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ORIGINAL ARTICLE

Seasonal and individual variability of lipid reserves in *Oithona similis* (Cyclopoida) in an Arctic fjord

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Abstract The lipid storage of the cyclopoid copepod *Oithona similis* was investigated from spring to late summer 2007 in Kongsfjorden (Svalbard, Norway). The volume of lipid droplets in each individual reflected the amount of stored wax esters. Seasonal changes of lipid storage coupled with informative inter-individual variability were thus obtained. The seasonal pattern showed an increase in lipid store during the spring bloom, starting before the chlorophyll *a* maximum for both copepodids stage V and females. Those reserves were used during the main reproductive event in June. Individual variability was very high, with a significant proportion of copepods having no droplet, while others were lipid rich. Because of the overlap of generation, females could have different age and feeding history, particularly in September. Consideration of intra-population variability in lipid storage using an optical approach has been shown to be important to understand *O. similis*'s ecology and life cycle.

Keywords *Oithona similis* · Lipids · Life strategy · Image analysis · Oil sac · Kongsfjorden · Svalbard

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Introduction

Small copepod species (<1 mm) play a key role in the functioning of the pelagic ecosystem. Highly abundant, they represent a link between the 'classical' and microbial food webs, as reviewed by Turner (2004). However, their importance and impact are still underestimated, mainly because of inappropriate sampling gears (Gallienne and Robins 2001). Among them, *Oithona similis* Claus 1866 (Cyclopoida) is a ubiquitous and cosmopolitan copepod, often the most abundant species of the mesozooplankton community from tropical areas to high latitudes. In the Southern Ocean, the importance of small copepods, such as *O. similis*, has been assessed by Atkinson and Sinclair (2000) from historical data. In this region, its annual production was greater than any of the biomass-dominant copepods (Fransz and Gonzalez 1995). In the same manner, *O. similis* is one of the most abundant mesozooplankton in the Arctic (Auel and Hagen 2002; Daase and Eiane 2007; Walkusz et al. 2003). Recently, the knowledge about the ecology of this species has increased, especially in the European Arctic region (Castellani et al. 2005a, b; Lischka 2006; Nielsen et al. 2002; Sabatini and Kiørboe 1994). An interesting feature of *O. similis* is that its life strategy differs from the large Arctic Calanoids, such as *Calanus* spp. It does not overwinter in diapause, but stays active in the upper layer of the water column (Conover and Huntley 1991; Lischka et al. 2007). This species reproduces year-round (Ashjian et al. 2003; Sabatini and Kiørboe 1994), and shows two major reproductive events at our study site (Lischka and Hagen 2005). Thus, *O. similis* could play a key role in the Arctic at particular periods of the year, i.e., autumn and winter when other species are undergoing diapause (Lischka and Hagen 2005), and act as stabilization factor of planktonic communities as hypothesized by Paffenhöfer (1993).

The European sector of the Arctic Ocean undergoes a strong influence of Atlantic water masses flowing through the Fram Strait, which is predicted to increase with global climate change (Cottier et al. 2005; Schauer et al. 2004). In this context, the role of smaller taxa, small copepods and other microzooplankton, could become more important (Hansen et al. 2003). Since cyclopoid species show generally less specialization than calanoids (Paffenhöfer 1993), they are able to survive over an extended range of environmental conditions, being thus less affected by water masses modifications even though it has been speculated that *O. similis* fitness at high latitudes was limited by low temperatures (Ward and Hirst 2007). Therefore, this species could actually benefit from a rising temperature in the Arctic. A clear increase of the relative abundance of *O. similis*, among other small copepods, has already been underlined in Kongsfjorden, west coast of Spitsbergen, from 1996 to 2002 (Hop et al. 2006).

In arctic regions, zooplankton has adapted to the high seasonality of primary production in storing large amounts of lipids as energy reserves (Falk-Petersen et al. 1990; Hagen and Auel 2001). These reserves ensure the winter survival and the reproductive capacity of populations the next spring (Falk-Petersen et al. 2006; Lee et al. 2006). Hence, arctic zooplankton represents a highly energetic food source, which supports the productivity of the higher trophic levels. At the individual level, looking at lipid stores gives insights into the physiological state of the organism and the population fitness.

