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ORIGINAL PAPER

Seasonal changes in digestive enzyme (trypsin) activity of the copepods *Pseudocalanus minutus* (Calanoida) and *Oithona similis* (Cyclopoida) in the Arctic Kongsfjorden (Svalbard)

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Abstract Seasonal activities of the digestive enzyme trypsin were measured between August 1998 and May 1999 to study different nutritional strategies of the two copepods *Pseudocalanus minutus* and *Oithona similis* in the Arctic Kongsfjorden (Svalbard) using a highly sensitive fluorescence technique. Stage-, depth- and season-specific characteristics of digestive activity were reflected in the trypsin activity. *P. minutus* females and stage V copepodids (C) had highest trypsin activities in spring during reproduction (197.5 and 145.7 nmol min^{-1} ng C^{-1}, respectively). In summer stages CIII–V and in autumn stages CIV and V had high activities (80–116 nmol min^{-1} ng C^{-1}) in the shallow layer (< 100 m) presumably as a consequence of prolonged feeding before descending to overwintering depth. Trypsin activities at depth (> 100 m) in summer and autumn were low in stages CIII and CIV (29–60 nmol min^{-1} ng C^{-1}) and in winter in all stages in both layers (20–43 nmol min^{-1} ng C^{-1}). Based on low trypsin activity, males most likely did not feed. In *O. similis*, the spring phytoplankton bloom did not significantly affect trypsin activity as compared to the other seasons. *O. similis* CV and females had high trypsin activities in summer in the deep stratum (304.5 nmol min^{-1} ng C^{-1}), which was concomitant with reproductive processes and energy storage for overwintering. In autumn, stage CV and female *O. similis* had significantly higher activities than stage CIV (130–152 versus 78 nmol min^{-1} ng C^{-1}), which is in accordance with still ongoing developmental and reproductive processes in CVs and females. Comparisons of both species revealed different depth-related responses emphasizing different nutritional preferences: the mainly herbivorous *P. minutus* is more actively feeding in the shallow layer, where primary production occurs, whereas the omnivorous *O. similis* is not as much restricted to a certain depth layer, when searching for food. *P. minutus* had lower levels of trypsin activity during all seasons. In contrast to *P. minutus*, higher enzyme activities in males of *O. similis* suggest that they continue to feed and survive after fertilization of females.

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Introduction

The calanoid copepod *Pseudocalanus minutus* (Krøyer) and the cyclopoid *Oithona similis* (Claus) are two widely occurring marine zooplankton species. The first species is a neritic form distributed in boreal and Arctic waters (Corkett and McLaren 1978, and references therein), and the latter species is one of the most abundant and ubiquitous metazoans in the world's ocean (Paffenhöfer 1993; Gallienne and Robins 2001). Both species are abundant in Svalbard waters (Kwasniewski 1990; Weslawski et al. 1991).

Pseudocalanus spp. are predominantly herbivorous, while *Oithona* spp. have been termed carnivorous, herbivorous,

omnivorous, detritivorous as well as coprophagous (Corkett and McLaren 1978; González and Smetacek 1994, and references therein). Considering their different feeding ecology, specific adaptational strategies to the Arctic environment with its pronounced seasonal, and therefore strongly fluctuating, food regime are to be expected.

To elucidate variation in physiological processes under changing nutritional conditions, the determination of various types of digestive enzyme activities has proven successful in pelagic organisms such as copepods, euphausiids, and fish larvae (e.g. Harris et al. 1986; Hirche 1989; Hassett and Landry 1990; Mayzaud et al. 1992; Ueberschär 1995; Meyer et al. 2002). The production of digestive enzymes is regulated by ingestion and by metabolic requirements of the consumer (Båmstedt et al. 2000). The former is the theoretical basis for the use of enzyme activities as proxies of feeding activity levels. While changes in the food environment are usually poorly reflected in enzyme activity, extremely high or low feeding activities are clearly resolved (Båmstedt et al. 2000).

Trypsin-like enzymes are widely occurring proteolytic enzymes in vertebrates and invertebrates such as copepods. Copepods maximize protein ingestion through selective feeding (Libourel-Houde and Roman 1987; Cowles et al. 1988). Hence, trypsin activity can serve as a sensitive index of digestive activity and may elucidate life-cycle strategies. Moreover, alterations in enzyme activity have also been observed in decapods as a response to ontogenetic changes, i.e. increased activities associated with higher energy turnover in later developmental stages or as a result of a change in the function and relative size of the digestive tract (Lovett and Felder 1990; Lemos et al. 1999). However, the digestive metabolism in general is complex and the measurement of activities of a specific enzyme does not differentiate between its synthesis, secretion, and an enzyme pool stored in cells. Moreover, it is presumed that only secretion reflects the instantaneous digestive activity (Mayzaud 1986). Consequently, only the "digestive potential" of a given enzyme during a particular time can be determined in homogenates of whole individuals (Head and Harris 1985).

