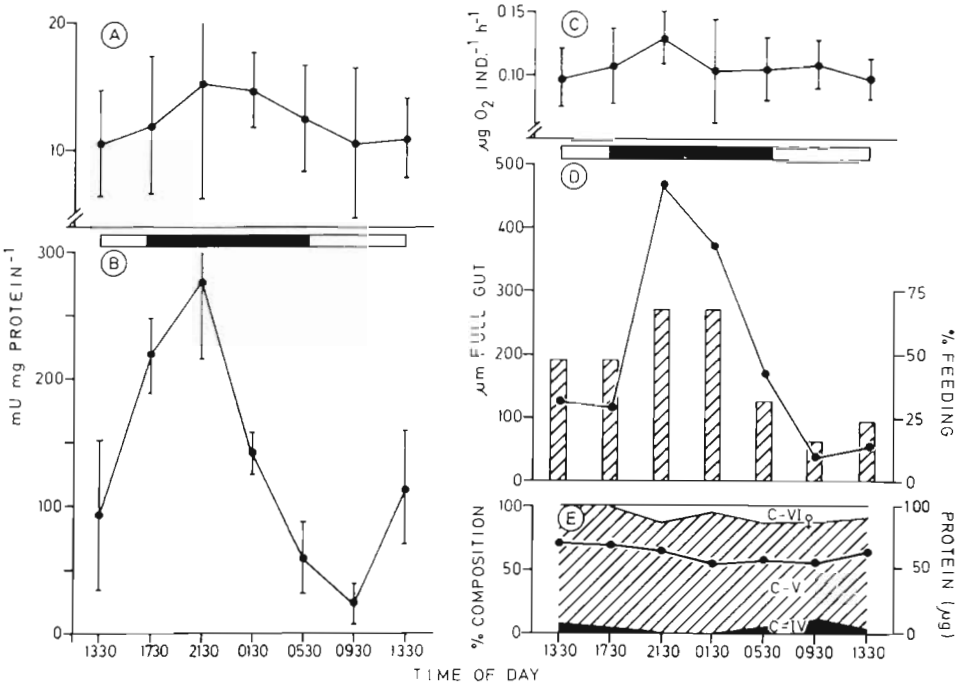

Fig. 3. *Calanus finmarchicus*. Diel variations in metabolic parameters and feeding indices of Stage V copepodites in Kosterfjorden, 28–29 Sep 1981. (A) Specific activity of amylase. (B) Specific activity of trypsin. (C) Respiration rate as given by the ETS activity. (D) Average gut fullness (curve) and proportion of feeding animals in the population (histogram). (E) Relative composition of developmental stages in the population and average individual protein content of Stage V copepodites. Vertical bars: 95 % confidence limits; dark region of horizontal bars: period when sun was below horizon

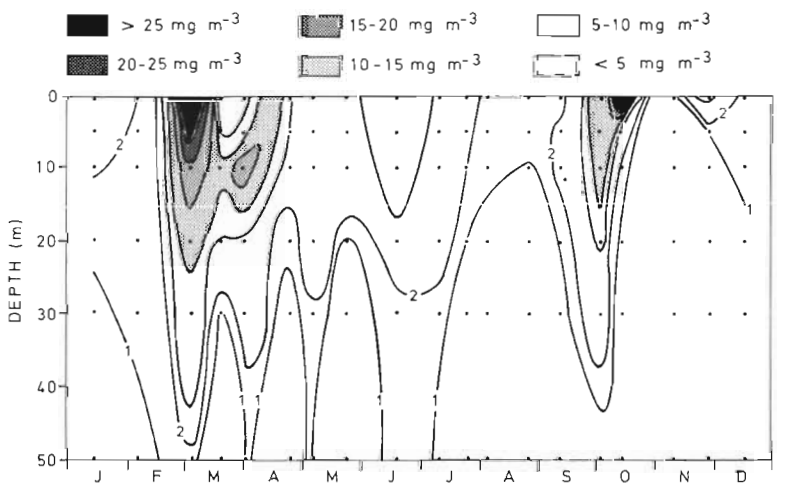

Fig. 4. Isopleth diagram of chlorophyll concentration (chlorophyll *a* + *b* + *c*) in Kosterfjorden during 1981. Data from B. Rex unpubl.

Obviously, our understanding of environmental regulation of zooplankton enzyme activity is still premature, but the requirements of the animals and the quality of the food are factors that have great influence in the processes, as suggested by the 'assimilation law' (Samain et al. 1981, 1985, Harris et al. 1986). Such a 'law' can explain most of the variation occurring in the natural environment, but appears to be of very limited value as a predictive tool. For example, a number of authors have shown positive correlations between food availability and enzyme activity, both for mixed zooplankton (e. g. Boucher & Samain 1974, 1975, Boucher et al. 1976, Mayzaud & Conover 1976), and for single species (e. g. Mayzaud & Poulet 1978, Cox 1981, Cox & Willason 1981, Hirche 1981, 1983). In contrast, Hasset & Landry (1983) found that *Calanus pacificus* had highest enzyme activity when food level was at a minimum. These seemingly contradictory results are simply explained by the 'assimilation law', where a positive relationship between substrate and enzyme activity indicates food limitations, a negative or uncorrelated one food saturation. Samain et al. (1981) suggested that the enzyme activity is mainly determined by the metabolic need for different substrates, thus optimizing the composition of assimilated food by regulating the activity of the different enzymes in response to the composition of the ingested food. This theory implies that food with low content of a given substrate should induce increased activity of the corresponding enzyme and vice versa. If this were true then carnivorous

species (feeding on food with high protein content) should not necessarily show higher protease activity than herbivorous species (feeding on food with high carbohydrate content) or vice versa for carbohydrase activity. Furthermore, as there is no trend of differences in relative protein and carbohydrate content among carnivores and herbivores (cf. Båmstedt 1980) the food requirements should not differ systematically between the 2 groups.