Lipid stores in zooplankton are essentially made from neutral lipids, wax esters and triacylglycerols, and can be quantified by several methods (reviewed in Lee et al. 2006). Optical estimation of lipid droplets has been shown to be a useful alternative tool to biochemical methods. Quicker and cheaper, they are non-destructive and give information on the location of the reserves in the organism. Additionally, population features are deduced from individual measurements, which give information on the intra-population variability. First developed for freshwater Cladocerans, the amount of lipid droplets was evaluated by a simple visual index, ranging from 0 to 3 (Goulden and Hornig 1980). Later, Arts and Evans (1991) studied the seasonal changes of lipid reserves in freshwater calanoid copepods and showed that both lipid droplets volume measurements and biochemical analysis gave concordant results. In small marine calanoids, a lipid index calculated as the percentage of the lipid sac area on the prosome area has been proposed by Norrbin (1991). The lipid sac volume measurement was also used to quantify the amount of storage lipids in *Calanus finmarchicus* (Miller et al. 1998). When lipid storage is rather diffuse, Nile Red staining has been shown to be a useful tool for the relative quantification of neutral lipids, either for the determination of the droplets area (Carman et al. 1991), or using directly the fluorescence intensity of stained lipid droplets (Tankersley 1998). Using Nile Red properties, a spectrofluorometric absolute quantification of neutral and polar lipid has been achieved by Alonzo and Mayzaud (1999).

In this study, we discuss the suitability of an optical-digital method, and the usefulness of Nile Red staining, for the investigation of the lipid reserves in *O. similis*. The seasonal development of these energetic reserves in an Arctic fjord is presented at the population level, considering the individual variability.

Materials and methods

The fieldwork took place from April to September 2007 in Kongsfjorden, a fjord on the west coast of Spitsbergen (Svalbard, Norway). Our sampling station was located in the middle zone of the fjord (78°57.0′N 11°56.4′E), where the bottom depth is of *ca.*300 m (Fig. 1).

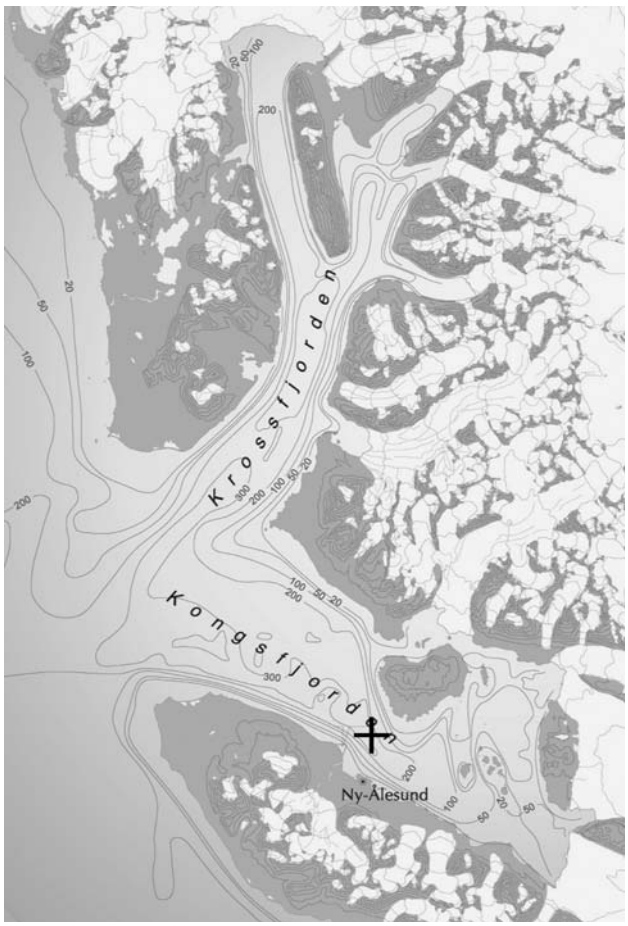

Fig. 1 Map of Kongsfjorden (79°N), West coast of Spitsbergen (Svalbard, Norway). *Cross* sampling station

Chlorophyll *a* and temperature measurements

Once a week from May to September, the chlorophyll *a* (Chl*a*) concentration was monitored down to the minimum depth of 200 m, with a Seapoint Chlorophyll Fluorometer (Seapoint Sensors, Inc., Exeter, USA). Data acquisition rate was of one measurement per second, corresponding to a vertical accuracy of 0.2–0.7 m. As post-treatment, no smoothing function was applied to the data, but spikes were removed. In April, the chlorophyll *a* was measured from water samples at discrete depths (surface water, 5, 10, 20 and 30 m). Triplicates of 0.7–1 L of sea-water were filtrated on GF/F filters and stored at −80°C. The extractions have been conducted in 90% acetone in the dark during 5 h and the fluorescence of Chl*a* and phaeopigments in the extracts was measured using a Turner Design 10 Fluorometer (Lorenzen 1966).

Zooplankton sampling

At fifteen dates from the 18th of April to the 24th of September, zooplankton was collected with a 200-µm mesh size WP2 net hauled vertically from 200 m to the surface, at a speed of 0.75 m s^{-1}, and the samples were stored in formalin (4% vol. final concentration). On five separate dates—22 April, 3 May, 29 May, 3 July and 8 September—the WP2 net was towed gently within the 50 upper meters of the water column, at boat drifting speed. The zooplankton caught was kept alive in 10 L of surface seawater, at in situ temperature until sorting for in vivo observation and, at the latest date, Nile Red staining and biochemical analysis.

