Polar Biology

Fatty acid composition of the post larval daubed shanny (*Leptoclinus maculatus*) during the polar night

Svetlana N. Pekkoeva[1], Svetlana A. Murzina[1], Zinaida A. Nefedova[1], Stig Falk-Petersen[2,], Jørgen Berge[3,4,5], Ole J. Lønne[4], Nina N. Nemova[1]

Abstract

Recent discoveries of high levels of biological activity in the Arctic marine ecosystems during the polar night raise questions regarding the ecophysiology of the pelagic postlarval daubed shanny *Leptoclinus maculatus*. Of special interest in the composition of the lipid sac – a unique feature not found in other Arctic fishes. Analysis of the fatty acid content of major classes of lipids as membrane - total phospholipids (PL) and storage – triacylglycerols (TAG) in the different postlarvae stages during the polar night is presented in this work for the first time. A high levels of monounsaturated fatty acids (MUFA) (70%-74% of the total fatty acids (FA) of TAG) was found in the TAG of the L3-L4 postlarvae stages, among which 20:1n-9 (28%) and 22:1n-11 (27%-29%) FA are trophic biomarkers of the zooplankton *Calanus* spp. Among the polyunsaturated FA (PUFA) the n-3 FA dominated (22:6n-3 – in the PL, and 18:4n-3 – in the TAG). A decrease in the SFA and an increase in the MUFA in the structural PL during the transition from the postlarval to the demersal stage was observed. Our analyses of the FA composition of the lipid sac suggest that it continues to actively hunt for its preferred prey item *Calanus* spp. during the polar night.

Keywords: *Leptoclinus maculatus*, lipid sac, fatty acids, lipid classes, adaptation, polar night, Arctic, Spitsbergen

Introduction

Pelagic high latitudes marine animals are known to store lipids as an adaptive strategy to environmental changes on different time scales (Falk-Petersen et al. 2004, 2009). The daubed shanny, *Leptoclinus maculatus* (Fries, 1838), is a common demersal fish in the Arctic (Makushok 1979; Meyer Ottesen et al. 2014). The life history involves a pelagic juvenile stage that last from three to five years (Meyer Ottesen et al. 2011), and its body (dry weight) consists of 40% lipids (Falk-Petersen et al. 1986; Murzina 2010; Pekkoeva et al. 2017a; Meyer Ottesen et al. 2018). The abdominal part of the body of the pelagic postlarvae contains a morphophysiological structure hereafter referred to as a “lipid sac” (Falk-Petersen et al. 1986; Murzina 2010), which stores lipids as triacylglycerols.

The lipid sac is considered an adaptation for growth and development in an environment with strong seasonal changes in the food supply (Murzina 2010; Pekkoeva et al. 2017a, b). The lipid sac begins at the pectoral

Svetlana N. Pekkoeva

e-mail: pek-svetlana@mail.ru; +79535266012

[1]Institute of Biology of the Karelian Research Centre of the Russian Academy of Sciences, Petrozavodsk, Russia;

[2]Akvaplan-niva AS, Fram Centre, N-9296, Tromsø, Norway;

[3]UiT The Arctic University of Norway, Department of Arctic and Marine Biology, Tromsø, N-9037, Norway;

[4]The University Centre in Svalbard, N-9171 Longyearbyen, Norway;

[5]Norwegian University of Science and Technology, Centre for Autonomous Marine Operations and Systems, Tromsø, Norway.

fins and runs to the anus. It is marked by a series of melanophores and consists of large, closely packed lipid vacuoles surrounded by a simple membrane (Falk-Petersen et al. 1986; Murzina 2010; Meyer Ottesen et al. 2011). A specific feature of the lipid sac of the daubed shanny is that its storage lipids are homogenous and do not accumulates in fat cells (adipocytes) as they do in adipose tissue (Falk-Petersen et al. 1986; Murzina 2010). For example, the adult Antarctic notothenioid fish *Pleuragramma antarcticum* has intermuscular lipid structures, which are lipid sacs that consist of several white adipocytes with a large lipid droplet, and whose key function is to maintain the neutral buoyancy of the fish in the absence of a swim bladder (Eastman and DeVries 1989).

