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Lipid composition of the Antarctic fish *Pleuragramma antarcticum*. Influence of age class

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Abstract

Larvae and juvenile stages of *Pleuragramma antarcticum* have been collected in the Dumont D'Urville Sea (East Antarctica) during summer 2008 on board the TRV *Umitaka Maru* during the CEAMARC survey. Detailed analyses of their lipid class and fatty acid compositions were carried out. *P. antarcticum* showed a pronounced ontogenic lipid accumulation with increasing size. Larvae displayed a dominance of polar lipids (83% of total lipids) and low percentage of triglycerides (7%). Conversely juveniles showed an increasing accumulation of triglycerides (up to 72.4%). The fatty acid composition of polar lipids remained rather stable between stages with 22:6n−3 and 20:5n−3 as dominant contributors. The relatively minor ontogenic changes, e.g. increase of mono-unsaturated and decrease of C18 polyunsaturated fatty acids, may reflect the influence of differences in diet. Triglycerides showed that all three age classes are well segregated in term of fatty acid composition. Larvae triglycerides are characterized by significant percentages of 16:0, 20:5n−3, 20:6n−3 and to a minor extent 18:4n−3, which suggest a prymnesiophyte based diet. Juveniles are characterized by larger percentages of C20:1 and C22:1 acids, considered as markers of *Calanus* type copepods. The increasing contribution of 18:1n−9 in the triglycerides of the older juveniles suggests a gradual and increasing shift from a copepod dominant diet to an euphausiid dominant diet. Fatty acid trophic markers pattern suggests a shift from a phytophagous and omnivorous diet for larvae to a carnivorous diet for juveniles.

Keywords: *Pleuragramma antarcticum*; Age class; Lipid composition; Fatty acid trophic markers

1. Introduction

Like all high latitude ecosystems, Antarctic shelf waters are characterized by extremely low temperature, strong seasonality in light regime yielding strongly pulsed primary productivity. As a result, most planktonic animals have developed adaptive strategies with massive lipid accumulation to cope with food scarcity (Lee et al., 2006). In the case of the silverfish *Pleuragramma antarcticum*, this strategy is coupled buoyancy adaptation based on triglycerides accumulation in intermuscular lipid sacs (Devries and Eastman, 1978). The significance of the lipid content at early stages of

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P. antarcticum remains unclear. Lipid content varies with life stages from 12% to 48% of dry weight in larvae and adult respectively (Reinhardt and Van Vleet, 1986), and ranged between 20 and 47% in juvenile and adult (Friedrich and Hagen, 1994). Changes of lipid content with age class has been considered by Hubold and Hagen (1997) who showed that total lipid were relatively stable in year 1 size group and increased in juvenile stages between 50 and 70 mm.

Compositional data of lipid classes is limited. The study of Reinhardt and Van Vleet (1986) indicated a dominance of polar lipids in larvae associated with triglycerides as the main neutral lipid. Wöhrmann et al. (1997) reported on the lipid composition of juvenile stages while Hagen et al. (2000) reported on that of adult stages. High percentages of triglycerides associated with significant amount of wax esters characterized adult stages.

The present study has a dual purpose: 1) clarify the relationships between lipid and early stages of the life cycle of *P. antarcticum* and 2) using the detailed fatty acid composition of each lipid class attempt to evaluate the main role of the different categories.

2. Material and methods

The specimens have been collected on the shelf of the Dumont d'Urville Sea (East Antarctica) at station 10 (66°S, 143°E) between 28 January and 12 February 2008 on board the TRV *Umitaka Maru* during the CEAMARC survey. Larval and juvenile stages of *P. antarcticum* have been caught using a Rectangular Midwater Trawl (RMT 1 + 8) and an International Young Gadoid Pelagic Trawl net (IYGPT), respectively. All stages were divided according to size and frozen in liquid nitrogen before shipment back to France, where samples were kept at −80 °C. Individuals for size distribution were fixed in 5% formaldehyde. Shrinkage due to chemical preservation of samples was considered negligible. Population structure was based on measurements of 253 individuals to the nearest mm.