Lipid droplet observation on formalin-fixed copepods

The fifteen formalin preserved samples were examined under an Olympus SZH10 stereomicroscope with transmitted light. For each sample, 20–30 individuals of both females and copepodites V were picked out and the presence or the lack of lipid droplet was noted. Males were too scarce to be studied over the season.

In vivo lipid droplet observations

Within the day following each of the five catches, successive aliquots of the sample were carefully poured through a sieve of 1-mm mesh size to remove the biggest zooplankton species and then through a 63-µm mesh to retrieve the size class of interest. Different developmental stages from the species *O. similis* were sorted out alive under stereomicroscope; 27–60 females and 8–37 copepodites V (CV) and additionally in July, 15 CIV and 9 CIII. After a light anesthesia using tricaine methanesulfonate (MS222, Sigma-Aldrich), sorted organisms were individually observed under a microscope; either a Leica DM 2500 microscope or an Olympus IMT-2 inverted microscope. Pictures were taken using a MotiCam 1000 digital camera and its associated software Motic Image Plus (capture resolution 640 × 512 pixels), except in April, when a LEICA camera and its associated software were used instead (capture resolution 2,088 × 1,550 pixels). Specific calibrations were performed for each setting using a micrometric scale (final resolution, 0.73–1.18 µm $pixels^{-1}$).

The copepods from the September sample were stained alive with the hydrophobic fluorescent dye Nile Red (Sigma-Aldrich) before observation. A stock solution of Nile Red at 0.125 g L^{-1} in acetone was prepared the week before use and stored in the dark. Several batches of five *O. similis* were placed in small dishes with filtered seawater (0.45 µm) and Nile Red was added to a final concentration of 2.5 10^{-3} g L^{-1}. After 5-min staining, the solution was diluted with filtered seawater and the individuals were immediately observed. The concentration of dye and the exposure time for staining have been chosen after testing the fluorescence signal obtained under different conditions: a higher concentration deformed the copepod's shape and a longer exposure time did not increase fluorescence levels. The stained copepods were viewed for yellow-gold fluorescence of the neutral lipids using an epifluorescence microscope LEICA DM 2500 (excitation wavelength of 450/500 nm, emission >528 nm) according to Greenspan et al. (1985). The image capture was realized with fixed exposure time and gain to allow comparison between the fluorescent levels of different pictures.

Picture analysis and estimation of lipid reserves

For both non-stained and stained samples, pictures were analyzed using the Image J 1.40 software (a public domain software developed by W.S. Rasband, US National Institutes of Health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/, 1997–2008). From each picture, prosome and lipid droplet(s) area were measured in dorsal view by visual adjustment using the polygon selection tool. From the shape of the selected areas, the major (M) and minor (m) axis of the best fitting ellipse were determined. The volume was then calculated both for the droplets and the prosome, assuming an oblate spheroid shape (Arts and Evans 1991), using the following equation:

$$V = \frac{4}{3}\pi\frac{M}{2}\left(\frac{m}{2}\right)^2$$

Lipid content of each individual was computed by adding the volume of all its droplets, and we assumed that staining the September sample did not affect the area measurement.

In addition, for Nile Red stained organisms, pictures were converted to grey scale with pixel values ranging from 0 to 255. The droplet(s) integrated fluorescence was then computed from the intensity values of all pixels contained in the droplet area.

Biochemical analysis

Oithona similis females from the September sample were also sorted out for lipid biochemical analysis. Three samples were pooled depending on the size of the lipid droplet in the copepod: no visible droplet, small droplet (ca. 2,000 μm^2) and big droplet (ca. 9,000 μm^2)—96, 71 and 63 individuals, respectively. The copepods of each sample were concentrated alive by gentle filtration through small filters made from a 50-μm mesh size net, and immediately frozen at −80°C. The samples remained deep frozen until analysis. The filters were rinsed with water to take the copepods off and the lipid extractions were conducted immediately in chloroform and methanol following Bligh and Dyer (1959). The copepods were homogenized using a Potter homogenizer (glass/teflon) at 0°C. The solvent was separately preserved at 0°C, while the copepod carcasses were extracted two more times following the above protocol. The total lipid samples obtained were kept under non-oxidant atmosphere (N_2) at −80°C until further analysis. To investigate the lipid classes composition, two pseudo-replicates from each sample were analyzed by thin-layer chromatography, flame ionization detector using an Iatroscan MK V TH10 (Iatron, Japan; Ackman 1981), solvent systems followed Mayzaud et al. (1988). Calibration was carried out using commercial standards that approximate the lipid composition of the samples. The results presented are the mean values of the two pseudo-replicates (variability <5%), and are shown in percentage of the total lipid weight (sum of all classes). The ratios wax esters/polar lipids and triacylglycerols/polar lipids have been calculated, because the amount of polar lipids is supposed to remain stable within an organism, independently from the lipid reserve metabolism (accumulation or degradation), and thus brings insight on the absolute neutral lipid content.

