Changes in digestive enzyme activities in the Arctic copepod *Calanus glacialis* during ontogenetic vertical migration

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Abstract

Zooplankton communities in Arctic shelf regions are dominated in terms of biomass by the calanoid copepod *Calanus glacialis*. During the winter months their metabolic rate decrease while they reside at deep water layers. In late winter, it migrates to the surface where the spring generation develops. To date, it is not fully understood what regulates the activity of the copepods and how it coincides with food availability. We sampled *C. glacialis* in a high Arctic fjord in monthly intervals for a one year cycle and determined proteinase and lipase/esterase activities in relation to food availability and depth distribution of the copepods. By substrate SDS-PAGE (sodium dodecylsulfate polyacrylamide gel electrophoresis), we tackled changes in specific isoforms. We found clear seasonal enzyme activity patterns; activities of individuals in winter were reduced by at least 75 % as compared to those feeding in spring. SDS-PAGE showed a high heterogeneity of lipolytic enzymes in *C. glacialis*, which reflects an extensive accumulation and metabolization of internal lipid. One band of proteolytic activity was found and it intensified with the onset of the algal blooms. Females and younger developmental stages showed high digestive enzyme activities when they resided in surface water and low activities when they were in deep water. High enzyme activities were closely correlated to the onset of ice algae bloom in spring. However, the copepods descended in autumn even though food was still available. These results suggest that *C. glacialis* should be benefit from an earlier ice-break up on thus, earlier algal blooms in the season, but that the copepods would not benefit from long lasting phytoplankton blooms.

Keywords: overwintering, *Calanus glacialis*, Arctic, substrate SDS-PAGE, proteinase, lipase/ esterase

Introduction

Copepods of the genus *Calanus* dominate zooplankton communities in northern high latitudes (Jaschnov 1970). In Svalbard waters, *C. glacialis* accounts for up to 80 % of the biomass of these communities and links primary production and higher trophic levels (Falk-Petersen et al. 1990; Blachowiak-Samolyk et al. 2008; Søreide et al. 2008). During its one to two year life cycle, *C. glacialis* performs ontogenetic vertical migration and overwinters in deep water and actively feed and develop in surface waters in spring and summer (Smith and Schnack-Schiel 1990; Kosobokova 1999). The overwintering state is commonly referred to as diapause (Conover 1988). During diapause, copepods arrest their development (Hirche 1991, Scott et al. 2000), they do not feed but rely on internal energy reserves (Hirche 1996, Conover and Huntley 1991). In Svalbard waters, *C. glacialis* usually enters diapause in late July/ early August (Søreide et al. 2010; Daase et al. 2013). The length of the life cycle and the timing of diapause differ among populations from different ecosystems, and have been related to water temperatures (Kosobokova 1999; Niehoff and Hirche 2005), light (Morata & Soreide 2013) and food availability (Søreide et al. 2008, 2010; Daase et al. 2013). Warmer temperature may shorten the duration of diapause and of the reproduction period in *Calanus* spp. (Kosobokova 1999; Niehoff & Hirche 2005; Pierson et al. 2013). The photoperiod may alter the metabolic activity of overwintering copepods, with in turn may hamper their survival (Morata & Soreide 2013).

The timing of food availability is critical for *C. glacialis*, since the overwintering copepods rely on the lipid reserves from its algae diet during the productive season in spring and summer. *C. glacialis* converts low-energy

carbohydrates and proteins of the algae into high-energy wax esters (Lee et al. 2006; Falk-Petersen et al. 2009). The reproduction and growth success of the copepods depends decisively on the quantity and quality of the algae diet. Females fuel gonad maturation and spawning based on the ice algae and phytoplankton bloom, while the younger developmental stages rely on the phytoplankton bloom to grow and develop. With a changing climate, the sea-ice cover shrinks and ice-free seasons become longer (Gough et al. 2004; Comiso et al. 2008; Stroeve et al. 2012). This will change the underwater light regime and severely impact the timing and intensity of primary production (Arrigo et al. 2008; Kahru et al. 2011). This, in turn, may disturb the match between the reproduction and development of *C. glacialis* and the timing of the bloom events. The consequences of such a scenario for Arctic shelf ecosystems are yet unpredictable. This makes it important to understand how *C. glacialis* regulates its digestive activity in order to predict its ability to adjust to climate driven changes in the algal food regime.

Digestive enzymes link food uptake with the biochemical transformation and assimilation of the ingested components (Mayzaud 1986). Different enzyme classes catalyse the hydrolysis of alimental components, i.e. proteinases hydrolyze peptide bonds (Mayzaud 1986) and lipases/esterases cleave the ester bonds of carboxylic acids (Luppa and Andrä 1983). At present, the methods only allow for the measurement of maximum potential activities, but not the actual *in situ* activity.. Moreover, enzyme activities do not always respond linearly to food supply but may be influenced by other factors, e.g. metabolic requirements and feeding history (Hassett and Landry 1983; Roche-Mayzaud et al. 1991). It is thus difficult to use digestive enzyme activities as a proxy for feeding activity in copepods (Oosterhuis and Baars 1985). However, when digestive enzymes are studied in a seasonal context or compared at different experimental conditions, they can provide detailed insight on the relation between food occurrence and uptake by the copepods history (Boucher and Samain 1974; Hassett and Landry 1983). Accordingly, several laboratory studies, in which either food quantity or quality was manipulated, showed how distinctly enzyme activity can change with dietary conditions (Harris et al. 1986; Kreibich et al. 2008, 2011; Freese et al. 2012). Studies addressing seasonal aspects of digestive enzyme activities in copepods and especially in *Calanus* spp. are rare, but indicate less activity during winter than during summer (Hirche 1981, 1983; Tande and Slagstad 1982; Båmstedt 1988; Hassett and Landry 1990). Hallberg & Hirche (1980) have shown in overwintering non-feeding *C. finmarchicus* and *C. helgolandicus* that cells in the gut epithelium, which are believed to produce digestive enzymes, were reduced in winter, thus limiting the ability to digest dietary components even if food were available. However, in order to effectively exploit the short productive season for reproduction and growth, it is important that the copepods can respond immediately when ice algae or pelagic algae become available in spring (Kosobokova 1990; Niehoff et al. 2002; Søreide et al. 2010). After winter, the copepods thus need to regain a certain level of digestive enzyme activity. In *C. hyperboreus*, it has been shown that digestive enzyme activity increased already in late winter and this allows the copepods to assimilate dietary components immediately when food becomes available and feeding starts in spring (Head and Conover 1983). *C. hyperboreus* is considered primarily a deep-water species since it spends most of its life cycle over the deep-sea basins of the Arctic oceans below 1000 m water depth (Scott et al. 2002; Auel et al. 2003). Also *C. finmarchicus* overwinters in deep water of the open ocean, but also in shelf regions (Hirche 1983, Dale et al. 1999). In contrast, *C. glacialis* overwinters mainly in shelf regions above 500 m water depths (Dale et al. 1999). Information on digestive enzyme regulation on the shelf species are lacking.

