

Article

# Tracing the Food Web of Changing Arctic Ocean: Trophic Status of Highly Abundant Fish, *Gasterosteus Aculeatus* (L.), in the White Sea Recovered Using Stomach Content and Stable Isotope Analyses

Genelt-Yanovskaya A.S.<sup>1</sup>, Polyakova N.V.<sup>2</sup>, Ivanov M.V.<sup>1</sup>, Nadtochii E.V.<sup>1</sup>, Ivanova T.S.<sup>1</sup>, Genelt-Yanovskiy E.A.<sup>1,3</sup>, Tiunov A.V.<sup>2</sup>, Lajus D.L.<sup>1</sup>

<sup>1</sup>Saint Petersburg State University, 199034 Saint Petersburg, Russia

<sup>2</sup>A. N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, 119071 Moscow, Russia

<sup>3</sup>Zoological Institute Russian Academy of Sciences, 199034 Saint Petersburg, Russia

\*Correspondence: anndemch@gmail.com

**Abstract:** Studies of dietary preferences of migratory species are of great importance as these species connect food webs of habitats across the migration route and thus represent trophic relationships between the spatially disjointed communities. Here we describe dietary preferences of threespine stickleback *G. aculeatus* in the White Sea during the spawning season using stable isotope and stomach content analyses. Both analyses indicated that during the spawning season, when sticklebacks spend most of the time in the inshore, their diet significantly consist of benthic species in contrast to the start of the spawning season when fishes migrating from the offshore are feeding on zooplankton. Also, we show that stickleback eggs contribute greatly to the diet of both male and female fishes. Using Bayesian mixing modelling we show that dietary preferences in females were broader than in males, and more variable during the spawning season. Males fed on eggs almost while guarding their nests. Both stomach contents and isotope signatures demonstrate that by the end of the spawning season sticklebacks again increase consumption of plankton, and isotope analysis proved to be more reliable tool to trace this change than stomach content analysis. Our results show that stable isotope and stomach content analyses well supplement each other in understanding of seasonal changes in dietary composition of stickleback.

**Keywords:** threespine stickleback; *Gasterosteus aculeatus*; stomach content analysis; stable isotope analysis; fish diet; the White Sea; boreal fish; Subarctic.

## 1. Introduction

Arctic marine communities are vulnerable to climatic oscillations and anthropogenic pressures. Here the recent warming is occurring at a rate that is more than twice compared to the global rate [1,2]. As a result, today's Arctic ecosystem is a rapidly changing environment where some species, especially widely distributed, can benefit by taking new niches, while others can be stressed by facing non-optimal conditions [3]. As a result, temperate communities are predicted to shift northwards into polar regions [4]. Subarctic ecosystems, such as the White Sea, are currently thus under specific attention to monitor how the ongoing global change will be reflected in the structure of communities inhabited by both boreal and arctic species. The White Sea is a semi-enclosed marine area mostly located to the south from the Arctic Circle and connected to the Barents Sea via the narrow Gorlo strait [5]. Additionally, the White Sea is a marine area characterized by relatively low anthropogenic impacts, even commercial fisheries are minor compared to the adjacent Barents Sea [6–8].

Small fishes play fundamental roles in pelagic ecosystems, as they transfer energy and nutrients to higher trophic levels [3]. The White Sea population of the threespine stickleback *Gasterosteus aculeatus* has been the subject of extensive research in recent years, as abundance of this small fish is growing here in line with other populations across northern Europe [9–14]. Stickleback has negligible commercial significance and has been targeted by fisheries only during periods of high abundance. Sticklebacks play a critical role in the marine ecosystem and often recognized as a species forming the ‘wasp waist’ in a food web [15], as being responsible for a remarkable energy flow between lower trophic levels (e.g., planktonic communities) and higher trophic levels including top predators. In recent decades threespine stickleback along with herring *Clupea sp.* are the most abundant fishes in the White Sea [11,13]. It is evident that its role of so massive species in trophic chains must be very high, but information on that is very limited [16,17].

Stable isotope analysis is among the most informative methods for studying trophic relationships of organisms, diet and trophic positions, sources of primary production, origin of particular organic matter in an ecosystem and type of ecosystem itself [18–23]. Values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  represent the major energy flow pathways at lower trophic levels, offering a time-integrated measure of the organism’s trophic position, accounting for temporal and spatial variation in feeding at multiple levels of the food web, and detecting trophic interactions that are otherwise unobservable, as stomach contents can differ from the material actually assimilated by an organism [24]. Stable isotope analysis does not require assumptions of prey trophic levels, thus can be applied at lower trophic levels as well [25]. Additionally, knowledge of trophic position of populations within species allows differentiation between cryptic species or revealing previously unknown aspects of their biology [26].

The present study aims on the assessment of trophic status of the White Sea threespine stickleback during spawning period with the specific reference to habitat heterogeneity. We used two complimentary methods – analyses of stomach content and stable isotope composition of nitrogen and carbon in the fish tissues and their prey organisms. By comparing results of these two approaches, we expect to better understand the role of *G. aculeatus* in energy flows between the open sea and inshore communities of the White Sea. As the ongoing climate changes strongly affects Arctic marine ecosystems, studies of diet of widely distributed and highly abundant fish such as threespine stickleback spawning in the inshore and wintering in the open sea is important for predicting future changes in the biota.

## 2. Materials and Methods

### 2.1 Study area and field sampling

Samples of threespine stickleback were collected at four sites near the Education and Research station “Belomorskaya” of the Saint-Petersburg State University in the Kandalaksha Bay of the White Sea. Samples for the main dataset (three inshore sites) were collected between June and July 2016 during the long-term monitoring studies of *G. aculeatus* (Figure 1). These sites represented various types of stickleback spawning grounds (Table 1) [13,16,27,28]. Inshore samples were collected in three periods of stickleback spawning season, particularly (i) beginning of the season (start of the first decade of June), (ii) middle (end of the second decade of June) and end (start of the first decade of July).

In early June 2019, before the start of spawning of stickleback, an offshore sample was collected at the center of Chupa Inlet entrance of Kandalaksha Bay (*CIE*) at 950 meters from the shoreline and above approx. 50 meters depth.

