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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