Most enzyme studies measured quantitative enzyme activities only. This approach, however, may mask shifts between isoenzymes while the total activity does not change (Kreibich et al. 2011). As the capability to synthesize different enzymes may reflect physiological capacities of a species to respond to lipids derived from food of varying quality or from tissue, we combined total activity measurements with substrate SDS-PAGE (sodium dodecyl sulphate polyacrylamide). With SDS-PAGE, enzymes are separated according to their molecular weight by gel electrophoresis (Laemmli 1970). By using a substrate, e.g. casein (proteinases) or 4-methylumbelliferyl butyrate (lipases/esterases) this method visualizes specific enzymes classes and patterns. The number and location of bands of active proteolytic or lipolytic enzymes on the gels, can change considerably with different feeding conditions (Kreibich et al. 2008; Freese et al. 2012) and over one day as related to the feeding cycle (Guérin and Kerambrun 1982; Kerambrun and Champalbert 1993).

To predict to what extent *C. glacialis* is able to respond flexible to changes in the timing and composition of algal blooms, this study aimed to increase our understanding of physiological aspects of the feeding biology of this species. *C. glacialis* was sampled monthly in Billefjorden, a high-Arctic sill fjord in Svalbard waters, from July 2012 to July 2013. We chose Billefjorden, because the inner fjord is separated from the outer water masses by a sill of about 40 to 50 m water depth, which minimizes advection processes (Nilsen et al. 2008) and thus, provides the unique opportunity to follow one population over a year (Arnkværn et al. 2005; Grigor et al. 2014). By sampling in monthly intervals this study aimed to determine the timing of changes in digestive enzyme activities and patterns and assess if this is related to food availability. in different seasons.

Material and methods

Sampling area and sample processing

From July 2012 to July 2013, *C. glacialis* was sampled monthly in Billefjorden (78°40′N; 16°40′E) in Svalbard waters. Billefjorden is a high-Arctic sill fjord, which is located on the west-coast of Svalbard (Nilsen et al. 2008). The maximum water depth of the fjord is about 190 m. A sill of 40 to 50 m depth restricts the exchange of water masses. In the sampling year, the fjord was ice-covered from February until June 2013 with water temperatures ranging from -1.7 to 5.0 °C in the surface. Below 100 depth, the temperature was around -1 °C year-round. Physical data were obtained by applying a SAIV SD204 CTD or a SBE Seabird Electronics CTD. The chlorophyll *a* concentration in the water column was determined fluorometrically with methanol as an extraction solvent after Holm-Hansen and Rieman (1978). Chlorophyll *a* data were provided by Miriam Marquardt (University Centre in Svalbard (UNIS)). Depending on the sea-ice conditions the sampling location was either accessed by boat (RV Helmer Hanssen, KV Svalbard, Farm), by inflatable boat or by snowmobile. Copepods were collected with a WP-3 or WP-2 closing plankton net with a vertical messenger operated closing mechanisms (Hydro-Bios, Germany) (1000 µm and 200 µm mesh size, respectively) from 180 m to 100 m depth in autumn and winter (from July 2012 to February 2013 and in July 2013) and from 50 m depth to the surface in spring and summer (in July 2012 and from March to June 2013), depending on where animals occurred in the highest abundances. Before sampling for biochemical analysis, copepod abundance was roughly estimated by eye and later on, in the laboratory, abundances were determined from community samples, which were conserved in a sea water - 4% formaldehyde solutions. Immediately after capture, the samples were either

processed in a controlled temperature room onboard a research vessel or transported to the laboratories of UNIS. Copepods of copepodite stage IV (CIV), V (CV) and adult females (CVIF) were sorted alive under a stereo-microscope at ambient temperature and snap-frozen in liquid nitrogen. Individuals were transported in seawater and depending on the means of transport, the time lag between sampling and sorting of individuals ranged between 2 to 24 hours. In *Calanus* spp. it takes between one and two weeks before digestive enzyme activities change significantly (Hassett and Landry 1983; Head and Conover 1983) and thus, within the time frame of 24 hours changes in enzyme activities should be negligible. Samples were stored at -80 °C until further analysis.

Analyses of enzyme activity

Digestive enzyme activities, i.e. proteinase and lipase/esterase activities, were determined in triplicates in each sample, which contained ten individuals each. All samples were briefly homogenized with a micro pestle in 200 µl ice-cold 0.1 M Tris/HCl (supplemented with 10 mM $CaCl_2$) buffer at pH 7.0. Homogenates were centrifuged at 15,000 g at 4 °C for 15 min (Thermo Scientific, Heraeus Fresco 17). In order to gain optimal activity results and to prevent protein denaturation at the same time, assay temperatures and pH were chosen close to the optima for the respective digestive enzymes. In calanoid copepods, proteinase activity peaks at 40/50 °C and pH 6/7 and lipase/esterase activity is the highest at 30 °C and pH 7 (Knotz et al. 2006; Solgaard et al. 2007; Freese et al. 2012). Enzyme activities were calculated per mg dry mass (DM). To determine DM, individual copepods were placed in pre-weighed Sn-cartridges and dried for 48 h at 60 °C. Afterwards, DM was determined gravimetrically. This was done for each sampling occasion and DM of copepods was related to the respective individuals, which were sampled for enzyme activities.

Proteinase activity

Total proteinase activity (EC 3.4.21-24) was measured after Saborowski et al. (2004), modified after Kreibich et al. (2008). Twenty µl of sample or 20 µl buffer for the controls were pre-incubated on a thermo shaker for 5 min at 30 °C. Subsequently, 5 µl azocasein (1 % in deionized water, Fluka BioChemika, 11615) were added to the reaction tubes, which were incubated for another 60 min at 30 °C. Fifty-microliter trichloroacetic acid (TCA, 8 % in deionized water) were added to stop the reaction, and samples/controls were centrifuged at 15,000 g at 4 °C for 15 min to obtain supernatants, which then were transferred into an ultra micro-cuvette (Hellma 105.203-QS). The optical density of the supernatants was measured with a spectrophotometer (Thermo Scientific, UV1) at 366 nm (dE_{366}) and recorded with the software VisionLite (Version 2.2).

