J. Northw. Atl. Fish. Sci., Vol. 30: 47–60

Northern Shrimp (*Pandalus borealis*) Recruitment in West Greenland Waters Part II. Lipid Classes and Fatty Acids in *Pandalus* Shrimp Larvae: Implications for Survival Expectations and Trophic Relationships

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Abstract

Lipid class and fatty acid compositions were determined in shrimp larvae (*Pandalus borealis* and *P. montagui*) collected along transects across banks on the West Greenland shelf in June 1999, May and July 2000. The lipid class contents were investigated as indices of larval shrimp lipid condition and food type. Fatty acid compositions were investigated for lipid biomarkers to establish trophic relationships between larval shrimp and potential prey.

Phospholipids were the dominant lipid class in all six pelagic development stages of larval *P. borealis* and *P. montagui*, accounting for 80 to 92% of the total lipid.

In all six stages the contents of free fatty acids were more abundant than triacylglycerol. With increasing larval size from hatching to a carapace length of ~2.3 mm, there was a decreasing trend in the contents of hydrocarbons attributed to reduced amount of phytoplankton in the diet. Triacylglycerol (TG) content to wet weight ratio was investigated as index of nutritional larval condition and survival potential. Proportions of larvae with TG indices >0.2 were relatively high in May 2000 and June 1999, but generally low in July 2000.

The fatty acids 16:0, 16:1n-7, 18:0, 18:1n-9, 18:1n-7, 20:5n-3, and 22:6n-3 were major lipid components of the larvae and their mean proportions varied within relatively small ranges between larval stages. The dominant fatty acids were 16:0, 20:5n-3, and 22:6n-3 with average proportions of about 17%, 20%, and 13%, as percentage of total fatty acids. Biomarkers for algae (16:0, 16:1n-7, 18:1n-7, 20:5n-3, diatoms: elevated 16:1n-7/16:0 ratios), flagellates (18:0, 18:4n-3, 22:6n-3), and zooplankton (18:1n-9, 20:1n-9, 22:1n-11) indicate that larval *P. borealis* and *P. montagui* are omnivorous.

ZI larvae in high concentrations at coastal and fjord stations in May 2000 and most larvae (ZIV–ZVI) from July 2000 were low in lipid. Larvae from June 1999 had relatively high lipid contents probably due to better feeding and growth history. Our results suggest variability in growth and survival expectations of larvae related to variations in larval food availability between years.

Keywords: biomarkers, condition, fatty acids, larval shrimp, lipids, *Pandalus*

Introduction

The food pathway in the pelagic marine ecosystem originates in the phytoplankton whose organic constituents are transformed by animals through food webs and different trophic levels (Nielsen and Hansen, 1995; Kiørboe, 2001). Lipid classes and fatty acid compositions generally characterise marine species or groups of organisms and they can be used to assess nutritional condition, survival expectations and trophic relationships at the different developmental stages (Lee *et al.*, 1971a; Sargent and Whittle, 1981; Sargent *et al.*, 1987; Sargent and Falk-Petersen, 1988; Kattner *et al.*, 1994; Anger, 1998; Harding and Fraser,

1999; Reuss and Poulsen, 2002). Lipid biomarkers have been found to be useful in the analyses of predator-prey relationships in food web studies (Graeve *et al.*, 1994a,b; Kharlamenko *et al.*, 1995; St. John and Lund, 1996; Graeve *et al.*, 1997; Desvilettes *et al.*, 1997; Falk-Petersen *et al.*, 2000; Parrish *et al.*; 2000; Hamm *et al.*, 2001; Arts *et al.*, 2001). Ratios of 16:1n-7/16:0 have been positively correlated to lipid condition indices of juvenile fish, indicating a positive effect on growth and survival of diatom based food chains (St. John and Lund, 1996; Pedersen *et al.*, 1999). St. John and Lund (1996) found juvenile cod in better lipid condition in frontal areas, dominated by diatoms, than outside frontal areas, characterized by various flagellates.

Relatively little is known about lipid contents, and trophic relationships of field collected Pandalid shrimp larvae (Stickney and Perkins, 1981; Harrison, 1990; Hopkins *et al.*, 1993; Ouellet *et al.*, 1995; Anger, 1998). According to Anger (1998) field studies of larval growth and development should be intensified to resolve uncertainty as to what extent laboratory data can be extrapolated to life in the natural environment. Distribution studies of larval shrimp have found wide larval dispersal and no association with specific hydrographic characteristics (Ouellet *et al.*, 1990; Pedersen and Rice, 2002; Pedersen *et al.*, 2002). This suggests that studies of food availability, physiological rates, and biochemical condition of individual larvae in specific water masses might be most important in determining survival and recruitment variability. According to Ouellet *et al.*, (1992, 1995) a biochemical (lipid-based) condition index [i.e. larval triacylglycerols (TG) content (µg): wet weight (mg) ratio x 1000] can reflect survival potential of larval *P. borealis* cohorts. This condition index may prove to be useful in assessing ecological factor(s) involved in recruitment processes of *P. borealis* populations.

Today the fishing industry as well as the economy in Greenland is mainly dependent on a large northern shrimp fishery, based on stable recruitment of new year-classes to the shrimp stock (e.g. Christensen and Vestergaard, 1993; Buch *et al.*, 2002). Understanding processes and dynamics of the lower trophic levels of the marine pelagic food web during the productive period are one of the keys to understand changes in community structure and variability in recruitment success of shrimp and fish in the marine ecosystem (e.g. Cushing, 1989, 1995; Anderson, 2000; Pedersen *et al.*, 2002).

The main purpose of this study was to investigate: 1) lipid class contents of different development stages of *P. borealis* and *P. montagui* larvae, 2) triacylglycerol (TG ww^{-1}) as indices of nutritional condition, and 3) fatty acid compositions to establish trophic relationships. Questions we seek to answer are: Do lipid condition and fatty acid composition differ between the two dominant *Pandalus* species? Do specific prey (i.e. diatoms *versus* flagellates) carry a water-mass specific food web signal (fatty acid composition), which can be traced and related to good or bad lipid condition indices (survival expectations) in larval shrimp? Is variability in larval shrimp growth and survival coupled to variability in hydrographical processes and plankton production?

Materials and Methods

Sampling

Fresh *P. borealis* and *P. montagui* larvae were picked from zooplankton samples at selected stations during four research cruises carried out in June 1999, May 2000, and July 2000 between 63°50'N and 66°50'N on the West Greenland shelf (see Pedersen *et al.*, 2002). Stations were selected along transects (coast to offshore) to investigate variation in lipid contents of the larvae in relation to changes in the environment (temperature, salinity and abundance of potential larval food). The larvae were frozen individually in argon atmosphere in 2 ml Eppendorf vials. On the June 1999 survey, the vials were frozen within few minutes after sampling and stored in a freezer (-25°C). On three other surveys the vials were frozen in liquid nitrogen (-196°C) for later determination of lipid contents in the laboratory. At the end of all cruises, the samples were immediately transferred to a biofreezer (-80°C) until analysis.

Biochemical analysis

Lipid extraction was performed on individual larvae for 24 hours in 1.5 ml chloroform/methanol (2:1 v/v) at -20°C with a known quantity of internal standard added (Ketone: Hexadecan-3-one). Additional lipid extraction of larvae for 24 hours in 1.5 ml chloroform/methanol (2:1 v/v) at -20°C did not produce further lipid from the larvae. The amount of internal standard was adjusted after the weight (lipid content) of the larvae. After lipid extraction the sample was centrifuged for 2 minutes (4000 rpm). The supernatant (lipid solution) was transferred to a 10 ml glass vial and the solvent was evaporated under a steam of argon gas. The retained lipid was then

redissolved in hexane, transferred to a small glass vial, hexane evaporated under an argon flow and the dried lipid extract was stored at -80°C until further analysis.