Head & Conover (1983) studied the enzymatic response of *Calanus hyperboreus* offered a variety of foods, and found that the copepod secreted a suite of digestive enzymes, unrelated to the food composition. Feeding experiments on fish have also indicated that there is a very complex relationship between quality and quantity of the food ingested and the activity of different digestive enzymes (cf. Kawai & Ikeda 1971, 1972, 1973a, b, Onishi et al. 1976, McLeese & Stevens 1982). Recently however, Pedersen et al. (1987) showed that the intestinal trypsin content of larval herring *Clupea harengus* was strongly correlated with the prey abundance and intestinal content of prey, whereas pancreatic trypsinogen content was more or less unrelated to these factors.

Mayzaud & Poulet (1978) suggested that neritic herbivorous zooplankton are in a continuous process of acclimation to the food environment, since environmental changes occur with higher frequency than the time sufficient for the enzyme system to acclimatize. These authors suggested that between 24 h and 6 d was required for full acclimation of the digestive system, and that more rapid changes in the food environment would cause a saturation-type response of the enzyme system. Such a functional relationship is not in accordance with the results on diel variability in enzyme activity of *Calanus finmarchicus* from northern Norway (Tande & Slagstad 1982) or my own results on *C. finmarchicus* from Kosterfjorden (Fig. 3), the latter indicating a synchronous variation in trypsin activity and feeding activity, and a less pronounced but parallel variation in amylase activity and respiration rate. A similar but less pronounced pattern has been reported by Harris & Malej (1986) for trypsin and laminarase activities of *Calanus helgolandicus* from the western English Channel.

Different responses in relation to seasonal and diurnal events may be explained by a 2-level regulating mechanism. The long-term level is determined by the number of enzyme-storing cells (B-cells) in the gut epithelium whereas the short-term level is regulated by direct enzyme production as a response to feeding events. The potential limit of enzyme production at any given time is determined by the actual abundance of B-cells. Hallberg & Hirche (1980) have shown a positive correlation between number of B-cells in the gut epithelium and enzyme activity of *Calanus finmarchicus* and *Calanus helgolandicus*. Nott et al. (1985) elegantly showed that *C. helgolandicus* uses up part of its digestive epithelium with the B-cells during feeding and that this epithelium is renewed during non-feeding periods. They suggested that the supply of epithelium cells actually may be the limiting factor for the duration of feeding.

The postulated existence of a 2-level regulating mechanism could explain why the herbivores in Kosterfjorden did not respond with high enzyme activity during the first part of the spring bloom; development of specific cell types which increases the digestive capacity of the animals may take considerable time. In contrast, during the productive season of the year it must be an advantage to have a high digestion capacity (many B-cells) and regulate the enzyme activity by controlled synthesis of the different enzymes, thereby making it possible to adapt to short-term variations in the food supply. The diel rhythm shown in Fig. 3 is probably an example of such a short-term regulating mechanism. In a study on the arctic copepod *Calanus glacialis* from a fjord on the west coast of Spitsbergen, Båmstedt (1984) showed that the absence of a diel rhythm in feeding activity in mid summer was also associated with stochastic variations in enzyme activity.

Several authors have detected a significant diel feeding rhythm uncorrelated with the digestive enzyme activities in copepod species such as *Calanus glacialis, C. hyperboreus, C. finmarchicus, Centropages hamatus, C. typicus, Pseudocalanus elongatus* and *Temora longicornis* (Head et al. 1984, 1985, Baars & Oosterhuis 1984). By combinations of low and high abundance of food components and low and high food requirements such apparently contradictory results may be explained by the 'assimilation law' of Samain et al. (1981). However, strong evidence is lacking for this model, except from a laboratory study on *Artemia* (Samain et al. 1985), and therefore it still appears somewhat speculative.

In conclusion, the incorporation of information on digestive enzymes into studies of the nutritional ecology of zooplankton may give additional information on the environment/organism relationships, but results may easily be misinterpreted. The high interspecific variation makes the interpretation of results from multispecies samples difficult. Variations in enzyme activities within herbivores/omnivores and carnivores, respectively, are high, reducing the possibility of finding significant, general differences between these groups. Our present knowledge points to several possibilities for the environmental regulation of activities of digestive enzymes in zooplankton, where non-quantitative differences in 'nutritional strategy'

and short-term and long-term rhythms in feeding activity of species may be interpreted. However, any further generalisations must await further comparative studies. In particular, determinations of ingestion and assimilation should be performed in food regimes varying from starvation to saturation conditions and enzyme activities recorded concomittantly. Such controlled laboratory experiments should help to explain seemingly contradictory results and test the 'assimilation law' by Samain et al. (1981).

Acknowledgements. I thank Ingrid Dahlin and Birgitta Klefbohm for technical assistance, Hans-Jürgen Hirche and 2 anonymous referees for comments on the manuscript and Neil Swanberg for linguistic corrections. This project was financially supported by grants from the Swedish Natural Science Research Council (NFR).

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This article was presented by Dr H. R. Skjoldal; it was accepted for printing on February 2, 1988