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ORIGINAL PAPER

Lipid components as a measure of nutritional condition in fish larvae (*Pleuragramma antarcticum*) in East Antarctica

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Abstract *Pleuragramma antarcticum* is a key component of the neritic assemblages in the Antarctic coastal waters. Larvae of this species were sampled from 2008 to 2011 in the Dumont d'Urville Sea (East Antarctica). The lipid class composition [triacylglycerols (TAG), cholesterol (Chol) and polar lipids (PL)] of larvae was measured to assess the larval condition. The total amount of lipids was linearly related to the quantity of structural polar lipids, suggesting that growth is favored over lipid storage. The TAG:Chol ratio showed interannual variability in the condition of fish larvae, probably related to prey availability. Nevertheless, the essential fatty acids composition of polar lipids illustrates that larvae with low levels of TAG:Chol could be either growing or under starvation. Only the combination of a low TAG:Chol ratio and low polar lipids content, which can also be mobilized during starvation periods, allowed identification of larvae in poor condition. This lipid condition index should be of great assistance to evaluate the probability of survival of *P. antarcticum* larvae in long-term monitoring. It has widespread applicability and should also be useful in the diagnosis of nutritional condition in other species.

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Introduction

In Antarctic, the quantification of the stock–recruitment relationships remains a difficult problem in fish ecology. One of the reasons is that early life stages, as a critical part of the life history of fishes, are still underexplored (Cushing and Horwood 1994). Predictions of recruitment success using fish larvae abundance alone assume that all individuals have the same probability of surviving. However, while it is well recognized that the outcome of the larval stage clearly impacts recruitment (Doherty and Williams 1988; Hjort 1914), the hypothesis relating recruitment directly to larval abundance alone is an oversimplification. To understand recruitment variability, it is necessary to study the factors that determine survival during the early life stages.

The two major sources of larval mortality are starvation and predation (Leggett and Deblois 1994). The hypotheses that implicate starvation as a principal cause of mortality are Hjort's "critical period" hypothesis (Hjort 1914), which explicitly links larval survival and subsequent recruitment to food abundance during the transition of larvae from endogenous to exogenous feeding, and Cushing's "match/mismatch" hypothesis in which food limitation during the entire larval period is a major regulator of larval survival and recruitment (Cushing 1972). In addition, food-mediated changes in larval growth, condition and performance are likely to alter the frequency and intensity of predation on fish larvae (Leggett and Deblois 1994).

The probability of the survival of individuals can be estimated directly from various measurements of larval condition (Ferron and Leggett 1994). At the whole organism level, morphometric indices focus on detecting changes in external body shape which relate to condition (Shelbourne 1957). At the tissue and cellular level, changes in condition are detected through histological analysis reflecting the fact that the tissue of starved fish larvae differs from that of well-fed larvae (Umeda and Ochiai 1975; Ehrlich et al. 1976). Finally, biochemical condition can be assessed by quantifying chemical constituents used as the energy substrates (lipids) or by measuring physiological rate indicators (proteins, nucleic acids, digestive enzymes) that are known to vary in relation to the nutritional status of the animal.

The lipid condition index is based on the principal that larval development in many marine organisms is largely dependent upon energy reserves, which correspond in most cases to triacylglycerols (TAG). When the derived energy from exogenous feeding exceeds the immediate metabolic demands of the larvae, then the excess of energy can be stored as TAG (Fraser 1989). In contrast, when this energy is insufficient to maintain the basal metabolism of the larvae, endogenous TAG is preferentially catabolized (Ehrlich 1974). The concentration of TAG typically declines during starvation, as known in anchovy (*Engraulis mordax*) (Hakanson 1989a), herring (*Clupea harengus*) (Tocher et al. 1985) and the Atlantic cod (*Gadus morhua*) (Fraser et al. 1988). Such observations suggest that lipid components could be an indicator of nutritional condition. One of the advantages of the TAG index is that it adjusts quickly to changes in food availability or quality if compared to morphometric condition indices.

Because the TAG content is dependent on larval size or body mass, the TAG content must be expressed relative to body size, standardizing by body weight. Alternatively, TAG could be expressed relative to structural lipids, which are also correlated with larval size. The main structural lipids used to standardize TAG are cholesterol (Chol) and polar lipids (PL), which are both important membrane components. Most studies to date have used the triacylglycerols:cholesterol ratio (TAG:Chol) because cholesterol is correlated with larval size and is not catabolized during starvation and thus is independent of nutritional condition (Fraser et al. 1988; Fraser 1989). Compared to Chol, there is a greater variability in PL content as they can eventually be catabolized under starvation (Hakanson 1989a) and could represent an important energy source in eggs and some larval fish (Evans et al. 1998; Copeman et al. 2008; Laurel et al. 2010). The TAG:Chol ratio reflects the ability of larvae to withstand variation in food availability in that larvae with high TAG:Chol ratios are interpreted to be in better condition and have a greater potential to survive subsequent starvation events than larvae with low TAG:Chol ratios.

Pleuragramma antarcticum is the dominant pelagic fish species in the high-Antarctic shelf waters (Hubold 1984) where it can represent up to 90 % of the fish biomass. This species is the only notothenioid in which all developmental stages are pelagic. Eggs are associated with sea ice (Vacchi et al. 2004), while larvae are omnivorous and forage on phyto- and mesozooplankton (Giraldo et al. 2011; Tavernier el al. 2011). Juveniles appear to be carnivorous, foraging mainly on copepods when they are young and switching to euphausiids later in their development to the adult stage (Mayzaud et al. 2011). Juveniles and adults of the Antarctic silverfish occupy an intermediate trophic level and probably exert a top–down control on mesozooplankton and a bottom–up control on top predators. Together with ice-krill, this species can be considered as an indicator of the quality of the pelagic neritic environment.

