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A laboratory study on digestive processes in the Antarctic krill, *Euphausia superba*, with special regard to chitinolytic enzymes

Abstract Feeding experiments of 9, 14 and 20 days duration were carried out on the Antarctic krill, *Euphausia superba*. Two groups were fed with the chitinous diatom *Cyclotella cryptica* and the non-chitinous green algae *Dunaliella bioculata*, respectively. A control group remained unfed. The time courses of the activities of endo- and exochitinase in the stomach and the midgut gland were compared with those of the digestive enzymes protease, cellulase (1,4-β-D-glucanase) and laminarinase (1,3-β-D-glucanase). Specific activities of all enzymes were higher in the stomach than in the midgut gland. Characteristic time courses of activity were evident after 4 days. In starved animals, enzyme activities decreased to a minimum after 4 days and recovered within 14 days to initial values. In the stomach, the activities of endo- and exochitinase increased when krill were fed on *Cyclotella*. For animals fed with *Dunaliella*, activities stayed constant or decreased slightly. The results confirm chitinases as digestive enzymes and, therefore, the capability of krill to utilize various food sources.

Introduction

The Antarctic ocean is an extreme environment and organisms living there require biological specializations and, particularly, physiological and biochemical adaptations. An important organism in the Antarctic ecosystem is the Antarctic krill, *Euphausia superba*. Krill represent the main food source for predators such as whales, seals and birds. As filter feeders, krill feed mainly on phytoplankton. Primary production in Antarctic waters is, however, characterized by high seasonal and spatial variation (El-Sayed and Taguchi 1981; El-

Sayed and Weber 1982) and, potentially, *E. superba* can also change to carnivorous feeding at low phytoplankton densities or during the Antarctic winter (Price et al. 1988; Lancraft et al. 1991; Huntley et al. 1994; Atkinson and Snÿder 1997; Pakhomov et al. 1997). Krill are able to graze on patchy food sources very efficiently (Hamner et al. 1983). Appropriate activity levels of digestive enzymes are a prerequisite to ensure rapid digestion and resorption under a variable food regime.

In addition to several glucanases and proteases (Mayzaud et al. 1985; McConville et al. 1986), high activities of endo-chitinase (poly-β-1,4-(2-acetamido-2-deoxy)-D-glucosid-glucanohydrolase) and *N*-acetyl-β-D-glucosaminidase (here NAGase) have been found in the digestive tract of krill (Buchholz 1989). Both enzymes are also present in the integument, where they show moult-related patterns of activity (Buchholz and Buchholz 1989). In the stomach and the midgut gland (hepatopancreas), however, the activities are not affected by the moult cycle. Previous field investigations support the hypothesis of a digestive function of both enzymes (Buchholz and Saborowski 1996).

Chitin consists of amino sugars and is, therefore, of considerable nutritive value. Diatoms of the genus *Thalassiosira* contain substantial amounts of chitin as "spines" (McLachlan et al. 1965) and at certain times can dominate the Antarctic phytoplankton (Johansen and Fryxell 1985; V. Smetacek, personal communication). Krill could, therefore, make use of this rich food source, and the presence of a set of readily available enzymes would enhance their physiological capability. A possible induction of the chitinases, similar to that reported for other enzymes (Mayzaud and Conover 1976; Cox and Willason 1981), would demonstrate their relation to the food supply, and identify them as digestive enzymes.

The aim of the present work was to study the response of chitinases in the krill's digestive organs to different feeding conditions in comparison to various typical digestive enzymes. The protocol included feeding chitinous and non-chitinous algae, respectively. The

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activity patterns of the chitinases were investigated in relation to protease, cellulase, laminarinase, gut fullness and the gross composition of the diet. The results are discussed in relation to the digestion physiology of krill and Antarctic ecological conditions.

Materials and methods

Phytoplankton cultures

Strains of the chitinous diatom *Cyclotella cryptica* (Bacillariophyceae, Centrales) and the green alga *Dunaliella bioculata* (Chlorophyceae) were obtained from the algal collection of the University of Göttingen (Dr. Schlösser). Both species were cultured during the expedition Met 11/4 (21.12.1989–18.01.1990) on board the research vessel FS Meteor. Semi-batch cultures were maintained at 20–25 °C in a seawater/nutrient solution according to Guillard and Ryther (1962) and were exposed to a 16:8 h light:dark cycle.

For the determination of the dry weight of algae, 800 ml of the culture was centrifuged for 10 min at 4000 g. Several pellets were transferred into 1.5-ml reaction tubes and were again centrifuged for 10 min at 8000 g. The supernatants were removed and the remaining material was lyophilized to constant weight (Christ, Beta A).

Determination of substrate concentrations in the cultured algae

Soluble protein and the substrates cellulose and laminarin were determined and expressed relative to the dry weight of algae samples. Lyophilized samples of algae were resuspended in a total volume of 1 ml 0.2 M Citrate-Phosphate Buffer (CPB), pH 5.5, and homogenized on ice by ultrasonication (Branson, Sonifier B12) for 3×15 s with a break of 20 s each time. The cell-disrupter was adjusted to 30% of maximal energy. Algal chitin as "spines" was isolated from fresh cells according to McLachlan et al. (1965) and stored at −80 °C for further analysis. For *D. bioculata*, which does not contain chitinous spines, lyophilized algae were analysed.