Lipase/esterase activity

Lipase/esterase (carboxylic ester hydrolases; EC 3.1.1) activities were measured after Knotz et al. (2006). As substrate, 10 µl 4-methylumbelliferyl butyrate dissolved in dimethyl sulfoxide (5 mmol L^{-1}, MUF-butyrate, Fluka BioChemika, 19362; DMSO, AppliChem A3608) was added to 20 µl sample or 20 µl buffer (controls) and 470 µl 0.1 M Tris/HCl (supplemented with 10 mM $CaCl_2$) buffer at pH 7.0. Standard curves were made with the fluorescent compound 4-methylumbelliferone (MUF, Sigma M1381) in DMSO (15.625 to 1000 µmol l^{-1}). Samples and controls were incubated in the dark on a thermo shaker at 25 °C for 30 min. Enzyme activities were

calculated from the fluorescence, which was determined with a NanoDrop 3300 at 360 nm (excitation) and 450 nm (emission) and recorded with the software ND-3300 V 2.7.0. Autolysis of MUF-butyrate was measured and subtracted from the assay-results.

Substrate SDS-PAGE

To reveal proteinase and lipase/esterase enzyme activity bands, proteins were separated by discontinuous substrate sodium dodecyl sulphate polyacrylamide gel electrophoresis (substrate SDS-PAGE) modified after Laemmli (1970) and Kreibich et al. (2011) for lipase/esterase and after Freese et al. (2012) for proteinase patterns. Sample homogenates were diluted 1:2 with sample buffer (25 % 0.5 Tris/HCl buffer at pH 6.8, 0.02 % bromophenol blue, 30 % glycerine, 4 % SDS) and were applied on Mini-gels, which consisted of a running gel (1.5 M Tris/HCl buffer at pH 8.8) and a stacking gel (0.5 Tris/HCl buffer at pH 6.8). To make proteinase bands visible, 0.05 % casein fluorescein isothiocyanate from bovine milk (FITC-casein, Sigma C0528) was pipetted in a concentration of 0.5 mg/ml into the still liquid running gel before the run. In order to determine the molecular weight, the detergent SDS was used to separate proteinases and lipases/esterases according to their size and thereby the molecular weight was determined. To minimize denaturation of the enzymes, the samples were neither treated with mercaptoethanol nor heated before applying on the gels. Any differences in the intensity of bands on the gels result from intraspecific differences in the protein content. Ten µL of sample and 5 µL of a molecular marker (Roti®-Mark Standard, Roth T851) were applied onto the gels. The running conditions for two (proteinase and lipase/esterase) gels were 300 V, 30 mA and 2 °C for approximately 1 h in a vertical gel electrophoresis chamber (Hoefer, Mighty Small II SE 250), which was filled with electrode buffer (25 mM Tris/HCl buffer with 0.192 M glycine and 0.1 % SDS, pH 8.3).

After the run, the gel for the documentation of the lipolytic activity patterns was first placed in 2.5 % Triton X 100 (in 50 mM phosphate buffer at pH 8.0) for 30 min and then washed in 50 mM phosphate buffer at pH 8.0. Thereafter, the gel was incubated in a 100 µM MUF-butyrate solution in 50 mM phosphate buffer at pH 8.0 for approximately 10 min (Díaz et al. 1999). The proteinase gel was washed in 2.5 % Triton X 100 (in 0.1 M Tris/HCl (supplemented with 10 mM $CaCl_2$) pH 8.0), rinsed with deionized water and placed in 0.1 M Tris/HCl (supplemented with 10 mM $CaCl_2$) pH 8.0 for 120 min. Then, the lipase gel was stained in a Commassie brilliant blue (CBB) G®-250 solution (5 % aluminium sulphate, 10 % ethanol, 2 % phosphoric acid) and the proteinase gel was stained in a CBB R®-250 solution (50 % methanol, 7 % acetic acid). Gel images were taken with a gel documentation system (Gel Doc™ EZ Imager, Bio Rad) under UV light (for lipolytic enzyme patterns) and under transmission light (for proteolytic enzyme patterns and documentation of markers). Analysis was performed with Image Lab 5.0 software (Bio-Rad).

Water-soluble protein content

To assess the presence of tissue degrading proteinases, water-soluble protein content was determined in the sample homogenates after Bradford (1976) using a Bio-Rad protein assay (BIO-RAD 500-0006). Bovine serum albumin was used (0 to 0.1 mg/ml) as a standard. Samples were diluted 1:27 in distilled water and 50 µl diluted sample was added to 250 µl protein assay, which was diluted 1:5 in distilled water. The assay was incubated for

15 min at 25 °C in a 96-well plate and the absorbance was measured at 600 nm and 25 °C with a Synergy HTX Multi-Mode Reader and the software KC4 3.4 Rev. 21.

Statistical analysis

Statistical analysis was performed using the free software R 3.0.1. The Shapiro-Wilk test was applied to test data for normal distribution. For normally distributed data, which showed variance homogeneity, one-way ANOVA was applied and followed by Tukey post-hoc tests. For non-normally distributed data, Kruskal-Wallis tests were used and followed by Tukey post-hoc tests. Spearman Rank Order Correlation was performed in order to identify dependencies between digestive enzyme activities and Chlorophyll *a*. Five % ($\alpha = 0.05$) was determined as level of significance. Results were regarded as statistically significant and the null hypothesis was rejected, if the p-value was lower than the α-level.

Results

Digestive enzyme activity over one year

In July 2012, when our study started, the copepods began to migrate from the surface to deeper water layers. They were therefore found in relatively high abundances throughout the water column at that time. We used this opportunity to compare individuals from the upper 50 m and from deep waters and found that the proteinase activity was high in CV from surface waters (5.6 ± 0.3 dE_{366} h^{-1} mg DM^{-1}) and low in CV captured at depth >100 m (1.3 ± 0.3 dE_{366} h^{-1} mg DM^{-1}). For the rest of the year, until December 2012, most CV were found below 100 m and their proteinase activities remained low. Also in CIV, sampled in October 2012 during a cruise with RV Helmar Hanssen, the specific enzyme activity was low (Fig. 1). In January and February 2013, before the copepods began their ascent to the surface, the specific proteinase activities in CV were slightly, but not significantly, higher as compared to those in December (Fig. 1a). The individual activity (Fig. 1b), however had not changed, and thus the increase can be attributed to a loss in body carbon which we observed at that time. In January and February, females were abundant at depth, possibly due to molting of the CV. Their activities were as low as those of the CVs (Fig. 1a, b). From March to May 2013, the numbers of CV were too low for measuring enzyme activities. We therefore focused on CIV and CVIF, which then were the most abundant stages. From January to March, the proteinase activities of both developmental stages were low (specific enzyme activity below 1.5 dE_{366} h^{-1} mg DM^{-1}). Ice algae developed at the end of March and later, in April, the chlorophyll *a* concentrations in the water column increased. Coincidently, the proteinase activities had increased in April, being significantly higher than in all other months (one-way ANOVA $p<0.05$, Tukey post hoc test). At that time, the copepods had migrated upwards and were found mainly above 50 m. Females almost vanished during April while CIV were still abundant at the end of April and in May 2013 and could thus be analyzed. In both months, the specific activities of the CIV were higher as compared to all other stages. In June and July 2013, CV again reached high abundances. They concentrated in the upper water column and in June their specific proteinase activity was significantly higher as compared to all other sampling days during the rest of the year (one-way ANOVA $p<0.05$, Tukey post hoc test). In July 2013, most of the CV had already descended and