In this study, the TAG:Chol condition index of Antarctic silverfish (*P. antarcticum*) larvae was complemented by the study of the fatty acid composition of PL. Our hypothesis was that "poor condition" might be reflected at the membrane level by different ratios of essential fatty acids. As for all vertebrates, in fish, essential fatty acids are required to normal growth and development: docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), and are also linked to survival: arachidonic acid (AA, 20:4n-6) (Sargent et al. 1993). Finally, whether larval condition varied interannually or spatially was analyzed.

Materials and methods

Sampling

Samples were collected in the Dumont d'Urville Sea (East Antarctica) during the CEAMARC surveys (Collaborative East Antarctic Marine Census) of the Census of Antarctic Marine Life (Hosie et al. 2011) and the French IPEV-ICO^{2}TA program (Integrated Coastal Ocean Observations in Terre Adélie) (Koubbi et al. 2011). Fish larvae were collected during the austral summer 2008 from the TRV "*Umitaka Maru*" using pelagic trawls (International Young Gadoid Pelagic Trawl, IYGPT, and rectangular midwater trawl, RMT 8 + 1) (Moteki et al. 2011) and from the RV "l'Astrolabe" during summer in 2009–2011 using a double frame bongo net (500 µm) (Table 1) (Koubbi et al. 2011).

Samples were collected at 29 stations along different transects from the Mertz Glacier Tongue (MGT) to the Adelie Bank and from the coast to the continental shelf (Fig. 1). Larvae were sorted and identified on board and measured to the nearest 0.1 mm with digital calipers (standard length, SL). *P. antarcticum* up to 30 mm SL were

Table 1 Sampling years, number of stations and fish larvae used for this study

Years	Month	Number of stations	Fish larvae (*n*)
2008	February	7	89
2009	January	8	96
2010	January	9	104
2011	January	5	20

considered to be larvae (Hubold 1984; Kellermann 1987; Koubbi et al. 2011). All samples were frozen in liquid nitrogen on board and kept at −80 °C in the laboratory.

Lipid analysis

Entire frozen specimens were placed on crushed ice and brought to 0 °C. Size (standard length = SL) and wet weight (WW) were measured prior to lipid extraction. Lipid extraction followed the method of Bligh and Dyer (1959) modified by Mayzaud et al. (2007). 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 the addition of chloroform and NaCl 0.7 % (w/v) with a final solvent ratio of methanol–chloroform–water of 2:2:1.8 (v/v/v). The total extract was concentrated under vacuum using a rotary evaporator. Extracts were stored in liquid nitrogen during the cruise and at −80 °C at the laboratory.

Total lipid (TL) content was determined gravimetrically. Lipid classes were quantified using a chromatographic separation coupled with FID (flame photometric detection) on an Iatroscan MK V TH 10. For this, 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 performed in duplicate. Lipid classes (polar and neutral lipids) were separated by chromatography, 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.

In order to have enough material to analyze the fatty acid (FA) composition of PL, larvae were pooled (7–30 individuals) according to their TAG:Chol ratio. Lipid classes were isolated by preparative TLC with the hexane/diethyl ether/acetic acid 170:30:2.5 (v/v), and the band of polar lipids (PL) was then scraped off and eluted. Lipid classes were visualized using dichlorofluorescein, and identification was achieved by comparison with standard mixtures. Fatty acids from PL were subsequently converted into methyl esters with 7 % boron trifluoride in methanol (Morrison and Smith 1964). Gas chromatography (GC) of all fatty acid methyl esters (FAME) was carried out on a 30 m length 9 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). In addition, FAME samples were hydrogenated to confirm fatty acid determination. 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).

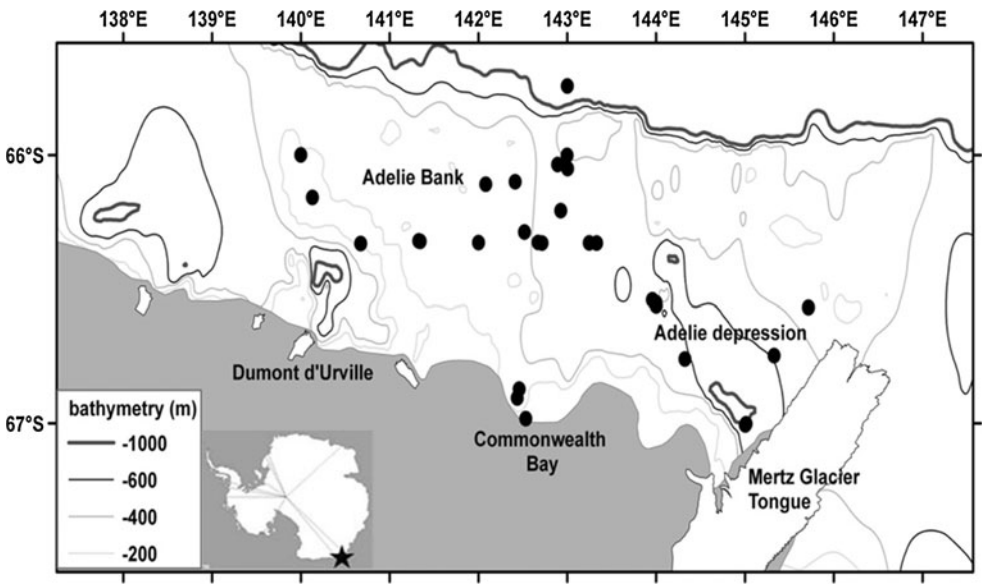

Fig. 1 Sampling stations from 2008 to 2011. The Antarctic continent is indicated in *gray* and isobaths in *black*

Validation experiment

Gut content may have an effect on the TAG measure when entire fish larvae are analyzed (Lochmann et al. 1995). However, dissection of *P. antarcticum* larvae is difficult. The liver lies upon the stomach, and handling can damage tissues or accelerate lipid degradation during the thawing process. To test whether there was a significant contribution of the stomach to the lipid signature of *P. antarcticum* larvae, we limited dissections to a relatively small number of individuals ($N = 30$, 10 individuals per year between 2008 and 2010) and compared the lipid composition of larvae with and without the stomach for each year for the same sampling station.