Soluble protein was determined according to Bradford (1976) using the BioRad microassay.

Cellulose (1,4-β-D-glucan) was hydrolysed by cellulase (Sigma C-2415). The reducing sugars were determined using 3′5′-dinitrosalicylic acid. In order to denature algal enzymes, the samples were heated for 10 min at 100 °C prior to analysis. A 100-μl sample was added to 100 μl of 0.2 M CPB, pH 5.5 in 1.5-ml reaction tubes. The reaction was initiated with 100 μl cellulase solution (1 U ml^{-1} in 0.2 M CPB, pH 5.5). The tubes were incubated for 2 h at 37 °C, with continuous stirring. After incubation, the tubes were centrifuged for 5 min at 15,000 g. The supernatant (250 μl) was added to 250 μl of 0.1 M K/Na-P-Buffer, pH 6.8. After the addition of 3′5′-dinitrosalicyl acid solution and 5 min of incubation at 100 °C, the reaction was stopped by cooling in ice-water. The absorption was read at 546 nm. Standards with cellulose (Sigma C-6288) suspended in 0.2 M CPB, pH 5.5, were run in parallel.

Laminarin-like glucans (1,3-β-D-glucans) were determined as described for cellulose, except that laminarinase (Sigma L-5272) with an activity of 1 U ml^{-1} replaced the cellulase. Standards were run in parallel with laminarin (Sigma L-9634).

The amount of chitin in algae was measured by acid hydrolysis of isolated spines and subsequent determination of amino sugars. For the acid hydrolysis, the dried spines were resuspended in 0.5 ml distilled water by sonication (Branson, Sonifier B12) for 3×15 s, interrupted by breaks of 20 s. Fifty microlitres of the suspension was incubated with 950 μl of 6 N HCl at 100 °C for 6 h. After incubation, the solution was transferred into small glass vials and evaporated under a stream of air. The residue was dissolved in 500 μl borate buffer, pH 9.2. Fifty microlitres of this solution were assayed for amino sugars according to Morgan and Elson (1934), modified by Saborowski et al. (1993). Blanks were run in parallel as well as standards containing purified chitin (Sigma C-3641).

Collection of krill

Antarctic krill, *E. superba*, were caught north of the Antarctic peninsula during the cruise Met 11/4 (21 December 1989 to 18 January 1990). Sampling was carried out with a Bongo net (2×63 cm, mesh width 320 μm) near Elephant Island (60 °S57.75 27 °W07.50) and a Rectangular Midwater Trawl, RMT 1 + 8 (Roe and Shale 1979) near King George Island (62 °S05.95 57 °W39.00 and 62 °S44.77 59 °W06.43). All samples were obtained from depths between 60 m and the surface. Immediately after capture, the animals were transferred into tanks containing fresh, aerated seawater. After 24 h the most active animals were selected for the feeding experiments.

Feeding experiments

In order to cover a maximum range of experimental time scales, three feeding experiments of different duration were carried out. Within each experiment, three groups of animals were maintained separately in aerated tanks. One group was fed with the diatom *C. cryptica*, the second with the green alga *D. bioculata*, and the third group was not fed. The experiments were carried out at 0–2 °C and illumination was provided on a 16:8 h light:dark cycle. The maintenance tanks (closed systems) had been filled with fresh seawater from the ship's seawater supply system. This surface water had been filtered through 3-μm membrane filters (Sartorius, 11302-293-G) using a pressure filter system (Sartorius, SM 16277). During the experiments the water in the tanks was exchanged at least every other day. Faecal pellets and moults were removed frequently. Krill were frozen and stored at −75 °C until analysis. Feeding with algae started 1 day after the tanks had been stocked with krill. Fifty millilitres of algal culture (approximately 1.75 mg dry weight, DW) were added per animal per day.

Experiment 1 lasted for 20 days and was carried out in three tanks of 40×60 cm and 20-cm height. Each tank was equipped with 24 PVC containers (volume 1 l), each containing 1 animal. The bottoms of the containers were punctured to ensure equal water levels in all containers and to expose the animals to the same water conditions. Experiment 2 (14 days) was carried out with 45 krill in each of 3 basins of $40 \times 30 \times 30$ cm. The animals were not separated as described above. In experiment 3 (9 days), 135 krill were maintained in each of 3 basins of $40 \times 60 \times 60$ cm. A subsample of 15 animals was removed after 2, 4, 6 and 9 days. The volume of the water was reduced proportionally after each sampling in order to maintain a constant density of krill.

Only a representative number of animals from each feeding experiment was analysed for enzyme activities. Thus, the numbers of animals used for feeding experiments and the numbers analysed differed in the three experiments.

Enzyme determination

The fullness of the three digestive organs, stomach, midgut gland and gut, was determined visually according to Morris et al. (1983) on frozen animals prior to the dissection of the stomach and the midgut gland for biochemical analysis. The organs were transferred into reaction tubes and 1 ml 0.2 mol l^{-1} of citrate/phosphate buffer (CPB, pH 5.5) was added. Homogenization was performed on ice with an ultrasonic cell disrupter (Branson, Sonifier B-12, microtip 101-148-063) at 30% of maximum energy with three bursts of 15 s with a break of 20 s inbetween. The homogenates were centrifuged at 15,000 g and the supernatant was used for analysis.