285 at depths >100 m their proteinase activity was as low as that at of the overwintering CV of the previous
286 generation.
287
288 In general, the lipase/esterase activities followed a similar pattern and accordingly, proteinase and lipase/esterase
289 activities correlated significantly in all three stages (CIV: Spearman Rank Order Correlation (SR): 0.90, $p <$
290 0.001; CV: SR: 0.83, $p < 0.001$; CVIF: SR of 0.92 $p < 0.001$). In July 2012, the lipase/esterase activity was
291 relatively high (221.3 ± 43.2 nmol h^{-1} mg DM^{-1}) in CV captured at the surface, while it was low in those, which
292 had already descended to depth >100 m (178.4 ± 38.8 nmol h^{-1} mg DM^{-1}). During the rest of the year, the
293 lipase/esterase activities were low at about 120 nmol h^{-1} mg DM^{-1} in CV as was the activity in the CIV captured
294 in October 2012 at depth >100 m (Fig. 2a). In January and February, when the specific proteinase activity had
295 increased, also the specific lipase/esterase activities in the CV increased (one-way ANOVA $p<0.05$, Tukey post
296 hoc test). The activities in females at that time were in a similar range (around 150 nmol h^{-1} mg DM^{-1}). In March,
297 when only females and CIV were present in high numbers, the specific lipase/esterase activity of the CIV was
298 less than half of that of the females and the CV in January and February. In early April the activities had
299 increased significantly in both females and CIV (one-way ANOVA $p<0.05$, Tukey post hoc test), and in CIV
300 they reached maximum values in May (351.5 ± 25.4 nmol h^{-1} mg DM^{-1}). Overall maximum activities, however,
301 were found in the CV from the upper 50 m in June 2013 (458.4 ± 29.7 nmol h^{-1} mg DM^{-1}). In July, when most of
302 the CV had migrated to below 100 m depth, the activities were significantly lower than in June (one-way
303 ANOVA $p<0.05$, Tukey post hoc test), but still higher than during autumn and winter 2012 (Fig. 2a, b).
304
305 Enzyme pattern unraveled by substrate SDS-PAGE
306
307 Substrate SDS-PAGE was applied to reveal if the composition of isoenzymes was different among the stages of
308 *C. glacialis* or seasons. We sampled the different stages whenever abundant, and thus we managed to cover all
309 seasons, i.e. late summer (August, CV), autumn (October, CV), winter (January, CV and females), spring
310 (March CIV and May, CIV) and again summer (June, CV). Most samples did not show any proteolytic bands
311 and the variety of isoenzymes was poor as we found only one band at 23 kDa. This band was found in CV in
312 June (Fig. 3 a) and CIV (Fig. 4b) in May, when they resided in the upper 50 m. Interestingly, the proteolytic
313 band appeared in females already in January when they still inhabited deeper waters. In April, when females
314 were feeding in surface waters, the band became more intense compared to January (Fig. 4a).
315
316 In contrast to proteolytic activity, lipolytic activity was found in all samples, and the intensity of the bands varied
317 among the CV from the different seasons (Fig. 3b). When the CV resided in surface waters, activity was the
318 highest in June and numerous bands were found between 30 and 169 kDa. In August, when CV were captured
319 below 100 m, there was only one major band of activity at 169 kDa. Minor bands were found at 117 kDa and
320 between 30 and 40 kDa. In October and January, all bands were more intense, suggesting higher lipolytic
321 activity, which was reflected in net enzyme activities (Fig. 2). Like in CV, lipolytic activity bands in females
322 were visible between 30 and 169 kDa in January and intensified in early April (Fig. 5a). In CIV in March, the
323 lipolytic activity was reflected by only one major band of lipolytic activity at 169 kDa, while in May, there were
324 major activity bands between 30 and 169 kDa (Fig. 5b).
325

Water-soluble protein content

The water-soluble protein content ranged between 30 and 70 µg indv.$^{-1}$ in CIV, 60 and 85 µg indv.$^{-1}$ in CV from July 2012 to February 2013 and 35 and 50 µg indv.$^{-1}$ in CV in June/July 2013 and 35 and 90 µg indv.$^{-1}$ in CVIF (Table 1).

Discussion

The digestive activity of *C. glacialis* followed a clear seasonal pattern with high values when the copepods were feeding in surface waters and low values when they resided at overwintering depth. Similar depth- and season-related patterns in digestive activity were previously found in the mainly herbivorous calanoid copepod *Pseudocalanus minutus* in Svalbard waters (Lischka et al. 2007). In *C. finmarchicus* and *C. helgolandicus,* the numbers of so called B-cells, which are located in the gut epithelium and which are responsible for the production of digestive enzymes, are reduced during winter (Hallberg and Hirche 1980). These species would thus not be capable to efficiently digest food even if algae were available. Other authors, in contrast, suggested that *C. finmarchicus* feeds on microzooplankton and detritus during winter (Marshall and Orr 1958). Their study, however, focused on a population in the Clyde Sea, where food availability does not cease completely. In the Arctic, winter-feeding of the mainly herbivorous *C. glacialis* is not likely for the population that overwinters in deep waters and minimum lipase and proteinase activities in our study should reflect the basic digestive potential without feeding. Low digestive enzyme activity at starvation has previously been shown in other copepod species and was explained as a metabolic adjustment to save energy (Hassett and Landry 1983). In *Temora longicornis* from the Southern North Sea, for example, the proteinase activity in females starving for only three days had decreased to 25 % of that of feeding females (Kreibich et al. 2008). As compared to this small copepods species, which lives in a habitat with continuous food supply, *C. glacialis* kept relatively high lipolytic and proteolytic potentials over the entire winter with approximately 25 % and 10 %, respectively, of the maximum activities. According to Hassett & Landry (1983) such strategy might be advantageous for copepods that live in environments with strong variations in food supply. This should also be true for *C. finmarchicus* and *C. helgolandicus*, as they too overwinter at great depths without food. Hirche (1983), however, found trypsin and amylase activities close to zero in overwintering individuals and up to 20-fold higher values in feeding individuals. Also Lischka et al. (2007) found trypsin activities of less than 13 % in the non-diapausing species *P. minutus* during winter compared to summer. However, it has to be kept in mind that both studies (Hirche 1983; Lischka et al. 2007) measured activities of specific enzyme classes while we studied the total proteolytic and lipolytic activities as not to exclude potential enzymes. It is thus possible that the comparably high proteolytic activities, which we measured, reflect the potential of proteinases other than trypsin, e.g. metalloproteases, which are involved in the degradation of tissue proteins and which were detected in *C. finmarchicus* (Solgaard et al. 2007). However, we did not observe any significant changes in water-soluble protein content during winter, which suggests a minor role of tissue degrading metalloproteases. In *C. glacialis*, SDS-PAGE revealed a proteolytic activity band at 23 kDa. It needs to be noted that single activity bands presented on the gels may be subunits of the same enzyme, however, the molecular weight of 23 kDa has been defined to be typical for isoforms of both trypsin and chemotrypsin and both cleave dietary proteins (Saborowski et al. 2004; Teschke and