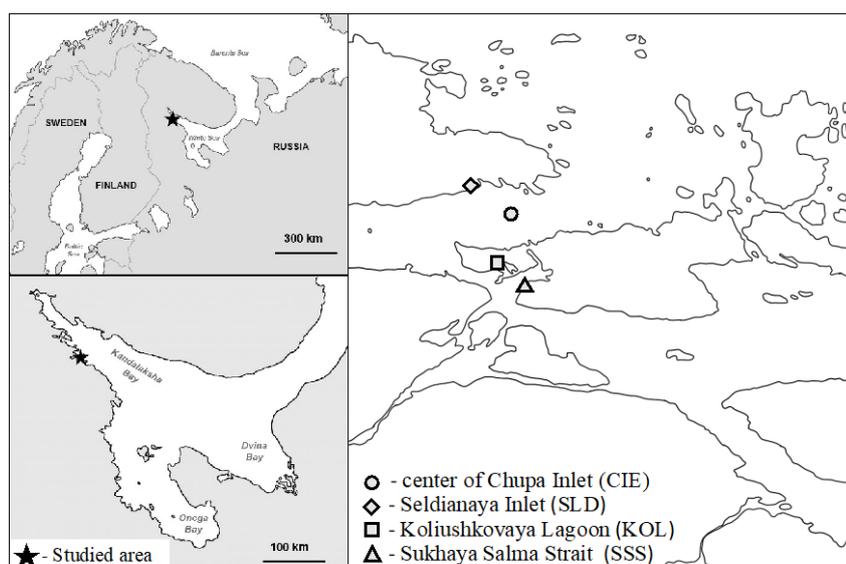


Figure 1. The study area

Table 1. Characteristics of abiotic and biotic conditions of the inshore sampling sites (see text for references).

Variable	Seldianaya Inlet (SLD)	Koliushkovaya Lagoon (KOL)	Sukhaya Salma Strait (SSS)
Geographical coordinates	66.33822° N, 33.62149° E	66.31307° N, 33.64644° E	66.31208° N, 33.65021° E
General description	Triangular inlet 120 x 240 m with wide entrance and shallow top. Average depth is 3.0 m	Isolated lagoon 200 x 540 m with average depth 1.5 m	Open strait with slope 6–8 cm/m in the study area
Tide amplitude, m	up to 2.5	up to 0.3	Up to 2.5
Surface water temperature at sampling in 2016, °C	May – 12, July – 20	May – 14, July – 22	May – 12, July – 20
Surface salinity at sampling in 2016, ppt	May – 23, July – 24	May – 15, July – 20	May – 21, July – 19
Bottom type	Stony littoral and muddy sublittoral zones	Muddy littoral and sublittoral zones	Stony littoral and muddy and sandy sublittoral zones
Aquatic vegetation	Fucoids in the littoral zone, dense eelgrass <i>Zostera marina</i> beds with dry biomass 1 kg/m <sup>2</sup> and projective cover – up to 100 %	Eelgrass beds near the sea entrance with dry biomass up to 0.1 kg/m <sup>2</sup> and projective cover up to 30 %, filamentous algae	Fucoids in the littoral, eelgrass with dry biomass up to 0.003 kg/m <sup>2</sup> in sublittoral zone

Inshore samples were collected using a beach seine with length and high of wings 7.5 and 1.5 m respectively, a mesh-size was 5 mm from knot to knot in the wings and 1 mm

in the codend. In few cases, sticklebacks were caught using hand nets. Samples at the off-shore site was collected using the surface twin trawl with characteristics similar to the beach seine. In 2016, simultaneously with fish samples, we also collected planktonic and benthic samples to analyze stable isotopes of main putative prey items for stickleback. Qualitative planktonic samples in one replicate were collected with a plankton net (size 93 mm) by filtering the surface water and consisted of mixture of zooplankton and phytoplankton organisms inhabiting the shallow water near the low water level mark. Intertidal benthic invertebrates were collected using benthic rectangular dredges.

## 2.2 Laboratory analyses

All fish were weighed ( $\pm 0.01$  g), measured for total length (TL) ( $\pm 0.1$  mm) and sexed by observing the gonads. Boneless and skinless muscle tissue samples were individually frozen for further stable isotope analysis. Other specimens for stomach content analysis were fixed with 4% formaldehyde. For stomach content analysis, all zooplankton organisms were identified to the possible lowest taxonomic unit and counted ( $Q_i$ ). The best-preserved specimens of each prey item (up to 10 individuals) were measured with a micrometer eyepiece scale (up to 0.03 mm) for calculations of their biomass ( $I_i$ ). The individual masses of prey organisms were determined based on their body length [29,30] or ready-average mass [31], and then summed up to obtain the total mass of particular prey item. In total, 264 *G. aculeatus* individuals were analyzed for stomach content analysis.

For stable isotope analysis, we have analyzed 175 samples from inshore sites (SLD, KOL, SSS). Stable isotope samples from CEO were not collected. Among them, 90 samples were sticklebacks equally represented by males and females (45 and 45 individuals respectively). Thus, each of three periods of spawning season was represented by 15 *G. aculeatus* specimens of each sex per site. Additionally, 85 analyzed samples were taken from putative planktonic and benthic prey objects. Planktonic samples were not subjected to taxonomical species identification and thus were analyzed totally. Organisms from benthic samples were pooled by high-level taxonomic units (e.g. Polychaeta, see Figure 4 for details) to achieve sufficient biomass for the stable isotope analysis.

All samples were dried for 48–72 h at about 50 °C. After drying, samples were put into small tin capsules and weighed using a Mettler Toledo MX 5 balance with an accuracy of  $\pm 1\mu\text{g}$ . At least (174 samples) in three replicates of each type of samples were prepared and analyzed.