Statistics

StaTAGraphics Centurion XV, SPAD and R were used to analyze data. Relationships among size, weight and lipid content were established using simple linear regression. Significance of the regression was tested by the analysis of variance, ANOVA. Comparison between different samples was analyzed by ANOVA followed by Tukey's HSD comparison tests. The Kruskal–Wallis test followed by the Fisher (LSD) post hoc test was used where nonparametric tests were most appropriate. Residual effect of size over the TAG:Chol ratio was described using quantile regression (0.05, 0.01, 0.02) [Koenker and Bassett (1978)]. Cluster analysis on the fatty acid composition of polar lipids was made using the coordinates of the first axes of a correspondence analysis.

Results

Size, weight and lipid components

The standard length (SL) of *P. antarcticum* larvae ($n = 309$) ranged from 8.7 to 23 mm and the wet weight (WW) from 3.8 to 47 mg. The relationship between WW and SL was described by a log–log function (Eq. 1):

$$\log(\mathrm{WW}) = -0.96 + 1.7 \times \log(\mathrm{SL}), \quad \mathrm{ANOVA}, F = 823.41, p < 0.05, R^2 = 0.72. \tag{1}$$

Larvae were significantly larger in 2008, but no difference in SL was detected among the other 3 years (Fig. 2. ANOVA, $F = 288.49$, $p < 0.05$, Tukey HDS). Size differences in *P. antarcticum* larvae collected in 2008 can be explained by the delay of 1 month of the CEAMARC cruise relative to other years (Koubbi et al. 2011).

Total lipid content (TL) ranged from 1.2 to 4 % of WW. PL are the major constituent (average 94.6 %) followed by

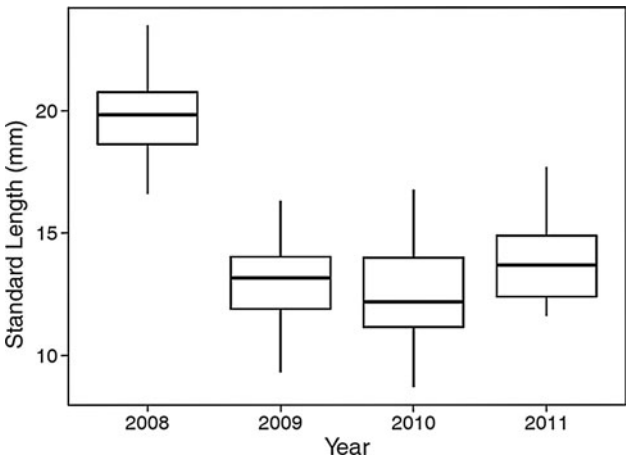

Fig. 2 Boxplot of the standard length (SL, mm) for each year

Chol (~2.4 %) and TAG (~1.8 %). In some samples, free fatty acid (FFA) and diacylglycerols (DG) were identified but represented less than 1 % of TL. Wax esters were not present (Table 2).

The amount of the membrane lipids, Chol (Eq. 2) and PL (Eq. 3) were correlated with SL (mm), as is expected for structural lipids:

$$\mathrm{Chol}\,(\mu g) = 0.54(\mathrm{SL}) - 2.15, \quad \mathrm{ANOVA}, F = 151.05, p < 0.05, R^2 = 0.33 \tag{2}$$

$$\mathrm{PL}\,(\mu g) = 20.92(\mathrm{SL}) - 58.79, \quad \mathrm{ANOVA}, F = 240.55, p < 0.05, R^2 = 0.43 \tag{3}$$

A weaker correlation existed between TAG and SL (Eq. 4)

$$\mathrm{TG}\,(\mu g) = 0.57(\mathrm{SL}) - 3.36, \quad \mathrm{ANOVA}, F = 47.37, p < 0.05, R^2 = 0.13 \tag{4}$$

The contribution of the main lipid classes (PL, Chol and TAG) to TL content was analyzed standardizing lipids (μg) by SL (mm). PL were the dominant constituent influencing changes in TL (Eq. 5) (Fig. 3). Triacylglycerols contributed very little to total lipid changes (Eq. 6), while cholesterol (Eq. 7) levels were low relative to other lipid fractions and constant irrespective of TL content (Fig. 3).

$$\mathrm{PL}\,(\mu g\,mm^{-1}) = 0.93(\mathrm{TL}) + 0.08, \quad \mathrm{ANOVA}, F = 15215, p < 0.05, R^2 = 0.99 \tag{5}$$

$$\mathrm{TG}\,(\mu g\,mm^{-1}) = 0.02(TL) - 0.18, \quad \mathrm{ANOVA}, F = 94.38, p < 0.05, R^2 = 0.24 \tag{6}$$

$$\mathrm{Chol}\,(\mu g\,mm^{-1}) = 0.01(\mathrm{TL}) + 0.20, \quad \mathrm{ANOVA}, F = 45.41, p > 0.05, R^2 = 0.13 \tag{7}$$

Triacylglycerols:Cholesterol ratio

Cholesterols were used to standardize TAG content because they are related to SL and are stable during

Table 2 Mean size ± standard deviation, wet weight (WW), % of total lipids TL and % of lipid classes per year