Saborowski 2005). We aimed at comparing the digestive enzyme activity pattern in copepods captured in different seasons. It remains, however, open which enzymes exactly contribute to these pattern as a detailed biochemical analysis of single bands was beyond the scope of our study.

In comparison to other crustaceans, such as the crabs *Cancer pagurus* and *Maja brachydactyla*, which showed several proteolytic activity bands in the range from 20 to 70 kDa (Saborowski et al. 2004; Andrés et al. 2010), *C. glacialis* exhibits little variety in proteolytic isoenzymes since SDS-PAGE shows only one band at 23 kDa. This activity band was also found in a previous study on *C. glacialis* in Billefjorden in November 2009 (Freese et al. 2012**)**. In contrast, we found a higher heterogeneity of lipolytic enzymes compared to proteolytic enzymes which may reflect the importance of the lipid metabolism in *C. glacialis*. During the productive season, individuals cleave diet-derived lipids and during overwintering they metabolize their internal lipid stores, which are mainly wax esters (Lee et al. 2006; Falk-Petersen et al. 2009). Lipases are classified as either digestive lipases, which cleave nutritive lipids or intracellular lipases, which cleave triacylglycerides in tissue lipids (Vihervaara and Puig 2008; Rivera-Pérez et al. 2010). In comparison to *C. glacialis*, the variety of lipolytic activity bands was less in the North Sea copepod *Temora longicornis,* which relies more on its protein metabolism (Kreibich et al. 2011).

The mechanisms that control timing and duration of dormancy in winter and activity during the productive Arctic season are controversially discussed. Some authors suggest that internal cues, i.e. threshold levels of total lipid content or hormones (Irigoien 2004; Clark et al. 2013) regulate onset and end of overwintering while others discuss environmental conditions such as temperature and light (Hirche 1996; Niehoff and Hirche 2005; Kosobokova 1990; Miller et al. 1991; Varpe et al. 2007). Since the seasonal cycle in the food regime is most prominent in Arctic ecosystems, also food availability has often been suggested to have a major influence on the timing of diapause (Søreide et al. 2010; Hirche 1981; Mayzaud and Poulet 1978). In our study, the digestive activities increased in females and CIV of *C. glacialis* in late March/early April, when ice algae developed, and high digestive enzyme activities corresponded to the phytoplankton bloom. A close correlation between the start of the algal spring bloom and high digestive enzyme activity was also found in the non-diapausing species *P. minutus* (Lischka et al. 2007). This contrasts the results from a previous study conducted in *C. hyperboreus* in which evidence for the internal regulation of enzyme synthesis prior to food supply as has been shown (Head and Conover 1983). Similarly, enzyme activities in the CV at the surface, in July 2012 were relatively high indicating that the copepods were still actively feeding, while the activity of the CV at depth was only half of that of individuals from the surface. These results suggest thatthe decrease in digestive enzyme activity is the result of starvation in *C. glacialis* at depth rather than that of internal regulation. It has to be noted that the copepods migrated downward and upward, respectively, in only a few weeks while our sampling interval was approximately one month. As we covered an entire year, sampling at higher frequency was not possible due to logistical constraints and to closely follow the changes in digestive activity during these transition phases in July/August and March/April would be essential to precisely estimate the timing of physiological changes.

Conclusion

Our study showed a clear seasonal pattern in digestion of *C. glacialis*, with high digestive activities in individuals in surface waters during the productive season and low activities in diapausing individuals in deep

waters. . High correspondence between activity levels and food abundance suggests that digestive enzyme synthesis was related to food availability, rather than being regulated internally or being triggered by the vertical distribution of the copepods in the water column. The copepods ascended well before algae developed and, because feeding probably stimulated increased digestive activity, *C. glacialis* should be capable to exploit earlier phytoplankton blooms. The descent started, however, before food supply had ceased in surface waters suggesting that the copepods would not benefit from phytoplankton late in the season.

Acknowledgements

We thank the crew and the scientists of the RV *Helmer Hanssen* and of the small motorboat Farm for their support during the cruises. For providing chlorophyll *a* data we thank Miriam Marquardt. Our manuscript benefited from constructive comments of Dr. Reinhard Saborowski. This research was part of the project CLEOPATRA II: Climate effects on food quality and trophic transfer in the Arctic marginal ice zone, funded by the Research Council of Norway (Project ID 216537). DF was financed from the Helmholtz Graduate School for Polar and Marine Research (VH-GS-200). Part of the fieldwork was financed by an Arctic Field Grant (Project ID 227555).