The primary function of the lipid sac in the daubed shanny is considered to be energy storage; however, lipids also contribute to buoyancy (Falk-Petersen et al. 1986; Murzina 2010). The melanophores and light refraction through the lipid droplets in the pelagic larvae are believed to make them inconspicuous to predators (Falk-Petersen et al. 1986; Pekkoeva et al. 2017a). This provisory organ is formed in the postlarvae stage of the daubed shanny as they begin feeding. Its size and lipid content increase during the prolonged pelagic larval development (three to five years). The lipid sac is resorbed at the demersal juvenile stage (Meyer Ottesen et al. 2011). To date, lipid sacs have only been found in Arctic-dwelling fish of the family Stichaeidae (Falk-Petersen et al. 1986; Murzina 2010; Meyer Ottesen et al. 2011).

Leptoclinus maculatus post larvae, feeding mainly on *Calanus* spp., has an important role in the Arctic food web transferring high energy lipids to fish, sea birds and mammals (Barrett 2002; Hovde et al. 2002; Weslawski et al. 2006; Labansen et al. 2007). Recent studies have revealed high biological activity in the Arctic marine ecosystems during the polar night, despite the absence of visible light (Berge et al. 2015a, b). Daubed shanny post larvae, are a visual predator, believed to feed intensely on *Calanus* spp. copepod during summer and winter, to sustain them during the polar night with little or no food. In this paper, we present new data, on the fatty acid composition of phospholipids as structural lipid type and triacylglycerols providing energy from the unique lipid sac. The data are discussed in relation to the trophic possession, development and function of the bio-membrane during the polar night.

Materials and methods

Sampling

Daubed shanny larvae were collected during the polar night, 14-20 January 2014, from the research vessel *Helmer Hanssen* in fjords on west coast Svalbard (79ºN) with MIK net, pelagic- and bottom trawl. The sea temperature was between 0.6 and 1,9 ºC and salinity approx. 34.8 psu. (Table 1)

The stages of development were defined according to the classification of Meyer Ottesen et al. (2011). The five developmental stages (L1, L2, L3, L4, and L5) were identified based on the morphological and physiological characteristics such as size, weight, colour and body pigmentation, as well as conditions of the lipid sac. During the expedition, the L4* development stage was distinguished from the L4 and L5 stages by a darker body colour, larger body and gills and the presence of a large lipid sac.

Lipid extraction

The lipid sacs of the L3, L4, and L4* development stages was dissected out from the fresh fish as soon as possible in cold conditions (Fig 1, Table 2). To prevent degradation of complex lipids, samples were fixed in 96% ethyl alcohol (10 ml in each) mixed with 0.001% of BHT as antioxidant (Murzina et al. 2013). In the laboratory,

total lipid was extracted from the samples stored in chloroform/methanol (2:1, v/v) by the method of Folch et al. (1957) and further threated as outlined by Murzina et al. (2013).

Thin-layer chromatography (TLC).

TLC was used for qualitative and quantitative determination of individual lipid classes as total phospholipids (PL), triacylglycerols (TAG), cholesterol (Chol) and cholesterols (Chol esters). Fractionation of total lipids was performed on ultrapure glass TLC Silica gel 60 F_{254} Premium Purity plates (Merck, Germany). The petroleum ether-diethyl ether-acetic acid (90:10:1 by volume) solvent system was used. After drying, the chromatogram was developed in iodine vapor.