Statistical analysis

Due to the non-normality of lipid droplets size distributions, we used non-parametric statistics for comparisons. The droplets volume in copepodids V and females over the season was compared with the Friedman's ANOVA by ranks, whereas the median test was used for date to date comparisons. ANOVAs were performed to investigate the prosome length variability. We calculated Pearson correlations to compare prosome length and droplet area for each date and stage. These analyses were performed using the statistical software STATISTICA 4.3 (Statsoft).

Results

Neutral lipid storage location

For all examined copepods, Nile Red fluorescence was restricted to one or few delimited areas in the copepods prosomes (Fig. 2a) and did not reveal any diffuse storage. Thus, the neutral lipids appeared to be stored most of the time in one ellipsoidal droplet representing up to 24% of the prosome volume. This main droplet was located at the posterior part of the prosome when small (Fig. 2b), and grew towards the front when larger. Additional droplets were observed mainly when the principal droplet was relatively large, and usually located either in the anterior part of the prosome or on each side of the major lipid droplet. All samples taken as a whole: 27% of the copepods had no visible lipid droplet, and 88% of the copepods having a droplet had only one.

Nile red versus natural light observation

Both integrated fluorescence and area of each droplet were measured on the September organisms, and a linear regression between the two parameters gave a significant correlation coefficient ($R^2 = 0.98$, $P < 0.0001$). We can safely assume that the fluorescence measurements provided similar information to the optical ones. Hence, estimation of the neutral lipid content using droplet area is the only valid measurement.

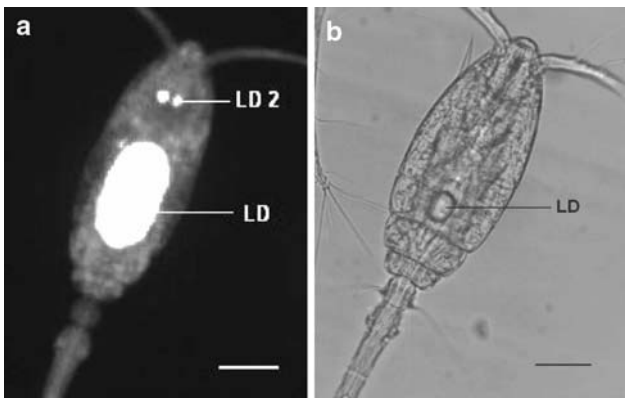

Fig. 2 *Oithona similis* females showing lipid droplets inside the prosome. **a** Nile red stained organism observed using an epifluorescence microscope, picture converted to grey scale. **b** Live organism observed using a transmitted light microscope. *LD* main lipid droplet, *LD2* additional lipid droplet. *Scale bar* 100 μm for both pictures

Lipid composition of the droplets

Comparing the neutral lipid composition of copepods with and without droplet, the major difference was the increase of the wax esters proportion when the droplet was larger, from 5.22 to 37.11% of the total lipid weight (Table 1). In addition, the wax esters/polar lipids ratio showed clearly the same increase, from 0.09 to 0.89. Triacylglycerols (TAG) contributed to the total lipid weight to a lesser extent, but their proportion was significant (5.66–8.74%). The highest TAG value occurred when the droplet volume was minimal; however, the TAG/polar lipids ratio remained relatively stable (0.10–0.15) whatever the droplet size and even if there was no droplet. So the decrease observed in TAG relative proportion when the droplet volume was bigger seemed to be an artefact due to the parallel increase in wax ester content. A reasonable assumption is that lipid droplets in *O. similis* are made from wax esters.

Seasonal patterns

The chlorophyll *a* (Chl*a*) concentration (Fig. 3) revealed a spring bloom of moderate intensity (4 μg Chl*a* L^{-1}). Chl*a* accumulation, as a proxy of phytoplankton biomass, started around 25 m depth from the 5 May and sank, with a later maximum around 80 m the 15 May. Later on, several smaller summer blooms (2–2.5 μg Chl*a* L^{-1}) occurred from the end of July onwards, in the 25 first meters of the water column. Those later blooms did not sink because of the established pycnocline (unpublished data).