Lipid classes

For analysis of lipid class content, the lipid extract was resuspended in hexane and 1.4 µl of the sample was spotted on a SIII-Chromarod for quantification using flame ionization detection with a model MK-5 IATROSCAN TLC/FID analyzer (Iatron, Laboratories Inc.) using Autospotter (SES 3200). For separation of the lipid classes, two different solvent systems were used to obtain two chromatograms per rod as described by Parrish (1987). The first solvent system, hexane:diethyl ether:formic acid (99:1:0.05), was used in a double development of 25 min followed by 20 min. Each rod was then scanned to after the internal standard (3-HEXADECANONE, Sigma product number: H 7504H) peak to obtain the first chromatogram, which included: hydrocarbons (HC), wax esters (WE), methyl esters (ME) and the internal standard (KET). After a second 40-min development in hexane:diethyl ether:formic acid (80:20:0.1) the second chromatogram was obtained, containing peaks of triacylglycerols (TG), free fatty acids (FFA), alcohols (ALC), free sterols (ST) diacylglycerols (DG), and phospholipids (PL) plus non lipid material (NLM). After the second development the scan was continued to the end of the rod. However, for 30 random selected lipid samples from July (mainly stage ZV larvae) the scan was only continued to the lowest point behind the DG peak. These samples were developed in a third solvent system (40 min in chloroform:methanol:water, 15:10:1, v/v/v, S. Jónasdóttir, DIFRES, pers. comm.) to obtain a third chromatogram separating the PL and NLM. In average we found less than 4% NLM in the PL+NLM peaks. In order to test for overlaps of ST and DG (1,3-DG and 1,2-DG) peaks, and improve their separation, we tried an improved separation method described by Striby *et al.* (1999) on several lipid samples. However, for some unknown reason we were unable to separate mixtures of our standards of 1,3-DG and ST, and we gave up using this time-consuming extra separation method. Therefore, our estimated ST amounts may contain some 1,3-DG or 1,2 DG (see Striby *et al.*, 1999), although we did find separate peaks of 1,3-DG and 1,2 DG in the lipid samples. Before and between developments, the rods were humidified over saturated NaCl in order to maintain constant development qualities.

The quantification of each lipid constituent was based on calibration curves prepared from solutions of standard lipids obtained from Sigma Chemical Company (product number-name): HC (N 4129-NONADECANE), WE (A 5885-ARACHIDIC ACID ARACHIDYL ESTER), TG (T 5016-TRISTEARIN), FFA (P 0500-PALMITIC ACID), 1,2 and 1,3-DG (D 8894-DIOLEIN and D 9019-DISTEARIN), ALC (P 3647-PHYTOL), ST (C 866-CHOLESTEROL), PL (P 5014-PHOSPHATIDYLCHOLINE, DIHEPTANOYL). For each shrimp larva the analysis for lipids was run twice in order to minimize errors. In general, an average of the two calculations of lipid contents was used. In a few cases one of the calculations was unreliable and rejected.

Fatty acids

Fatty acid contents of selected individual larvae collected in May 2000 (stage ZI and ZII) and July 2000 (stage ZIV, ZV, and ZVI) were derived from all extracted lipids, whereas the extracted lipids of larvae of the June 1999 samples (stage ZIII and IV) were fractionated in three classes: neutral lipids (NL) free fatty acids (FFA) and phospholipids (PL). Fractionation was performed on Sep-Pak columns (Millipore) eluted with solvents of increasing polarity. Fatty acids were converted to methyl esters (FAME) by saponification and methylation with methanol and boron trifluoride as outlined in Refsgaard *et al.* (2000), but scaled down to the small lipid amounts of larval shrimp. To separate the aqueous and organic solvents the sample was mixed with 3 ml saturated NaCl solution and 1 ml *n*-heptane. The upper heptane phase containing the fatty acids was transferred to gas chromatograph (GC) vials (method modified from Whyte, 1988). Analysis of individual fatty acid methyl esters was carried out on a HP 5890 gas chromatograph (Hewlett-Packard, Avondale, PA), equipped with a flame ionisation detector and an Omegawax 320 fused silica capillary column (0.32 mm × 30 m × 0.25 µm; Supelco, Bellefonte, PA). Splitless injection of 1–4 µl was performed. The injection and detection temperatures were 200°C and 240°C, respectively. The initial oven temperature program was (1) 80°C maintained for 1½ min, (2) ramp up to 160°C at a rate of 40°C min^{-1}, (3) a further increase to 240°C at a rate of 3°C min^{-1} and (4) final temperature maintained for 12 min. The helium carrier gas flow was 21 cm s^{-1}. Fatty acid methyl esters were identified based on a comparison with retention times of standards obtained from Sigma and Larodan,

and standard runs of saturated even and odd chained fatty acids. Quantification was performed by a Perkin Elmer NCI 900 integrator with Turbochrom 4.0 software (Perkin Elmer Inc.). Mass spectrometry was not used to confirm identities of individual fatty acids.

Data analysis

Discrimination between *P. borealis* and *P. montagui* was based on the size criteria given in Pedersen *et al.* (2002). Lipid class content (HC, WE, TG, FFA, ALC, ST, DG and PL) relative to wet weight of individual larvae was investigated for variation among species, size, stage, sampling station, transect, and month using multi- and one-way ANOVA (SAS, 1985; GLM procedure).

We chose to differentiate larvae of TG condition index ww^{-1} below and above 0.2, because 0.2 may be a critical level for larval shrimp survival (Ouellet *et al.*, 1992, 1995). Associations among potential larval food densities (integrated fluorescens values, number m^{-2} of copepod eggs, -nauplii, and copepodites, and other invertebrate larvae), larval shrimp density (number m^{-2}), and lipid condition indices were investigated using Sperman rank correlations analysis. Potential larval food, and shrimp larvae concentrations are reported in Pedersen *et al.* (2002).

Cluster analysis (Bray-Curtis similarity) and Principal Components Analysis (PCA) were performed to evaluate the similarities and groupings of the fatty acid frequency compositions of individual shrimp larvae by lipid classes (NL, FFA, and PL for June 1999 larvae, only), species, zoeal stage, station, and sampling month using the software PRIMER v. 5.0 (Clarke and Warwick, 1994).

Results

Lipid class content

The lipids of the zoeal stages of *P. borealis* and *P. montagui* were dominated by phospho-lipids (Tables 1 and 2). WE and ALC were present in low amounts in all stages. For ZI and ZII larvae, the second most abundant lipid class was HC followed by ST, FFA, TG, and 1,2DG. For ZIII and ZIV larvae from June, the second most abundant lipid class was FFA followed by ST, HC, TG, and 1,2DG. For ZV, ZVI, and VII from July, ST was the second most abundant followed by FFA, DG (1,2 and 1,3), HC, and TG (Appendix figure).

There was a marked difference in lipid contents between the June 1999 and July 2000 larvae, the amounts of lipids being much higher in June 1999 (Tables 1 and 2). Total lipid of ZIV larvae from June 1999 were in fact higher than of stage ZV larvae from July 2000, due to higher amounts of FFA, PL, and TG. The relative high FFA content found in the larvae collected in June 1999 could indicate enzymatic hydrolysis of PL due to improper freezing and storage of the larvae samples (Ohman, 1996). Pandalids are known to have high enzymatic activity. In June 1999, live larval shrimp were frozen in argon atmosphere in a -25°C freezer for 2 weeks before transferred to a biofreezer after the cruise. This may have caused lipid degradation and FFA formation.

Lipid condition indices

The individual ratios of lipid content to wet weight by larval size and lipid class showed trends and variations. With increasing larval size from hatching to a carapace length (cl) of ~2.3 mm there was a decreasing trend in the HC lipid content. From a larval size above ~2.5 mm cl the ST to wet weight ratio showed an increasing trend. The TG, 1,2DG, FFA, ALC, and PL contents to wet weight ratios with larval size showed variations with no clear trends.