The activities of chitinase and NAGase were determined according to Saborowski et al. (1993). Activities of total protease, cellulase and laminarinase were determined as described by Buchholz and Saborowski (1996) and Donachie et al. (1995), respectively. Enzyme activities as referred to in the following were normalized to fresh weight (ΔA min^{-1} mg^{-1} fresh weight).

As a control, we also determined the specific activities of the enzymes investigated in the phytoplankton cultures. Fifty millilitres of culture suspension was centrifuged at 4000 g. The pellets were resuspended in a small volume of culture medium and transferred into preweighed reaction caps (1.2 ml). After centrifugation at 15,000 g, the supernatant was discharged and the fresh weight of the algae was determined. The sediment was homogenized and processed as described for animal tissue samples.

Statistics

Pairwise comparison of data sets was performed with the Mann-Whitney U-test. The results are presented in the figures as means and 95% confidence intervals (95%CI).

A rank-correlation analysis was carried out on the data of enzyme activity in the stomach and the midgut gland during experiment 3. The strength of the association was expressed by the Spearman correlation coefficients as calculated by the computer program SYSTAT (Wilkinson 1989). The significance level was set at $P \leq 0.05$.

Results

Phytoplankton cultures

The biomass of *C. cryptica* cultures was 34.8 ± 1.6 mg DW l^{-1} and that of *D. bioculata* 38.3 ± 2.1 mg DW l^{-1} ($n = 7$). The amount of laminarinase digestible glucans was higher in *C. cryptica* ($8.7 \pm 0.9\%$DW) than in *D. bioculata* ($4.6 \pm 1.4\%$ DW). The cellulose content was nearly identical in the two species: $21.0 \pm 3.1\%$ DW in *C. cryptica* and $20.2 \pm 1.9\%$ DW in *D. bioculata*. The water-soluble protein content of *C. cryptica* was $15.2 \pm 2.5\%$ DW and that of *D. bioculata* $12.5 \pm 0.6\%$ DW. The amount of chitin in *C. cryptica* was $11.6 \pm 2.9\%$ DW while no chitin was found in *D. bioculata*.

No significant activity of the enzymes investigated was found in the phytoplankton cultures. Low activities of chitinase in *Cyclotella* and *Dunaliella* and of laminarinase in *Dunaliella* cultures were recorded but these were within the range of error for the assay.

Morphometric data and organ colour index of maintained krill

The largest animals were used in experiment 1 (Table 1). Accordingly, the percentage of juveniles was smallest

there. Specimens used in experiments 2 and 3 were obtained from the same catch and were, therefore, of similar length and weight.

The colour index of the digestive organs was related to the amount of food ingested during the feeding experiments (Fig. 1). The starved animals had significantly lower values than the fed animals. Within all experiments the values in fed animals were similar and ranged between 3 and 6.

Enzyme activities

Experiment 1 (Fig. 2)

A control group was frozen immediately after the catch. Before feeding started, a further control group was frozen at time $t = 0$. The experiment was terminated after 20 days.

Stomach chitinase and NAGase increased between capture and the beginning of feeding. After 20 days of the experiment chitinase and NAGase activities were significantly reduced in starved animals, but showed no distinct differences in animals fed *Cyclotella* or *Dunaliella*. A similar pattern was seen for the midgut gland, although in this case NAGase decreased significantly between capture and the start of the experiment. Animals fed with *Cyclotella* had higher chitinolytic activities than animals fed with *Dunaliella*.

In the stomach, protease and laminarinase activities of fed animals were higher than those in starved animals. Highest cellulase activities were evident in animals fed with *Dunaliella*. In the midgut gland, animals fed with *Cyclotella* had the highest activities of all three enzymes. Laminarinase activity in starved animals was higher than in animals fed with *Dunaliella*.

Experiment 2 (Fig. 3)

As in experiment 1 there was an increase in chitinase and NAGase between capture and the beginning of feeding. After 14 days there were only slight differences in chitinase activity between the three groups while NAGase was significantly higher in animals fed with *Cyclotella* than in animals fed with *Dunaliella*. In the midgut gland chitinolytic activities were significantly higher in animals fed with *Dunaliella*.

Stomach protease and cellulase levels were lower in animals fed with *Dunaliella*. Starved animals had slightly elevated levels of laminarinase. In the midgut gland, most enzyme activities of fed and starved animals were

Table 1 Length, fresh weight (means $\pm$ SD, each) and sex distribution (% juveniles, females and males) of krill maintained in the feeding experiments

	n	Length (mm)	Fresh weight (mg)		Sex (% j/f/m)	
Experiment 1	60	39.3 $\pm$ 4.2	466.8 $\pm$ 217.5	5	54	41
Experiment 2	25	34.3 $\pm$ 1.9	259.7 $\pm$ 45.9	20	37	43
Experiment 3	135	34.2 $\pm$ 1.9	264.6 $\pm$ 42.2	19	33	48