The stable isotope analysis (SIA) was performed according to standard methods [32] using a Thermo Delta V Plus isotope mass spectrometer (Thermo Scientific, United States) equipped with an element analyzer (Thermo Flash 1112) at the Joint Usage Center of A.N. Severtsov Institute of Ecology and Evolution of RAS (Moscow, Russian Federation). Isotopic composition of C and N in organic matter was expressed in  $\delta$ -notation relative to international standard ( $\nu\text{PDB}$  for carbon and the atmospheric  $\text{N}_2$  for nitrogen) (1).

$$\delta(\text{‰}) = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 103 \quad (1)$$

where  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{15}\text{N}/{}^{14}\text{N}$ . Samples were analyzed with reference gas calibrated against IAEA (Vienna, Austria) reference materials USGS 40 and USGS41. The drift was corrected using an internal laboratory standard (casein). The standard deviation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in the laboratory standard was  $\pm 0.2\text{‰}$

## 2.3 Data analysis

For fish, percent number ( $\%Q_i$ ), percent biomass ( $\%I_i$ ), and percent frequency of occurrence ( $\%F_i$ ) were calculated, along with the index of relative importance (IRI<sub>i</sub>) and percent IRI ( $\%IRI_i$ ) of each their prey items [33,34] using the following equations (2, 3):

$$IRI_i = (\%Q_i + \%I_i) \cdot \%F_i \quad (2)$$

$$\%IRI_i = [IRI_i / \sum(IRI_i)] \times 100 \quad (3)$$

Comparison of stomach contents between male and female sticklebacks was implemented using one-way PERMANOVA analysis using Bray-Curtis similarity index, and SIMPER test. Based on the stomach content data the D-index was also calculated, indicating the number of taxa from stomach content significantly contributing to the diet of fish [35].

Feeding intensity was measured as index of fullness ( $FI, \%$ ) calculated (4) at first for each individual, and then averaged per species [36].

$$FI = 100 \frac{WS}{TW} \quad (4)$$

where  $WS$  is the total weight intestinal tracts/stomachs contents and  $TW$  is the total weight of fish.

Trophic position was calculated by two methods. The first is more well-known [19,20] and based on difference of  $\delta^{15}N$  content in tissues of consumer and prey (also called the base) (5):

$$trophic\ position = (\delta^{15}N_{consumer} - \delta^{15}N_{base})/a + 2 \quad (5)$$

where  $a$  is a diet enrichment factor (3.2 for fish and their eggs; 3.4 for invertebrates) and 2 is the trophic level of the baseline organism (in our case it is the sample with the minimal isotope signature in each spawning season) [37]. Further, on these values are called as "observed".

The second method is based on stomach contents and stable isotope values of prey organisms [19] allowing to assess expected trophic position (6):

$$trophic\ position = \sum(I_i/T_i) + 1 \quad (6)$$

where  $I_i$  is the percent of biomass of prey item  $i$ , and  $T_i$  is the trophic position of prey item  $i$ , based on literature data on feeding ecology [19]. In the following sections, we will call these values as "expected".

To estimate proportion of each diet component we used Bayesian mixing models, which were performed in MixSIAR package in R [38]. To calculate the model, we prepared a set of data including (i) mean values of  $\delta^{13}C$  and  $\delta^{15}N$ , its standard deviation or standard error for predator (in our case these are males and females of threespine stickleback); (ii) mean values of  $\delta^{13}C$  and  $\delta^{15}N$ , its standard deviation or error for each prey organism; (iii) a set of trophic discrimination factors, which are calculated with the following equations (7; 8) [39].

$$\Delta^{13}C = \delta^{13}C_{predator} - \delta^{13}C_{diet} \quad (7)$$

$$\Delta^{15}N = \delta^{15}N_{predator} - \delta^{15}N_{diet} \quad (8)$$

where  $\delta^{13}C$  and  $\delta^{15}N$  are the carbon and nitrogen isotope values derived from the predator's tissue.

Statistical analyses were performed using standard spreadsheets software (MS Excel 2013), STATISTICA v7.0 and PAST v.411. Separate factorial ANOVA analyzes were run on individual parameters (factors: Sex, Period, Site; input data were  $\delta^{13}C$ ,  $\delta^{15}N$  and observed trophic position - TP). Fisher's post hoc comparisons were used to assess differences between sites, sexes and period of spawning season. Generalized linear models

(GLM) were used to evaluate factors affecting the intensity of adult stickleback feeding at spawning sites.

### 3. Results

#### 3.1 Feeding intensity

Microscopic analysis of randomly sampled sticklebacks revealed no individuals with empty stomachs. Feeding intensity of fishes, measured as index of fullness (*FI*, ‰) is shown on Figure 2. Further comparison of *FI* using generalized linear models (GLMs) revealed that *period of spawning season* had significant effect on *FI* ( $p=0,025$ ). Other studied factors (*Standard Length*, *Site and Sex*) alone did not have significant effect.

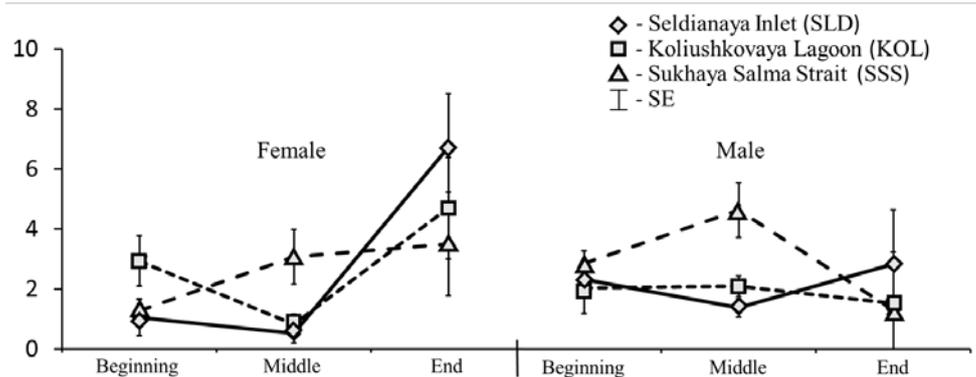


Figure 2. Feeding intensity of threespine stickleback during the spawning season. The Y-axis represent stomachs fullness index *FI* (‰). The dots and bars represent the sample means and standard errors (SE).

Two factor combinations had significant effects, particularly (1) *Period of spawning season & Site* ( $p=0,003$ ) and (2) *Period of spawning season & Sex* ( $p=0,001$ ). The post-hoc test indicated that during the beginning of the spawning, *FI* does not differ significantly between males and females at all sites studied. At the end of the spawning period, females demonstrated significantly higher *FI* than males at *KOL* and *SLD* ( $p=0,007$  и  $0,027$  respectively).