Seasonal variation of lipid droplet occurrence in *O. similis* CV and females is presented in Fig. 4. The percentage of individuals having droplets followed the same pattern in fixed organisms than for in vivo observations for the two stages; except at one date for CV, when only eight individuals were observed in vivo. An abrupt increase, starting before the peak of phytoplankton biomass, was seen both in CV and females. This percentage notably decreased during the Chl*a* maximum in CV (Fig. 4a), then increased and remained relatively constant from July to September (87–100%). In females, the decrease occurred right after the Chl*a* maximum and this trend continued during the summer, except one high value in August, with

Table 1 Proportions of neutral lipids in *Oithona similis* females from September 2007, sorted according to their lipid droplets size

Droplet size	*n*	WE		TAG	
		% total lipids	/polar lipids	% total lipids	/polar lipids
Large	96	37.1	0.89	5.6	0.14
Small	71	10.7	0.18	5.8	0.10
None	63	5.2	0.09	8.7	0.15

n number of individuals per sample, *WE* wax esters, *TAG* triacylglycerols

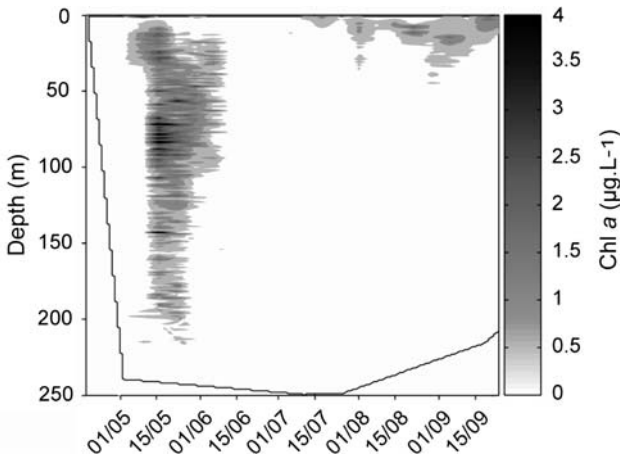

Fig. 3 Seasonal variability of chlorophyll *a* concentration (μg L^{-1}) from April to September 2007 at the sampling station

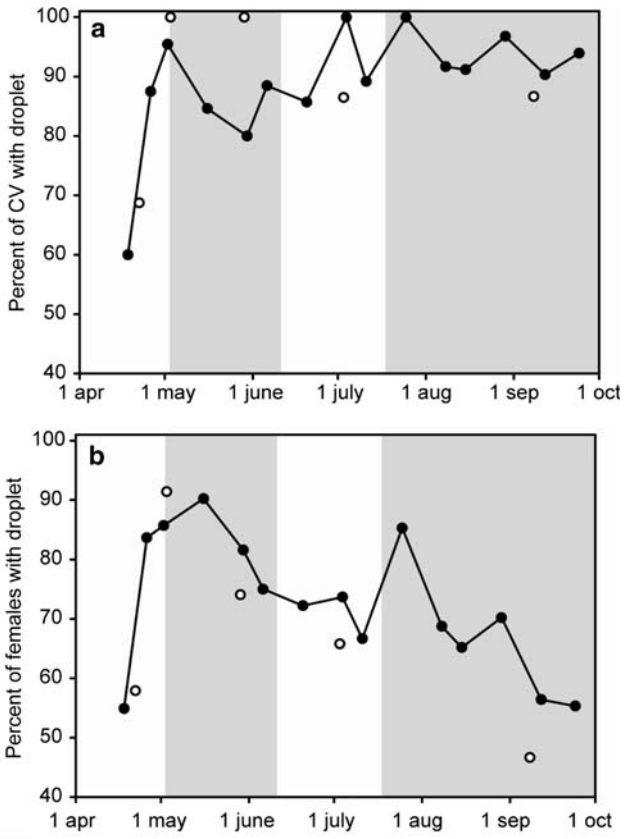

Fig. 4 *Oithona similis.* Seasonal changes of the percentage of individuals with lipid droplet from April to September 2007. **a** Copepodids stage V, **b** females. *Black circles* correspond to formalin-fixed samples, *white circles* to in vivo observations. *Grey undergrounds* represent periods of high chlorophyll *a* concentration

only half of females containing a lipid droplet in September (Fig. 4b).

Although the percentage of CV having droplets showed a seasonal trend, the median volume of this lipid droplet (Fig. 5a) remained stable throughout the season around an overall median of 0.35 $mm^3 ind^{-1}$. The maximal values, however, were twice as high in July and September as they were earlier in the season (ca. 1.2 instead of 0.6 mm^3 ind^{-1}). The droplet volume distribution around the median was rather symmetric except at the end of May. At this date, the distribution was shifted towards higher values, whereas the percentage of CV having a droplet showed a decrease. Concerning females having droplets (Fig. 5b), all medians but one were homogeneous (overall median, 0.05 mm^3 ind^{-1}). At the end of May, the median was significantly higher than the others (median test, $P < 0.001$), meaning that the median volume increased in spring and decreased in summer. The distributions around the medians were asymmetric in favor of low values, especially in September although the maximal value was the highest.