Fig. 1 Colour indices of the stomach (*ST*), the midgut gland (*MG*), and the gut (*GU*) of animals used in the feeding experiments (means and 95% CI, *n* = 4–16 of individual organs)
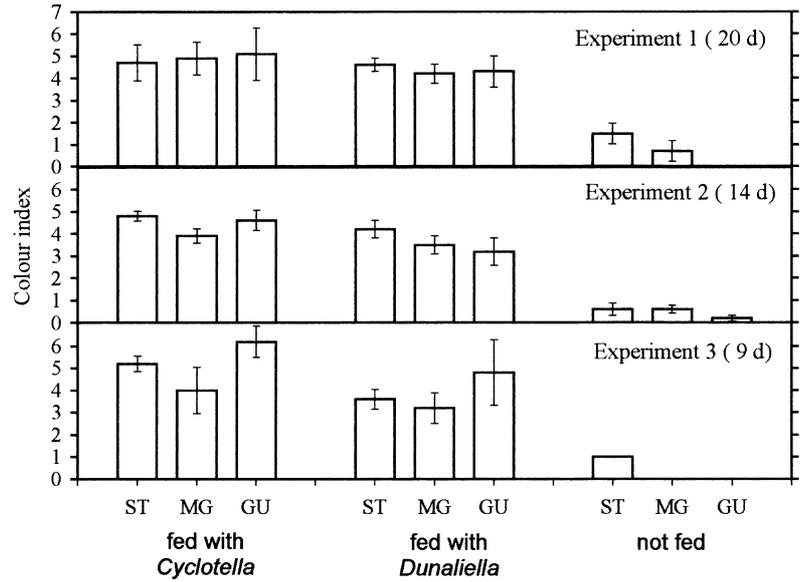
Fig. 2 Activities of digestive enzymes in the stomach and the midgut gland of krill maintained in experiment 1 (20 days). Krill were not fed (*Hu*), fed with *Dunaliella*(Du – chitin) or fed with *Cyclotella* (Cy + chitin). The *bar charts* show means and 95% CI of 4–16 individual organs. Values sharing the same *letter* are not significantly different within the respective plot (*P* ≤ 0.05)
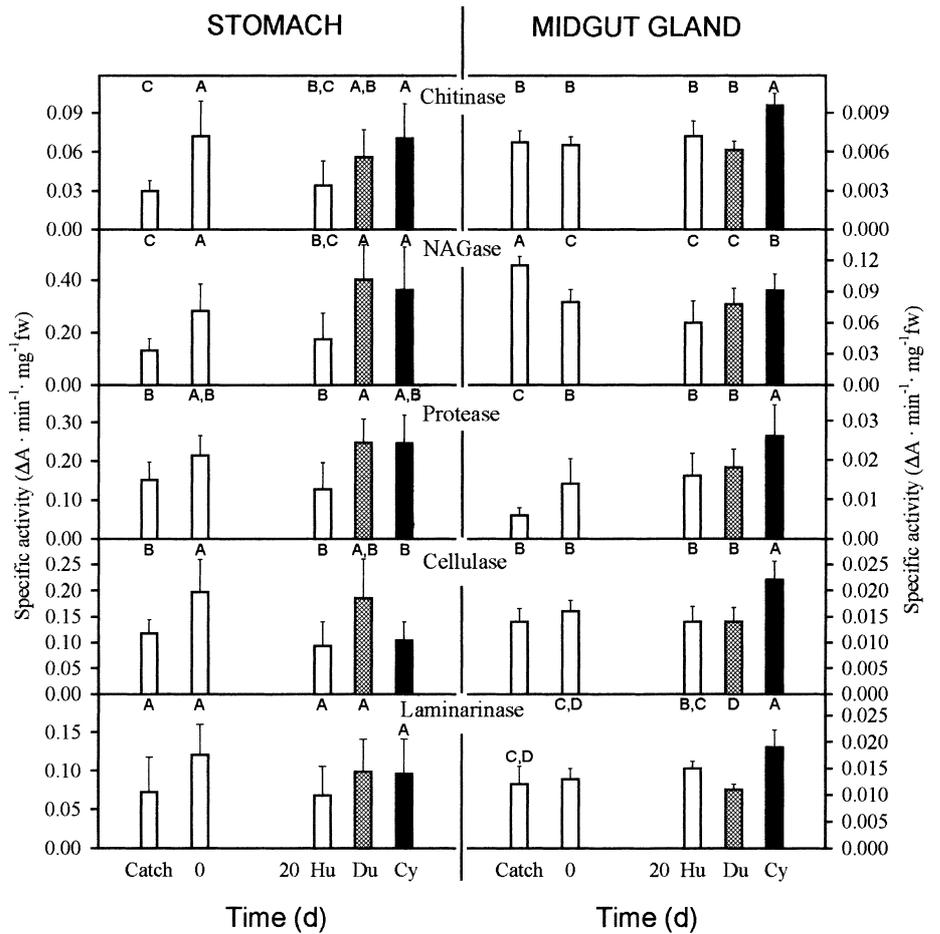

clearly higher than initial values. With the exception of protease, which was highest in starved animals, animals fed with *Dunaliella* had elevated activities at the end of the experiment which, however, were not statistically significant.