Years	*n*	SL (mm)	WW (mg)	% TL	% PL	% TAG	% Chol
2008	89	19.7 ± 2.1	24.1 ± 6.5	1.7 ± 0.5	94.3 ± 3.5	2.0 ± 1.5	2.5 ± 0.7
2009	96	13.0 ± 1.6	12.8 ± 3.8	2.2 ± 0.9	93.5 ± 3.9	2.3 ± 2.4	2.3 ± 1.3
2010	104	12.5 ± 1.9	10.3 ± 3.9	1.8 ± 0.6	95.8 ± 1.2	0.5 ± 0.5	2.5 ± 0.9
2011	20	13.6 ± 1.5	8.1 ± 3.1	2.7 ± 0.9	94.5 ± 2.4	1.5 ± 0.9	1.3 ± 0.7

PL polar lipids, *TAG* triacylglycerols, *Chol* cholesterol

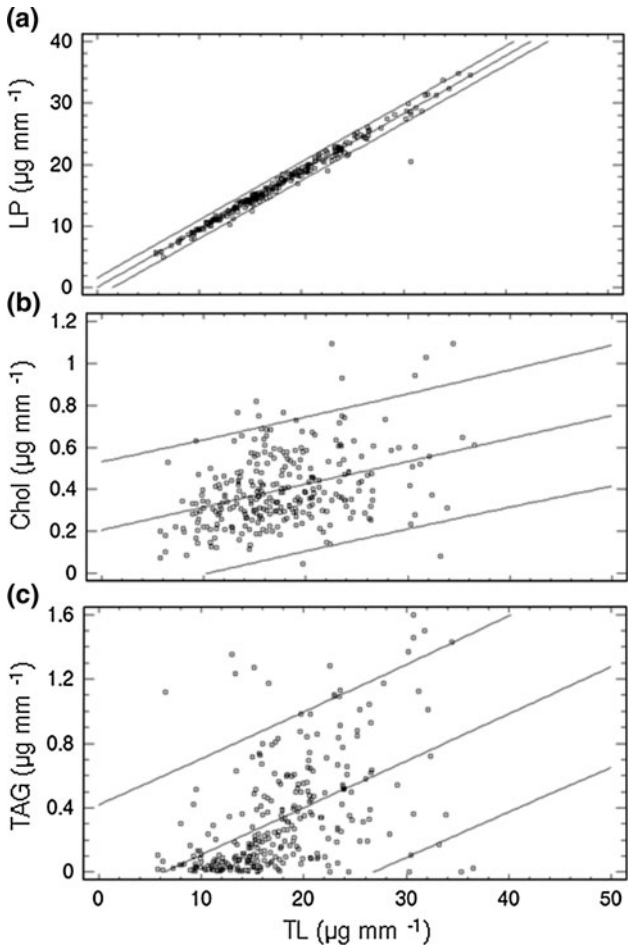

Fig. 3 Relationship between total lipid (TL) content and concentration of the main lipid classes. **a** LP: Polar lipids. **b** Chol: Cholesterol. **c** TAG: Triacylglycerols. Lipid content (µg) is standardized by SL (mm). All larvae sampled at the various locations were combined. Confidence intervals (95 %) are illustrated by the *continuous black line*

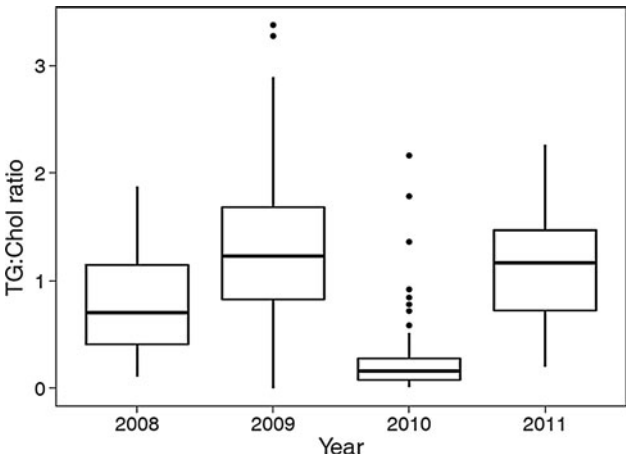

Fig. 4 Triacylglycerols:cholesterol ratio for cruises 2008–2011

starvation. The TAG:Chol ratio was calculated for each larva, and values ranged from 0.01 to 3.38 (Fig. 4). This large range of variation probably reflects all nutritional states from starvation to well-nourished larvae. TAG:Chol ratios differed among years except between 2009 and 2011 (Kruskal–Wallis test, $T = 155$, $p < 0.05$ for all years, Fisher (LSD) post hoc test).

The influence of gut content on lipid measurement was evaluated by comparing the TAG:Chol ratio of individuals with and without stomachs from the same sampling station ($n = 20$–30 individuals for each year with and without stomach). No significant differences were found reflecting that the stomach content of *P. antarcticum* larvae had no influence on lipid analysis (Mann–Whitney–Wilcoxon test, W value 0, −16, and 55 for 2008 to 2010, respectively, p value >0.05 for all years,).

Residual effect of size on the TAG:Chol ratio was analyzed using quantile regression for all data from 2008 to 2011. We focused on the lower quantiles (0.05, 0.1 and 0.2 % quantiles). Regression was significant for the three quantiles (Bootstrap method, t values = 3.76, 4.91 and 5.38 for quantiles 0.05, 0.1 and 0.2, respectively, $p < 0.001$ for all quantiles). The same quantile regression was made for all data but without 2008. Sizes of larvae of 2008 were larger (Fig. 2) and could have had an effect on the quantile regression. Regression was significant for the three quantiles (Bootstrap method, t values = 4.29, 3.58 and 3.12 for quantiles 0.05, 0.1 and 0.2, respectively, $p < 0.001$ for all quantiles). Interestingly, results from both models (with and without 2008) were almost identical. The graphical result for 0.1 quantile regression is illustrated in Fig. 5.