2.1. Lipid extraction and lipid class separation

Entire specimens were placed frozen on crushed ice and brought to 0 °C. Size (total length = TL) and fresh weight (WW) were measured prior to lipid extraction according to the method of Bligh and Dyer (1959). In the present study, the lipid extraction was achieved individually. Samples were homogenized mechanically and extracted twice with a one-phase solvent mixture of methanol–chloroform–water (2:1:0.8. v/v/v) and the phases were separated overnight by addition of chloroform and NaCl 0.7% (w/v) with a final solvent ratio: methanol–chloroform–water (2:2:1.8, v/v/v). The total extract was concentrated under vacuum using a rotary evaporator. Extracts were stored under nitrogen at −80 °C. Total lipid (TL) content was determined gravimetrically.

Lipid classes were quantified after chromatographic separation coupled with FID detection on an Iatroscan MK V TH 10. Total lipid extracts were applied to SIII chromarods using a SAS A4100 autospotter set up to deliver 1 μl of chloroform extract on each rod. Analyses were done in triplicate. Lipids classes (polar and neutral lipids) were separated using a double development procedure with the following solvent systems: n-hexane: benzene: formic acid 80:20:1 (v/v/v) followed by n-hexane:diethyl ether:formic acid 97:3:1.5 (v/v/v). The FID was calibrated for each compound class using commercial standards.

2.2. Fatty acid analyses

For fatty acids (FA) analysis, lipid classes were further isolated by preparative TLC with hexane:diethylether:acetic acid 170:30:2.5 (v/v), and the bands of polar lipids (PL) and triacylglycerols (TAG) were then scraped off and eluted. Lipid classes were visualized using dichlorofluorescein and identification was achieved by comparison with standard mixtures. Fatty acids from TL, TAG and PL were subsequently converted into methyl esters with 7% boron trifluoride in methanol (Morrison and Smith, 1964).

Gas chromatography (GC) of all fatty acids methyl esters (FAME) was carried out on a 30 m length × 0.32 mm internal diameter quartz capillary column coated with Famewax (Restek) in a Perkin–Elmer XL Autolab GC equipped with a flame ionization detector (FID). The column was operated isothermally at 185 °C for FAME. Helium was used as carrier gas at 7 psig. Injector and detector were maintained at 250 °C. Individual components were identified by comparing retention time data with those obtained from laboratory standards (capelin: menhaden oils 50:50). The level of accuracy is ±3% for major components, 1–9% for intermediate components and up to ±25% for minor components (<0.5% of total fatty acids).

2.3. Statistical analyses

Size weight and lipid weight relationships were computed on log transformed data and type 2 regressions (Sokal and Rohlf, 1981). Significance of means

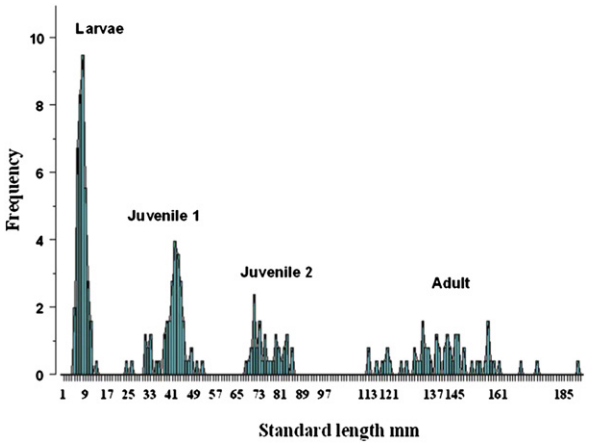

Fig. 1. Size frequency distribution of the *Pleuragramma antarcticum* collected at station 10.

was tested using non parametric test of Kruskall–Wallis. Computation was made using Stagraphics XVI software.

3. Results and discussion

3.1. Population structure

The size distribution of the population sampled showed four size classes (Fig. 1). Size intervals were 1–3 cm, 3–7 cm, 7–11 cm and 11–19 cm for class 1, 2, 3 and 4 respectively. From a biological point of view, individuals between 1 and 3 cm are larval stages, individuals between 3 and 7 cm and 7–11 mm are juvenile, and individuals of 11–19 cm are adults. This is consistent with the data reported earlier by Hubold (1984), Kellermann (1986), Hubold and Tomo (1989), Guglielmo et al. (1998), Hoddell et al. (2000) and Vacchi et al. (2004) for various sector of the Antarctic shelf.