Experiment 3 (Fig. 4)

In experiment 3 samples were taken after capture, at the beginning of feeding, and then after 2, 4, 6 and 9 days of feeding. In the stomach there was a slight increase of

Fig. 3 Activities of digestive enzymes in the stomach and the midgut gland of krill maintained in experiment 2 (14 days). Krill were not fed (*Hu*), fed with *Dunaliella* (Du − chitin) or fed with *Cyclotella* (Cy + chitin). Means and 95% CI of five individual organs. Values sharing the same *letter* are not significantly different within the respective plot ($P \leq 0.05$)
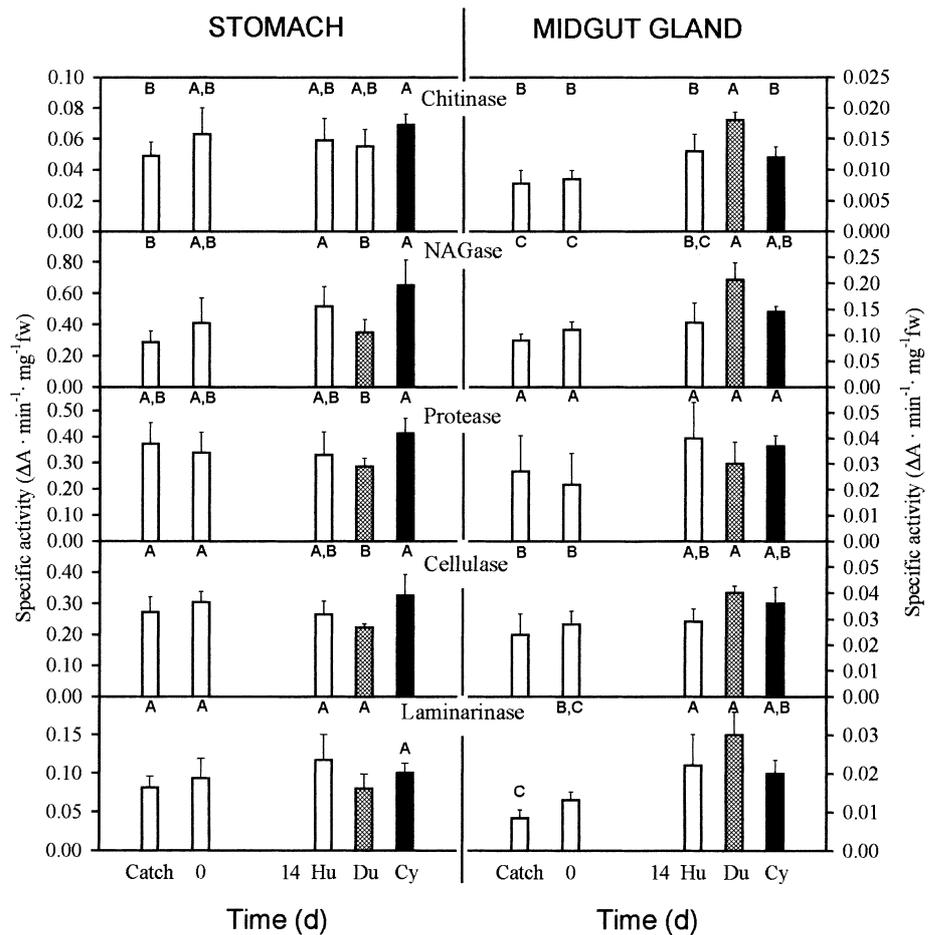
chitinase, as well as NAGase activity, between capture and the beginning of feeding as observed in experiments 1 and 2. In starved animals, chitinolytic activities decreased rapidly after $t = 0$ and reached minimal values at the 4th day. Thereafter, the activities increased again and reached about 50% of the initial values. Chitinase and NAGase activity in animals fed with *Dunaliella* decreased continuously and, after 9 days, were not significantly different from those in starved animals. In contrast, activity in animals fed with *Cyclotella* increased and final values were significantly higher than in animals fed with *Dunaliella* or starved animals. Maximum activities were reached at the 6th day, after which chitinase dropped towards initial values, while NAGase remained at a high level.

In the midgut gland, the time courses of activity were less distinct. Chitinolytic activities were similar in groups fed with both algae, but starved animals showed decreased activities.

In general, protease and cellulase in the stomach showed similar patterns as the chitinolytic enzymes. Laminarinase increased strongly after 6 days in both the stomach and the midgut gland. In the midgut gland, the time courses of enzyme activity differed less than in the stomach. In starved animals a decrease of activity to the 4th day of the experiment was observed. In the fed animals, the distinct trends reported for the stomach could not be seen.

Correlation analysis

The correlation analysis between activity levels in the stomach and in the midgut gland in experiment 3 showed statistically significant correlations only for protease in starved animals and in animals fed with *Dunaliella* (Table 2). Most enzymes were only weakly correlated and some negative correlations also appeared.