Year-round ecological studies in Arctic environments are rare (e.g. Weslawski et al. 1988; Kwasniewski 1990) because of its difficult accessibility. Seasonal studies of enzyme activities of copepods in the Arctic are lacking altogether, although such investigations are indispensable to understand the life-cycle strategies of Arctic copepods. The present study was carried out in Kongsfjorden to determine seasonal changes in trypsin activity, as a proxy of feeding activity, of *P. minutus* and *O. similis* during the course of a year from August 1998 until May 1999. The working hypothesis was that the predominantly herbivorous *P. minutus* would show more pronounced seasonal changes in digestive potential, indicated through trypsin activity, as adaptation to the Arctic environment with its extreme seasonality, then the omnivorous *O. similis*. The use of the highly sensitive fluorescence technique developed by Ueberschär (1988) allowed us to measure enzymatic activities in individual specimens of small copepod species such as *P. minutus* and *O. similis*. Our specific questions were: 1. Are there significant seasonal differences in trypsin activity between developmental stages and/or seasons for each species and between species, resolving extremes of feeding activity? 2. What are the implications of these findings in terms of their life-cycle strategies?

Material and Methods

Study area

Kongsfjorden is an Arctic fjord located on the western side of Svalbard at about 79° N and 12° E (Fig. 1). The solar radiation shows pronounced seasonal variations (Svendsen et al. 2002). Kongsfjorden has a relatively warm climate compared to other fjords at the same latitude which is mainly due to the influence of a branch of the North Atlantic current, the so-called West Spitsbergen Current, which carries relatively warm and saline water along the coast (Ingvaldsen et al. 2001; Svendsen et al. 2002). Sea-ice conditions are variable, but usually the inner part of the fjord is ice-covered from December until the beginning of June. Stable ice cover does not develop in the middle and outer part of the fjord in most years (Weslawski et al. 1994; Ito and Kudoh 1997). In winter 1998/99 the ice cover was unstable from December until February in the inner to the beginning of middle zone of the fjord (Hop et al. 2002), and a persistent fast-ice cover did not establish until the beginning of March, remaining until mid-May.

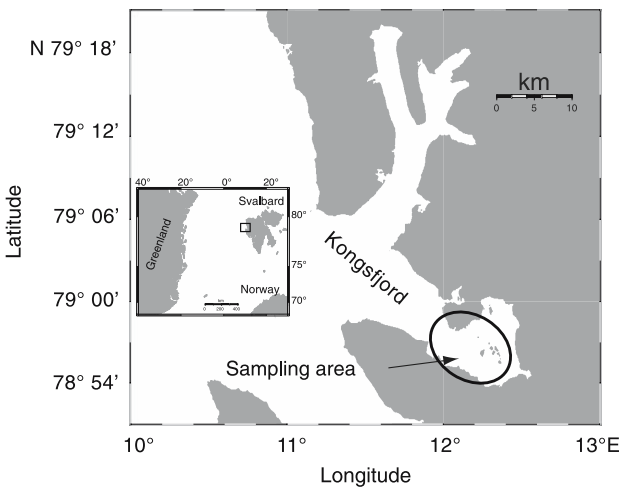

Fig. 1 Svalbard Archipelago with Kongsfjorden and sampling area

Field sampling

Zooplankton specimens were collected in Kongsfjorden (Ny Ålesund, Svalbard) during 13 sampling occasions between August 1998 and May 1999 (for details see Lischka and Hagen 2005). We used a modified Apstein closing net (100 µm mesh-size, 0.2 m^2 mouth opening) either on *R/V Jan Mayen* (University of Tromsø), a Norwegian coast-guard vessel (R/V *Lance*), a rubber dinghy, or from the sea ice via a drilled hole. Except on board *R/V Jan Mayen*, a hand-operated winch was used for the net hauls. Sampling took place in the deepest accessible part of the fjord (max. bottom depth 428 m, Ito and Kudoh 1997, Fig. 1) weather permitting, to allow collection of the deeper-living overwintering copepodite stages. Stratified samples (0–50, 50–100, 100–200, 200–300, 300–350 m) were taken from bottom to surface. Due to changing and sometimes harsh weather and ice conditions sampling could not be done at regular intervals and some depth strata could not always be sampled. In that case, lacking depths were added during the next sampling occasion of the same sampling period. Due to limited availability of specimens from certain depth strata and the need for sufficient collections of specimens for a set of different investigations (e.g. fatty acid composition, gut cell morphology) and for fast processing, only samples from depth strata with highest visual abundances of the required copepod species were chosen for the analysis.

During periods of ice formation, grease or slush ice (WMO 1970) formed in the water and the sample containers. Due to the risk of mechanical damage to the copepods when poured out for processing, sorting could not be done before ice had melted. In order to assure equal treatments, all samples — irrespective of the appearance of ice — were stored cool (4° C) in 5 L containers in ambient sea-water over night to allow melting of ice. Afterwards the most vital individuals of *P. minutus* and *O. similis* were sorted out as soon as possible (maximum 48 h after capture) in a cooled laboratory (ca. 0° C). Starvation experiments with *P. minutus* and *O. similis* from the Greenland Sea by Obermüller (1999) have shown that trypsin activities did not change significantly during the first five days of incubation in filtered sea-water at 5° C. Separated by species and stages, individuals were transferred to Nalgene cryo-vials, shock-frozen in liquid nitrogen, and stored in a freezer at −80 °C prior to enzyme measurements.