#### 3.2 Stomach content

Diet of stickleback in open water site *CIE* before the start of spawning season (early June) consisted of 24 planktonic taxa, with prevalence of *Calanus glacialis* (50%) and Euphausiacea varia (30%) (Figure 3). The number of taxa in fish diet in the inshore zone between June and July was significantly greater - up to 33 species (ANOVA,  $F_{1,99}=36.79$ ,  $p<0.001$ ). On average,  $5.5\pm 0.6$  prey items were found in stomachs of fishes from inshore sites in contrast with  $3\pm 0.5$  prey items in stomachs of fishes from the offshore site. Diet of fish at inshore sites was characterized by notably higher D-index than the offshore site. At the offshore site *CIE* D-index varied per individual fishes between 1.4 and 1.6 in females, and between 1.7 - 2.2 in males. At the inshore sites, D-index varied between 3.1 and 3.8 in females, and between 2.7 and 3.7 in males.

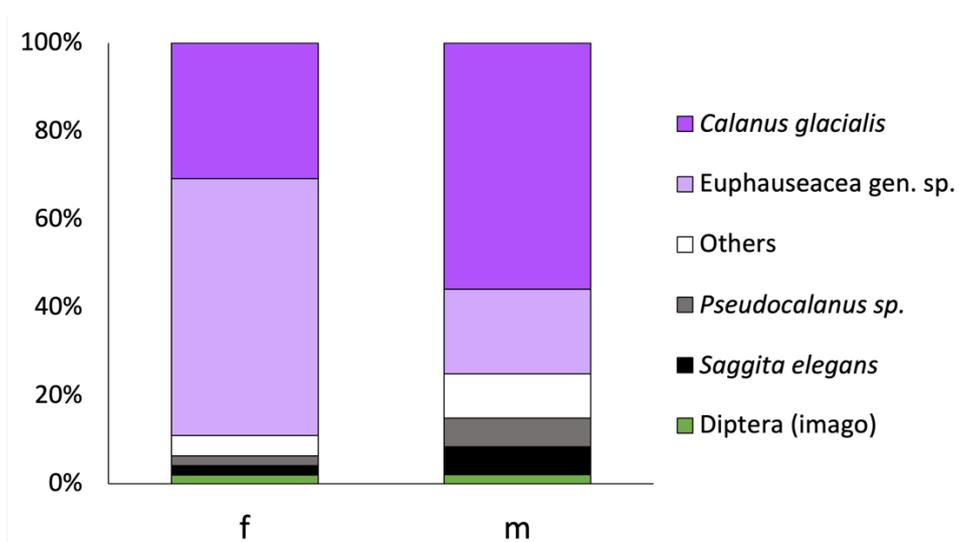


Figure 3. Stomach contents of female (F) and male (M) threespine stickleback at the offshore site CIE in the beginning of spawning period

According to stomach content analysis, stickleback eggs were the most important prey item of sticklebacks at the inshore sites during the whole spawning season, comprising more than 90% of stomach content in 25% of analyzed individuals. *G. aculeatus* demonstrated a switch from a planktonic to a benthic feeding, preying on polychaetes (up to 85%), pupae and larvae of Chironomidae, amphipods and imago stages of Diptera (Figures 3 and 5; Table 2).

Table 2. Stomach contents (IRI, %) of females (F) males (M) at the offshore and inshore sites.

Taxa	Offshore site		Inshore sites	
	IRI, % (F)	IRI, % (M)	IRI, % (F)	IRI, % (M)
Diatomeae <i>gen. sp.</i>	3.2	0.3	1.5	0.0
<i>Calanus glacialis</i>	<b>20.7</b>	<b>61.6</b>	-	-
<i>Oithona similis</i>	2.5	0.2	-	-
<i>Pseudocalanus sp.</i>	9.3	<b>19.7</b>	-	-
Copepoditii Copepoda	<b>16.0</b>	3.0	-	-
Euphauseacea <i>gen. sp.</i>	<b>33.9</b>	9.5	-	-
Gastropoda varia	0.0	0.2	5.8	0.5
Polychaeta varia	0.0	0.0	6.5	1.7
Amphipoda varia	0.5	0.0	1.7	0.5
Chironomidae varia	-	-	8.0	4.2
Diptera (imago)	1.1	1.0	1.8	1.8
<i>Gasterosteus aculeatus</i> eggs	-	-	<b>67.8</b>	<b>82.0</b>
Other planktonic food	12.7	3.4	5.6	9.3
Other benthic food prey	0.0	1.0	0.4	0.01

Based on taxonomic identification of prey items from stomach contents, diet of sticklebacks at the inshore sites throughout the season consisted of 25 taxa in female fishes and 17 taxa in male fishes (Table 2). Nevertheless, no significant differences were found between stomach contents of males and females at the inshore sites during the spawning season (PERMANOVA  $F=1.376$ ,  $p=0.24$ ). According to SIMPER test, overall average dis-

similarity was 38.48, i.e., less than 40%). On the contrary, diet of stickleback differed related between sexes at the offshore site sampled before the start of the spawning season (PERMANOVA  $F=1.376$ ,  $p=0.24$ ; SIMPER overall dissimilarity = 90.76).

### 3.3 Stable isotopes values in sticklebacks, benthic and planktonic invertebrates

In sticklebacks,  $\delta^{13}C$  values varied between  $-25.76\text{‰}$  and  $-19.34\text{‰}$  in males, and from  $-22.76\text{‰}$  to  $-19.34\text{‰}$  in females. The  $\delta^{15}N$  varied between  $11.19\text{‰}$  and  $13.65\text{‰}$  in females, and between  $12.07\text{‰}$  and  $13.82\text{‰}$  in males (Table 3). Difference in  $\delta^{15}N$  values between sexes was significant, differences of  $\delta^{15}N$  values in sticklebacks (sexes pooled together) between sites and periods of spawning season were not significant. Differences in  $\delta^{13}C$  carbon values between different sites were also significant, Koliushkovaya Lagoon (KOL) was different from other sites (ANOVA, post hoc  $p<0.01$ ) (Figure 5; Table 3, 4).

Table 3. Values and ranges of  $\delta^{13}C$  and  $\delta^{15}N$  and trophic position (TP, mean  $\pm$  SE) in males, females and eggs of *Gasterosteus aculeatus*.