Two lipid condition indices, 1) TG ww^{-1}x1000, and 2) HC+TG+FFA+ALC+DG ww^{-1} x1000 (hereafter referred to as the TG and Lipcon index, respectively), were investigated for variations among species, stage, sampling station, transect, and month using multi- and one-way ANOVA. The TG and Lipcon indices were significantly correlated in larvae collected in May 2000 and July 2000, but not in larvae collected in June 1999 (Fig. 1).

ZI larvae from May 2000 were not identified to species, and ANOVA of the TG indices (n = 146, r^2 = 0.48, F = 8.04, $p(p>F)$<0.001) showed no significant (p>0.05) effects of stage (ZI larvae: n = 142, and ZII larvae: n = 4) or sampling location (transect). However, a few stations had significant higher TG indices: 1) the ocean ward stations of transect 1 (TR1), 2) a station on the inner edge of Sukkertop Bank on transect 3 (TR3), and 3) two fjord stations.

ANOVA of TG indices from June 1999 (n = 63, r^2 = 0.55, F = 5.12, $p(p>F)$<0.001) showed significant (p<0.05) effects of species, stage, and sampling station. The smaller *P. montagui* larvae generally had higher TG indices than *P. borealis* larvae, and ZIII larvae having higher TG indices than ZIV larvae. A few stations west of the banks on Tr2 and Tr3 had significantly higher TG indices compared to the mean

TABLE 1. Comparison of wet weight, carapace length, and contents of lipid classes of "large" larvae, *P. borealis*, by zoeal stage, sampling month and year (mean±SE of N individuals). Zoeal stage I includes both *P. borealis* and *P. montagui* larvae. HC = hydrocarbons, WE = wax esters, TG = triacylglycerols, FFA = free fatty acids, ALC = alcohols, 1,3 DG = 1,3 diacylglycerol, ST = free sterols, 1, 2DG = 1,2 diacylglycerol, PL = phospholipids.

Zoeal stage	ZI	ZII	ZIII	ZIV	ZIV	ZV	ZVI
Month(year)	May(2000)	May(2000)	June(1999)	June(1999)	July(2000)	July(2000)	July(2000)
N	142	4	17	32	2	58	37
Carapace length (mm)	1.4 ±0.0	1.7 ± 0.1	2.5 ± 0.0	3.1 ± 0.0	2.7 ± 0.0	3.1 ± 0.0	3.7 ± 0.0
Wet weight (mg)	1.8 ±0.1	2.4 ± 0.3	6.3 ± 0.2	9.8 ± 0.3	6.6 ± 0.2	12.8 ± 0.3	18.4 ± 0.5
Lipid classes:							
HC (µg)	5.0 ±0.2	3.8 ± 0.3	3.0 ± 0.3	2.3 ± 0.2	4.1 ± 1.0	4.3 ± 0.2	5.2 ± 0.3
WE (µg)	0.2 ±0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.5 ± 0.3	0.4 ± 0.1	0.5 ± 0.1
TG (µg)	1.0 ±0.1	0.8 ± 0.2	3.2 ± 0.6	3.1 ± 0.3	0.6 ± 0.1	2.0 ± 0.1	3.0 ± 0.4
FFA (µg)	2.6 ±0.2	3.4 ± 0.4	36.5 ± 3.2	49.0 ± 4.6	4.3 ± 0.6	16.0 ± 1.2	21.1 ± 1.9
ALC (µg)	0.2 ±0.0	0.1 ± 0.1	0.6 ± 0.3	1.3 ± 0.3	1.0 ± 1.0	0.2 ± 0.1	0.7 ± 0.6
1,3DG (µg)	0.1 ±0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	5.7 ± 0.7	9.0 ± 1.4
ST (µg)	3.4 ±0.2	4.0 ± 0.7	12.2 ± 0.8	22.8 ± 1.5	19.9 ± 2.3	69.3 ± 3.3	100.6 ± 4.9
1,2DG (µg)	0.8 ±0.1	1.0 ± 0.3	1.5 ± 0.1	3.3 ± 0.3	3.3 ± 3.3	1.2 ± 0.6	4.7 ± 1.5
PL (µg)	81.7 ±5.5	87.7 ± 13.8	426.3 ± 27.7	926.0 ± 86.1	235.8 ± 98.3	713.7 ± 33.0	962.2 ± 74.6
Total lipid (µg)	94.8 ±6.0	101.1 ± 14.0	485.0 ± 27.9	1009.8 ± 87.9	269.3 ± 96.3	812.8 ± 35.4	1106.9 ± 75.2

TABLE 2. Comparison of wet weight, carapace length, and contents of lipid classes of "small" larvae, *P. montagui*, by zoeal stage, sampling month and year (mean±SE of N individuals).

Zoeal stage	ZIV	ZIV	ZV	ZVI
Month(year)	June(1999)	July(2000)	July(2000)	July(2000)
N	13	17	41	45
Carapace length (mm)	2.3 ±0.1	2.0 ±0.1	2.5 ±0.1	3.0 ±0.0
Wet weight (mg)	5.6 ±0.5	4.4 ±0.2	7.5 ±0.4	11.6 ±0.5
Lipid classes:				
HC (µg)	2.2 ±0.2	3.5 ±0.3	3.5 ±0.2	4.4 ±0.3
WE (µg)	0.0 ±0.0	0.1 ±0.1	0.3 ±0.1	0.4 ±0.1
TG (µg)	3.5 ±0.4	0.9 ±0.4	1.9 ±0.6	2.3 ±0.6
FFA (µg)	45.4 ±19.1	2.8 ±0.8	7.4 ±0.9	12.0 ±1.2
ALC (µg)	0.8 ±0.4	0.0 ±0.0	0.6 ±0.4	1.0 ±0.5
1,3DG (µg)	0.0 ±0.0	0.3 ±0.2	2.2 ±0.4	4.6 ±0.8
ST (µg)	10.9 ±1.5	10.2 ±1.1	27.9 ±2.9	51.5 ±4.2
1,2DG (µg)	1.2 ±0.2	4.3 ±0.5	1.8 ±0.4	5.8 ±1.1
PL (µg)	499.7 ±85.4	209.7 ±19.4	398.2 ±35.5	610.4 ±48.0
Total lipid (µg)	566.4 ±86.1	231.9 ±19.9	443.8 ±38.4	692.3 ±51.8

value. ANOVA of Lipcon indices from June 1999 (n = 63, r^2 = 0.56, F = 5.25, $p(p>F)$<0.001) showed significant effects of species, and station, but no significant effect of stage. There was a significant higher mean Lipcon index on the westernmost station of Tr3.

ANOVA of TG indices from July 2000 (n = 197, r^2 = 0.07, F = 0.58, $p(p>F)$<0.943) showed no significant (p>0.05) effects of species, stage, or sampling station. This result excludes one station (three larvae) with significant and exceptional high TG indices.

The proportions of larvae with TG indices >0.2 were relatively high in May 2000 and June 1999, but generally low in July 2000 (Fig. 2). In May and June, the proportions of larvae with TG indices >0.2 were highest on the western transect stations. ZIV larvae in June1999 were generally in better lipid condition than in July 2000, and the smaller *P. montagui* larvae were in better lipid condition than *P. borealis* (Fig. 3). In fact nearly all investigated *P. borealis* from July 2000 had a TG index below 0.2.