3.2. Lipid content and lipid class composition

Lipid content was estimated on a smaller sample (15 individuals) of larvae and juveniles. Results from Table 1 indicated that size of larvae ranged 1.8–2.5 cm, corresponding to a fresh weight of 12–28 mg and lipid contents of 0.3–0.7 mg ind^{-1} or 1.7–3.3% wet weight (WW). Juveniles of size 1 (Table 1) displayed size values between 5.2 and 5.6 cm, corresponding to a fresh weight of 69–881 mg and lipid contents ranging between 28.3 and 40.5 mg ind^{-1} or 4.2–4.6% WW. Juvenile of size 2 lengths showed sizes between 7.2 and 9.4 cm, wet weights between 2.04 and 6.11 g ind^{-1} and lipid contents between 86.2 and 446.8 mg ind^{-1} or 3.9–7.3% WW. The values recorded are in general agreement with the range of data recorded by Hubold and Hagen (1997), Hagen (1988) and Friedrich and Hagen (1994) who also observed an increase in lipid (29–41% dry weight) with fish size between 5.5 and 7.2 cm (Hubold and Hagen, 1997).

The log-linear relationship between wet weight and length and lipid and weight were established using pooled data of all larvae and juvenile. The regressions were highly significant ($R^2 > 0.99$) corresponding to the general equations:

$$\mathrm{WW} = 3.82 \log \mathrm{Size} - 3.75 \left(R^2 = 0.997; F_{1,19} = 8394;\ p = 0.00001\right)$$

$$Lip_{tot} = 1.16 \log WW - 1.83 \left(R^2 = 0.997; F_{1,19} = 3545;\ p = 0.00001\right)$$

Changes in lipid classes are presented in Table 2. Polar lipids and triglycerides are the two major constituents which showed a reverse pattern Supprimer of with age. Larvae displayed a dominance of polar lipids and low percentage of triglycerides. Conversely juveniles showed an increasing accumulation of triglycerides and intermediate levels of polar lipids. Sterols showed a similar percentage for all group of age at 4% of the total lipids. Wax esters, diglycerides and hydrocarbons were always minor and several times below detection level. Free fatty acids were low to undetectable, confirming the good condition of the samples analyzed.

Hagen (1988) reported high phospholipids and minor triglycerides fractions (values not given) for

Table 1
Median and range of length, wet weight and lipid content of *Pleuragramma antarcticum* larvae and juveniles.

	Standard Length (cm)	Wet Weight (mg/ind)	Lipids (mg/ind)	Lipids (%WW)	Lipids (%DW)[a]
Larvae					
Group 1 ($n = 6$)	1.9 (1.8–2.5)	19.2 (12.0–28.0)	0.4 (0.3–0.7)	2.3 (1.7–3.3)	23.1 (17.1–33.3)
Juveniles					
Juveniles 1 ($n = 3$)	5.4 (5.2–5.6)	793 (669–881)	35.4 (28.3–40.5)	4.5 (4.2–4.6)	37.2 (35.3–38.3)
Juveniles 2 ($n = 6$)	8.2 (7.2–9.4)	3420 (2040–6108)	170.2 (86.2–446.8)	5.3 (3.9–7.3)	44.1 (35.2–60.9)

[a] dry weight computed assuming 90% water content for larvae and 88% for juveniles (Friedrich and Hagen, 1994).

Table 2
Composition in lipid classes (% total lipid) of *Pleuragramma antarctica* in relation to developmental stage.

	HD	WE	FFA	TAG	ST	DG	PL
Larvae							
$n = 6$	1.05 ± 0.73	0.41 ± 0.51	1.43 ± 0.54	7.2 ± 3.0	4.1 ± 0.8	2.8 ± 3.5	83.0 ± 6.1
Juveniles							
Group 1^+ $n = 3$	0.04 ± 0.08	0.41 ± 0.24	nd	60.8 ± 1.6	4.5 ± 0.3	nd	34.3 ± 1.6
Group 2^+ $n = 6$	0.01 ± 0.03	0.60 ± 0.27	nd	72.4 ± 4.0	4.1 ± 0.9	1.2 ± 2.1	21.6 ± 3.7

Symbols: HD = hydrocarbons; WE = wax esters; FFA = free fatty acids; TAG = triglycerides; St = sterols; DG = diacylglycerols; PL = polar lipids; nd = not detected; ± SD = standard deviation; n = number of replicates.