Sex and spawning period	n	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$ range	$\delta^{15}N$ range	TP observed	TP expected
<i>Threespine stickleback Gasterosteus aculeatus</i>							
Females, beginning	15	$-22.15 \pm 0.101$	$12.58 \pm 0.140$	-22.76 to -21.51	11.19 to 13.24	$5.22 \pm 0.044$	4.2
Females, middle	15	$-21.02 \pm 0.108$	$13.02 \pm 0.087$	-21.57 to -20.33	12.41 to 13.65	$5.32 \pm 0.027$	4.2
Females, end	15	$-20.16 \pm 0.111$	$12.56 \pm 0.135$	-20.75 to -19.34	11.53 to 13.59	$5.04 \pm 0.042$	3.8
Males, beginning	15	$-22.05 \pm 0.272$	$12.96 \pm 0.138$	-25.48 to -21.26	12.07 to 13.8	$5.34 \pm 0.043$	4.4
Males, middle	15	$-21.24 \pm 0.131$	$13.05 \pm 0.117$	-22.1 to -20.64	12.11 to 13.75	$5.33 \pm 0.037$	4.1
Males, end	15	$-20.55 \pm 0.052$	$13.09 \pm 0.118$	-20.85 to -20.07	12.11 to 13.82	$5.21 \pm 0.037$	4.2
Stickleback eggs	3	$-22.45 \pm 0.315$	$12.63 \pm 0.104$	-22.81 to -21.95	12.47 to 12.75	$5.2 \pm 0.033$	
<i>Prey organism</i>							
Amphipoda	15	$-16.98 \pm 0.249$	$4.86 \pm 0.451$	-18.93 to -15.67	2.4 to 8.61	$2.66 \pm 0.124$	
Chironomidae	21	$-18.47 \pm 0.217$	$4.96 \pm 0.421$	-20.12 to -15.79	2.27 to 6.88	$2.73 \pm 0.125$	
Gastropoda	12	$-15.06 \pm 0.341$	$4.91 \pm 0.31$	-16.97 to -13.21	3.2 to 6.13	$2.65 \pm 0.095$	
Isopoda	3	$-16.27 \pm 0.166$	$5.76 \pm 0.177$	-16.49 to -16.03	5.6 to 6.05	$2.86 \pm 0.052$	
Oligochaeta	3	$-19.01 \pm 0.36$	$7.56 \pm 0.214$	-19.52 to -18.51	7.31 to 7.9	$3.56 \pm 0.063$	
Polychaeta	5	$-16.48 \pm 0.64$	$8.3 \pm 0.14$	-18.11 to -15.71	8.12 to 8.65	$3.2 \pm 0.567$	
Plankton	22	$-22.22 \pm 0.373$	$7.26 \pm 0.394$	-24.64 to -16.65	5.36 to 12.14	$3.39 \pm 0.114$	

Table 4. P-values resulted from three-way ANOVA (Sex, Period, Site) for  $\delta^{13}C$ ,  $\delta^{15}N$  and observed trophic position (TP) (significant values are marked in bold)

Variables	Factor		
	Sex	Period	Site
$\delta^{13}C$	0.12	<b>&lt;0.01</b>	<b>0.03</b>
$\delta^{15}N$	<b>&lt;0.01</b>	0.07	0.79
TP	<b>&lt;0.01</b>	<b>&lt;0.01</b>	0.86

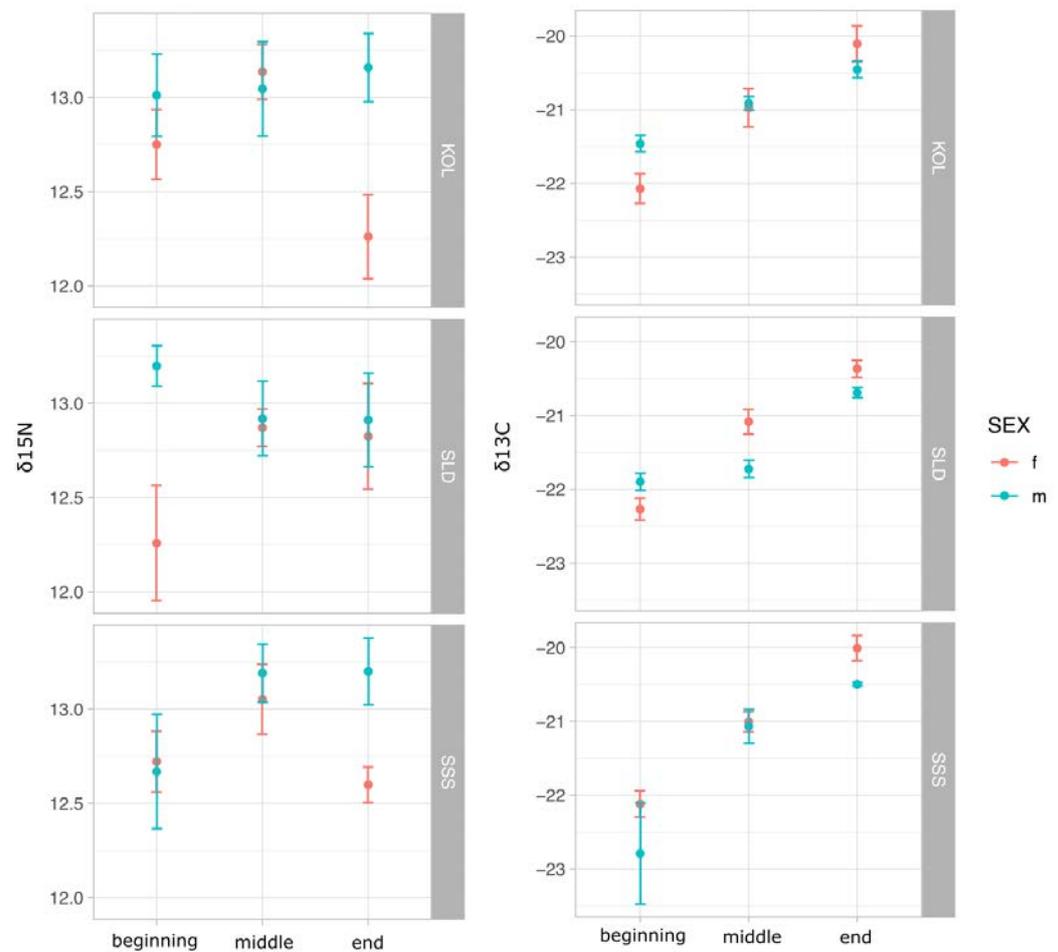


Figure 4. Mean ( $\pm$ SE) stable isotope values of carbon  $\delta^{13}\text{C}$  and nitrogen  $\delta^{15}\text{N}$  of male female sticklebacks during three periods of spawning season. See Figure 1 for site description.