Fatty acid compositions (FAC)

The larvae analysed for FAC were selected from stations in June 1999, May, and July 2000 (Fig. 2 – number of analysed larvae in brackets). Lipid samples of ZIII and ZIV larvae collected in June 1999 were fractionated in three classes: neutral lipids (NL) free fatty acids (FFA) and phospholipids (PL). Bray-Curtis

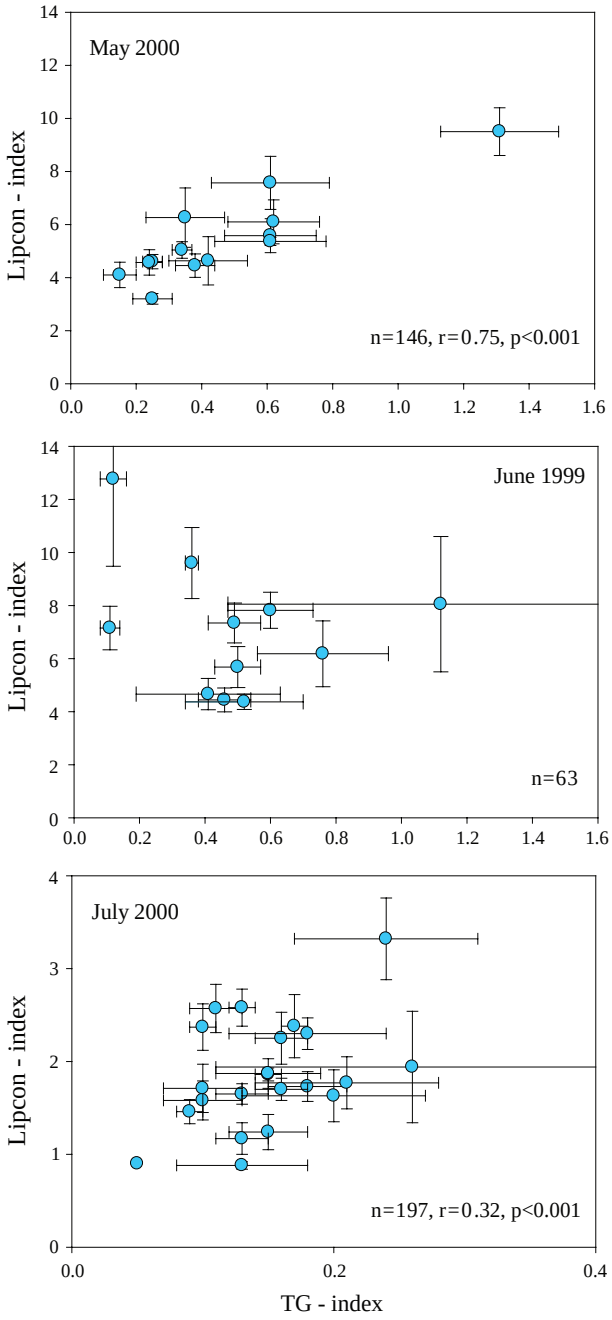

Fig. 1. Mean (±SE) TG-index *versus* mean (±SE) Lipcon-index in May 2000, June 1999, and July 2000. Significant correlation coefficients in May and July are shown.

similarity indices and PCA showed no clear or consistent differences between the FACs of individual larvae by the lipid classes NL, FFA, and PL. Therefore, FACs of individual larvae collected in May and July 2000 were analysed in total lipid, only. Hence, the comparisons of similarities and groupings of the FACs of individual larvae were made for total lipid.

The fatty acids 14:0, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 18:4n-3, 20:1n-9, 20:5n-3, and 22:6n-3 were major lipid components of the larvae (Table 3). Their mean proportions varied within relative small ranges between larval stages. There was no difference in fatty acid composition between *P. borealis* and *P. montagui*.

The dominant fatty acids were 16:0, 20:5n-3, and 22:6n-3 with average proportions of about 17%, 20%, and 13%, as percentage of total fatty acids. The FACs showed a high proportion of polyunsaturated fatty acid (PUFA) in all larval stages, mainly PUFAn-3 (Table 3). The proportions of the monounsaturated fatty acids (MUFA) 20:1n-9 and 22:1n-11 were generally highest in ZIV larvae collected in June 1999. C18-PUFA showed an increase from in average 2.9% in ZI to about 8% in ZIII-ZVI larvae. Contents of C18 fatty acids showed significant correlation with flagellate abundance and may be considered a biomarker for flagellates (see Reuss and Poulsen, 2002). ANOVA of proposed diatom biomarkers, ratios of 16:1n-7/16:0, 20:5n-3/18:4n-3, and 20:5n-3/22:6n-3, showed no significant effects of sampling month, stage, and species.

Bray-Curtis similarity indices of the FACs of individual larvae showed generally higher similarities among ZIV, ZV, and ZVI larvae, compared to ZI larvae (Fig. 4). Larvae were generally grouped by stage and sampling station. However, ZIV larvae from stations in June 1999 showed high similarities with ZVI larvae from stations in July 2000. The FACs of ZI larvae collected at a coastal and a fjord station differed from offshore collected ZI larvae from May 2000 (Fig. 4). The coastal and fjord stations both had high larval abundance, low TG condition, lower proportions of 16:0, 16:1n-7, 20:5n-3, 22:6n-3, and higher proportions of 18:0, compared to offshore collected ZI larvae.

Lipid content and potential larval shrimp food

The FACs of individual larvae from June 1999 and May 2000 were compared with FACs of plankton <300µm collected at the same station. For a detailed description of the FACs of plankton <300µm, see Reuss and Poulsen (2002). Bray-Curtis similarity between FACs of shrimp larvae and plankton <300µm were from 55 to 78% in June 1999, and from 55 to 68% in May 2000. In May, fatty acid compositions of shrimp larvae and plankton <300µm from two costal stations clustered (the innermost station of Tr1 and a station in the entrance to Godthåbsfjord, Fig. 2), and