young post larvae. Reinhardt and Van Vleet (1986) analyzed *P. antarcticum* larvae (length not indicated) but recorded 41% hydrocarbon, an unusually high percentage not supported by Hagen (1988) or the present study, which suggests contamination of the sample. The changes in lipid pattern with age, leading to increasing content of reserve lipids at 2^+ juveniles stage was reported by Hagen (1988) and for adults by Reinhardt and Van Vleet (1986) and Hagen et al. (2000), but while Reinhardt and van Vleet reported equal levels of wax esters and triglycerides in the flesh (dominant tissue), both studies by Hagen and collaborators indicated high levels of accumulation of triglycerides for entire individuals above 4–5 cm in length in accordance with the present data. Maximum triglycerides were recorded for adult of 12 cm in length (Hagen et al., 2000).

3.3. Fatty acids constitutive of the different lipid classes

3.3.1. Polar lipids

The fatty acid composition of the phospholipid fraction of the different growth stages of *P. antarcticum* is presented in Table 3. Polyunsaturated acids dominated in all stages with 22:6n−3 and 20:5n−3 as the main PUFAs. No significant changes between stages were observed for the total PUFAs or the sum of n−3 or n−6 acids. Saturated acid was dominated by palmitic acid (16:0) and to a minor extent stearic acid (18:0). Myristic acid as well as branched acids displayed low percentages in all stages. Monoenes were dominated by oleic acid (18:1n−9), as well as vaccenic acid (18:1n−7) followed by palmitoleic acid (16:1n−7). C20:1 isomers were also present but in minor proportions. Interestingly, the percentage of oleic acid, 20:1n−9 and 20:1n−7 increase significantly with growth stage. Unsaturated acids with 2–4 double bonds were always intermediate or minor components. The main fatty acids for these three groups were 18:2n−6, 16:3n−3, 18:3n−3, 18:4n−3 and 20:4n−6. In terms of changes with growth stages a significant decrease in 18:2n−6, 18:3n−3 and 18:4n−3 occurred from larvae to juveniles while 20:4n−6 showed the opposite trend. A cluster analysis based on Bray and Curtis similarity (Fig. 2) indicates homogeneity of structure for both juvenile group of age and different structure for the larval stages.

The high concentration of DHA and a relatively low EPA to DHA ratio displaying values <1 indicated both that DHA was preferentially incorporated into membrane lipids instead of being used for energy (Sargent et al., 2002). The enrichment of long chain n−3 polyunsaturated fatty acids and notably of DHA is involved in maintenance of the structural and functional integrity of biological membranes and development of neural and visual functions. Polar lipids are known to be strongly regulated and seem directly related with membrane requirements and specific pathways (reviewed by Sargent et al., 2002 and Dalsgaard et al., 2003). The relatively minor changes in fatty acid composition between larvae and juvenile may reflect the influence of differences in food source as fish have limited ability to synthesize phospholipids *de novo* and assimilate ingested phospholipids (Sargent et al., 2002; Tocher et al., 2008).

Comparison with earlier data is possible with the work of Reinhardt and Van Vleet (1986) on *P. antarcticum* larvae and Hagen et al. (2000) on adults. In larval stages Reinhardt and Van Vleet (1986) reported low percentages of DHA (12%) and almost traces of EPA (0.3%), which contrasts with the present data. The high value of 20:4 they reported suggests either an incomplete conversion toward higher n−3 or a typographic error with EPA at 10.3% and 20:4 at 0.3% and not the reverse. The dominant percentage of oleic acid (29.9%) and, to a minor extent 20:1 and, the relatively low value of palmitic acid (10.3%) are also at odds with our results. To what extent these differences could be associated with the contamination of their sample by hydrocarbons is difficult to assess. On the contrary, the pattern reported by Hagen et al. (2000) agrees quite well with the present data on juveniles, keeping in mind the older stages used in their study.

Table 3
Fatty acid composition (% total fatty acids) of the phospholipid fraction of the different growth stages of *Pleuragramma antarcticum*.