Because of the influence of size on the TAG:Chol ratio, the condition index analysis was split by size classes of equal number of larvae. Values of 25 % quantile per size class showed that the lower TAG:Chol ratios were below

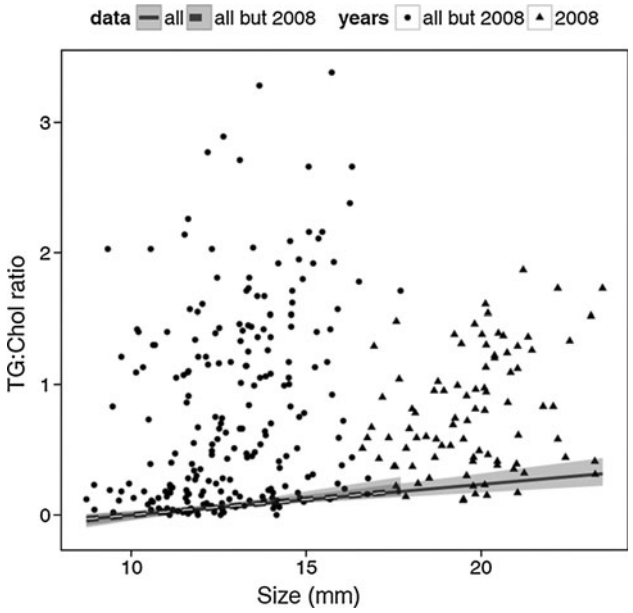

Fig. 5 Quantile regression. Effect of size (mm) on the ratio of triacylglycerol (TAG) to cholesterol (Chol). The first model used all data from 2008 to 2011 (*solid black line*). The second model used data from 2009 to 2011 (*spaced white line*). Quantile regression line of 0.1 is illustrated (*solid and spaced line*) with their 95 % confidence interval

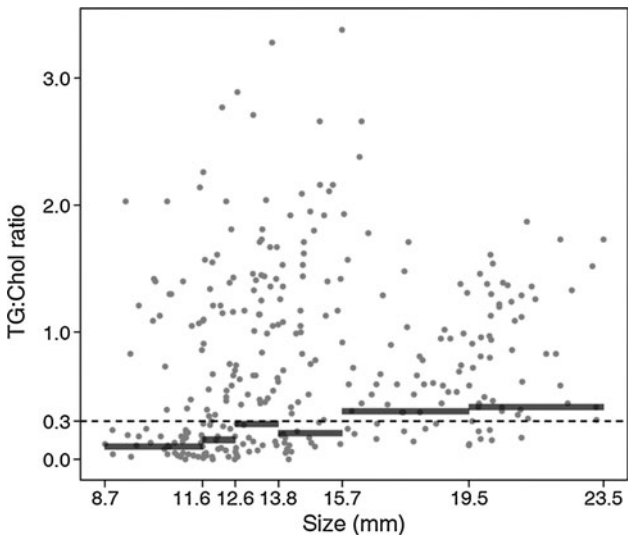

Fig. 6 Effect of size (mm) on the ratio of triacylglycerol (TAG) to cholesterol (Chol). Intervals of equal number of larvae are indicated on the *x* axes. The first quantile for each interval is illustrated by the *continuous black line*. The quantile values of larvae from 8.7 to 15.7 mm were below 0.3

the value 0.3. This value was used as an arbitrary threshold to determine the percentage of larvae in poor condition (Fig. 6).

The percentage of larvae below the TAG:Chol threshold of 0.3 decreases with increasing size classes, declining from 64 % for size classes below 11.6 mm to 9 % for larvae between 19.5 and 23.5 mm. As indicated in Table 3, the proportion of individuals with a TAG:Chol ratio below 0.3 was significantly different among size class intervals (G test, $G = 42.9$, $p < 0.05$).

Table 3 Number of individuals below the threshold TAG:Chol = 0.3 for size class intervals of equal number of larvae

Size class intervals	n	$n <$ TAG:Chol 0.3	Below 0.3 (%)
8.71–11.6	53	34	64
11.6–12.6	50	20	40
12.6–13.8	52	14	26
13.8–15.7	50	15	30
15.7–19.5	52	10	19
19.5–23.5	51	5	9

Proportions are also illustrated

Fatty acid composition of polar lipids

In order to determine whether the different ratios of TAG:Chol observed were also reflected at the membrane level, we analyzed the fatty acid composition of polar lipids (PL). Larvae were pooled according to their TAG:Chol ratio, and the fatty acid composition of PL was analyzed by a correspondence analysis followed by a cluster analysis (Fig. 7).

The polar lipid composition of larvae with mean TAG:Chol values below 0.3 was different from the others. These individuals (<0.3) had actually different ratios of essential fatty acids DHA:EPA as illustrated in Fig. 8 (Eq. 8).

$$\begin{aligned}&\text{DHA:EPA} = 1.48 - 0.16 \times \ln(\text{TAG:Chol ratio}),\\ &\text{ANOVA}, F = 21.82, p < 0.05, R^2 = 78.4.\end{aligned} \quad (8)$$

TAG:Chol ratio and decrease in polar lipids (PL)

Following Hakanson (1989a), an additional parameter was combined with the usual TAG:Chol ratio to better discriminate between individuals in poor condition and individuals likely to be experiencing net positive growth. In the present study, we calculated the 25 % quantile of PL content for each size class, which corresponded to a lower polar lipid content of about 20 % (when compared to the mean PL content per size class), as found by Hakanson (1989a). We assumed that only larvae with a TAG:Chol ratio <0.3 *and* a PL content below the value of the 25 % quantile per size class were under starvation and in poor condition. The percentage of larvae estimated in poor condition by these criteria is illustrated in Fig. 9. Within individuals with TAG:Chol <0.3, about half of them also displayed an amount of PL below the value 25 % quantile of its class size. There were great differences between the years using both methods. In 2008, 12 % of the larvae had a low TAG:Chol