Stable isotope values ( $\delta^{13}\text{C}$ ) differentiated potential prey items for *G. aculeatus* into two groups corresponding to planktonic and benthic taxa respectively (Table 3, Figure 5) with  $\delta^{13}\text{C}$  values in plankton lower than in benthos. From the beginning to end of spawning period, carbon  $\delta^{13}\text{C}$  values in the muscle tissues of sticklebacks significantly increased following changes of the diet from planktonic to benthic prey in both sexes.

#### 3.4 Comparing stable isotopes with stomach contents

Trophic position of stickleback significantly differed between sexes and spawning periods. Observed trophic position (TP) of male sticklebacks was slightly higher compared to females, but the entire range in opposite was a bit higher for the females (see Tables 3 & 4). Trophic position of entire population was significantly higher in the end of spawning position compared to the beginning and middle periods (ANOVA, post hoc all  $p < 0.001$ ). Yet, no significant differences between sites in heavy stable isotope content was found (Table 4). The expected trophic position tended to be lower than the observed position by 0.9-1.2 units with a median of 1 unit (Table 3).

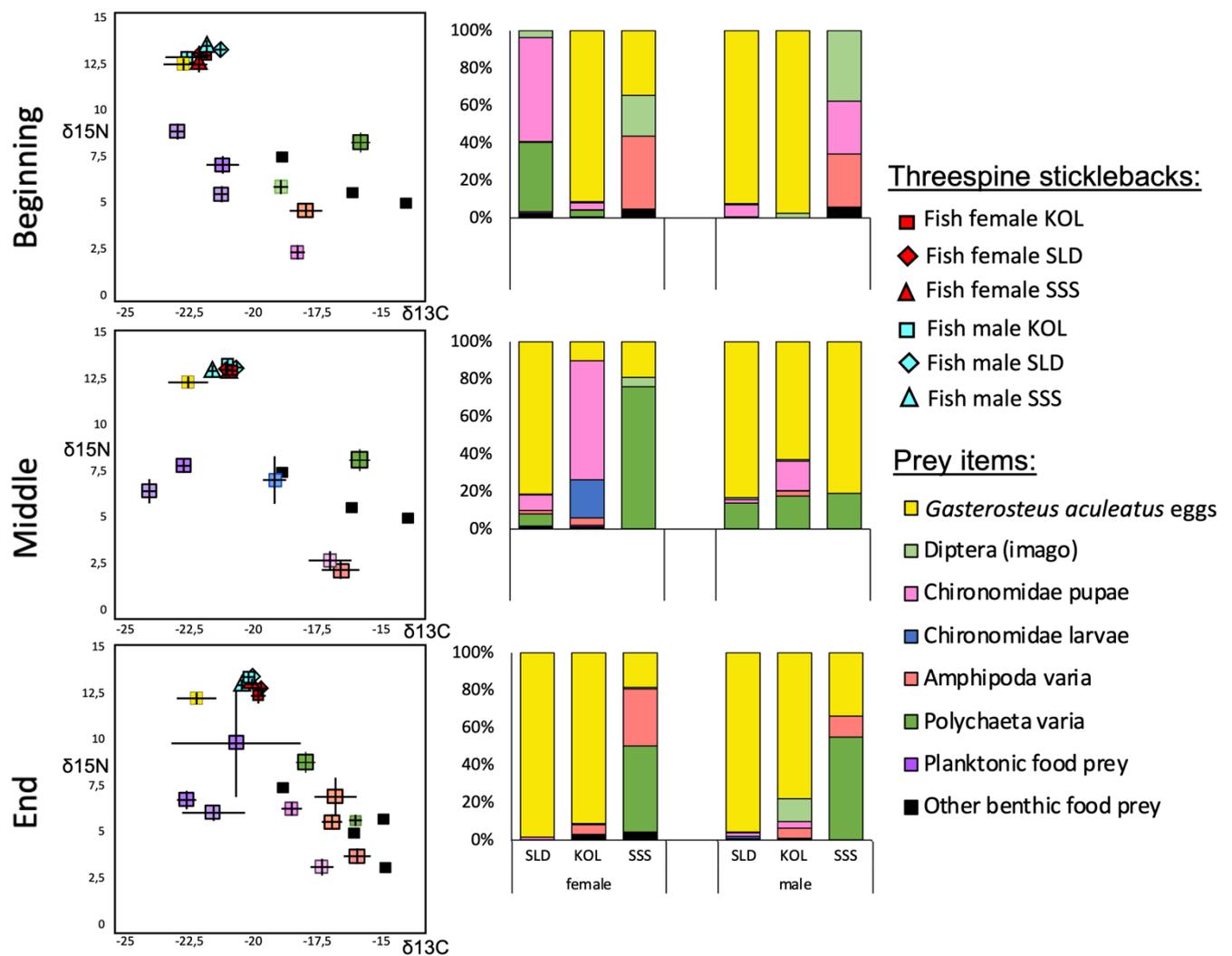


Figure 5. Mean ( $\pm$ SE) stable isotope values of carbon  $\delta^{13}\text{C}$  and nitrogen  $\delta^{15}\text{N}$  content (left column) and stomach contents (right column) of stickleback and their prey items during the spawning period (beginning, middle and end) at Seldianaya Inlet (SLD), Koliushkovaya Lagoon (KOL), Sukhaya Salma Strait (SSS) in 2016.