	Larvae $n = 2$	Juvenile 1^+ $n = 3$	Juvenile 2^+ $n = 6$
Size cm	2.10 ± 0.14	5.40 ± 0.20	8.18 ± 0.84
14:0	0.85 ± 0.15	1.31 ± 0.34	0.86 ± 0.24
ISO15:0	0.14 ± 0.03	0.07 ± 0.01	0.05 ± 0.01
15:0	0.30 ± 0.05	0.29 ± 0.03	0.16 ± 0.01
ISO16:0	0.31 ± 0.07	0.08 ± 0.01	0.06 ± 0.01
ISO17:0	0.74 ± 0.13	0.69 ± 020	0.55 ± 0.03
ANT17:0	0.34 ± 0.01	0.24 ± 010	0.18 ± 0.05
16:0	20.50 ± 1.67	21.94 ± 2.26	19.66 ± 0.44
17:0	0.58 ± 0.07	0.54 ± 0.15	0.52 ± 0.09
Phytanate	0.36 ± 0.00	0.33 ± 0.10	0.30 ± 0.04
ISO18:0	0.26 ± 0.03	0.32 ± 0.11	0.19 ± 0.02
18:0	3.85 ± 0.40	2.31 ± 0.10	2.79 ± 0.23
Σ *saturates*	28.63 ± 2.67	28.27 ± 3.08	25.48 ± 0.73
16:1n−7	2.86 ± 0.32	2.15 ± 0.27	2.02 ± 0.16
18:1n−9[a]	6.16 ± 0.04	6.67 ± 0.42	8.50 ± 0.53
18:1n−7	7.20 ± 0.74	5.25 ± 1.18	6.39 ± 0.52
18:1n−5	0.86 ± 0.11	1.23 ± 0.06	1.12 ± 0.11
19:1	0.29 ± 0.04	0.27 ± 0.05	0.26 ± 0.11
20:1n−9[a]	0.69 ± 0.06	2.87 ± 0.49	3.28 ± 0.72
20:1n−7[a]	0.12 ± 0.03	0.32 ± 0.06	0.35 ± 0.10
22:1n−9	0.21 ± 0.11	0.18 ± 0.09	0.41 ± 0.11
24:1	0.40 ± 0.27	0.55 ± 0.23	1.11 ± 0.18
Σ *monoenes*	18.98 ± 0.70	19.81 ± 0.52	24.00 ± 1.51
16:2n−6	0.18 ± 0.02	0.15 ± 0.15	0.14 ± 0.05
18:2n−9	0.42 ± 0.03	0.45 ± 0.11	0.39 ± 0.03
18:2n−7	0.15 ± 0.03	0.19 ± 0.05	0.18 ± 0.05
18:2n−6[a]	2.01 ± 0.15	1.07 ± 0.09	1.06 ± 0.11
18:2n−4	0.11 ± 0.04	0.15 ± 0.02	0.15 ± 0.05
20:2n−6	0.12 ± 0.02	0.10 ± 0.00	0.08 ± 0.02
Σ *dienes*	3.09 ± 0.25	2.18 ± 0.24	2.08 ± 0.22
16:3n−6	0.38 ± 0.05	0.51 ± 0.05	0.56 ± 0.08
16:3n−4	0.37 ± 0.03	0.27 ± 0.03	0.27 ± 0.03
16:3n−3	0.73 ± 0.01	0.61 ± 0.16	0.35 ± 0.11
18:3n−6	0.20 ± 0.01	0.18 ± 0.04	0.16 ± 0.02
18:3n−3[a]	0.80 ± 0.17	0.20 ± 0.00	0.19 ± 0.04
Sum trienes	2.60 ± 0.25	1.91 ± 0.20	1.64 ± 0.11
16:4n−3	0.37 ± 0.19	0.24 ± 0.05	0.18 ± 0.02
18:4n−3[a]	1.77 ± 0.09	0.62 ± 0.07	0.51 ± 0.12
18:4n−1	0.16 ± 0.01	0.13 ± 0.01	0.13 ± 0.02
20:4n−6[a]	0.59 ± 0.04	0.85 ± 0.05	1.17 ± 0.15
20:4n−3	0.26 ± 0.02	0.37 ± 0.05	0.27 ± 0.05
Σ *tetraenes*	3.71 ± 0.65	2.29 ± 0.16	2.36 ± 0.20
20:5n−3	16.89 ± 1.90	20.90 ± 0.54	17.87 ± 0.37
21:5n−3	0.22 ± 0.02	0.22 ± 0.02	0.21 ± 0.04
22:5n−6	0.10 ± 0.04	0.09 ± 0.03	0.11 ± 0.01
22:5n−3	0.57 ± 0.07	0.56 ± 0.10	0.67 ± 0.05
Σ *pentaenes*	17.78 ± 2.03	21.76 ± 0.68	18.86 ± 0.41
22:6n−3	25.19 ± 2.49	23.78 ± 2.88	25.58 ± 1.81
Σ PUFA	52.38 ± 3.37	51.92 ± 3.60	50.51 ± 1.80
Σ n−3	46.81 ± 4.22	47.50 ± 3.61	45.82 ± 1.87
Σ n−6	3.58 ± 0.12	2.94 ± 0.19	3.28 ± 0.34

[a] Larvae and pooled juveniles significantly different at $p < 0.05$.