Measurement of trypsin activity

Trypsin activity was measured according to the fluorometric assay method described by Ueberschär (1988) with the following three modifications: (1) Na-benzoyl-L-arginine-4-methylcoumarinyl-7-amide (BZ-L-Arg-MCA; BACHEM Biochemica GmbH, Heidelberg, I-1070) was used as substrate as Obermüller (1999) showed a 5–6 fold higher affinity of copepod trypsin for this substrate than for Na-carbobenzoxy-L-arginine-4-methylcoumarinyl-7-amide (CBZ-L-Arg-MCA, BACHEM, I-1130) which was used by Ueberschär (1988). (2) Two-sided metallized microcuvettes were used to increase the sensitivity of the measurement. (3) Cuvette temperature was adjusted to 30 °C for standard assays. Activity measurements occurred under optimum laboratory conditions with respect to temperature, pH, substrate concentration and specificity, homogenate concentration, etc. (Ueberschär 1988). Prior to analysis copepods were thawed, examined for damage and the cephalothorax length was measured using a stereo microscope. The whole procedure was carried out at 0 °C on crushed ice. Damaged individuals were excluded from analysis. For *P. minutus* CIII–VI, 1–3 individuals and for *O. similis* CIV–VI, 1–5 individuals were used for each measurement and transferred into Eppendorf tubes (1.5 ml) with 200 µl ice-cold TRIS-HCL buffer (0.1 mol l^{-1}, pH 8.0), homogenized with an Eppendorf micropistill, and centrifuged at 4 °C at 4110 × *g* for 60 min. The supernatants were subsequently used to measure trypsin activity. 500 µl of the substrate (0.2 mmol l^{-1} MCA in TRIS-HCL buffer) were added to 100 µl of the copepod homogenate in the metallized microcuvettes and mixed thoroughly (resulting in 0.17 mmol l^{-1} end concentration). The increase of fluorescence in each cuvette was recorded after 2, 4, 6, and 8 min at 380 nm (ex) and 440 nm (em) using a KONTRON SFM25 spectral fluorometer with a temperature-controlled automatic cuvette holder. The hydrolysis of the substrate MCA was calculated from the increase of fluorescence after every two minutes interval. The turnover of substrate MCA was calculated via a linear regression obtained from a standard curve of 4.5 to 247.5 nmol l^{-1} of pure fluorophore ($R^2 = 0.9961$). Trypsin activity was calculated in relation to ash-free dry mass and is expressed as the amount of hydrolyzed substrate MCA per minute and per ng of carbon (nmol min^{-1} ng C^{-1}). The following length-mass relationships were used to calculate the ash-free dry mass where M = mass in µg C and L = cephalothorax length in µm: *P. minutus*: $\log W = 2.7302 (\log L - 6.9121$ (Klein Breteler et al. 1982), and *O. similis*: $W = 9.4676 (10^{-7} * L^{2.16}$ (Sabatini and Kiørboe 1994). In total, 781 measurements were carried out on *P. minutus*, and 273 measurements on *O. similis*.

Data analyses

Data obtained from the different sampling events were grouped according to seasons as follows: May samples were categorized as “spring”, those from August/September as “summer”, those from November as “autumn”, and those from February/March as “winter”. For the detection

of differences in trypsin activity between water depths, samples were grouped in a shallow water- (0–100 m) and a deep-water group (> 100 m).

Data analyses were run using general linear models following factorial ANOVA designs (Zar 1996). The factors were species, depth stratum, season and stage. Not all stages or species were found at each depth stratum and season. The presence of each stage changed seasonally and with depth, reflecting inter- and intraspecific patterns of growth and behavior. This precluded the use of a four-way factorial design for hypothesis testing. However, the fact that several stages co-occurred in different depth strata and seasons allowed for three- or lower factorial designs to test for spatial, temporal and interspecific patterns of enzyme activity. They will be presented in the result section. After significant effects in the ANOVA we ran the Student–Newman–Keuls post hoc test (SNK-test).

Raw data were highly heterogeneous with errors not following normal distribution; logarithmic transformed data showed variance homogeneity and normality of residuals. Statistical tests proceeded using log $(X + 1)$ transformed data, with trypsin activity expressed per nmol min^{-1} ng C^{-1}. Data analysis was performed using Statistica™.

Results

Trypsin activities in *Pseudocalanus minutus* stages CIII–V ranged during the year between 15 and 116 nmol min^{-1} ng C^{-1} in shallow waters and between 20 and 197 nmol min^{-1} ng C^{-1} in deep water. For *O. similis*, stage CV and females could be analyzed during all seasons. Here, trypsin activities varied between 98 and 136 nmol min^{-1} ng C^{-1} in shallow water and between 56 and 312 nmol min^{-1} ng C^{-1} in deep water.

Pseudocalanus minutus

Trypsin activities measured for stages CIII–V in summer, autumn and winter were generally higher in summer and shallow waters than in winter and deep waters and increased from stage CIII to stage CV (Fig. 2). However, there was significant interaction between stage × depth × season (Table 1). Activities in stage CIII varied seasonally in shallow waters only: In autumn and winter, activities were similarly low in both depth strata, whereas activities in summer were significantly increased in shallow waters. In stage CIV, trypsin activity was high in summer and low in winter in both depth strata. In autumn, activities were significantly higher in shallow waters (Fig. 2, Table 1). In stage CV, activities decreased significantly from summer to winter irrespective of the depth stratum.