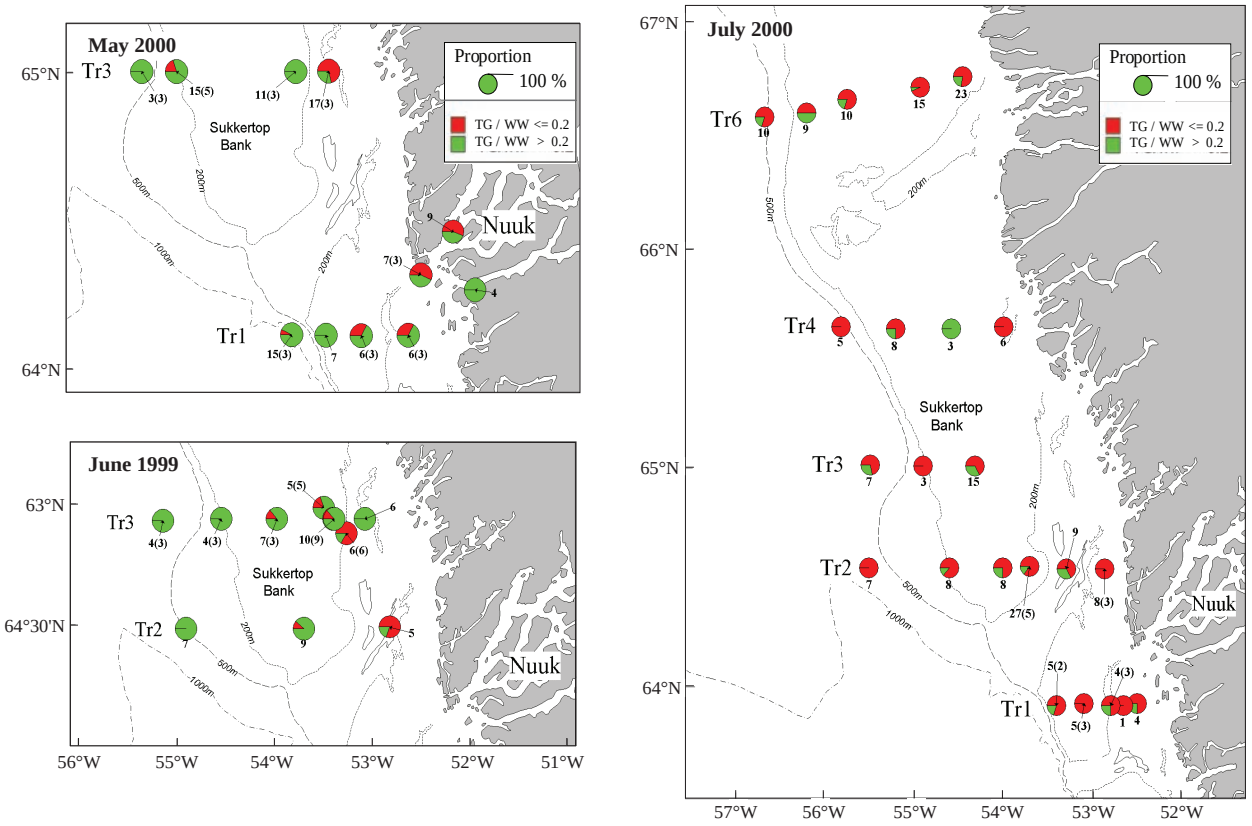

Fig. 2. Proportion of TG-indices in shrimp larvae from May 2000, June 1999 and July 2000. TG index > 0.2 (green), and TG index ≤0.2 (red). Number of samples (individual larvae) shown below dots. Number in brackets shows the number of larvae analysed for fatty acid composition.

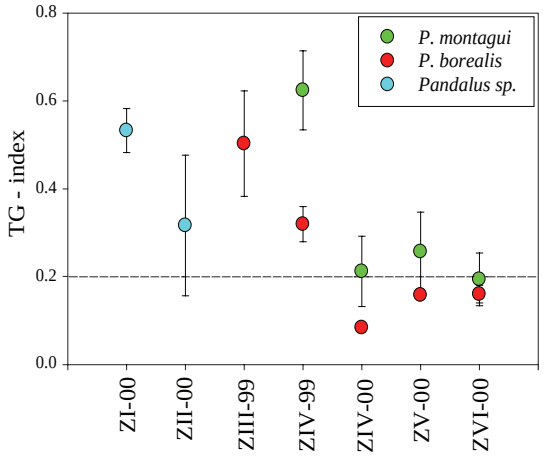

Fig. 3. Mean TG indices (±SE) by zoeal stage, sampling year, and species. Zoeal stage I and II includes both *P. borealis* and *P. montagui* larvae. TG 0.2 is critical level.

all other mainly offshore samples of shrimp larvae and plankton <300µm clustered.

There was a significant positive correlation between the diatom biomarker, 16:1n-7/16:0 ratio, found in larval shrimp and in plankton <300µm for May (n = 26, r = 0.41, p = 0.04) and June (n = 29, r = 0.74, p<0.01). For May, only, there was also a significant positive correlation between the percentages of C18 fatty acids in shrimp larvae and in plankton <300µm (n = 26, r = 0.45, p = 0.02).

In the shrimp larvae, the diatom biomarker, 16:1n-7/16:0 ratio, and the flagellate biomarker, %C18, showed no significant correlation in May, June or July. However, the diatom biomarker 20:5n-3/18:4n-3 ratio and %C18 showed significant correlations in May (n = 28, r = -0.38, p = 0.05), June (n = 29, r = -0.78,

TABLE 3. Fatty acid composition (mean mass % ± SD of N individuals) of total lipid of *P. borealis and P. montagui* larvae by species, zoeal stage and sampling month (year).

		P. borealis				*P. montagui*		
Zoeal stage	ZI	ZIII	ZIV	ZV	ZVI	ZIV	ZV	ZVI
Month(Year)	May(2000)	June(1999)	June(1999)	July(2000)	July(2000)	June(1999)	July(2000)	July(2000)
N	29	3	20	4	3	5	4	5
Carapace length (mm)	1.4 ± 0.3	2.4 ± 0.1	3.2 ± 0.1	3.2 ± 0.2	3.6 ± 0.0	2.4 ± 0.1	2.7 ± 0.3	2.9 ± 0.3
Wet weight (mg)	1.7 ± 0.7	7.0 ± 1.5	9.4 ± 2.4	13.7 ± 1.0	17.5 ± 2.0	5.5 ± 0.3	7.6 ± 1.9	11.5 ± 4.3
Fatty acid (mass %):								
13:0	1.9 ± 5.6	1.4 ± 0.4	0.4 ± 0.4	0.0 ± 0.0	0.5 ± 0.2	0.6 ± 0.9	0.0 ± 0.0	0.1 ± 0.3
14:0	2.4 ± 0.9	4.5 ± 0.4	5.3 ± 1.2	4.8 ± 0.5	4.6 ± 0.5	4.7 ± 0.6	4.2 ± 0.4	4.7 ± 0.4
16:0	17.2 ± 4.7	15.3 ± 1.5	19.7 ± 3.0	21.1 ± 4.5	19.0 ± 0.8	16.7 ± 1.3	21.8 ± 4.5	20.0 ± 4.9
17:0	1.3 ± 1.6	2.3 ± 0.1	2.4 ± 1.3	0.3 ± 0.2	0.4 ± 0.0	3.0 ± 1.6	0.5 ± 0.2	0.2 ± 0.2
18:0	7.2 ± 7.1	5.5 ± 2.1	4.1 ± 0.9	3.7 ± 1.2	2.9 ± 0.4	6.4 ± 2.2	3.9 ± 1.3	2.6 ± 1.7
20:0	3.5 ± 5.2	0.5 ± 0.8	0.3 ± 0.5	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.2 ± 0.1
%SAFA	33.3 ± 9.3	29.6 ± 2.4	32.2 ± 5.6	29.9 ± 6.0	27.4 ± 0.7	31.4 ± 2.6	30.7 ± 6.0	27.9 ± 5.6
16:1n-7	4.5 ± 2.3	4.7 ± 1.0	6.4 ± 2.8	5.7 ± 2.6	5.5 ± 0.7	4.1 ± 0.9	4.3 ± 2.3	7.0 ± 2.5
18:1n-9	5.9 ± 1.9	17.3 ± 14	7.3 ± 1.7	8.8 ± 4.3	8.9 ± 2.4	12.6 ± 8.4	10.4 ± 2.6	8.8 ± 3.3
18:1n-7	6.1 ± 2.4	3.9 ± 0.9	4.9 ± 1.0	4.7 ± 0.3	5.2 ± 0.2	4.0 ± 0.2	4.4 ± 2.0	3.2 ± 2.4
20:1n-9	0.7 ± 0.5	2.8 ± 0.7	3.6 ± 1.3	1.9 ± 1.4	2.1 ± 0.4	3.9 ± 0.9	1.5 ± 2.0	2.0 ± 1.3
22:1n-11	0.8 ± 1.3	0.2 ± 0.1	3.2 ± 2.6	1.0 ± 1.0	1.0 ± 0.2	1.6 ± 0.5	0.7 ± 0.3	1.2 ± 0.8
22:1n-9	0.2 ± 0.1	1.7 ± 0.9	0.6 ± 0.5	0.4 ± 0.1	0.3 ± 0.1	1.4 ± 1.5	0.2 ± 0.1	0.4 ± 0.3
24:1n-9	2.2 ± 3.1	0.5 ± 0.6	0.2 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.6	0.0 ± 0.0	0.0 ± 0.0
%MUFA	20.4 ± 6.9	31.1 ± 15	26.2 ± 6.2	22.5 ± 1.3	23.0 ± 1.1	28.1 ± 11	21.4 ± 3.7	22.6 ± 3.7
16:2	3.5 ± 5.1	0.2 ± 0.2	0.5 ± 0.4	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.6	0.3 ± 0.2	0.4 ± 0.3
18:2n-6	1.1 ± 0.8	3.1 ± 0.9	2.3 ± 0.3	2.9 ± 0.6	2.3 ± 0.1	2.4 ± 0.5	2.7 ± 0.3	2.9 ± 1.0
18:3n-3	0.3 ± 0.3	0.9 ± 0.3	0.7 ± 0.2	1.4 ± 0.4	1.5 ± 0.3	0.8 ± 0.2	1.4 ± 0.5	1.1 ± 0.4
18:4n-3	1.4 ± 2.0	4.2 ± 0.6	3.0 ± 1.6	4.5 ± 1.1	3.6 ± 0.8	6.2 ± 1.6	4.0 ± 2.0	2.6 ± 0.8
20:2n-6	1.3 ± 1.4	0.1 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
20:5n-3	20.8 ± 8.3	11.8 ± 5.8	17.4 ± 5.8	21.4 ± 5.3	24.1 ± 1.2	15.6 ± 5.3	23.0 ± 3.2	24.8 ± 3.2
22:5n-3	2.0 ± 2.3	0.2 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
22:6n-3	8.9 ± 4.0	13.7 ± 10	12.4 ± 4.6	13.6 ± 2.5	14.5 ± 1.0	11.1 ± 2.0	12.8 ± 1.1	13.9 ± 1.5
%PUFA n-3	34.4 ± 10	31.5 ± 13	34.6 ± 11	42.2 ± 6.5	44.9 ± 1.4	34.3 ± 8.1	42.5 ± 4.1	43.5 ± 4.5
% Unknown	5.5 ± 5.0	3.8 ± 0.7	3.2 ± 0.8	2.7 ± 0.7	2.9 ± 0.5	2.8 ± 0.6	2.8 ± 1.1	3.5 ± 1.9
%other*	2.5 ± 1.7	1.3 ± 0.5	1.4 ± 0.3	1.8 ± 0.2	1.6 ± 0.2	0.9 ± 0.5	1.7 ± 0.1	1.6 ± 1.6
18:1n-9/18:1n-7	1.3 ± 1.3	4.2 ± 3.0	1.5 ± 0.4	2.2 ± 0.6	1.8 ± 0.4	3.2 ± 1.9	2.6 ± 2.3	3.8 ± 2.8
16:1n-7/16:0	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.2	0.4 ± 0.2
20:5n-3/18:4n-3	25.8 ± 17	2.8 ± 1.5	7.1 ± 4.5	5.2 ± 2.4	7.0 ± 1.7	2.5 ± 0.6	7.1 ± 4.0	10.6 ± 4.2
20:5n-3/22:6n-3	2.8 ± 1.5	1.1 ± 0.6	1.5 ± 0.5	1.6 ± 0.1	1.7 ± 0.1	1.4 ± 0.3	1.8 ± 0.2	1.8 ± 0.3
C18PUFA/16:1n-7	1.0 ± 1.2	1.8 ± 0.5	1.1 ± 0.4	1.9 ± 1.2	1.3 ± 0.2	2.3 ± 0.5	2.4 ± 1.2	1.2 ± 1.0