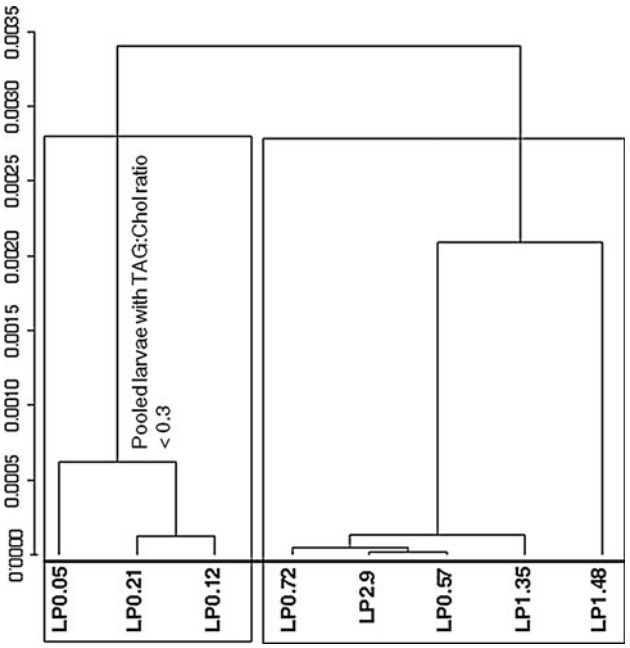

Fig. 7 Cluster analysis of the fatty acid composition of polar lipids (PL) according to the value of the TAG:Chol ratio. LP values indicate the mean TAG:Chol value of the pooled larvae

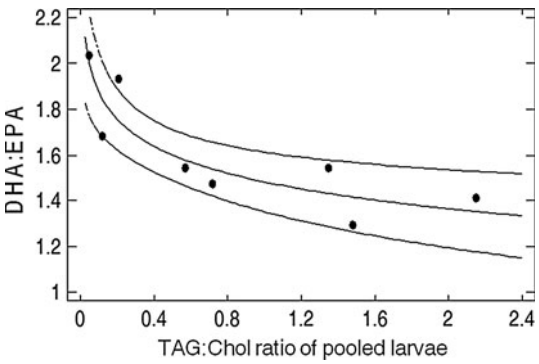

Fig. 8 Docosahexaenoic acid (DHA): eicosapentaenoic acid (EPA) ratio of pooled larvae according to the TAG:Chol ratios. Confidence intervals (95 %) are illustrated by the continuous *outer black line*

and 6 % were in poor condition when using both criteria. In 2009, the year with the highest TAG:Chol ratio, 4 % of the individuals had a TAG:Chol <0.3, but none of them showed a decrease in PL. The most striking example was larvae collected in 2010, in which 78.8 % of the individuals had a TAG:Chol <0.3 but only 32.7 % showed a decrease in PL. The number of larvae estimated in poor condition was the same for both methods in 2011 (2 %).

Condition index: spatial variability

Using the TAG:Chol ratio and the low content of polar lipids, the percentage of individuals in poor and good

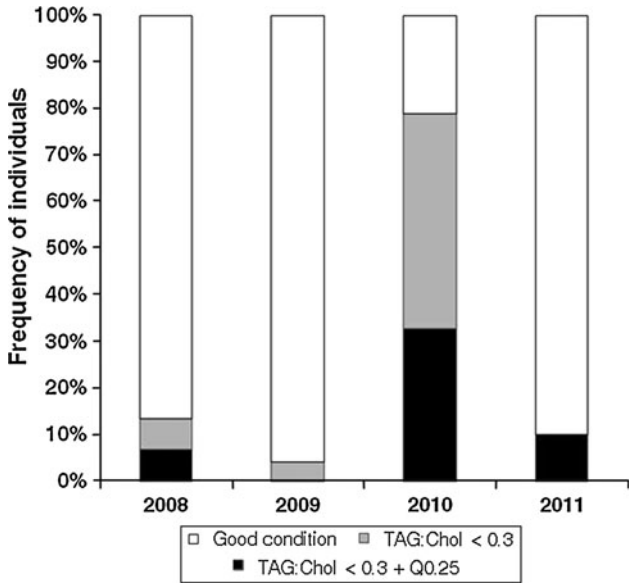

Fig. 9 Comparison of the two criteria used to detect larvae in poor condition. First method: TAG:Chol <0.3. Second method: TAG:Chol <0.3 and low polar lipids content (below the value of the 25 % quantile)

condition across years and sampling stations was highly variable (Fig. 10). Differences between sampling stations within years were significant (Mann–Whitney, $p < 0.001$), while particular stations or regions displayed great inter-annual differences. Stations close to the Mertz Glacier Tongue (MGT) or to Commonwealth Bay were characterized by a high percentage of individuals in poor condition in 2010, whereas in 2009 at the same stations, no individuals were found to be in poor condition. Maximum depth varied between 200 and 300 m for stations on the Adelie Bank to more than 1,000 m for stations in the Adelie depression (close to the MGT). Following maximum depth, stations close to the MGT were colder (−1.6 to −1.4 °C between 0 and 100 m) than stations on the Adelie Bank (−0.8 to −0.3 °C between 0 and 100 m) (Koubbi et al. 2011). Neither, temperature nor depth was correlated with the condition of fish larvae (Spearman, $S = 151.7$ and 266.9 for temperature and depth, respectively, $p > 0.05$ in both cases).