In spring, stage CIII was not found in sufficient numbers to allow analysis. Stages CIV and CV could only be analyzed from deep waters and showed activities of 47.4 nmol min^{-1} ng C^{-1} (SE 16.0 nmol min^{-1} ng C^{-1}) and 145.7 nmol min^{-1} ng C^{-1} (SE 43.9 nmol min^{-1} ng C^{-1}), respectively. Activity in spring was not significantly different from that determined in summer at depth (Two-way ANOVA: season: $F_{1,113} = 0.01$, $P >> 0.05$; stage: $F_{1,113} = 3.12$, $P = 0.07$; season × stage: $F_{1,113} = 0.19$, $P >> 0.05$).

Sufficient adults for analysis were collected in spring; females showed high activity (mean 197.5 nmol min^{-1} ng C^{-1}; SE 52.0 nmol min^{-1} ng C^{-1}), which did

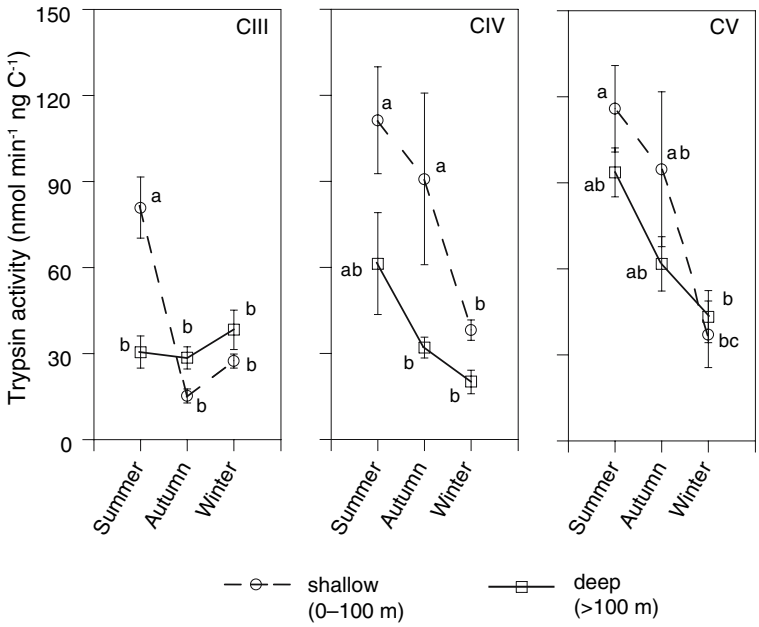

Fig. 2 *Pseudocalanus minutus*. Changes in mean trypsin activity in relation to season, depth level (shallow 0–100 m, deep > 100 m) and stage; error bars are standard errors. Treatment with same letters were not significantly different after Student–Newman–Keuls post hoc test (SNK)

Table 1 *Pseudocalanus minutus* three-way ANOVA to evaluate the role of stage (III, IV, V), season (summer, autumn, winter) and depth strata (shallow, deep) on enzyme activity

Source of variability	df	MS	F	p
Stage (St)	2	11.17	12.00	<0.001
Season (Se)	2	25.47	27.36	<0.001
Depth zone (D)	1	4.79	5.15	<0.025
St × Se	4	4.17	4.49	<0.005
St × D	2	4.79	5.14	<0.01
Se × D	2	3.53	3.79	<0.025
St × Se × D	4	3.63	3.90	<0.005
Error	705	0.93		

df degrees of freedom, *MS* mean squares, *F* Fisher statistic

not significantly differ from stages CIV or CV during that time. Activity in males was very low (mean 8.8 nmol min $^{-1}$ ng C^{-1}, SE 4.0 nmol min $^{-1}$ ng C^{-1}) and significantly lower than in CIV, CV, and females collected in spring and in deep waters (One-way ANOVA: $F_{3,34} = 10.7$, $P < 0.0001$).

Oithona similis

Stage CIV individuals were found mostly in autumn and winter. Trypsin activities in this stage (mean 89.2 nmol min $^{-1}$ ng C^{-1}, SE 21.2 nmol min $^{-1}$ ng C^{-1}) did not differ between depths or seasons (P values>>0.05). Activities in CV and females, the most common stages, differed between depth levels in summer (Table 2, Fig. 3a) with higher activities in individuals caught in deep waters; in autumn and winter trypsin activities did not differ among depths.

Table 2 *Oithona similis* three-way ANOVA to evaluate effects of stage (CV and females), season (summer, autumn, winter) and depth zone (shallow vs. deep) on trypsin activity (ng C) of stage V and females

Source of variability	df	MS	F	p
Stage (St)	1	2.16	3.29	0.071
Season (Se)	2	0.98	1.48	0.229
Depth zone (D)	1	11.28	17.16	<0.0001
St × Se	2	0.69	1.06	0.347
St × D	1	0.55	0.83	0.362
Se × D	2	6.16	9.37	<0.0005
St × Se × D	2	0.67	1.02	0.361
Error	198	0.66		