*Other components: 15:0, 16:4(n-3), 20:4(n-6), 20:3(n-3), 20:4(n-3). Mass spectrometry was not used to confirm identities of individual fatty acids.

p<0.01), and July (n = 16, r = -0.81, p<0.01). The diatom biomarkers showed correlations with each other only in June between 16:1n-7/16:0 and 20:5n-3/22:6n-3 (n = 29, r = 0.55, p<0.01), and in July between 20:5n-3/18:4n-3 and 20:5n-3/22:6n-3 (n = 16, r = 0.75, p<0.01).

We found no significant correlations between diatom biomarkers and the larval lipid condition indices (TG ww^{-1} and Lipcon ww^{-1}). However, in June 1999, highest 16:1n-7/16:0 ratios were found in larvae caught on the westernmost station of Tr3. Larvae from this station had relatively high TG- and Lipcon indices, the latter dominated by FFA. This station also showed the highest potential food concentrations of phytoplankton, copepod egg, nauplii, copepodites, and other invertebrate larvae (see Pedersen *et al.*, 2002), indicating a link between food concentration and larval shrimp lipid condition. Attempts to associate larvae lipid condition from May and July 2000 with indices of potential food concentrations failed. In May 2000, there was a significant negative correlation between TG indices and ZI abundance (n = 100, r = -0.41, p<0.001), but no such correlation for more developed larvae in June 1999 or July 2000.

Discussion

Lipid class contents

Phospholipids were the dominant lipid class in all six pelagic development stages of larvae, accounting for 80 to 92% of the total lipid. This is

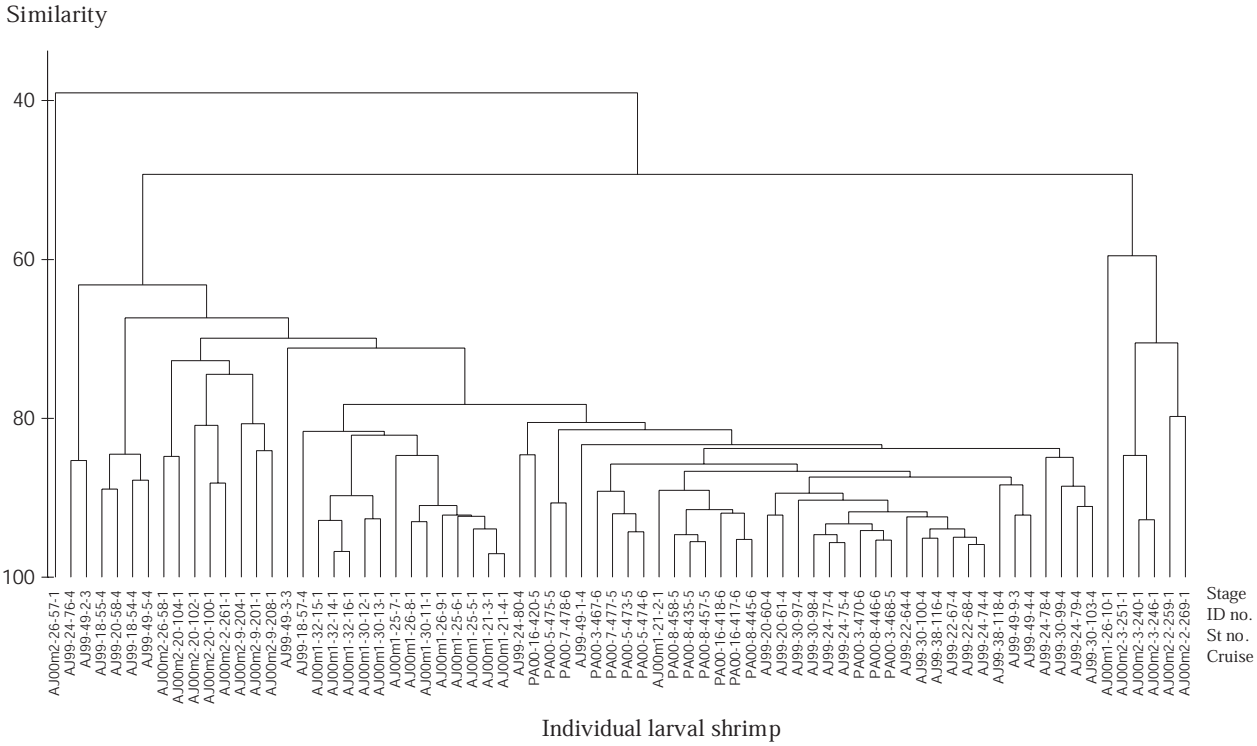

Fig. 4. Dendrogram of Bray-Curtis similarity indices of the fatty acid frequency compositions of individual shrimp larvae (no data transformation). The sample code is: Cruise-Station no.-Sample ID no.-Zoeal stage (1 = ZI, 3 = ZIII, 4 = ZIV, 5 = ZV, 6 = ZVI). Hence, stage number is closest to x-axis.