Results of Bayesian mixing modelling (Table 5) demonstrated that stickleback eggs are the main food resource for fishes during the whole spawning season. At the beginning of spawning, proportion of fish eggs in the diet spectrum was 99.4% for females and 99.9% for males; proportion of other prey in female's and male's diet are extremely low (<0.1%). In the middle of spawning season, fractions of benthic resources were slightly higher in females (4.8% – Polychaeta, 0.2% – Oligochaeta) and in males (1.4% – Polychaeta). Yet, the diet was still mostly consisting of stickleback eggs (94.8% for females and 98.5% for males). By the end of spawning, the diet of females was more diverse due to the higher fractions of benthic (29.1% totally) and zooplanktonic prey (14.6%), whereas fish eggs remain practically the only prey for males (99.9%).

Table 5. Predicted diet composition of threespine stickleback (%) in different stages of spawning period based on Bayesian mixing model performed with MixSIAR package in R. Range from 2.5% and 97.5% quantiles is in numerator, mean value is in denominator. Prey items with the highest contribution are marked in bold.

Diet	Female			Male		
	beginning	middle	end	beginning	middle	end
Amphipoda	<0.01 – 0.01 0.001	<0.01 – <0.01 <0.001	<0.01 – 0.03 0.005	– –	<0.01 – <0.01 <0.001	– –
Chironomidae	<0.01 – 0.01 0.001	<0.01 – <0.01 <0.001	<0.01 – 0.57 <b>0.164</b>	<0.01 – <0.01 <0.001	<0.01 – <0.01 <0.001	<0.01 – <0.01 <0.001
Stickleback eggs	<b>0.97 – 1.00</b> <b>0.994</b>	<b>0.80 – 1.00</b> <b>0.948</b>	<0.01 – 1.00 <b>0.563</b>	<b>0.99 – 1.00</b> <b>0.999</b>	<b>0.87 – 1.00</b> <b>0.985</b>	<b>0.99 – 1.00</b> <b>0.999</b>
Gastropoda	<0.01 – 0.01 0.001	<0.01 – <0.01 <0.001	<0.01 – 0.01 0.001	<0.01 – <0.01 <0.001	<0.01 – <0.01 <0.001	<0.01 – <0.01 <0.001
Isopoda	<0.01 – 0.01 0.001	– –	<0.01 – 0.07 0.009	<0.01 – <0.01 <0.001	<0.01 – <0.01 <0.001	<0.01 – <0.01 <0.001
Oligochaeta	<0.01 – 0.02 0.002	<0.01 – 0.03 0.002	<0.01 – 0.16 0.018	<0.01 – <0.01 <0.001	<0.01 – <0.01 <0.001	<0.01 – <0.01 <0.001
Polychaeta	<0.01 – 0.01 0.001	<0.01 – 0.17 0.048	<0.01 – 0.28 0.094	<0.01 – <0.01 <0.001	<0.01 – 0.13 0.014	<0.01 – <0.01 <0.001
Mixed zooplankton	<0.01 – 0.01 0.001	<0.01 – <0.01 <0.001	<0.01 – 0.50 <b>0.146</b>	<0.01 – <0.01 <0.001	<0.01 – <0.01 <0.001	<0.01 – <0.01 <0.001

#### 4. Discussion

Diet of marine and freshwater threespine stickleback has recently been extensively studied using the stomach content, stable isotope, and DNA metabarcoding analyses [40–42]. In the present study we provide description of changes in diet of sticklebacks during the migration to the seasonal spawning grounds (represented by an offshore site *CIE*) and between three periods of spawning season of sticklebacks, when fishes spend most of the time in the inshore. For this, we analyzed the stomach content using a combination of two methods - taxonomic identification and stable isotope analysis from fishes collected at three types of spawning grounds of *G. aculeatus* (see Materials and Methods for details).

At the spawning grounds, stickleback diet was more diverse than in the pelagic zone, which can be attributed to a shift from pelagic to benthic feeding. Based on the stable isotope analysis, *G. aculeatus* has the most constant trophic relationships during their life history with planktonic species. Before the spawning season the main food sources of sticklebacks caught at the Chupa inlet entrance (*CIE*) were *Calanus glacialis* and Euphausiacea (Figure 3). During the spawning season a temporary switch to benthic taxa occurs, which can be seen from the stomach content analysis of fishes caught inshore (Figure 4). At the inshore spawning grounds sticklebacks demonstrated nearly 2-fold increase in diversity of prey objects in the inshore area than in the offshore before the spawning season, and no planktonic taxa in stomach contents of the inshore fishes were found.

Stable isotope signature changed in muscle tissues during the summer season when fishes mostly spend time and presumably forage in the intertidal and upper subtidal zones. During this period, sticklebacks slightly changed their  $\delta^{13}\text{C}$  isotope signature from planktonic to benthic species. Previous studies have shown that juvenile sticklebacks first feed on benthic intertidal chironomid larvae and then switch to planktonic diets prior to migrating offshore [17,42]. Thus, both analyses indicate that the threespine stickleback *G. aculeatus* in the White Sea can be regarded as omnivorous fish species with opportunistic feeding behavior that feeds on the most available food source at the moment.

Diversity of food objects measured as D-index was found to be higher ca. 2.5-fold in females and about 2-fold higher in males in the inshore sites during the spawning season than in the offshore site before the start of spawning season. The diet of sticklebacks at the offshore site was mostly dominated by one prey species, later in the summer, at the inshore sites, 2-3 various prey items always prevailed in diet of fishes. In the middle of the

spawning season, no considerable variation of stickleback diet was observed despite some fish just arrived inshore whereas others already spent there few weeks. The latter data indicate that switch from the plankton to benthos occurs simultaneously among the whole local population.

Even though sticklebacks fed on a variety of benthic organisms, they mostly fed on stickleback eggs during most of the spawning season. Egg and larval cannibalism is already well known in threespine stickleback [13,43]. Eggs are rich in protein and fat, match the nutritional requirements of species, presumably restoring the female energy balance after spawning effectively [44]. Stickleback males are known to prey on eggs while removing the undeveloped ones [44,45], to sustain themselves during the nest guarding. It is also plausible that switching from water column planktonic to bottom benthic feeding in the middle of the spawning period is a result of foraging of both males and females around the nests. Finally, benthic organisms are larger than planktonic and thus are expected to provide more energy per individual consumed [46]. In general, feeding intensity does not differ between males and females in first weeks of the spawning, yet at the end of season females feed more intensively than males. Also, males did not show remarkable differences in feeding intensity throughout the season. Feeding intensity in females increases not due to the more variable diet but as a result of consuming higher proportion of eggs. In two sites, KOL and SSS, increase in feeding intensity was also a result of consuming larvae and pupae of chironomids and polychaetes.