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648
649 **Figure legends**
650
651 **Fig. 1** Specific proteinase activity (A; dE_{366} h^{-1} DM^{-1}) and activity per individual (B; dE_{366} h^{-1} $indv.^{-1}$) of *Calanus*
652 *glacialis* copepodite stages IV, V and adult females from July 2012 to July 2013 (n = 3 x 10 individuals,

653 presented as mean ± standard deviation, except for CIV begin April n = 2). In July 2012 and from March 2013 to
654 July 2013, *C. glacialis* was sampled in the upper 50 m and from August 2012 to February 2013 from 180 to 100
655 m water depth. From February to early June, the fjord was covered by ice. Chlorophyll *a* (Chl *a*, mg m^{-2}) was
656 integrated over the water column from 75 m to surface, except for July 2013 (from 35 m to surface). Ice algae
657 were present from mid-March to end of April/beginning of May. *d*E366: the optical density at 366 nm; DM: dry
658 mass
659
660 **Fig. 2** Specific lipase/esterase activity (A; nmol h^{-1} DM^{-1}) and activity per individual (B; nmol h^{-1} $indv.^{-1}$) of
661 *Calanus glacialis* copepodite stages IV, V and adult females from July 2012 to July 2013 (n = 3 x 10 individuals,
662 presented as mean ± standard deviation, except for CIV begin April n = 2). In July 2012 and from March 2013 to
663 July 2013, *C. glacialis* was sampled in the upper 50 m and from August 2012 to February 2013 from 180 to 100
664 m water depth. From February to early June, the fjord was covered by ice. Chlorophyll *a* (Chl *a*, mg m^{-2}) was
665 integrated over the water column from 75 m to surface, except for July 2013 (from 35 m to surface). Ice algae
666 were present from mid-March to end of April/beginning of May. DM: dry mass
667
668 **Fig. 3** Proteolytic activity bands (a) and lipolytic activity bands (b) in *Calanus glacialis* copepodite stage V at
669 different times of the year (August 2012: lane 2-4, October 2012: lane 5-7, January 2013: lane 8-10, June 2013:
670 lane 11-13) and the molecular marker (lane 1, 20-200 kDa)
671
672 **Fig. 4** Proteolytic activity bands in *Calanus glacialis* adult females (CVIF) (a) and copepodite stage IV (b). (a):
673 lane 1: molecular marker (20-200 kDa), lane 2-4: CIV from January 2013, lane: 5-7: CIV from begin of April
674 2013. (b): lane 1: molecular marker (20-200 kDa), lane 2-4: CVIF from March 2013, lane: 5-7: CVIF from May
675
676 **Fig. 5** Lipolytic activity bands in *Calanus glacialis* adult females (CVIF) (a) and copepodite stage IV (b). (a):
677 lane 1: molecular marker (20-200 kDa), lane 2-4: CIV from January 2013, lane: 5-7: CIV from begin of April
678 2013. (b): lane 1: molecular marker (20-200 kDa), lane 2-4: CVIF from March 2013, lane: 5-7: CVIF from May
679
680 **Table 1** Water-soluble protein content in *Calanus glacialis* copepodite stage IV, V and and adult females (CVIF)
681 from Billefjorden from July 2012 to July 2013. Individuals were sampled from 180 to 100 m from July 2012 to
682 February 2013 and in July 2013 and from 50 m to surface from March 2013 to June 2013 (mean ± SD, n = 3)
683

Water-soluble protein content [µg $indv.^{-1}$]			
	CIV	**CV**	**CVIF**
23/07/2012	N/A	69.3 ± 7.9	N/A
28/08/2012	N/A	65.4 ± 15.2	N/A
21/09/2012	N/A	87.1 ± 24.2	N/A
05/10/2012	64.4 ± 16.3	87.8 ± 4.3	N/A
06/11/2012	N/A	79.2 ± 17.9	N/A
04/12/2012	N/A	61.1 ± 6.4	N/A

10/01/2013	N/A	78.1 ± 8.1	91.9 ± 26.1
04/02/2013	N/A	73.3 ± 9.7	64.9 ± 3.3
13/03/2013	72.2 ± 16.4	N/A	58.0 ± 9.2
09/04/2013	35.4 ± 0.0	N/A	35.6 ± 2.2
07/05/2013	27.8 ± 1.8	N/A	N/A
17/06/2013	N/A	36.5 ± 0.2	N/A
23/07/2013	N/A	49.5 ± 3.3	N/A