Data were log transformed prior to the analysis

df degrees of freedom, *MS* mean squares, *F* Fisher statistic

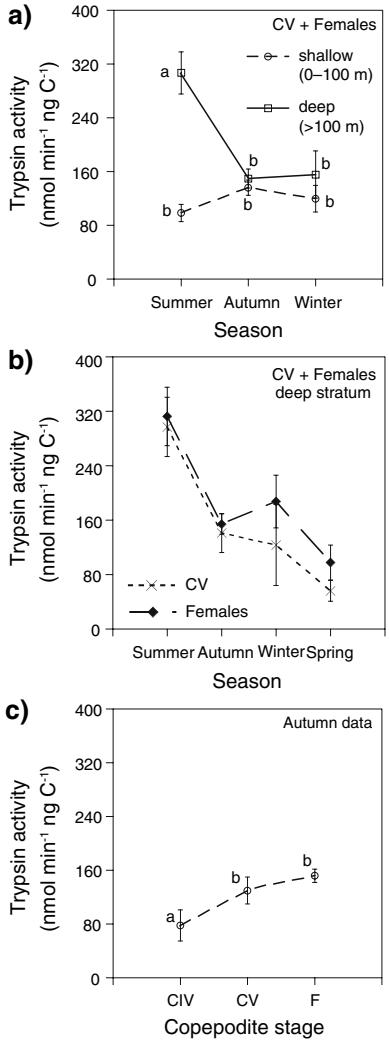

Fig. 3 *Oithona similis*. Changes in mean trypsin activity in relation to season, depth level (shallow 0–100 m, deep > 100 m) and stage, error bars are standard errors: **a** changes in relation to season and depth level in stage V and females (pooled data); **b** changes in relation to stage and season in the deep stratum; **c** stage-dependent patterns for autumn. In **a** and **c** treatment with different letters denote significant differences after SNK-test. In **b** there was a significant effect were: Stages: CV < Females; summer > autumn = winter > spring

In spring, enzyme activities could only be measured from females and stage CV individuals collected from deep waters. Trypsin activity in spring (Fig. 3b) was significantly lower than that observed in any other season (Two-way ANOVA: season: $F_{3,123}$ 14.84, $P < 0.0001$; season × stage: $F_{3,123} = 0.37$, P>>0.05).

Females from the deep stratum showed significantly increased trypsin activities as compared to stage CV from the deep stratum during all seasons (Two-way ANOVA: stage $F_{1,123}$ 4.50, P < 0.05, data were log-transformed for analysis).

In autumn, stage CIV had lower activities than CV and females (Fig. 3c; One-way ANOVA: $F_{2,118} = 12.43$,

$P < 0.00005$). The limited number of males measured (spring, summer, autumn) showed high individual variability in activity (mean 151.2 nmol min $^{-1}$ ng C^{-1}, SE 68.4 nmol min $^{-1}$ ng C^{-1}).

Comparisons between *Pseudocalanus minutus* and *Oithona similis*

Comparing stages CIV, CV, males and females in all seasons and both depths, *P.minutus* showed generally lower trypsin activities than *O similis*; this was most pronounced in males (Fig. 4a; Two-way ANOVA Species: $F_{1,799} = 41.52$, $P(0.0001$; Stage: $F_{3,799} = 16.49$, $P< 0.0001$; Species × Stage $F_{3,799} = 4.57$ $p < 0.01$).

For stage CV, the prevailing stage in both species, it was possible to do interspecific comparisons considering variability among seasons and depth strata (Table 3). This analysis revealed consistently lower trypsin activities in *P. minutus* as compared with *O. similis* (Fig. 4b, Table 3) by about 45% in summer and autumn and 68% in winter.

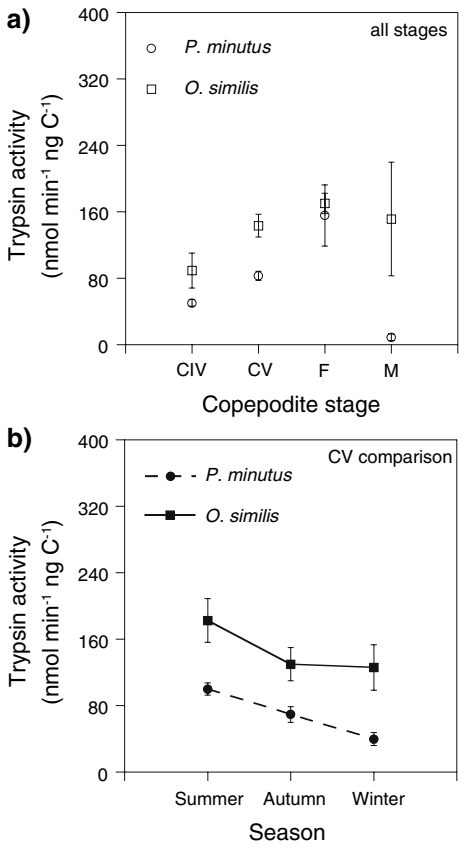

Fig. 4 Interspecific patterns of mean trypsin activity in relation to season and stage; error bars are standard errors. **a** Stage-dependent patterns (data of all seasons and depth pooled); **b** changes in stage V in relation to season. In all stages activity in *P. minutus* was significantly lower than in *O. similis*. In **b**, activity in winter was significantly lower than in summer and autumn (SNK- test)

Table 3 Three-way ANOVA to evaluate interspecific differences between *P. minutus* and *O. similis* and the effect of season (summer, autumn, winter) and depth zone (shallow vs. deep) on trypsin activity of stage V