Certain lipids (PL, TAG, Chol ester and wax esters) were quantified using the hydroxamate method that was modified by (Sidorov et al. 1972), which involves the formation of dark-brown complexes of trivalent iron ions with hydroxamic acid through ester bonding between the lipids and hydroxylamine (Walsh et al. 1965). The stain intensity was measured using a spectrophotometer (SF-2000, OKB "Spectr", Russia) at a wavelength of 540 nm. The quantitative determination of Chol was determined based on the method described by (Engelbrecht et al. 1974) using trichloroacetic iron dissolved in perchloric acid. The stain intensity was measured using a spectrophotometer at a wavelength of 550 nm. Lipid classes were identified according to the standards of the respective studied components (Sigma-Aldrich, USA; Avanti Polar Lipids, Inc., USA) taking into account the correspondence of the Rf values.

Gas chromatography

TAG and PL was analysed by gas chromatography. Material for fatty acid methylation of PL and TAG were scraped (spots) from the TLC plates. 0.1 mL of a solution containing 20 mg/10 mL (behenic FA, C22:0) (Sigma Aldrich, USA) in methanol was added as internal standard. Fatty acid methyl esters (FAME) were identified using a gas chromatograph "Chromatec-Crystal-5000.2" (Chromatec, Russia), with a flame ionization detector and a capillary gas chromatographic column Zebron ZB-FFAP (Phenomenex, USA).

The mobile phase was nitrogen. The separation mode was isothermal, the thermostat temperature of the columns was 200 °C, the temperature of the detector was 250 °C, and the temperature of the evaporator was 240 °C. Under these conditions, the methyl esters of fatty acids were divided according to their number of carbon atoms and double bonds. Chromatec-Analytik-5000.2 software (Chromatec, Russia) was used for recording and integrating the data. Fatty acids methyl esters were identified with standard mixtures of Supelco 37 Components FAME Mix (Sigma Aldrich, USA) and the lengths of the carbon chain and table constants were compared according Jamieson (Jamieson 1975). The research was carried out using the facilities of the Equipment Sharing Centre of the KarRC of RAS.

The results are given as means ± SE (standard error). Differences between means of total lipids, lipid-classes and fatty acids in the lipid sac were analysed by ANOVA (one-way) (ANOVA_$F_{1,25}$, $p=0,0250$). Differences were considered statistically significant at $p \leq 0.05$. A normal distribution was confirmed by (Shapiro–Wilk's W test, $p>0.05$).

Results

Fatty acid composition of triacylglycerols (storage lipids)

We observed large and continuous increases in size (Pekkoeva et al. 2018), volume (Figure 1, Table 1) and lipid content of the lipid sac during the development from L1 to L5 stages of development. The composition of TAG in the lipid sac of the daubed shanny at the L3, L4, and L4* developmental stages was dominated by the

MUFA, with 71%-74% of the total FA. Saturated (SFA) and the PUFA contributed much smaller amounts (Table 3). The dominant TAG FAs were the 20:1n-9 (27.5%–28.2% of the total FA) and 22:1n-11 (26.9%–29.3% of the total FA). In total, the 20:1n-9 and 22:1n-11 FA accounted for nearly 60% of the TAG. Among the PUFA, 18:4n-3, 20:5n-3, 22:6n-3 FAs were recorded, but their level was approximately equal or lower than 2% of the total FA. No significant differences in the content of these FA among ontogenetic stages were found. The SFA featuring high levels were 14:0 and 16:0 FA (up to 7%-8%).

Fatty acid composition of phospholipids (membrane lipids)

The FA composition of phospholipids (PL) at stage L3 showed a prevalence of SFA (58.1% of the total FA), whereas at stages L4 and L4*, the MUFA were dominant. A decrease ($p \leq 0.05$) in the SFA content (from 58.1% to 25.9% of the total FA) and a rise ($p \leq 0.05$) in the MUFA content (from 26.3% to 60.6% of the total FA) by stage L4* was observed. Among the MUFA in the PL, the 20:1n-9 and 22:1n-11 FA were prevalent, and their levels rose ($p \leq 0.05$) from 9.7% to 24.8% of the total FA and from 7.7% to 20.4% of the total FA by stage L4*, respectively. Remarkably, the content of the palmitoleic 16:1n-7 FA was higher in the TAG (6.2%–7.1% of the total FA) compared to the PL (2.0%–5.0% of the total FA). The prevalent PUFA in the lipid sac of the daubed shanny at stages L3, L4, and L4* were FA of the n-3 class (4.6%–7.7% of the total FA), primarily attributed to the 22:6n-3 and 20:5n-3 FA. The 16:0/18:1n-9 ratio in the PL was 2.4-2.8, which is higher than in the TAG (1.6–1.7). In the n-6 class, linoleic acid 18:2n-6 prevailed in both the PL and TAG.