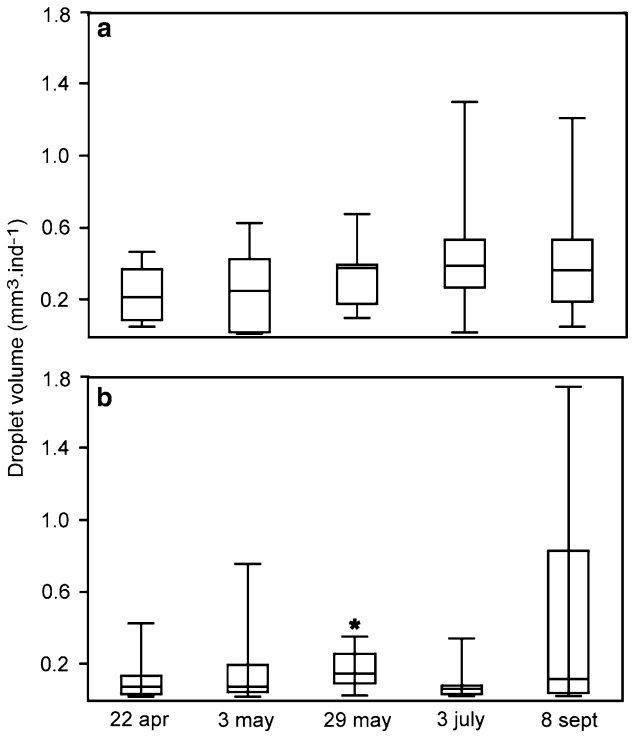

Fig. 5 *Oithona similis.* Seasonal changes of the lipid droplet volume, in vivo observations from April to September 2007. **a** Copepodids stage V, **b** females. *Boxes* limits represent the 25 and 75 percentiles around the median; *whiskers* extend from the maximal to the minimal values. *Asterisk* significantly higher median ($P < 0.001$). Copepods devoid of lipid droplet were excluded

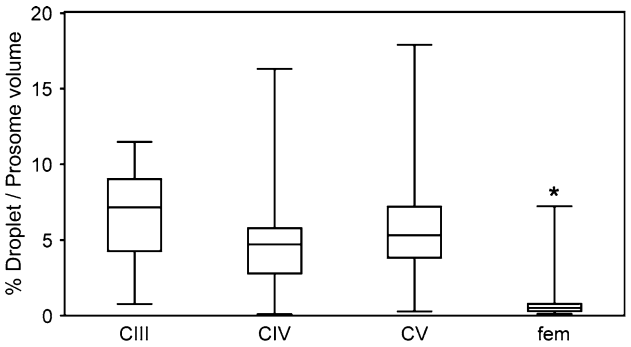

Fig. 6 *Oithona similis.* Percentage of lipid droplet volume/prosome volume in different copepodid developmental stages, in vivo observations in July 2007. *C III* to *V* copepodids stage III to V, *fem* females. *Boxes* limits represent the 25 and 75 percentiles around the median; *whiskers* extend from the maximal to the minimal values. *Asterisk* significantly lower median ($P < 0.001$). Copepods devoid of lipid droplet were excluded

Interstage variability

Data obtained in July showed that copepodids stored neutral lipid in a droplet at least from stage III (Fig. 6). The ratio between lipid droplet volume and prosome volume was not significantly different in CIII, CIV and CV (overall median, 5.20%), meaning that these copepodids accumulated lipid reserves in proportion to their growth. Conversely, females stored lower amount lipid than the three other stages (median test, $P < 0.0001$). Considering the whole period of study (Fig. 5), the difference between females and CV was confirmed, lipid droplets being significantly larger in the latest (Friedman ANOVA on ranks, $P = 0.02$).

Prosome length variability

The prosome length of the copepods was not correlated to the droplet area, except for females in September (Pearson correlation, $P < 0.01$). The prosome lengths in samples from different seasons were homogeneous in CV. In females, a significant variation was found (ANOVA, $P < 0.01$) due to lower values in April. The lipid content did not seem to be driven by the size of the copepods; thus, lipid droplets volume values are given per individual.

Discussion

Optical estimation of lipid reserves

In previous studies on marine planktonic organisms, Nile Red staining coupled with image analysis have been used successfully to estimate the total amount of neutral lipids (Carman et al. 1991; Tankersley 1998). In those cases, the

neutral lipids were allocated in small droplets throughout the body and Nile Red fluorescence signal from one focal distance was a good estimate of the total amount of neutral lipids, rather than the area measurement of all droplets. We observed that *O. similis* stored neutral lipids in one or a few big droplets, already observed using transmitted light. Moreover, we showed that the integrated fluorescence intensity was proportional to the area of the droplet and was not affected by its thickness. The main reason is probably that the depth of focus associated with the magnification required to observe *O. similis* is very narrow compared to the thickness of the droplet. The contamination of the fluorescence signal at one focal distance from the vertical diffusion of fluorescence emitted from other levels was not sufficient to reflect the thickness of the droplet. With respect to *O. similis*, this study showed that image analysis of pictures taken under transmitted light brought as much information as analysis of pictures obtained after fluorescent staining.