Source of variability	df	MS	F	p
Species (Sp)	1	31.57	31.92	<0.0001
Season (Se)	2	9.34	9.44	<0.0002
Depth zone (D)	1	1.71	1.73	0.189
Sp × Se	2	1.67	1.69	0.185
Sp × D	1	2.92	2.95	0.087
Se × D	2	1.64	1.65	0.197
Sp × Se x D	2	2.49	2.52	0.083
Error	302	0.99		

Data were log transformed prior to the analysis

df degrees of freedom, *MS* mean squares, *F* Fisher statistic

Discussion

This study analyzed and compared intra- and interspecific differences in levels of trypsin activity, a proxy of feeding activity levels (Båmstedt et al. 2000), in the copepods *P. minutus* and *O. similis* from the Arctic Kongsfjord. Intraspecific differences were mainly related to season and water depth, but also to developmental stages. The design of the study and results from previous studies suggest that the observed patterns reflect different digestive activities in relation to the species' life-cycle strategies rather than temperature effects related to water stratification. Since we measured enzyme activity at optimum laboratory conditions, our data reflect the digestive potential of trypsin, irrespective of the influence of in situ water temperature in situ activity.

Hallberg and Hirche (1980) showed that the developmental stage, season, feeding condition, sex and perhaps reproductive state affected enzyme levels in calanoid copepods (*Calanus finmarchicus*, *C. helgolandicus)* from the Gullmarfjord (west Sweden). Variation in trypsin activity in response to the spring phytoplankton bloom, in association with the spawning period as well as in different developmental stages of herbivorous calanoid copepods from Kosterfjorden (Sweden) and Balsfjorden (Norway) were also observed by, e.g., Tande and Slagstad (1982) and Båmstedt (1988). Enhanced enzyme activities in later developmental stages are also known from decapods associated with higher energy turnover due to ontogenetic changes or resulting from changes in function and relative size of the digestive tract (Lovett and Felder 1990; Lemos et al. 1999).

Pseudocalanus minutus

Pronounced seasonal variation in digestive potential reflects adaptive life-cycle traits in a high Arctic population of the

copepod *P. minutus*. Higher trypsin activities were generally found in shallow than deep waters in summer and autumn. These depth-related patterns are consistent with enzyme activities (trypsin, amylase) and distribution patterns of *Calanus finmarchicus* and *C. hyperboreus* in Fram Strait/Greenland Sea (Hirche 1989). In *P. minutus*, very low digestive potential in the deep stratum was correlated with lower water temperatures at this depth, especially in summer (Lischka and Hagen 2005). This suggests that CIII–V individuals of *P. minutus* from Kongsfjorden were already prepared for lower-level metabolic rates at depth and represented the first individuals of the overwintering stock (Hirche 1989). Likewise, copepodids III–V occurring in summer and autumn belong to the new generation and represent the principal overwintering stages in Kongsfjorden (Lischka and Hagen 2005).

P. minutus individuals in the shallow water layer, in contrast, likely prolonged feeding until sufficient energy reserves were accumulated to assure survival at depth during the winter period. This agrees with the finding that CIII–V individuals in the surface layers had lower wax ester amounts than those that had already migrated to deeper layers for overwintering (Lischka and Hagen 2007). The high standard errors of trypsin activities of stages CIII–V especially in summer and autumn reflect high individual variability during preparation for overwintering. This is probably caused by a delay in the adjustment of digestive activity in response to diminished feeding activity; later in winter standard errors were much lower. Since digestive enzyme activities in copepods do not change significantly over time scales of less than a day (Head et al. 1985; Hassett and Landry 1988), there should be a time lag between changes in feeding activity and the adjustment of enzymatic activity.

Trypsin activities in *P. minutus* species tended to be high in spring, summer and autumn, and low in winter. High trypsin activity in spring appeared to be related to the algal spring bloom and associated reproductive activity in Arctic *P. minutus*. Deep-dwelling CVs and females collected in spring had exceptionally high trypsin activities which suggest intense feeding on the spring phytoplankton bloom. This intense feeding period coincided with the time of development, final gonad maturation and a peak in reproductive processes (Lischka and Hagen 2005, 2007). Maximum trypsin activity during the spawning period was also found in adult *Calanus finmarchicus* females from Balsfjorden (northern Norway), whereas stage CV had ten times lower activities (Tande and Slagstad 1982).

Although we did not measure chlorophyll concentrations, the intense greenish coloration in our spring samples (May) from underneath the ice as well as at the ice underside and the high abundances of *Pseudocalanus* fecal pellets in net samples indicated high concentrations of phytoplankton and ice-algae, respectively. Bloom conditions and high trypsin activities in spring agree with previous studies describing *Pseudocalanus* spp. as mainly herbivorous and feeding avidly on ice-algae (Corkett and McLaren 1978; Bedo et al. 1990). The available prey during the spring phytoplankton bloom in Kongsfjorden consists predominantly of diatoms and the haptophyte *Phaeocystis pouchetii*, whereas diatoms prevail at the underside of the ice (Hop et al. 2002; Leu et al. 2006). Likely, *P. minutus* fed in the surface layer during night before the presumed diurnal downward migration during which they were sampled (Runge and Ingram 1991; Wiktor 1999). CVs and females indeed had high amounts of diatom marker fatty acids during the spring bloom period supporting high feeding activities (Lischka and Hagen 2007).