Discussion

The lipid sac is a provisory organ in daubed shanny larvae, where the substantial amounts of the TAG are stored (up to 68 % dry weight), while PL, cholesterol esters and cholesterol constitute lower amounts (Falk-Petersen et al. 1896; Murzina 2010; Pekkoeva et al. 2017a) (Table 2). TAG in the lipid sac of the daubed shanny postlarvae at all investigated stages (i.e., L3, L4, and L4*) demonstrated the MUFA as the prevailing FA (71%-74% of the total FA in TAG) (Table 3). *Leptoclinus maculatus* has an important role as an intermediate in the Arctic trophic chains, where it simultaneously acts as a predator and prey.

The MUFA is a main component of storage lipids (Tocher et al. 1985; Tocher 2003), and their structure makes them energetically important for maintaining the metabolic needs of the organism; therefore, MUFA are mainly used as sources of energy (Lloret et al. 2014). The FA composition of the lipids in fish is strongly dependent on the fatty acid composition of their diet (Dalsgaard et al. 2003; Tocher 2003, 2010; Arts and Kohler 2009; Nemova et al. 2015). In Arctic ecosystems *Calanus* spp. species are the most important herbivorous zooplankter (Søreide et al. 2006; Mayzaud et al. 2015) and are the main food item for many Arctic pelagic fish, including the daubed shanny post larvae. Species of the zooplankton *Calanus* also have a lipid sac, where lipids are stored (up to 70% dry weight) primarily as wax esters. *Calanus* copepods can *de novo* synthesize 20:1n-9 and 22:1n-11 FA, which are reliable trophic biomarkers (Dalsgaard et al. 2003; Sargent and Henderson 1986; Kattner and Hagen 1995). The dominant MUFA in *Calanus glacialis*, *C. finmarchicus*, and *C. hyperboreus* are 20:1n-9, 22:1n-11, and 16:1n-7 FA (Lee et al. 2006; Mayzaud et al. 2015), which are transferred up the food chain to fish, seabirds, and mammals (Falk-Petersen et al. 2007). Wax esters from copepods enter the body of fish during feeding and converted into membrane and reserve lipids). A structure like the lipid sac in polar animals are believed to be an adaptation to the cold environment and the seasonal variations in food availability. Daubed shanny is well adapted for reproduction and development in northern latitudes (Falk-Petersen et al. 1986; Meyer Ottesen et al. 2011, 2014; Murzina et al. 2012, 2013a, b). Some studies (Mecklenburg et al. 2011) have shown the expansion of its habitat in

the seas of the Arctic Ocean. The Kongsfjorden–Krossfjorden fjord system is particularly suitable for studies of effects of climate changes on ecosystems because it lies adjacent to both Arctic and Atlantic water masses (Hop et al. 2006). The proportions of copepods, the boreal *C. finmarchicus* and local Arctic *C. glacialis* (which the daubed shanny postlarvae actively feeding on) are varying seasonally and annually in Kongsfjorden depending on the timing and volume of Atlantic and Arctic water mass intrusions (Kwasniewski et al. 2003). *Leptoclinus maculatus* was identified as an indicator species along with other fish from the Arctic region for studying variations in the structure of ecosystems of high latitudes under climatic changes (Swanburg et al. 2015).