Lipid droplet volume was extrapolated from droplet area in dorsal view assuming a perfect ellipsoidal shape. This latter assumption could lead to a slight overestimation of lipid content in some particular cases. Indeed, thickness and width were very similar in small droplet but when droplet size increased, thickness seemed to reach a maximum before length and width probably because droplet growth in that direction was limited by the gut. However, this potential overestimation concerned a limited number of copepods.

The copepods have to be observed alive for an accurate measurement of the droplets size. Though formalin fixation is not likely to affect the size of the lipid sac, the prosome of fixed copepods is often less transparent and its shape can be damaged. However, good results were obtained when considering only the presence or absence of droplet inside the prosome.

Droplet location and composition

A histological study described two types of lipid deposits in calanoid copepods (Blades-Eckelbarger 1991). The copepods presented either a large, thin-walled lipid sac containing a single large deposit of intracellular lipid enclosed by a very thin rim of cytoplasm, or discrete lipid deposits as cytoplasmic droplets in cells near the anterior part of the mid-gut. Although we showed no evidence of any structure for accumulation of lipids, the lipid deposits observed in *O. similis* could be described as oil sacs with respect to these definitions.

Our results from September suggested that *O. similis*'s lipid sacs were made up of wax esters, which are known as the major storage lipids in high latitude species (Lee et al. 2006). Although, lipid classes present in *O. similis* have not been totally described yet, wax esters are known to be part of the lipid storages since Lischka and Hagen (2007) found significant seasonal variations of the total fatty alcohols amount, as wax esters estimate, in Kongsfjorden. In Antarctica, high amounts of fatty alcohols were found in *O. similis* males, but relatively lower in females (Kattner et al. 2003). In the present study, we showed that *O. similis* also contained triacylglycerols (TAG) in lower proportion, but this compound did not seem to be linked to the lipid sac. Similarly, Miller et al. (1998) showed that *Calanus finmarchicus* stores only wax esters inside the oil sac and concluded that TAG storage takes place elsewhere. Since we only investigated lipid sacs, we were not able to gather information about inter-individual variability or seasonal variability of TAG throughout the body, leaving open the question of their role as energy reserves in *O. similis*.

Accumulation of lipids at the onset of the spring bloom

We observed that the proportion of both females and CV with visible lipid reserves increased rapidly between 22 April and 3 May, with at least 90% of copepods containing lipid reserves at the later date (Fig. 4). Considering individuals having droplet, the median droplet volume did not change significantly during this period (although the maximal size did, Fig. 5), implying that this increase of lipid reserves occurred at the population level. Conversely, during the maximum of phytoplankton biomass around mid May, the increase of lipid storage in *O. similis* was observed at the individual level both in CV and females. The median size of the droplets increased while fewer copepods contained droplets, reflecting individuals with positive and negative energetic budget.

Strikingly, we showed an increase of lipid content starting even before the accumulation of phytoplankton biomass in the water column (from 5 May). This apparent mismatch supports the hypothesis that at least those two stages of *O. similis* took advantage of other food sources than the blooming algae to accumulate lipids. *O. similis* is known to have an omnivorous feeding regime, eating preferentially ciliates and other nano- and micro-zooplankton (Castellani et al. 2005a; Lonsdale et al. 2000), but also fecal pellets and detritus, although this matter is still under discussion (Reigstad et al. 2005; Svensen and Nejstgaard 2003). Moreover, in Kongsfjorden, the highest food quality in particulate organic matter (i.e., high proportion of polyunsaturated fatty acids) has been encountered before the maximum biomass in spring (Leu et al. 2006). This observation, supported by other in situ and experimental studies (respectively, Hayakawa et al. 1996; Parrish et al. 2005), indicates that during the onset of the bloom the algae prioritize polar lipid production for rapid growth. Thus, *O. similis* could have benefited from this high nutritional value and high production at the beginning of

the bloom to build up neutral lipid reserves. Similariy, females of *Calanus glacialis* and *Calanus finmarchicus*, seem to profit of the high nutritional quality in particulate matter prior to the peak of the bloom (Leu et al. 2006; Wold et al. 2007). But in these cases, the authors suggested that phospholipids rather than neutral lipids are synthesized first, in order to support egg production.