The correlation analysis between all stomach enzymes within one feeding condition (Table 3) showed, for starved animals, statistically significant relations of chitinase to NAGase, protease and cellulase. Almost perfect correspondence was found for NAGase-protease, NAGase-cellulase, and for protease and cellulase. The other correlation coefficients were comparatively high and were always positive. In animals fed with *Dunaliella,* again all correlation coefficients were positive and comparatively high. Five enzymes matched significantly. The weakest correlations among the enzymes were found between NAGase-cellulase and NAGase-laminarinase. In animals fed with *Cyclotella*, the correlation coefficients were lower and some negative

Fig. 4 Time courses of digestive enzymes in stomach and midgut gland of krill maintained in experiment 3 (9 days) (○ not fed, *circle partly filled* fed with *Dunaliella*, ● fed with *Cyclotella*; means and 95% CI, $n = 4$–15 individual organs)
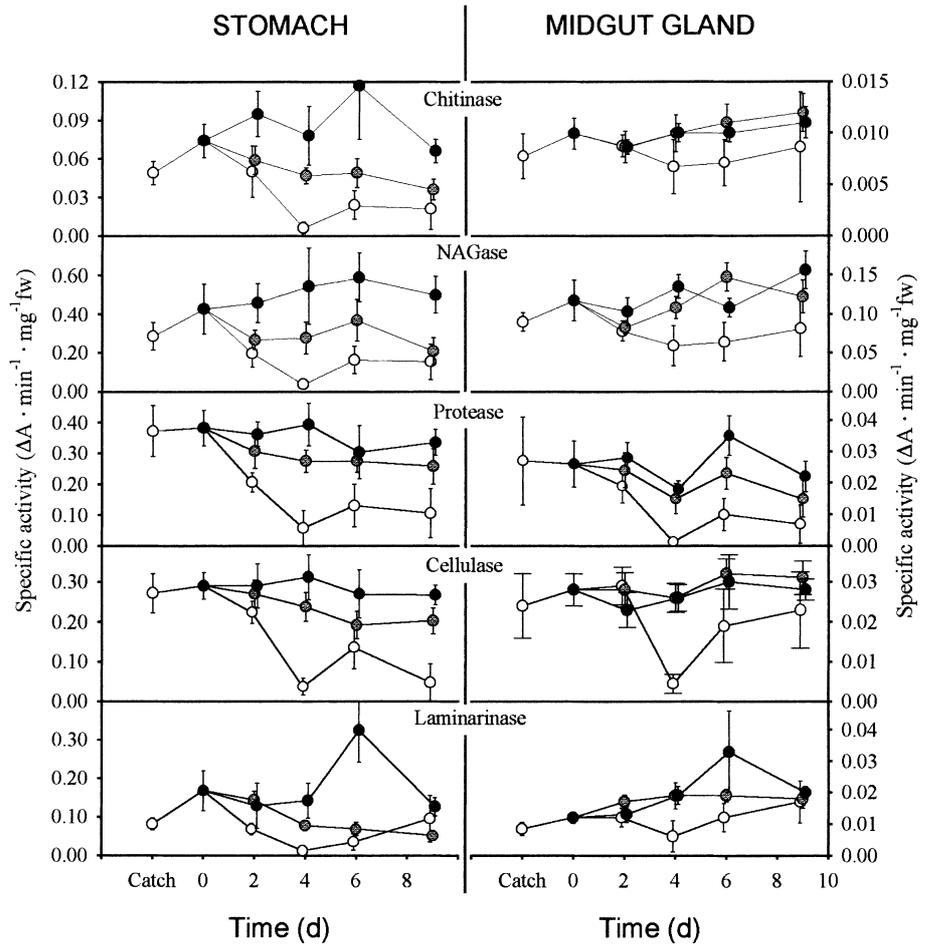
Table 2 Matrix of correlation coefficients (r_s) of enzyme activities between the stomach and the midgut gland of *Euphausia superba* under different feeding conditions in experiment 3 ($n = 6$, see Fig. 4)

	Chitinase	NAGase	Protease	Cellulase	Laminarinase
Hunger	0.829	0.829	0.943*	0.771	0.577
Dunaliella	−0.609	0.257	0.897*	−0.667	−0.638
Cyclotella	0.203	0.486	−0.657	−0.485	0.486

*$P< = 0.05$

Table 3 Matrix of correlation coefficients (r_s) between the enzyme activities in the stomach and in the midgut gland, respectively, of *Euphausia superba* under different feeding conditions in experiment 3 ($n = 6$, see Fig. 4)

	Stomach				Midgut gland			
	NAGase	Protease	Cellulase	Laminarinase	NAGase	Protease	Cellulase	Laminarinase
Hunger								
Chitinase	0.943*	0.943*	0.943*	0.600	0.771	0.543	0.886*	0.577
NAGase		1.000*	1.000*	0.657		0.771	0.657	0.334
Protease			1.000*	0.657			0.714	−0.030
Cellulase				0.657				0.334
Fed with *Dunaliella*								
Chitinase	0.638	0.868*	0.667	0.899*	0.829	−0.841*	0.754	0.754
NAGase		0.667	0.317	0.486		−0.435	0.754	0.522
Protease			0.899*	0.927*			−0.441	−0.868*
Cellulase				0.886*				0.529
Fed with *Cyclotella*								
Chitinase	0.714	−0.314	0.203	0.771	0.899*	−0.406	0.676	0.841*
NAGase		−0.371	−0.116	0.600		−0.714	0.493	0.543
Protease			0.841*	−0.086			0.058	0.086
Cellulase				0.203				0.638

*$P< = 0.05$

correlations appeared. The only significant positive relation was found between protease and cellulase.