In earlier studies (Pekkoeva et al. 2017a), we observed an increase in the content of 20:1n-9 and 22:1n-11 FA of the total lipids in the muscles of larvae of the L2 stage, likely in connection with the transition to a high-energy diet of *Calanus* spp. zooplankton from the phytoplankton-based diet at the L1 stage. These FA, which are derived from a *Calanus* diet, are mostly included in the TAG of the lipid sac (up to 28%-29% of the total FA) of L3, L4 and L4*. In this study, high amounts of 20:1n-9 and 22:1n-11 FA (up to 29% of the total FA) were found among MUFA of the TAG. In comparison, the content of MUFA in the TAG of muscles of the postlarvae (L3-L4* stages) is lower (61%-65% vs 71%-74% of the total FA in TAG) due to 20:1n-9 and 22:1n-11 FA (22%-25% and 17%-22% respectively) (Pekkoeva et al. 2019). The level of 16:1n-7 FA in the TAG of the lipid sac of the daubed shanny postlarvae is within 6%–7 % the total FA in the polar night, and 8% in the autumn (Falk-Petersen et al. 1986). The 16:1n-7 FA is known to be derived from food and used as a source of energy (Tocher 2003). The content of this acid was higher in the TAG (6.2%–7.1% of the total FA) than in PL (2.0%-5.0% of the total FA). Most of the dietary FA are incorporated into TAG, unaltered (Sargent et al.2002; Iverson 2009).

Thus, high levels of 20:1n-9 and 22:1n-11, as well as 16:1n-7 FA in the TAG of the lipid sac of postlarvae of the L3, L4, and L4* stages confirm feeding on *Calanus* spp., both in the pelagic zone and near the bottom during the polar night. A rise from 23.6% to 60.6% of the total FA was demonstrated for the MUFA content in the PL is due to the increase in 20:1n-9 and 22:1n-11 FA (from 9.7% to 24.8%, and from 7.7% to 20.4% of the total FA, respectively. The FA composition of food items of fish is known to influence to involvement of individual FA in the adaptive transformation of the organism's biomembranes in response to the environmental and food (Dalsgaard et al. 2003; Arts and Kohler 2009; Tocher 2010; Murzina et al. 2012b; Nemova et al. 2015). It has been suggested that increase hydrostatic pressure and low temperature effect the biomembrane (Velansky and Kostetsky 2008) as shown with the increase of MUFA with depth. In our previous research, we found that the MUFA/PUFA content in the TL is higher in the arctic *L. maculatus* (Fries 1838) (Isfjord, Spitzbergen) inhabiting at 0ºC temperatures at 206 m compared with the subarctic *Lumpenus fabricii* (Reinhardt 1836) (White Sea) collected from two habitats in the temperature range of 5.9–6.7ºC at depths down to 38 m (Murzina et al. 2013). Despite the known genetic determinacy, the FA composition of the PL may vary at early ontogenetic stages in fish in response to environmental factors (Tocher et al. 2008). The high dietary supply of 20:1n-9 and 22:1n-11 FA deposited in the lipid sac of daubed shanny can influence the FA composition of the PL, which are involved in the adaptation of biomembranes to extreme environments. A decrease (two-fold) of the SFA content in the PL (58.1%-26% of the total FA) in the lipid sac was detected, possibly due to a demand for a modification of the fatty acid composition. This implies a replacement of SFA with MUFA in the biomembranes in the ontogenetic transition from L3 to L4* to a demersal stage of life.

The content of PUFA is 2.5 - fold lower in the PL and seven - folds lower in the TAG of the lipid sacs compared to the MUFA content. The PUFA content in the PL is considerably lower in the lipid sac (up to 18.7% of the total FA) than in the muscles (up to 45% of the total FA) at these stages of postlarval development (Pekkoeva et

al. 2019), suggesting that they have a major role in maintaining the functioning of complex biomembranes. Polyunsaturated fatty acids of the n-3 class were found to prevail over n-6 PUFA in the PL and TAG in the lipid sac.