3.3.2. Triglycerides

The fatty acid composition of triglycerides is presented in Table 4. Monoenoic and saturated acids dominated with palmitoleic (16:1n−7), oleic (18:1n−9), vaccenic (18:1n−7), palmitic (16:0) and stearic (18:0) acids as main constituents. Myristic acid (14:0) as well as erucic acid (20:1n−9) becomes important components for both juvenile stages. Branch fatty acids (Iso and anteiso) are more or less constant in all age groups. Composition in PUFA is dominated by both EPA (20:5n−3) and DHA (22:6n−3) with a ratio EPA/DHA >1. Other PUFAs showed large variations with the group considered with 18:2n−6, 18:3n−6, 18:3n−3, 18:4n−3 showing percentages larger than 1% in larval stages, while they are small contributors in both juvenile age groups. A cluster analysis based on Bray and Curtis similarity index showed (Fig. 3) that all three groups are well segregated in terms of fatty acid composition.

Changes in triglyceride fatty acid composition are usually related to the type of trophic interactions developed by the organisms (Dalsgaard et al., 2003). Larvae triglycerides showed significant percentages of pamitoleic acid, EPA, DHA, 18:4n−3 and to a minor extent 18:3n−3, which suggest a phytoplankton based diet (Dalsgaard et al., 2003). These results confirmed the high degree of omnivory observed with gut content studies for *P. antarcticum* larvae during summer in the coastal waters of Terre Adélie in 2004 (Koubbi et al., 2007).

The absence of C16 PUFA markers and the relative importance of C18 PUFA are indicative of a prymnesiophyte based diet rather than a diatom base one (Graeve et al., 1994). Juveniles are characterized by larger percentages of C20:1 (n−9, n−7) and C22:1 acids, considered as markers of *Calanus* type copepods. These acids are present in the wax esters of *Calanoides acutus* as well as in the triglycerides of

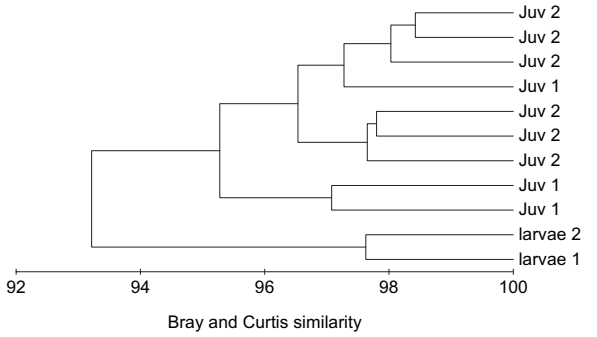

Fig. 2. Cluster analysis of the polar lipid fatty acid composition of the different larval and juvenile stages using the Bray and Curtis similarity index.

Table 4
Fatty acid composition (% total fatty acids) of the triglyceride fraction of the different growth stages of *Pleuragramma antarcticum*.