684

685 **Figures**

686

687 **Fig. 1**

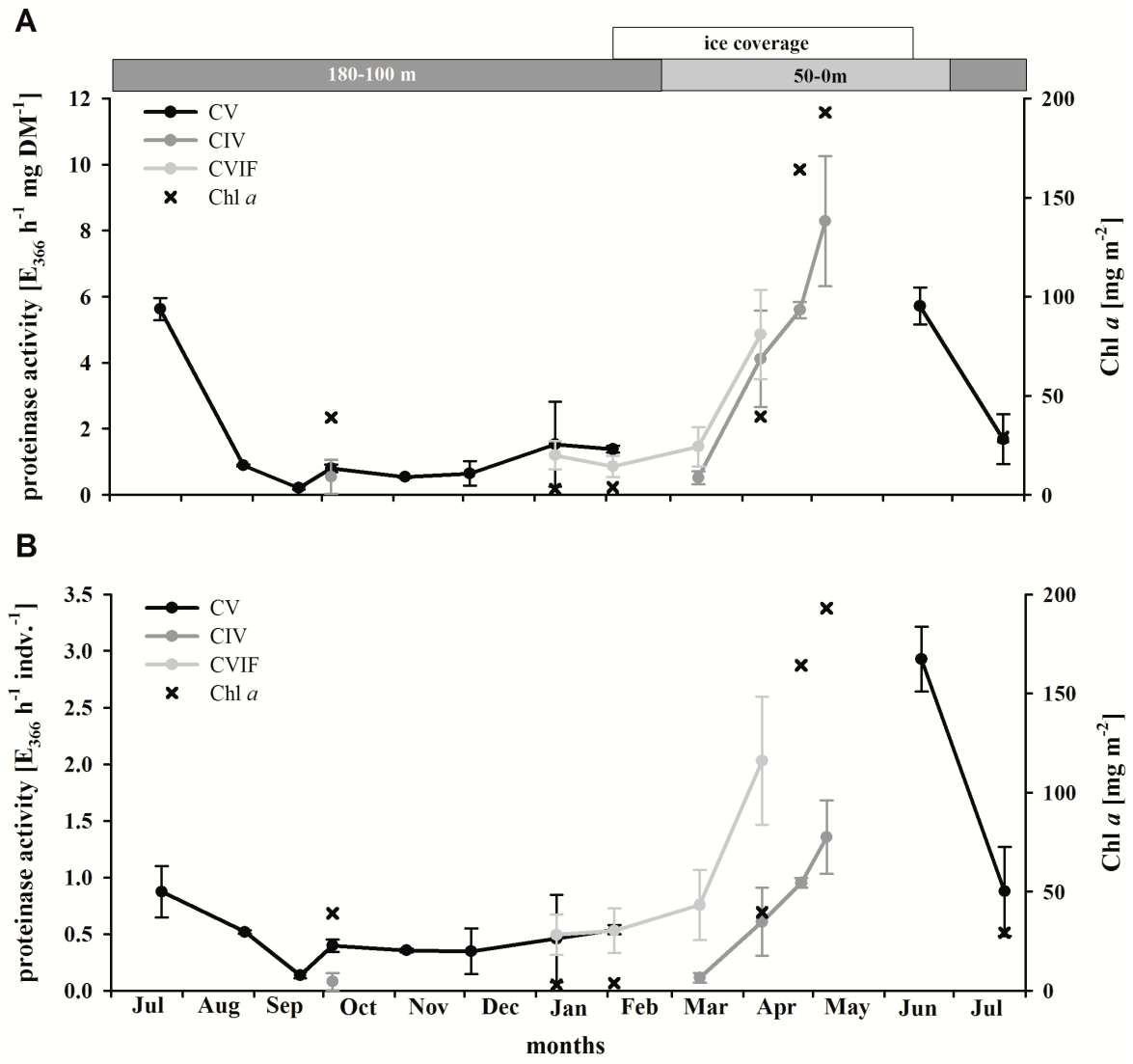

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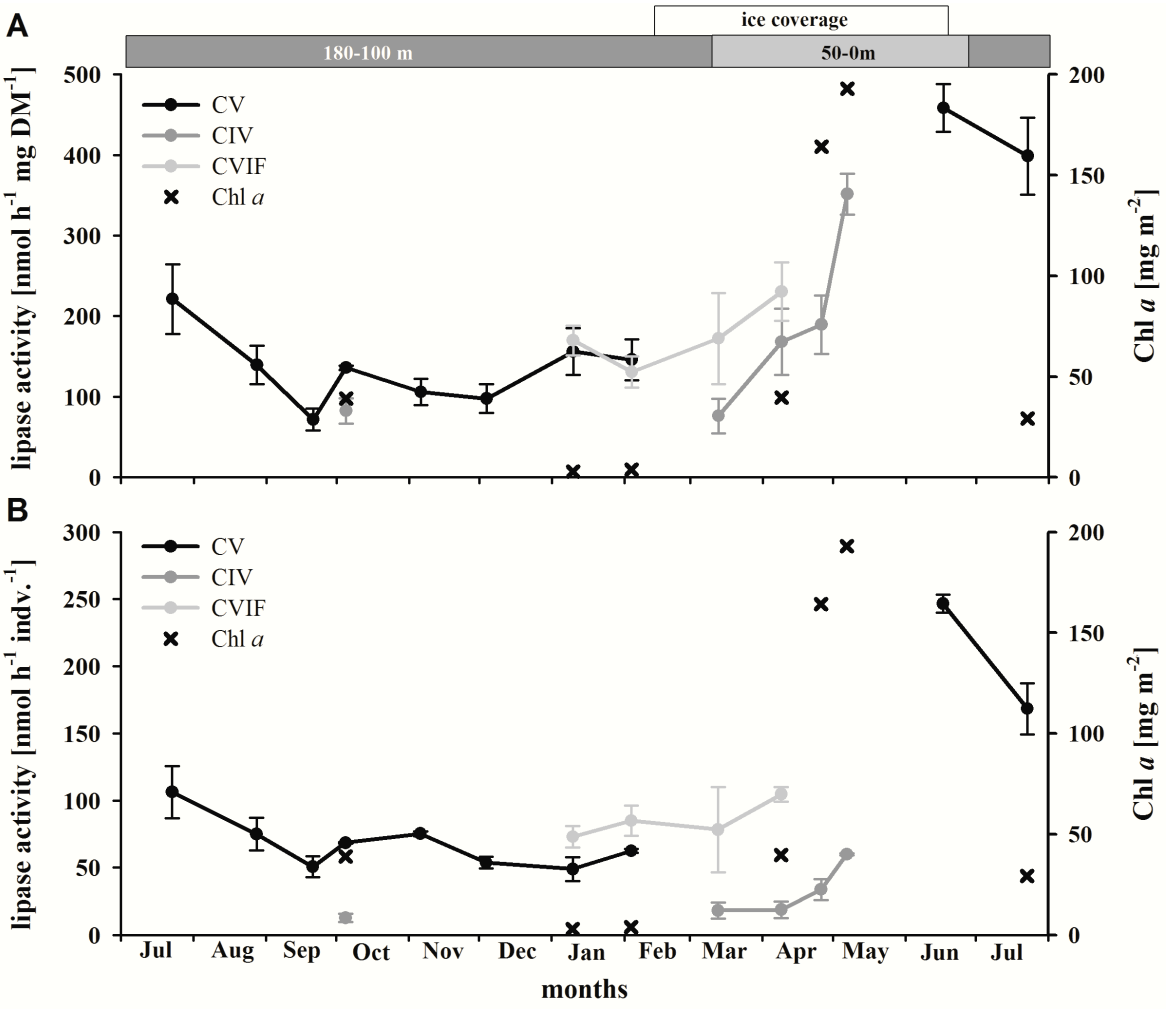
Fig. 2