In the midgut gland only a few statistically significant correlations were found as compared to the stomach. In starved animals these were between chitinase and cellulase. In animals fed with *Dunaliella*, significant correlations appeared between chitinase and protease as well as between protease and laminarinase; however, these were negative. In animals fed with *Cyclotella*, the courses of chitinase and NAGase, as well as chitinase and laminarinase, matched significantly and were positively correlated.

Discussion

The algae used for the feeding experiments needed to be easily cultured during the expedition, to have high growth rates, and to contain different amounts of chitin. Prior to the expedition, attempts were made to grow several arctic diatom species. The cultures had rather low growth rates, however. Thus, we decided to use boreal species which could be maintained at higher temperatures and which could sustain high growth rates and high densities in culture. The green alga *D. bioculata* was chosen as a non-chitinous species and the diatom *C. cryptica* was selected because of its high chitin content (Herth and Zugenmaier 1977). *Cyclotella* contains chitinous spines at the edge of the valves (Herth and Barthlott 1979). A description of the chitin-synthesizing apparatus of diatoms is given by Herth (1978). These typical "pores" or "strutted processes" are also used as criteria in species determination. Johansen and Fryxell (1985) found several *Thalassiosira* species in Antarctic waters using these criteria. Thus, chitin-containing diatoms are common in the Antarctic Ocean. Opalinski et al. (1997) found that krill prefer *Thalassiosira* species as food items.

Chitin in *C. cryptica* amounted to 11.6% of the dry weight, corresponding to 4 mg l^{-1} of culture. *D. bioculata* contained no chitin. In comparison, McLachlan et al. (1965) reported a chitin content of 31–38% in *Thalassiosira fluviatilis* and Lindsay and Gooday (1985) determined 4.9 mg chitin l^{-1} in cultures of the same species.

The food offered was accepted well as determined by the organ fullness indices. These were in the same range or higher than those for field samples, indicating a sufficient food supply during the experiments. However, the evaluation is not without problems. A certain element of subjectivity cannot be avoided and changing food composition can cause differences in colour and intensity. Empty organs have a slight yellowish coloration, corresponding to an index between 1 and 2, which explains the values for starved krill.

Containers for the maintenance of krill have to be large enough to allow the animals to move freely in the water and to ensure unhindered filtering. However, for longer experiments it is necessary to separate specimens, because cannibalism may occur (Ikeda and Dixon 1982). We observed cannibalism only occasionally when krill were maintained in small tanks and starved for more than 3 weeks. Otherwise there were no distinct behavioural differences between animals maintained in groups and those kept separately. Therefore, the maintenance conditions appeared to be favourable and should not have affected the results to a great extent.

Previous investigations of digestive enzyme activities in pelagic crustaceans have been restricted to extracts of whole animals (e.g. Mayzaud and Poulet 1978; Mayzaud and Mayzaud 1981; Hassett and Landry 1982; Tande and Slagstad 1982) or the midgut gland only (McConville et al. 1986). The complexity of the digestion process and interactions between midgut gland and the stomach can be explained only when both organs are investigated separately (Buchholz 1989). Furthermore, the adaptation of enzyme activity in the digestive organs to different feeding conditions requires a certain time. It is difficult to define appropriate experimental periods in advance, however. Thus, we designed our experiments to cover a range from 3 weeks in experiment 1 to a few days in experiment 3.

Relatively little difference was observed in the enzyme activity of the three differently fed groups in experiments 1 and 2. McConville et al. (1986) reported similar results for 1,3-β-D-glucanase in krill. Hassett and Landry (1982) suggested that high activities of digestive enzymes at low food concentrations are an adaptation to a patchy and thus unpredictable food supply. If a rich patch is found, krill are able to make use of this situation immediately. This suggestion is supported by our results. However, during the highly productive summer, starvation periods of 2–3 weeks seem unlikely, particularly as krill are fast and persistent swimmers (Kils 1983). Therefore, krill should be able to leave areas with low food concentrations to locate better food sources. Accordingly, variations of enzyme activity on a shorter time scale seem possible. We observed this in experiment 3. As in experiments 1 and 2, enzyme activity in the digestive organs increased during the short acclimation period before food was given. This increase probably corresponded to the organs being emptied. A filled stomach weighs 1–2 mg depending on the size of the krill, a totally empty one only 20–30 µg. Consequently, the specific activity increases. Thereafter, activities in the stomach of the starved krill decreased considerably, whereas activities in the fed groups either increased steeply or at least remained at the same level. Towards the end of experiment 3, values approached the situation described in experiments 1 and 2. The activity patterns in the midgut gland were similar, but differences between starved and fed specimens were less distinct.

The temporal pattern of enzyme activity described above can be explained by the morphology and function of the digestive tract. Although a detailed study on the microscopic anatomy of the krill stomach exists (Ullrich et al. 1991), histological and cytological investigations of

the midgut gland of euphausiids are rare and there have been no such investigations for *E. superba*. The following explanation is, therefore, based on the well-characterized digestive tract of systematically closely related decapods. Vogt (1985) described the histology and cytology of the midgut gland of *Penaeus monodon* and, in more detail, that of *Astacus astacus* (Vogt 1994), permitting to draw a general functional model of the digestive tract.