Male *P. minutus*, in contrast, had significantly lower trypsin values in spring supporting previous conclusions that adult male *P. minutus* do not seem to feed, like males of other copepod species (*P. acuspes*: Norrbin 1994; *Calanus hyperboreus*: Head and Conover 1983). Non-feeding adult males of *Euchaeta norvegica* and *Chiridius armatus* also showed minimal trypsin activities relative to actively feeding stages (Båmstedt 1988). In contrast, comparatively low trypsin activities in spring CIVs from the deep layer suggest that they were starting to feed, but still mainly relied on their lipid reserves as supported by low diatom marker fatty acids in this stage suggesting that the spring bloom was not intensely used (Lischka and Hagen 2007).

Oithona similis

Comparatively low variability in trypsin activity between seasons and depth strata reflects the opportunistic life-cycle trait of *O. similis*. For *O. similis*, stage CV and females predominated in summer (Lischka and Hagen 2005), and their highest trypsin activities were registered in the deep stratum; in individuals collected at other seasons enzyme activities did not change with depth stratum. Hence, high nutritional requirements in summer seem to be satisfied best in the deep stratum which may be related to the feeding mode of this species: Species of the genus *Oithona* are ambush feeders and prefer motile prey (Lampitt and Gamble 1982; Drits and Semenova 1984; Uchima and Hirano 1986). According to Nakamura and Turner (1997) *O. similis* prefers flagellates, heterotrophic dinoflagellates and ciliates. Flagellates and dinoflagellates predominate during the summer/autumn phytoplankton bloom in Kongsfjorden (Okolodkov et al. 2000). Furthermore, *Oithona* spp. feeds on *Calanus* fecal pellets (González and Smetacek 1994; Nielsen and Sabatini 1996) and prefers to stay below the halocline in the Baltic Sea and in association with the pycnocline in the Skagerrak (Hansen et al. 2004; Maar et al. 2006). Kongsfjorden specimens from the deep

stratum resided below the pycnocline and *Calanus* spp. copepods were abundant in both water layers during this time (Lischka and Hagen 2005; Lischka unpublished data). Thus, this species could also have exploited sinking fecal pellets of calanoid copepods or marine snow aggregates (Maar et al. 2006).

In the summer and early autumn, major events occur in *O. similis*' life cycle, making high feeding rates necessary. First, high summer trypsin activities in *O. similis* coincided with the second main reproductive peak in Kongsfjorden in August/September (Lischka and Hagen 2005) and suggested high feeding activities to afford reproduction. Experimental evidence shows that fecundity of *Oithona* spp. (*O. similis* and *O. marina*) from the Kattegat (Denmark) is strongly limited by food availability or composition (Sabatini and Kiørboe 1994). In the North Sea, for example, the specific egg production rate of *O. similis* was positively correlated to the occurrence of protozooplankton (Nielsen and Sabatini 1996). Thus, good feeding conditions for *O. similis* from Kongsfjorden must have prevailed during this time. Second, at the same time accumulation of new storage lipids (wax esters) for the next overwintering season was observed (Lischka and Hagen 2007). Hence, *O. similis* had high nutritional needs during summer as reflected in high trypsin activities.

Trypsin activities in *O. similis* had decreased by autumn but did not reach minimum levels until the next spring. In autumn, intermediate activity levels likely reflect nutritional requirements due to continuing developmental and reproductive processes (Lischka and Hagen 2005). In spring,