	Larvae $n = 3$	Juvenile 1 $n = 3$	Juvenile 2 $n = 6$
14:0[a]	1.16 ± 0.72	8.31 ± 1.60	9.63 ± 1.44
ISO15:0	0.30 ± 0.16	0.32 ± 0.06	0.46 ± 0.09
ANT15:0	0.17 ± 0.10	0.09 ± 0.01	0.12 ± 0.02
15:0	0.40 ± 0.22	0.36 ± 0.06	0.37 ± 0.06
ISO16:0	0.31 ± 0.06	0.09 ± 0.02	0.12 ± 0.02
ISO17:0	0.76 ± 0.09	0.44 ± 0.06	0.46 ± 0.09
ANT17:0	0.62 ± 0.10	0.10 ± 0.01	0.12 ± 0.02
16:0	10.95 ± 2.29	10.23 ± 1.12	11.77 ± 2.15
17:0[a]	1.84 ± 0.64	0.29 ± 0.09	0.55 ± 0.28
Phytanate	0.70 ± 0.98	0.25 ± 0.23	0.17 ± 0.13
ISO18:0	0.30 ± 0.06	0.15 ± 0.01	0.11 ± 0.02
18:0[a]	5.20 ± 2.43	0.70 ± 0.07	0.66 ± 0.12
Σ *saturates*	23.51 ± 6.35	21.53 ± 3.28	24.75 ± 3.91
14:1n−5[a]	0.03 ± 0.04	0.31 ± 0.08	0.33 ± 0.07
16:1n−7	8.05 ± 0.27	8.58 ± 0.57	10.53 ± 1.06
16:1n−5	0.87 ± 0.28	1.19 ± 0.13	1.19 ± 0.09
18:1n−9	8.82 ± 0.63	9.32 ± 2.94	12.54 ± 2.53
18:1n−7	6.58 ± 0.27	3.96 ± 1.26	5.22 ± 1.11
18:1n−5[a]	0.92 ± 0.13	1.09 ± 0.10	1.24 ± 0.12
20:1n−11[a]	0.02 ± 0.03	0.17 ± 0.07	0.11 ± 0.02
20:1n−9[a]	1.83 ± 0.72	18.35 ± 3.19	14.39 ± 4.57
20:1n−7[a]	0.31 ± 0.27	2.77 ± 0.34	2.25 ± 0.33
22:1n−13 + 11[a]	0.93 ± 0.27	7.68 ± 1.85	7.94 ± 2.75
22:1n−9[a]	0.97 ± 0.26	4.08 ± 0.66	5.71 ± 1.27
22:1n−7[a]	0.10 ± 0.01	0.38 ± 0.14	0.70 ± 0.18
Σ *monoenes*	31.36 ± 1.57	58.87 ± 2.49	63.71 ± 5.42
16:2n−6[a]	0.38 ± 0.05	0.24 ± 0.01	0.25 ± 0.01
16:2n−4[a]	0.21 ± 0.05	0.47 ± 0.06	0.49 ± 0.03
18:2n−9	0.50 ± 0.15	0.36 ± 0.08	0.40 ± 0.06
18:2n−7	0.14 ± 0.02	0.15 ± 0.04	0.16 ± 0.04
18:2n−6[a]	2.70 ± 0.82	1.45 ± 0.09	1.39 ± 0.05
18:2n−4	0.10 ± 0.13	0.18 ± 0.03	0.19 ± 0.03
Σ *dienes*	4.26 ± 1.07	3.00 ± 0.14	2.99 ± 0.17
16:3n−6[a]	0.89 ± 0.58	0.34 ± 0.04	0.26 ± 0.05
16:3n−4	–	0.10 ± 0.01	0.12 ± 0.03
16:3n−3[a]	0.53 ± 0.06	0.15 ± 0.02	0.20 ± 0.03
18:3n−6[a]	1.32 ± 0.87	0.35 ± 0.06	0.20 ± 0.02
18:3n−3[a]	1.35 ± 0.20	0.33 ± 0.02	0.34 ± 0.04
Σ *trienes*	4.68 ± 1.93	1.46 ± 0.13	1.21 ± 0.09
16:4n−3[a]	0.32 ± 0.03	0.10 ± 0.01	0.09 ± 0.01
16:4n−1	0.24 ± 0.01	0.44 ± 0.10	0.53 ± 0.22
18:4n−3[a]	7.49 ± 1.14	1.61 ± 0.21	1.55 ± 0.42
18:4n−1	0.57 ± 0.27	0.26 ± 0.02	0.36 ± 0.08
20:4n−6[a]	0.23 ± 0.01	0.15 ± 0.03	0.12 ± 0.02
20:4n−3	0.20 ± 0.05	0.41 ± 0.09	0.16 ± 0.08
Σ *tetraenes*	9.04 ± 1.41	3.02 ± 0.39	2.82 ± 0.62
20:5n−3[a]	14.67 ± 3.40	7.25 ± 0.99	3.36 ± 1.31
21:5n−3[a]	0.50 ± 0.10	0.22 ± 0.03	0.08 ± 0.04
22:5n−3	0.42 ± 0.17	0.37 ± 0.08	0.07 ± 0.04
Σ *pentaenes*	15.59 ± 3.67	7.86 ± 1.09	3.52 ± 1.39
22:6n−3[a]	11.64 ± 3.57	4.29 ± 0.77	1.02 ± 0.67

[a] Larvae and pooled juveniles significantly different at $p < 0.05$.