Very low level of 18:2n-6 and 18:3n-3 FA in the PL and TAG in the lipid sac (≤2.3% of all FA in the total lipids) was observed. However, moderate to low levels of 22:6n-3, 20:5n-3, and 18:4n-3 prevailed, which is typical of marine organisms at high latitudes (Sargent et al. 2002; Burri et al. 2012; Mayzaud et al. 2015), who derive the FA from their food. The PL were dominated by 22:6n-3 (up to 4.6%), and the TAG by 18:4n-3 (up to 2.0%). The 22:6n-3, 20:5n-3 FA are known to be essential for marine predaceous fish and are supplied in large amounts in food, whereas 18:2n-6 and 18:3n-3 FA are less important for growth and development. Marine fish have low capacity to convert these FA into highly unsaturated FA (Sargent et al. 1995; Tocher 2003).

Conclusions

We present, for the first time, data on the FA composition of the membranes and storage lipids in the lipid sac of the postlarvae stages (L3, L4 and L4*), of the daubed shanny from the polar night. A distinctive feature of the FA profile of the lipid sac in young fish of the daubed shanny is that the TAG and PL contain high levels of the MUFA, primarily 20:1n-9 and 22:1n-11 FA, which are biomarkers of the zooplankton *Calanus* spp.. The high content of these FA in the daubed shanny larvae indicate that *Calanus* copepods are the main food source for juveniles during the polar night. These data are important for the analysis of the putative pathways of the FA transformations and transfer in the food web of the Arctic ecosystem. Data on the fatty acid composition of the TAG and PL in the lipid sac of postlarvae daubed shanny developing under polar night conditions can contribute to the understanding of the role of lipids in the early ontogenetic ecological-biochemical adaptations of this Arctic fish species whose life cycle has, so far, been poorly studied.

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Compliance with Ethical Standards

The authors declare no conflict of interest and that all applicable institutional, national or international guidelines for the use and care of animals were strictly followed in the present study.

Conflict of interest: The authors declare no conflicts of interest.

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357 acids, the values of that did not exceed 1%: 12:0; 15:0; 17:0; 20:0; 24:0; 16:1(n-5); 18:1(n-5); 22:4(n-6); 22:3(n-3);
358 22:4(n-3) FA.
359 **Fig 1** *Leptoclinus maculatus* early life stages (L1, L2, L3, L4, L4*, L5) (Pekkoeva et al., 2018).
360 **Fig 2** Map of sampling of the *Leptoclinus maculatus* postlarvae in fjords (Kongsfjord) west coast of Svalbard (79ºN)
361 (Google Earth)
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364 **Table 1** Data of sampling of the *Leptoclinus maculatus* postlarvae in Kongsfjord (Svalbard)

Stage	L1	L2	L3	L4	L4*	L5
Samples	6	30	40	30	12	17
Length, cm (Pekkoeva et al., 2018)	3.4	5.8	6.5	7.5	8.9	9.0
Equipment	MIK net	Pelagic trawl			Bottom trawl	
Depth of sampling, m	30	125			>130	
Temperature, °C	0.60–1.90	0.90–1.10			0.60–0.80	
Salinity, psu	34.8	34.8			34.7-34.8	

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Table 2 The content of lipid classes (% of dry weight) in the lipid sac of postlarvae *Leptoclinus maculatus* of different developmental stages (L3, L4, L4 *) from Kongsfjord. c – the value significantly differs from that of stage L3 ($p \leq 0.05$), d – the value significantly differs from that of stage L4 ($p \leq 0.05$).