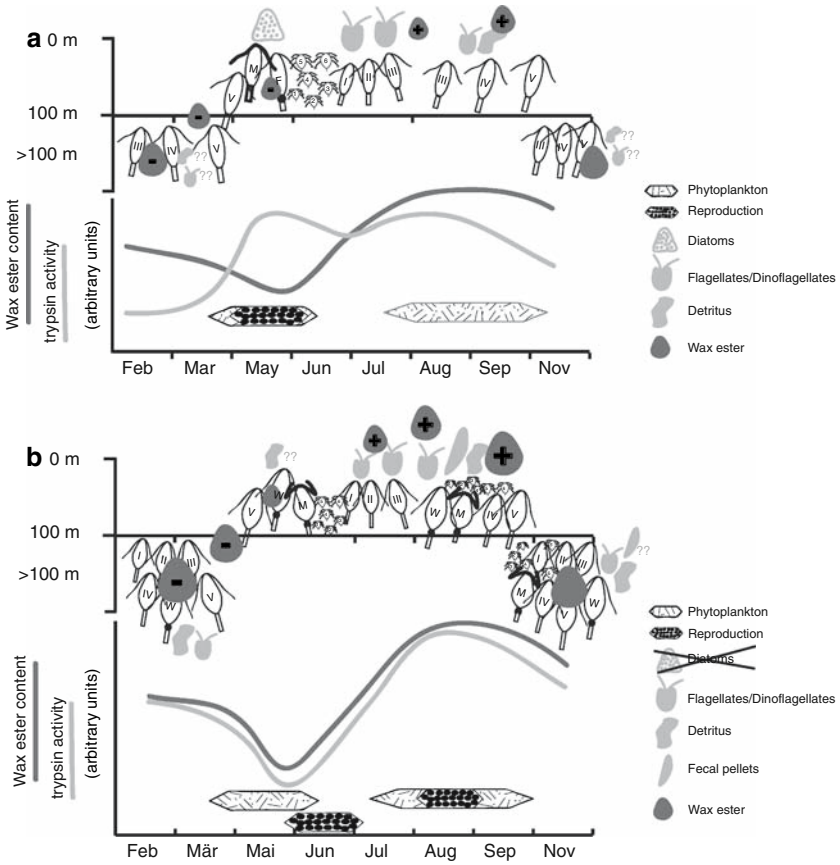

Fig. 5 Conceptual diagram of the life-history strategies of *P. minutus* (**a**) and *O. similis* (**b**) combining stage development (dominant stages shown during a particular time), (main) reproductive period(s), vertical distribution (overwintering stages migrate to depth > 100 m), seasonal changes in wax ester content and trypsin activity, as well as dominant nutrition as indicated from seasonal variations of fatty acid compositions (Lischka and Hagen 2005, 2007). Phytoplankton bloom periods were assumed from literature (Okolodkov et al. 2000; Hop et al. 2002; Leu et al. 2006). *P. minutus* fuels final gonad maturation and reproduction by the spring phytoplankton bloom (indicated through reproduction period drawn within the phytoplankton bloom) consisting mainly of diatoms (Leu et al. 2006), the summer phytoplankton bloom is used to build up depot lipids in the overwintering stages (new generation). *O. similis* fuels the first main reproductive peak in spring by accumulated lipids (hence, reproduction is not drawn within the phytoplankton bloom), whereas the second peak in summer is fuelled by the summer phytoplankton bloom (with reproduction drawn within the phytoplankton bloom) and at the same time accumulation of new storage lipids in overwintering stages occurs. Whereas the spring (diatom) bloom seems to be essential for the reproductive success of *P. minutus*, *O. similis* does not seem to utilize the spring bloom but affords the first reproductive peak by lipid reserves. The overwintering stages of both species migrate to depths > 100 m and are characterized by significantly higher wax ester levels as compared to those occurring in the surface layer

trypsin activity was low in spite of the concurrent diatom-dominated phytoplankton bloom in Kongsfjorden (Hop et al. 2002). This mismatch is consistent with previous findings that *Oithona* spp. is an omnivorous/carnivorous/coprophagous species (Paffenhöfer 1993, and references therein; González and Smetacek 1994) and that *O. similis* feeds little on diatoms (Marshall and Orr 1966). Thus, *O. similis* does not directly depend on the euphotic layer for food. Apparently, the first main reproductive period in June is fueled by lipid reserves (Lischka and Hagen 2007; Fig. 5).

Species comparison

The species studied here, *P. minutus* and *O. similis*, differed in overall levels of trypsin activity and in depth-related responses. These differences emphasize the different nutritional preferences and life-cycle strategies of the two species. The predominantly herbivorous *P. minutus* depends on surface phytoplankton blooms correlated with high trypsin activities in shallow waters in summer and autumn. In contrast, the omnivorous *O. similis* is less restricted to the euphotic layer and algal bloom period for food, and consequently digestive potential shows less (seasonal) variability (except in summer).

The overall higher level of trypsin activity of the smaller *O. similis* is consistent with the principle of increasing specific metabolic rates with decreasing organism size, e.g. respiration rates of differently sized copepod species (Mayzaud et al. 2002a, b). However, recent findings indicate extremely low respiration rates in *O. similis* (Castellani et al. 2005). As Lampitt and Gamble (1982) suggested for *O. nana*, *O. similis* might save energy due to its usually motionless behavior (Drits and Semenova 1984; Paffenhöfer 1993; Hwang and Turner 1995) resulting in low respiration rates despite relatively high trypsin activities and nutritional needs.

Finally, differences in trypsin activities in males confirmed previous assumptions that male *P. minutus* most likely do not feed (Norrbin 1994), while *O. similis* males may feed (Eaton 1971) and hence may have a longer life span after fertilization of the females.

To summarize, the digestive activity of *P. minutus* seemed to be closely connected to phytoplankton blooms in spring and summer/autumn in the shallow water in Kongsfjorden. In contrast, *O. similis* seemed to feed intensely during the summer bloom, but also fed more actively during autumn and winter than *P. minutus* (Fig. 5). These behaviors reflect the mainly herbivorous character of *P. minutus* in contrast to the omnivorous/carnivorous character of *O. similis*. Furthermore, the present results support the findings from earlier investigations on the timing of reproduction, fatty acid compositions as well as accumulation of storage lipids (wax esters) (Lischka and Hagen 2005, 2007): *P. minutus* has one reproductive peak in spring associated with high amounts of diatom marker fatty acids, suggesting intense feeding on the spring bloom. The new generation accumulates storage lipids by utilizing the summer/autumn flagellate/dinoflagellate bloom. In contrast, *O. similis* reproduced year-round fuelled by continuous omnivorous/carnivorous feeding, reflected in its specific marker fatty acids, and utilization of wax ester reserves (Fig. 5). Furthermore, consistently higher digestive activities year-round in *O. similis* as compared to *P. minutus* support the concept of higher metabolic/nutritional needs of the smaller species.

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