A
ice coverage
180-100 m
50-0m
CV
CIV
CVIF
Chl a
lipase activity [nmol h-1 mg DM-1]
Chl a [mg m-2]
B
lipase activity [nmol h-1 indv.-1]
Jul
Aug
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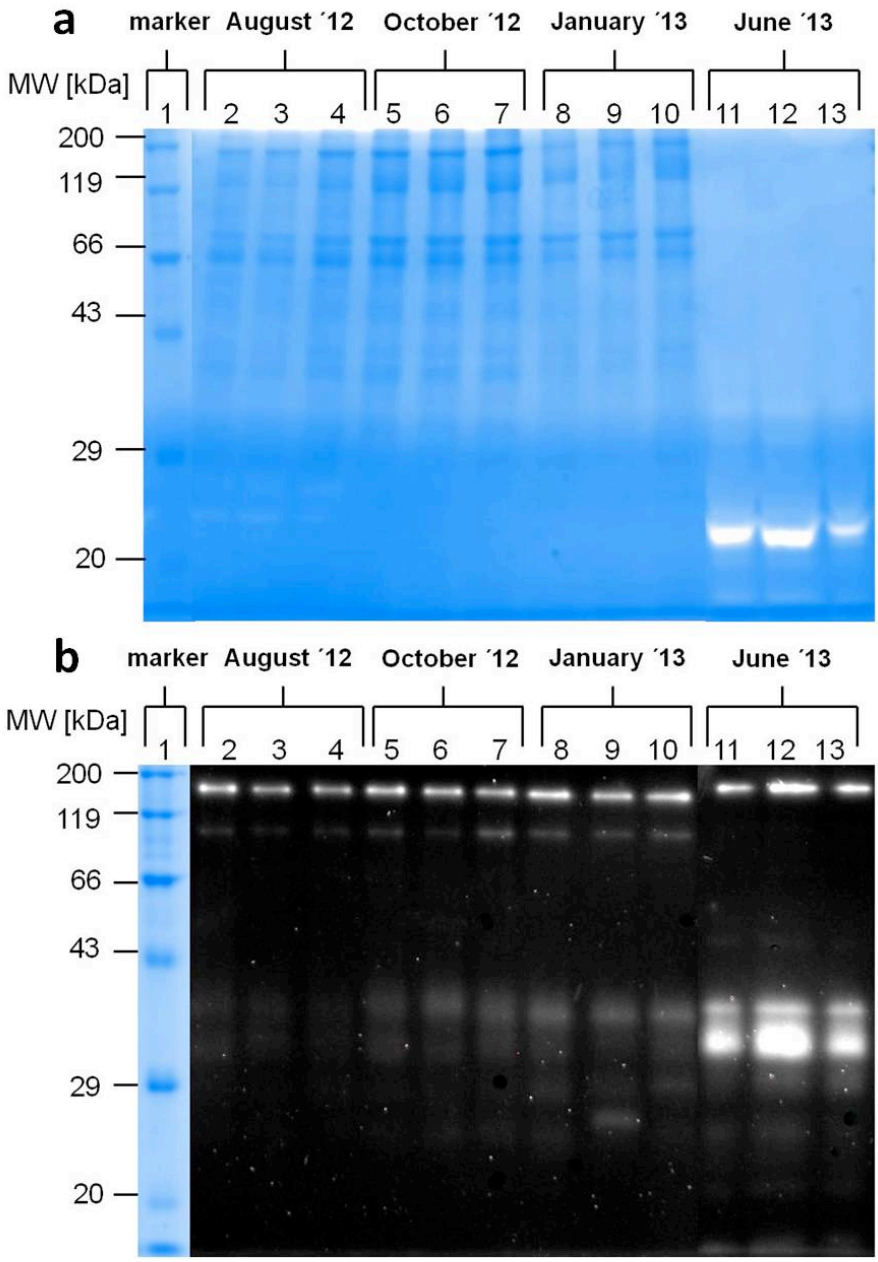
months

Fig. 3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marker
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MW [kDa]
1 2 3 4 5 6 7 8 9 10 11 12 13
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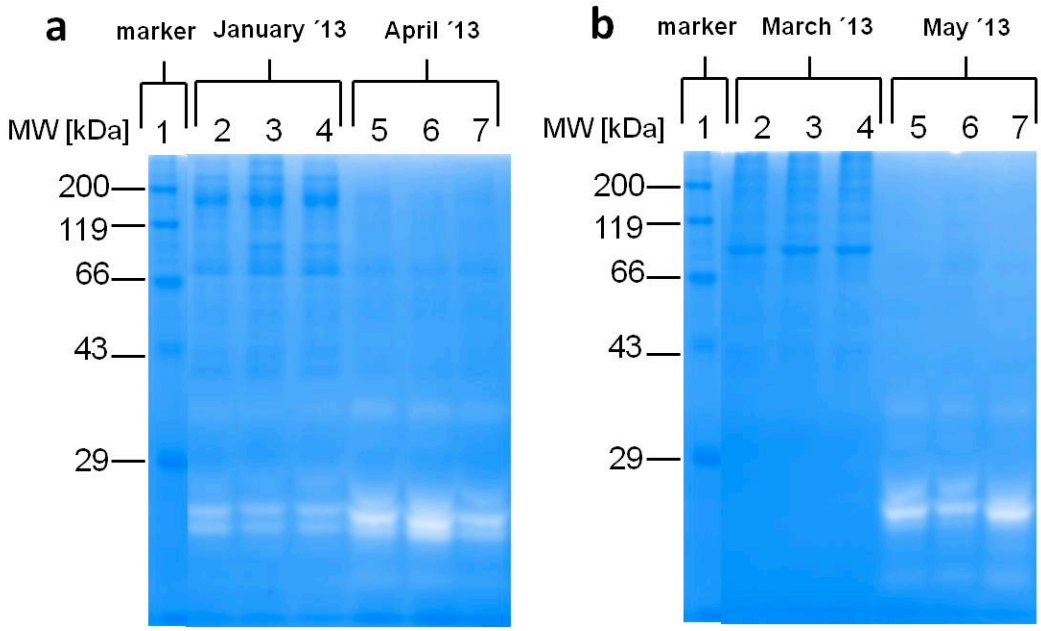
721 **Fig. 4**



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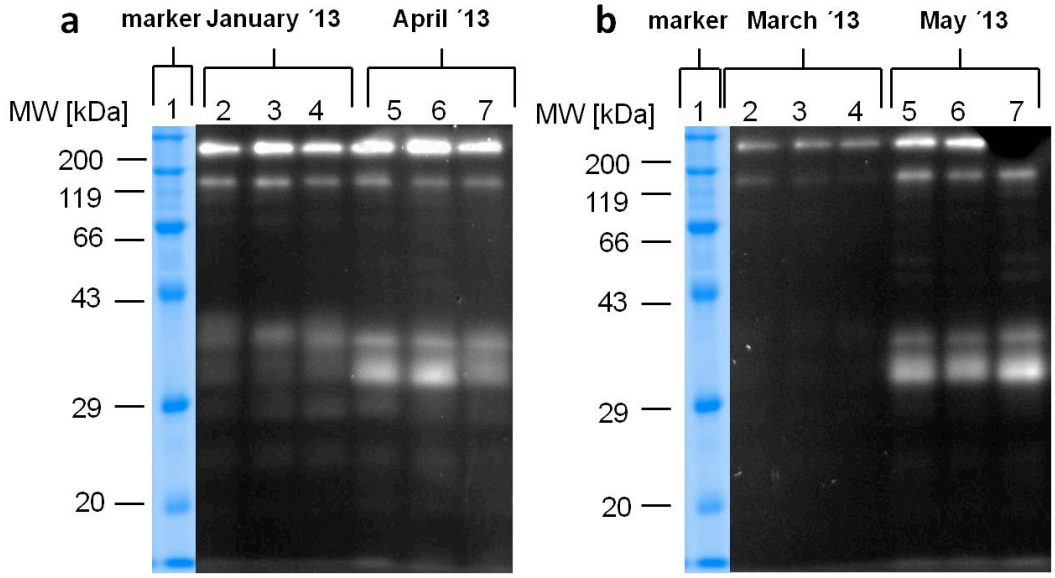
725 **Fig. 5**



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