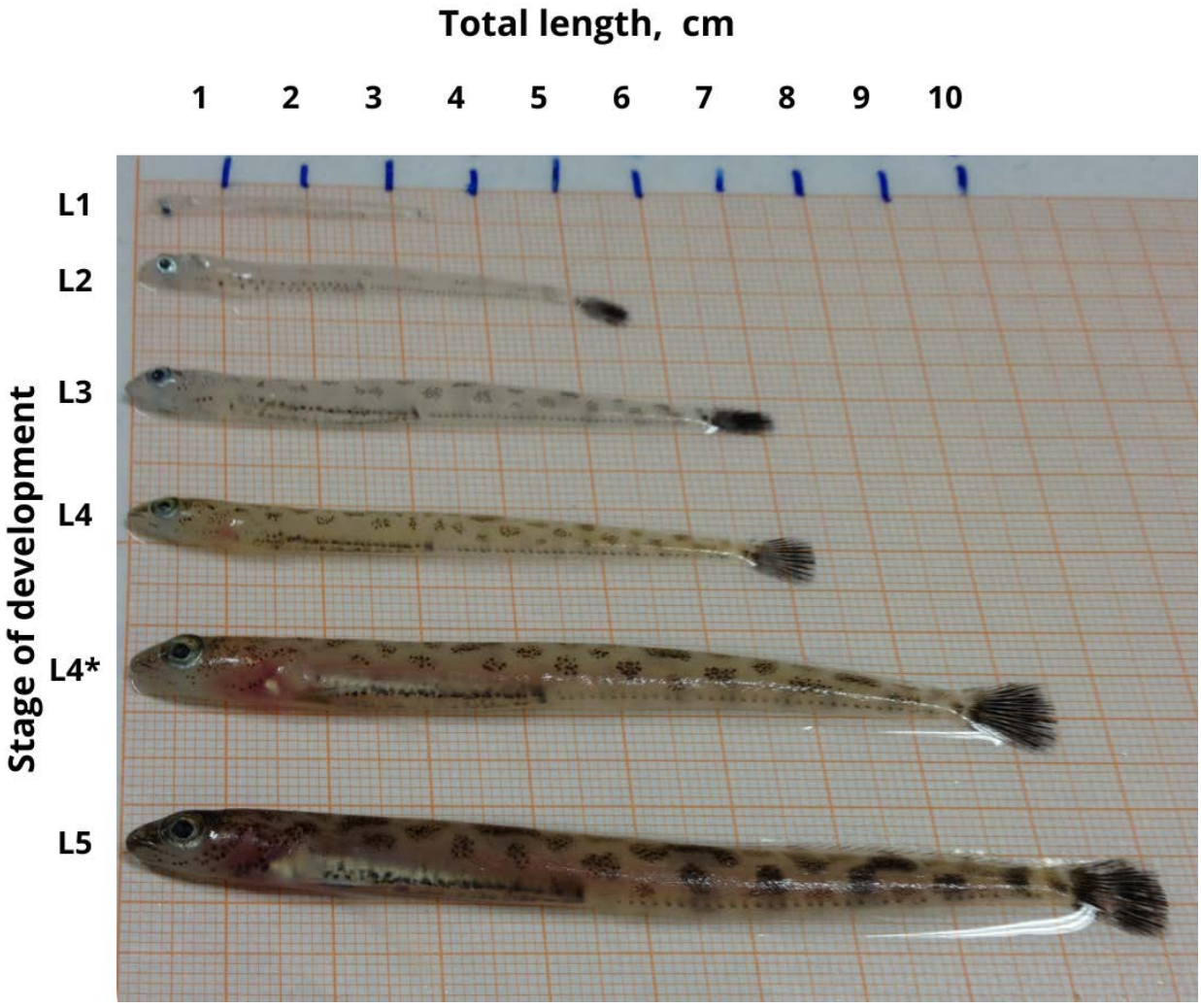
Stage of development	L3	L4	L4*
Total lipids	75.97±2.79	84.63±1.92 ^{c}	92.32±0.82 ^{cd}
Dry mass	24.03±2.79	15.37±1.92 ^{c}	7.68±0.82 ^{cd}
Total lipids / Dry mass	3.16	5.51	12.02
Structural lipids			
Phospholipids	0.84±0.31	13.11±4.78 ^{c}	31.01±5.36 ^{cd}
Cholesterol	3.42±0.35	11.31±4.36 ^{c}	4.37±0.89 ^{d}
Energetic lipids			
Triacylglycerols	68.75±2.47	55.15±5.83 ^{c}	55.95±5.76 ^{c}
Cholesterol esters and wax esters	2.96±0.42	4.06±1.09	1.01±0.30 ^{cd}

Table 3 The content of some fatty acids in the composition of phospholipids and triacylglycerols in the lipid sac of the daubed shanny postlarvae. Abbreviations: SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. c – the value significantly differs from that of stage L3 ($p \leq 0.05$). The samples contained other fatty acids, the values of that did not exceed 1%: 12:0; 15:0; 17:0; 20:0; 24:0; 16:1(n-5); 18:1(n-5); 22:4(n-6); 22:3(n-3); 22:4(n-3) FA.