similar to findings in field collected *P. borealis* larvae (ZI and ZII) in Gulf of St. Lawrence (Ouellet *et al*., 1995) and adults from NE Greenland (Graeve *et al*., 1997), but higher than found for age 1+ in the north Norwegian Balsfjord (Hopkins *et al*., 1993). More surprising was the high contents of HC, which has not previously been reported from lipid content studies of *P. borealis* larvae (Ouellet *et al*., 1992,1995) although reported for age 1+ (Hopkins *et al*., 1993). The decreasing trend of HC content with increasing larval size may reflect change in food and feeding. HC can consist of pristane (C19), C21:6, beta-carotene and others (Lee *et al*., 1971b; Gatten *et al*., 1979; Volkman *et al*., 1980; *www.chiron.no*). Pristane is a derivative of the phytol side chain of chlorophyll and C21:6 is a major HC in phytoplankton as is beta-carotene. HC in larval shrimp may therefore be an indication of phytoplankton feeding. The decreasing HC content with larval size could be an indication of reduced importance of phytoplankton in the diet. This is supported by food preference experiments performed by Rasmussen *et al*. (MS 2000) with *P. borealis* larvae on mixed diet. They found the larvae to feed on all food items, but the food selection seemed size dependent. Larvae in stage ZI and ZII had higher clearance rate for algae than for other items offered, while ZIII lost interest in planktonic algae (Rasmussen *et al*., MS 2000).

Larvae from June 1999 showed the highest FFA content, but also the highest TG, PL, and total lipid content (Table 1), indicating better lipid condition and higher feeding activity compared to larvae from July 2000. We suggest that the relatively high FFA content of the larvae from June 1999 was due to high feeding activity and anabolism (a model of lipid dynamics in Decapods is given on page 7 in Harrison (1990)). An investigation of a low number of shrimp larvae stomachs showed generally more stomach content in larvae from June than from July. This indicates that part of the elevated FFA content may originate from the stomach content. However, some FFA could also come from lipid degradation due to improper freezing storage, as noted in Results. Unlike in this study, Ouellet *et al*. (1995) found high content of DG and they suggested that in situations with low availability of FFA, the major flux from DG is directed in to PL synthesis. Our findings of relatively high FFA contents

may suggest a direct relationship between food availability, contents of FFA and PL.

Lipid condition and feeding

Larval TG content correlate with physiological condition of a larva (Håkonson, 1984; Fraser, 1989; Ouellet *et al*., 1992, 1995; Harding and Fraser, 1999), and when exogenously derived energy exceeds the metabolic demand of a larva, the excess energy is accumulated as TG. We found a high proportion of larvae with low TG index (<0.2) at stations with dense ZI concentrations in May and at nearly all stations in July for ZIV, ZV, and ZVI larvae indicating low food availability and feeding. According to Harding and Fraser (1999) a TG / ST ratio of 0.1 can be considered as the minimum survival threshold for lobster larvae to the next moult. For shrimp larvae in this study, the used minimum survival threshold of TG-index = 0.2 equals a TG / ST ratio of ~0.1. The increasing trend of the ST ww^{-1} ratio of larvae above ~2.5 mm cl indicates a downward trend of TG / ST ratios with size relative to the TG-index used in this study. Laboratory studies found downward trends in TG / ST ratios due to starvation during development of shrimp and lobster larvae (Mourente *et al*., 1995; Harding and Fraser, 1999). The generally low lipid condition indices of the shrimp larvae from July 2000 (high proportion of TG-index <0.2) suggests low food availability and starvation (Fig. 2 and 3). *P. borealis* larvae have been suggested to settle during stage ZV (Astthorsson and Gislason 1991). In this study we caught both ZV and ZVI larvae, suggesting that these larvae were searching for food in the pelagic (Pedersen *et al*., 2002). A possible explanation could be that in years with food limitation larvae in low lipid condition stay in the pelagic in search for food. If the larvae do not reach a certain lipid condition level they may not settle and starve to death. The smaller *P. montagui* larvae were generally in better lipid condition than *P. borealis* (Fig. 3). This could indicate that the smaller larvae had an advantage over larger larvae at the time of sampling due to e.g. better food availability or less energy requirement.

Ouellet *et al*. (1995) suggested that the differences observed in the TG index among sampling sites reflect trophically induced variability which can be associated with different survival expectations at moult for ZI and ZII larvae. For crustaceans, a minimum storage of energy reserves during each development stage is critical for successful moulting and survival from one stage to the next (Ouellet *et al*., 1992; Harding and Fraser, 1999). We were unable to relate variable TG indices to within moult cycles. 22 larvae from July 2000 were identified as being close to moulting and their mean TG- and Lipcon indices were not significant different from the means of 178 larvae that were not close to moulting.

The fatty acid composition of shrimp larvae reflects to a certain extent, the food and feeding history of the larvae. The most recent feeding will be reflected in the fatty acid composition of recently ingested FFA and in neutral lipid reserves, mainly TG, while the fatty acid composition of PL reflect the food and feeding history over a longer feeding period. We were unable to find clear differences in the fatty acid composition of NL, FFA, and PL, which may indicate similar food composition over short and longer time periods. Neutral lipid reserves were generally found in low amounts, whereas phospholipids, mainly membrane fatty acids (20:5n-3, 22:6n-3, and 16:0), were far dominating. The latter supports the low dependence on lipid reserves. When larval food is in excess the surplus energy seems to be used for growth and/or stored as PL mainly.

Lipid biomarkers, food web and lipid condition

The use of fatty acids as biomarkers is based on studies of the lipid composition of marine phyto- and proto-plankton, which show that the fatty acid compositions are characteristic of the different taxa (Falk-Petersen *et al*., 1998; Reuss and Poulsen, 2002). Of the important phytoplankton in polar waters, the diatoms tend to be rich in 20:5(n–3), 16:1(n–7), and C16 PUFA but deficient in C18 PUFA, whereas the flagellates tend to be rich on 18:4n–3, 18:5n–3, and especially 22:6n–3 and deficient of 16:1(n–7) (Falk-Petersen *et al*., 1998). The Haptophyceae *Phaeocystis pouchetii* that often dominate the blooms in polar waters are rich on C18 PUFA, especially 18:4n–3 and 18:5n–3, together with 20:5n–3 and 22:6n–3, while 16:1n–7 is found in very small amounts (Hamm *et al*., 2001). According to Falk-Petersen *et al.* (2000) oleic acid, 18:1n–9, is a major fatty acid of most marine animal lipids. 18:1n–7 is also frequently found in large quantities deriving from the elongation of 16:1n–7 that is likely to originate substantially from phytoplankton. This means that 16:1n–7 and 18:1n–7 in animal lipids tend to reflect phytoplanktonic dietary input, whereas 18:1n–9 reflects animal dietary input. The 20:1n–9 and 22:1n–11 units, present in very large amounts in calanoid copepods, are considered to be formed by *de novo* biosynthesis in these animals as the major site of the formation of 20:1n–9 and 22:1n–11 units in the marine food web

(Falk-Petersen *et al.*, 2000). Relating the above findings to the fatty acid compositions of the shrimp larvae in this study (Table 3), we find that larval *P. borealis* and *P. montagui* in development stage ZI to ZVI can be considered to be omnivorous. The fatty acid compositions of plankton <300µm in May and June showed relatively high similarity with the fatty acid compositions of shrimp larvae. ZI larvae had lower 18:1n-9/n-7 ratio and ZIII to ZVI larvae had higher contents of 18:1n-9 and 20:1n–9. Therefore, phytoplankton was a more important component in the diet of ZI larvae, whereas animal food became more important in the diet of ZIII to ZVI larvae.