The stomach and midgut gland form a functional unit and are connected by a funnel. In the anterior region of the stomach (cardia) the food is ground and mixed with digestive enzymes from the midgut gland. In the posterior part of the stomach (pylorus) the crushed food is filtered and the filtrate is passed on into the midgut gland (Ullrich et al. 1991). The midgut gland consists of a system of blind-ending tubules which again consist of a unilayer epithelium with special cells for nutrient resorption (R-cells) and cells for enzyme synthesis (F-cells). Digestive enzymes are released directly into the tubuli-lumina. In contrast to mammals, enzymes are stored extracellularly in active form in the lumen of the digestive organs (Vogt et al. 1989). In crustaceans, the transfer of digestive enzymes from the midgut gland into the stomach may be facilitated by a net of muscle fibres surrounding the tubules. During the cyclic digestion process the food extract is pumped from the pylorus into the tubules of the midgut gland. Here nutrient absorption takes place and digestive enzymes are released into the lumina of the tubules. By contraction of the muscle fibres these enzymes are pumped actively from the tubules through the funnel into the pylorus. This description is also in close accordance with detailed investigations by Powell (1974) on thalassinid crustaceans (see also Dall and Moriarty 1983; Icely and Nott 1992). While feeding, digestion proceeds as described above. As digestion progresses, the stomach is emptied. When no additional food is ingested, the release of enzymes into the stomach is diminished and consequently the enzyme activity decreases. Simultaneously, enzyme biosynthesis in the midgut gland decreases but continues at a lower level. The continuously synthesized enzymes are released into the tubules and, it is suggested, they are transported passively through the funnel into the stomach where they accumulate. Consequently, enzyme activities in the stomach increase again when specimens starve for a longer period.

This explanation is supported by the results of the correlation analysis. High coefficients and most of the significant correlations were found in the stomachs of starved animals, which indicated the simultaneous course of activity of at least a number of digestive enzymes (see also Fig. 4). In comparison, the correlation matrix of the midgut gland enzymes from starved animals was more inconsistent as a result of diverging activity patterns of the enzymes. Additionally, with the exception of protease, no correlation was found between enzymes from the stomach and the midgut gland, indicating different time courses of enzyme activity.

Our results also indicate that the appearance of digestive enzymes is to a certain extent selective. Selective enzyme induction has been reported for many vertebrates and invertebrates. However, investigations with marine zooplankton gave variable results. Cox (1981) and Cox and Willason (1981) were able to induce laminarinase in feeding experiments with *Euphausia pacifica* and *Calanus pacificus* and Roche-Mayzaud et al. (1991) reported comparable results on a set of enzymes in the copepod *Acartia clausi*. Head and Conover (1983) also induced protease, laminarinase and amylase by feeding algae to *Calanus hyperboreus*. However, when particulate laminarin was fed, no induction occurred. In contrast, Hassett and Landry (1982) reported decreasing activities of laminarinase, cellobiase and maltase in *Calanus pacificus* fed with varying amounts of algae. Van Wormhoudt et al. (1980) demonstrated in *Palaemon serratus* that maximum specific activities of amylase and protease did not coincide with maximum substrate concentration but appeared at intermediate levels. Furthermore, variation in the response of digestive proteases of *Penaeus vannamei* to different levels and sources of protein were related to animal size (Lee et al. 1984).

Field investigations have also been inconsistent. Mayzaud and Conover (1976) reported a significant correlation between the activities of several digestive enzymes in total zooplankton catches and the composition of particulate matter in the water-column. In *E. superba*, however, the correlation between the potential nutrient composition and some related enzymes was negative (Mayzaud et al. 1985).

Our correlation analysis showed an equidirectional course of the two chitinolytic enzymes chitinase and NAGase, as well as chitinase with laminarinase in the midgut gland, the site of enzyme synthesis, when krill was fed with *Cyclotella* (chitinous) but not when fed with *Dunaliella*. These patterns reflect the content of the substrate of the algal food source. Besides the possession of chitinous spines, *Cyclotella* contained more 1,3-β-D-glucan than *Dunaliella*. Laminarin is one of the major carbohydrates in many phytoplankton species, particularly in diatoms, and thus a rich energy source for herbivorous plankton feeders. Enzymes cleaving laminarin are common in many zooplankton species. The ability to increase enzyme activity in response to the substrate concentration can be considered an efficient adaptation to environmental conditions, typical for Antarctic waters, which require rapid and effective nutrient utilization. In this respect it appears to be useful to maintain high levels of the whole set of digestive enzyme activity but, additionally, to increase the activity of specific enzymes for the utilization of extra amounts of substrates.

The activity patterns of chitinases in starved and fed specimens are similar to those of other digestive enzymes. Chitinases are capable of utilizing valuable nutrients from algal sources but are just as important when krill switch to carnivorous feeding. The decrease of activity in the stomach during starvation can be explained by diminished secretion from the midgut gland into the

stomach. The relatively high and constant activities in the midgut gland seem to provide a stock of digestive potential that can be released when a food patch is met.

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