Our results underlined an increase of neutral lipids in spring, whereas Lischka and Hagen (2007), who conducted a seasonal survey in the same fjord in 1998–1999, concluded that *O. similis* does not take advantage of the spring bloom. After a decrease during winter, they noted the lowest wax ester content in spring (first half of May) for both CV and females. These differences could come from the different investigation methods used. We benefited from a better time resolution and looked into individual variability, allowing us to follow the lipid dynamics in greater details. We could also relate these differences to the contrasting environmental conditions during the two studies. The winter was cold and the fjord filled with sea ice when the previous study took place, while the temperatures were milder and the fjord ice-free during ours. *O. similis* could have thus exhibited two different life strategies, underlining its high adaptive capacity to various food conditions.

Lipid reserves and reproduction

Between the end of May and the beginning of July, we observed a decrease of both median droplet size and droplet occurrence in females (Figs. 4b, 5b). This high lipid consumption was most probably associated with an energetic demand from egg production, since June corresponds to the main reproduction peak of *O. similis* at this location (Lischka and Hagen 2005). It seems, from our study, that reproduction in June relied on newly accumulated lipids rather than last autumn's reserve as Lischka and Hagen (2007) concluded.

From this earlier study, the second reproduction peak (August–September and presumably October) is supported directly by the summer phytoplankton blooms and wax esters are stored at the same time, reaching maximal values in August–September (Lischka 2006). If we looked at our data in terms of average droplet volume, we would observe a clear increase between July and September as well. But from a median point of view, the lipids reserves were stable and the proportion of copepods having droplets was decreasing during the summer. Our individual-based study revealed huge intra-population variability in September. The low droplet median volume in females was hiding a high inter-individual variability due to a limited number of individuals with very big droplets, while almost half of the population had no droplet at all. This observation could be interpreted as some females stopping reproduction or taking a better advantage of the summer blooms than others or, more probably, as a significant input of newly molted females from the lipid rich CV stock. In other words, this could be associated with the overlap of two generations: rich CV that just developed into females and the "old" females that are still reproducing. Moreover, Sabatini and Kiørboe (1994) showed that *O. similis* females are rather long-lived (up to at least 50 days). The lipid rich females could be the youngest ones and could reproduce in autumn, when this species is known to undergo a major reproduction event (Lischka and Hagen 2005). Even if this copepod is known to remain active during winter, the food is supposed to be limiting and this huge lipid reserve could also help to survive the winter and ensure next year generations (Lischka et al. 2007).

Lipid reserves as life-cycle strategy or short-term adaptation?

Comparably, a study on *Calanus finmarchicus* from the Georges Bank showed that this copepod is either lipid poor or lipid rich at the end of the productive season. In this case, individuals with large lipid reserves are ready to overwinter in deep layers, whereas individuals that are not rich enough will stay active in the upper layer (Miller et al. 2000). In the case of *O. similis*, the cohorts are not synchronous due to the permanent production of eggs throughout the year (Ashjian et al. 2003; Lischka and Hagen 2005). Thus, the identified stage of a copepod does not necessarily indicate its age, and this is especially true for females which may live longer. The extreme intra-population variability could therefore be linked to the year-round reproduction of this species rather than to two different overwintering strategies. Similarly, Norrbin (1991) noticed less seasonality in visible lipid storage of *Microcalanus* than in *Pseudocalanus*, and hypothesized that it could be related to its smaller size. Because small copepods have higher weight-specific metabolic rates, they need a constant food supply, leading to a longer period of activity. The presence of wax esters, as long-term energy reserves, is typical in species which show a drastic seasonality in their life cycle (Falk-Petersen et al. 2006; Lee et al. 2006). However, *O. similis* has the ability to store wax esters although it feeds omnivorously and continuously, as identified by Lischka and Hagen (2007). The lipid stores observed in the present study might be an adaptation to short-term food variability rather than a seasonal pattern.

Importance of individual variability in ecological studies

Using classical biochemical methods to study small copepods like *O. similis*, the need of sufficient amount of

material leads to a loss of information about individual variability, tens to hundreds of animals being pooled for one analysis. Here, using image analysis, we observed not only seasonal variation in *O. similis* lipid content but also got informative individual variability.

Overall values of lipid content are meaningful when looking from a food supply point of view for upper trophic levels, as it represents the average energetic value of the ingested food, although the heterogeneity of the preys is surely significant. But investigating the lipid reserves of a population at the individual level brings new insights on the life strategy of the species and allows a better understanding of the pelagic ecosystem (Båmstedt 1988).

Conclusion

In this study, we underlined new features on *Oithona similis*'s ecology, opening questions on its management of lipid reserves, which seemed to differ from the traditional seasonal pattern described in *Calanus spp.* The image analysis method used here appeared to be a very good tool especially for the small sized copepods, because questions at this level of variability cannot be answered using the traditional biochemical analysis.

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