Compared to *P. borealis* eggs and age 1+ from Balsfjord (Hopkins *et al.*, 1993), the larvae in this study contained higher amounts of PUFAn-3. PUFA, 20:5n-3 and 22:6n-3, cannot be synthesized *de novo* or in the required amount by most animals although essential for normal growth and survival (Bell and Sargent, 1996; St. John *et al.*, 2001; Arts *et al.*, 2001). In tiger prawn (*Penaeus esculentus*), Dall *et al.* (1992) found no clear evidence that 20:5n-3 and 22:6n-3 are essential and the essential 20:4n-6 could be synthesized from 18:2n-6. However, for larval shrimp (*Crangon* spp.) low amounts of PUFA may be interpreted as a consequence of unfavourable nutritional conditions (Kattner *et al.*, 1994). ZI larvae from a coastal and a fjord station in May 2000 both had high larval abundance, low TG condition, lower proportions of 16:0, 16:1n-7, 20:5n-3, 22:6n-3, and higher proportions of 18:0, compared to offshore collected ZI larvae. At these two stations, the fatty acid compositions of plankton <300µm showed the same trend as in larval shrimp, indicating flagellate dominance at these two stations (Reuss and Poulsen, 2002). Therefore, poor food quality could be the reason for the observed low lipid condition of shrimp larvae at coastal and fjord stations in May 2000 (Fig.2).

The correlations between 16:1n-7/16:0 ratios in larval shrimp and in plankton <300µm for June and May, and the correlations between percentages of C18 fatty acids in larval shrimp and in plankton <300µm for May, indicate that fatty acid biomarkers can be used as food web tracers in diatom dominated versus flagellate dominated water columns. Although elevated larval lipid condition indices at the westernmost station of Tr3, we were unable to find clear links between water regimes, plankton community structure (biomarkers), zooplankton concentrations, and larval shrimp condition indices. The low number of plankton and larval shrimp samples investigated with low resolution in time and space is probably the main reason for this. However, the links between the physical processes, plankton productivity and larval shrimp distribution over the West Greenland shelf are complex and strongly influenced by variability in climate (wind) and currents (Poulsen and Reuss, 2002; Pedersen *et al.*, 2002). Reuss and Poulsen (2002) found fatty acids to be insufficiently specific to satisfactorily describe the composition of the plankton community. Fast changes in the physical-biological environment for the shrimp larvae due to turbulence will weaken the possibility of establishing clear links between larval shrimp lipid condition, food concentrations, and food web biomarkers.

Conclusion

Based on low lipid condition indices, we conclude that ZI larvae in high concentrations at coastal and fjord stations in May 2000 and most larvae from July 2000, were starving and had low probability of surviving the next moult. Lipid contents of larvae from June 1999 were more variable as were the physical environmental conditions, but generally the larvae were in higher lipid condition and more advanced in development compared to the larvae caught in July 2000 (Pedersen *et al.*, 2002). This was most probably due to better feeding and growth history of the larvae caught in June 1999. Our results suggest variability in growth and survival expectations of *P. borealis* and *P. montagui* larvae related to variations in larval food availability between years. This is supported by the observation of a large 1999 and small 2000 year-class in the annual shrimp assessment survey. Hence, this study supports the hypothesis forwarded by Anderson (2000) that year-class strength variability of Pandalids mainly is determined during the pelagic larval phase by bottom-up processes and variability in the timing of zooplankton production.

Acknowledgements

Thanks to Greenland Institute of Natural Resources, the crews on R/V *Adolf Jensen* and R/V *Paamiut*. Special thanks to Claus C. Simonsen for excellent cooperation during the field program. Thanks also to the lipid laboratory at the Danish Institute for Fisheries Research (DIFRES) - Marlene Carlsen, Karin Bohn Jensen, Jack Melbye, and Inger Hornum for their effort with our lipid samples. Sigrun Jónasdóttir was helpful with the lipid separation methods. Special thanks to Benny Jensen (DIFRES) for help in the analysis of fatty acid contents, and the interpretations of the results. Especially, one of three

anonymous reviewers gave helpful guidance for improvements of an earlier version of the manuscript. This work was based on economic support from the Danish Research Councils Committee on North Atlantic Research, Project no. 9803018.

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Appendix 1: Figure showing lipid composition of *Pandalus* sp. larvae.

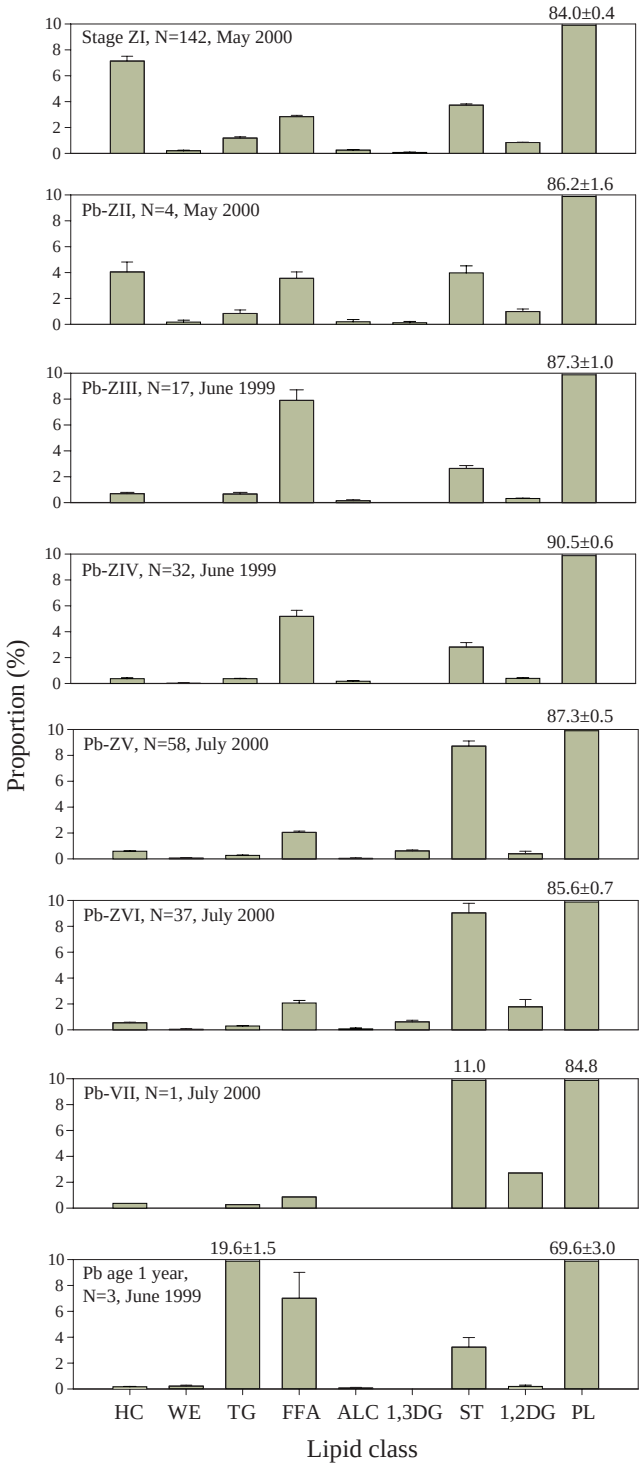

Fig. 1. Percent lipid composition of *P. borealis* (Pb) larval stages ZI to ZVI, VII, and 1 year old. Stage ZI includes also *P. montagui* larvae. Mean of N individuals, standard error indicated on columns. HC = hydrocarbons, WE = wax esters, TG = triacylglycerols, FFA = free fatty acids, ALC = alcohols, 1,3DG = 1,3 diacylglycerol, ST = free sterols, 1,2DG = 1,2 diacylglycerol, PL = phospholipids.