Discussion

Robust indicators of larval condition could be extremely useful tools in developing a clear understanding of how the environment may regulate the growth dynamics and survival of marine species. In this study, we developed of a lipid-based condition index for the dominant pelagic fish *P. antarcticum* and found significant differences in the condition of larvae between 2008 and 2011. *P. antarcticum*

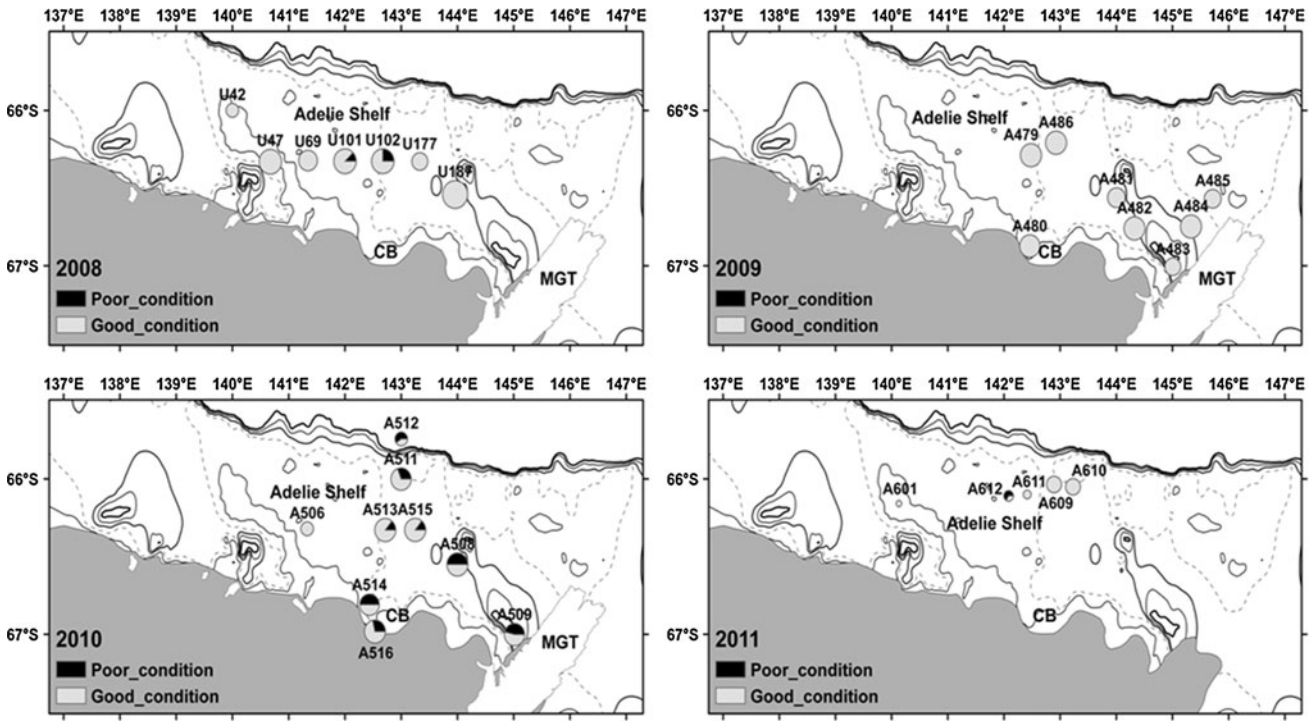

Fig. 10 Spatial and temporal variability in larval condition. Individuals considered in poor condition had a TAG:Chol ratio <0.3 and showed a lower LP content (below 25 % quantile LP per size class). A maximum of 15 larvae were available for each sampling station

larvae exhibit moderate to low lipid levels, with a mean value for all years considered in the present study of 2.1 % WW. The lipid composition of *P. antarcticum* larvae was similar to that reported for the same species at the region by Mayzaud et al. (2011) and Tavernier et al. (2011), who also found total lipids to be dominated by PL followed by TAG and Chol.

The potential contribution of gut contents to contribute to the measurement of lipid levels was analyzed for larvae from 2008 to 2010, but differences in the TAG:Chol ratio of individuals with and without stomachs were not significant. Lochmann et al. (1996) found that gut content can account for a 5- to 20-fold overestimation of triacylglycerols content. However, larvae in rearing experiments often show lipid values, not representative of the conditions of wild larvae (Hakanson 1989a; Ehrlich 1975). Furthermore, the main prey item of *P. antarcticum* larvae in terms of both mass and volume is copepods rich in wax esters. As suggested by Tavernier et al. (2011), the lack of wax esters in the lipid class composition is indicative of a negligible contribution of gut contents in the "whole-body" lipid signatures of this species.

Triacylglycerols are an energy store that can be directly linked to a potential for withstanding starvation events. Triacylglycerols in *P. antarcticum* juveniles and adults are stored in various tissues including muscle, liver or subdermal tissue (Eastman and DeVries 1989). TAG was standardized using Chol which represents the most stable lipid class because it is related to wet weight and standard length and is not catabolized under starvation (Hakanson 1989a; Fraser 1989). However, there was a residual effect of larval size over the TAG:Chol ratio. Dependence of the TAG:Chol ratio on size was well illustrated using quantile regressions. Individuals with low TAG:Chol ratio (below 0.3) were found from all sizes between 9 and 21 mm, but most of them were from the smallest size classes, with a maximum of individuals (64 %) between 8.7 and 11.6 mm. The effect of size on the TAG:Chol ratio was evident in all years and regardless of absolute larval size. Disproportionate mortality associated with small larvae in poor condition is a plausible mechanism to explain why the largest size classes contain fewer individuals with low TAG content and, therefore, with low TAG:Chol ratios.