	Phospholipids			Triacylglycerols		
Stage	L3	L4	L4*	L3	L4	L4*
14:0	2.6±0.2	5.5±1.0	6.6±0.5^{c}	7.0±0.1	6.5±0.4	6.0±0.2
16:0	13.2±0.9	11.2±1.0	9.9±0.2^{c}	8.2±0.2	7.7±0.4	7.5±0.1
18:0	6.7±03	3.3±0.6	2.1±0.2	0.9±0.1	0.7±01	0.8±0.0
∑SFA	58.1±2.7	31.6±2.7^{c}	25.9±1.4^{c}	18.9±0.4	16.7±1.0	16.2±0.4^{c}
16:1n-7	2.0±0.2	3.5±0.4	5.0±0.3	6.2±0.2	6.4±0.2	7.1±0.2
18:1n-9	4.7±0.3	4.5±0.3	4.1±0.1	5.3±0.1	4.6±0.3	4.6±0.1
20:1n-9	9.7±0.8	19.4±3.1	24.8±1.4^{c}	27.5±0.2	28.0±0.4	28.2±0.3
22:1n-11	7.7±0.7	16.5±2.7	20.4±1.5	26.9±0.6	29.3±0.7	27.8±0.7
∑ MUFA	26.3±1.9	49.7±6.3^{c}	60.6±3.3^{c}	70.7±0.6	73.7±0.4	73.1±1.0
18:2n-6	2.3±0.1	1.8±0.3	1.4±0.2	2.2±0.1	1.9±0.1	1.9±0.1
20:4n-6	0.1±0.0	0.3±0.1	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
∑ n-6 PUFA	2.3±0.1	5.1±1.1	3.4±0.5	3.0±0.2	2.6±0.1	2.7±0.1
18:3n-3	0.4±0.1	0.2±0.0	0.2±0.0	0.8±0.0	0.5±0.1	0.5±0.1

18:4n-3	0.8±0.1	0.5±0.1	0.7±0.1	1.9±0.1	1.7±0.4	2.0±0.4
20:5n-3	1.5±0.4	0.8±0.3	0.5±0.1	1.0±0.1	0.7±0.2	1.0±0.3
22:6n-3	4.6±0.5	2.8±0.4	2.0±0.2	1.6±0.1	1.3±0.2	1.5±0.3
∑ n-3 PUFA	7.7±0.9	6.3±1.1	4.6±0.6[c]	5.8±0.3	4.8±0.7	5.7±1.2
∑ PUFA	15.6±1.1	18.7±3.6	13.5±1.9	10.4±0.5	9.7±0.6	10.7±1.3
∑ n-3/∑ n-6	3.4±0.3	1.3±0.1	1.4±0.1	2.0±0.1	1.8±0.3	2.0±0.4
∑SFA/∑PUFA	3.7	1.7 [c]	1.9 [c]	1.8	1.7	1.5
18:3n-3/18:2n-6	0.2	0.1	0.1	0.3	0.2	0.3
16:0/18:1n-9	2.8	2.4	2.4	1.6	1.7	1.6

381 **Fig 1**



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384 **Fig 2**


N80°30'
N79°30'
Kongsfjord
Svalbard
N78°30'
E 7°30'
E 12°30'
E 17°30'
E 22°30'
E 27°30'
E 32°30'
N77°30'
N76°30'
Image Landsat / Copernicus
Image IBCAO

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