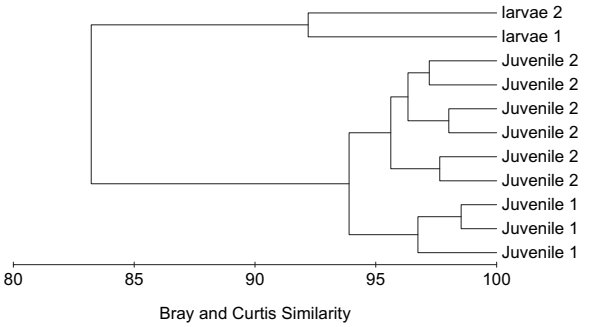

Fig. 3. Cluster analysis of the triglycerides fatty acid composition of the different larval and juvenile stages using the Bray and Curtis similarity index.

Calanus propinquus, species abundant on the Adelie shelf (Hecq and Volkaert, 2007), which suggest a dominant copepod based regime, in agreement with the conclusion of Olaso et al. (2004) on *P. antarcticum* feeding pattern. Comparison with the earlier report by Hagen et al. (2000) illustrates the shift in trophic interaction with age. Indeed, the fatty acid profile of adult stage showed major differences with the present data with larger proportion of myristic, palmitic and oleic acid and smaller percentages of C20 and C22 monoenes. They assign the increase level of oleic acid to the incorporation of 18:1n−9 from wax esters of *Euphausia crystallorophias* as acid (Kattner and Hagen, 1998) and *Thysanoessa macrura* as alcohol (Hagen and Kattner, 1998; Mayzaud et al., 2003). The increasing contribution of 18:1n−9 in the triglycerides of the Juvenile 2^+ individuals strongly suggest a gradual and increasing shift from a copepod dominant diet to a euphausiid dominant diet for older stages.

P. antarcticum is one of the few notothenioid which are adapted to the pelagic life mode. Since it lacks a swim bladder, it has developed an alternative to achieve neutral buoyancy through the combination of skeletal demineralization and lipid deposition in large subcutaneous and intermuscular lipid sacs (Eastman, 1993). Triglycerides have been shown to constitute the accumulation (Devries and Eastman, 1978), and it is still unclear whether or not stored lipids are metabolically active and supply energy to maintain body functions. Intracellular accumulation of triglycerides in fat cells (Eastman and Devries, 1982) would be the active form. From theoretical and model considerations, Maes et al. (2006) concluded that lipids are metabolically inactive uncoupling fat reserves from metabolism. Hence, lipid deposition from diet source should be the main process involved resulting in high degree of preservation of the structure of prey

triglycerides. The shift in fatty acid composition observed during ontogeny and between juvenile and adult (Hagen et al., 2000) have two explanations at least: 1) lipid in sacs are not totally uncoupled from metabolism and a certain degree of turn over allows changing signature or 2) the relationship between lipid sacs appearance, intracellular adipocytes and the degree of skeletal ossification (Devries and Eastman, 1978) yields differential turn over in relation with the dominance of active adipocytes, with small specimens with no subcutaneous and intermuscular sacs showing high turn over rate, medium sizes with moderate number of sacs showing intermediate turn over rates and adults with extensive development of lipid sacs and little turn over.

4. Conclusion

The present results on the composition and role of lipids in *P. antarcticum* larvae and juvenile fills an important gap in our knowledge of the biology of this central species in the Antarctic coastal ecosystem. Growth shift in the fatty acid of structural polar lipid was observed, suggesting different requirements for larval and juvenile stages. The definition of the trophic interactions confirmed that younger stages do feed in part on phytoplankton during the intensive bloom period, but carnivory on small copepod prey items remains the main source of energy. This conclusion is reinforced by the likelihood that regulation of density by triglyceride filled lipid sacs is minimal in larvae and young juveniles. However, our knowledge of the lipid metabolism of this species remains very limited despite its importance in the functioning of the Antarctic shelf food web. Further work is needed on the role of lipid during ontogeny and reproduction to clarify the relative importance of reserve and structural lipids in egg production, egg viability (fecundity), as well as early larval development (recruitment).

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