The commonly used TAG:Chol ratio indicated large interannual differences for *P. antarcticum* larvae. The sampling year appeared as the main parameter explaining the TAG:Chol variability in fish larvae. This characteristic is supported by the results of Tavernier et al. (2011) for *P. antarcticum* larvae collected in 2007 from the same region. They calculated the TAG:Chol ratio for pooled larvae (of 10–20 individuals) from each of the seven sampling stations. The mean TAG:Chol ratio across stations was 1.05 in 2007, with a small standard deviation of ±0.1, indicating a consistent condition of the fish larvae population across stations. The TAG:Chol ratio might be correlated with food web characteristics such as zooplankton abundance.

P. antarcticum larvae are omnivorous (Giraldo et al. 2011; Vallet et al. 2011 Tavernier et al. 2011), feeding mainly on copepods and phytoplankton, so abundances of total copepod were taken as a proxy of prey availability. The lowest TAG:Chol ratios were found in 2010, corresponding to the lowest copepod densities (mean = 212 ind/m^3 unpublished data), and the high values in 2009, corresponding to the highest copepod abundances (mean = 470 ind/m^3 unpublished data).

The TAG:Chol ratio reflects the potential of individuals to withstand starvation events, but does not necessarily represent a direct indicator of poor condition because it cannot differentiate between individuals that are actually in poor condition and those who have an adequate intake of food resource but are allocating more of their energy for growth and, therefore, manifest a relatively low TAG content. As shown in this study, a tight linear relationship between total lipids and PL suggests that *P. antarcticum* larvae favor growth over lipid storage, and growing larvae are likely to contain lower amounts of TAG relative to body size. The same result was illustrated by ratios of essential fatty acid in PL. Individuals with low TAG:Chol values had higher levels of DHA:EPA suggesting that most of the individuals pooled have a good intake of these essential fatty acids allowing active growth and then were not in poor condition. Lower TAG levels for larvae with positive somatic growth are supported by results on anchovy larvae (Hakanson 1993) which indicated that there are different interannual growth rates for the same species and that faster growing larvae had lower amounts of lipid. However, the precise relationship between growth and lipid content is likely to be species-specific, and this should be taken in consideration when analyzing condition indices. It seems that the only reasonable inference in considering the TAG:Chol ratio is that the potential to resist the effects of environmental stress for organisms with low TAG content (or low TAG:Chol ratio) is limited (Lochmann et al. 1995).

A key question is how to differentiate between those organisms that are allocating most of their net energy intake for growth (and, therefore, could have low TAG content and low TAG:Chol ratio) but are otherwise well fed, and those who are actually suffering starvation and/or are in poor nutrition. Hakanson (1989a, 1989b) demonstrated that after starvation, anchovy larvae show an immediate decrease in the TAG along with a more gradual decrease in PL. Rearing experiments with anchovy larvae indicated a threshold effect; larvae with a decrease in polar lipid content of 20 % of the mean for its size class, and with a TAG:Chol ratio smaller than 0.2, were considered in poor condition. The limit value of TAG:Chol ratio for anchovy larvae is close to the one proposed for *P. antarcticum* larvae using quantile analysis. The 25 % quantile of PL per size class corresponds to the 20 % decrease in PL suggested by Hakanson (1989a). The definition of larvae in poor condition used in this study is not absolute but should allow useful comparison between the four sampling years and between sampling stations within the same year. Comparing the percentage of individuals estimated in poor condition using the TAG:Chol ratio alone and when adding low PL levels suggests that the commonly used TAG:Chol ratio criterion may lead to an overestimation of the proportion of individuals in poor condition. The lipid condition index using TAG:Chol ratio and PL content was useful to better target individuals in poor condition and should be widely applicable specially for slow-growing larvae that favor growth over lipid storage.

Individuals estimated to be in poor condition (TAG:Chol ratio and PL) came from different sampling stations, indicating that all the ocean habitats are not equivalent in promoting growth and survival within the same year. Neither temperature nor depth seemed to have an effect on the condition index since larvae in poor conditions were sampled at different depths and temperatures. In 2010, 47 % of individuals estimated to be in poor condition were sampled near Commonwealth Bay (stations 514 and 516) and near Buchanan Bay (station 509) close to the Mertz Glacier tongue (Fig. 10). Koubbi et al. (2011) found high larval abundance at the same stations and suggested that coastal areas with deep canyons are favorable for spawning grounds and development of early larvae. Surprisingly, in 2009, no larvae were judged to be in poor condition, including individuals collected near the MGT (A483) and close to Commonwealth Bay, which had relatively high TAG:Chol ratios (TAG:Chol = 1.96 and 1, respectively). Koubbi et al. (2011) also showed that in 2010, larval abundance was around 20 times the abundance in 2009. Thus, the poor condition of individuals in 2010 might be density-dependant and could be connected to the lower copepods abundance observed. Ours results suggest that interannual variability in TAG:Chol ratios is greater than the magnitude of spatial variability at the scales examined in this project.

Conclusion

Important differences were observed in the percentage of *P. antarcticum* larvae estimated to be in poor condition when the criterion for condition was based on the TAG:Chol ratio alone or when the TAG:Chol ratio was considered together with a decrease in PL. These differences could be particularly important to assess the condition of the population. The TAG:Chol ratio alone has the potential to overestimate the percentage of individuals in poor condition associated with lipid-based indices. Therefore, we suggest that the TAG:Chol ratio should be considered

together with a decrease in polar lipids, as was suggested by Hakanson in 1989. Our results show that differences in the physiological condition of larvae are better explained by interannual variability than geographic features. Interannual variation in the condition index may be of great assistance in predicting the survival of *P. antarcticum* larvae in long-term monitoring, and attempts to test the link between larval condition and recruitment to the adult population are warranted. This study supports the hypothesis that *P. antarcticum* may serve as an important indicator of spatial and temporal changes in the marine environment because of its intermediate trophic position (Tavernier et al. 2011). The essential fatty acids of individuals with low TAG:Chol ratio and low PL content might be useful to determine the different levels of starvation and those individuals close to the point of no return.

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