Enrichment of  $\delta^{13}\text{C}$  in sticklebacks is presumed to be associated with the greater foraging in the intertidal rather than limnetic habitats [47,48]. Following this assumption, we can assume that both males and females shifted their feeding depending on spawning period and site. Firstly, in the end of the spawning period fishes slowly switch feeding behavior from benthic to planktonic feeding. Secondly, significant difference of  $\delta^{13}\text{C}$  between sites indicate that sources of organic matter in SEL and KOL during the start of spawning are different, where the intertidal type of organic matter prevails within the lagoon and pelagic – in the inlet. In the middle of spawning period fish from KOL and SEL shifted towards more littoral type, while SSS remained more pelagic. By the end of spawning, all sites became the intertidal type of organic matter supply. Apart from the White Sea, stickleback from the Baltic Sea coastal area mostly use pelagic derived carbon as a basic resource [21]. Our data indicates that isotope signatures of threespine stickleback changes during migrations from pelagic to inshore communities. Isotope signatures respond to change of the diet quite slowly. While most of the isotope studies were focused on static description of diet, several studies demonstrated that isotope signatures of migratory marine species vary during the season depending on foraging area [49,50].  $\delta^{15}\text{N}$  in females varied greatly than in males (12.4-13.1 and 12.8-13.2 respectively). This indicates that diet of females is more variable due to their higher foraging activity during the spawning period [51]. On the contrary, no sex-biased differences in  $\delta^{13}\text{C}$  concentration were found, which can be a consequence of overlapping between foraging habitats of male and female sticklebacks [48], although many researchers report higher proportion of benthic organisms in male diet which is associated with their sexual dimorphism [52–56].

In our study isotope trophic positions of amphipods (2.66) and gastropods (2.65) were the lowest among all organisms, while sticklebacks along with their eggs had the highest values (from 5.04 to 5.33 for fish and 5.20 for eggs). Similar results were obtained during the summer season in the northern Barents Sea [57]. However, trophic position of fish from that study (from 3.3 to 4.4) did not correspond to our observations but were similar to expected (3.8-4.4). Herring is the direct stickleback food competitor [58], and its trophic position in the Barents Sea was 3.4 [57]. In Canadian lakes trophic position of threespine stickleback (3.7) is in line with other predatory fishes (3.5 to 3.7), while trophic positions of Clupeids (alewife) and Salmonids (whitefish – 3.2) varied between 3.0 and 3.5 [25]. Authors also mention that all predatory fishes demonstrated high variability of trophic positions or even several trophic levels. This variability was explained by opportunistic feeding of fishes in various lakes differing by food organisms [25].

Our data show that trophic position of threespine stickleback may vary between sexes and spawning periods. Site and Period significantly affected  $\delta^{13}\text{C}$  signature, but the differences were observed in females only, as diet of males was relatively similar during the season. In both sexes TP was lowest at the end of the spawning season, probably due to shift from benthic to plankton feeding. The latter caused a decrease of  $\delta^{13}\text{C}$  values. These changes were clearly traced using the stable isotope analysis. Before the spawning, diet of both sexes almost completely consisted of zooplankton and other pelagic prey [51]. In spawning period, the trophic position of males is higher than females. According to IRI index, males prefer eggs of the same species and their diet include significant amount of other prey sources only in particular sites. Male sticklebacks are known to guard nests and due to decreased home range thus have less access to various prey items. On the contrary, females forage over larger distances in the area after spawning [51]. Our results correspond with other authors. For example, Reimchen et al. (2016) [48] has shown that male sticklebacks inhabiting streams and lakes of Western Canada also had higher trophic position than females in each locality. According to the authors, this dietary shift in males could emerge when pre-reproductive males shift from an offshore pelagic niche to an in-shore littoral niche. However, experimental results obtained by Grey (2000) [59] did not confirmed the fact that isotope analysis is able to demonstrate clear seasonal change in stickleback's diet.

Comparison between stomach content and stable isotopes using Bayesian mixing models implemented in MixSIAR confirmed the initial assumption that the most important food source of adult sticklebacks during the whole spawning period are stickleback eggs (56-99% for females and 99% for males). According to the model, predicted proportion of prey sources differed from observed, and this deviation was found in female diet. While females increased consumption of benthic organisms during the season, male stickleback diet does not change during the season. Possibly, mixing model generates overestimation of prey items in fish diet due to the data generalization [59], because one of three sites in each spawning period usually altered from others, especially in eggs proportion in a diet. On the other hand, Bayesian model can be a tool which illustrates an average or theoretical diet structure and cannot reveal slight differences between adjacent water areas and their inner variability, what is considered to be for the usual stable isotope analysis [48]. According to Vander Zanden & Rasmussen (1996) [25], stomach contents is only a "snapshot of the fish diet". Thus, it unnecessarily should repeat results obtained during the traditional data processing. Estimates of dietary trophic position require assumptions of the trophic position of prey items, which can be an additional source of errors [24]. Prey also can take remarkably variable trophic position [25]. Accuracy of mixing models also depends on whether diet tissue discrimination factors for a species are appropriate [59–61]. In turn, trophic discrimination factors (TDF) values are influenced by phylogeny, tissue type, diet of consumer, isotopic signature of food source, and the error associated with the measurement of TDF within a species [59]. Nevertheless, application of Bayesian mixing model generally confirmed initial expectations of diet variability in sticklebacks during the spawning season and variation in dietary habits between males and females.

## 5. Conclusions

Using a combination of stable isotope analysis and stomach content analysis we demonstrate that dietary preferences of threespine stickleback *Gasterosteus aculeatus* in the White Sea changes at least seasonally between planktonic and benthic feeding during the species life history. *G. aculeatus* is a widespread boreal fish species that experience global population expansion in recent decades [13,62]. Being a migratory fish, stickleback is supposed to transfer energy between the offshore and inshore communities and serve as indicator of long-term changes in both coastal and open sea ecosystems. The White Sea *G. aculeatus* spend most of time between autumn and late spring in the offshore areas and consume planktonic species. This was clearly observed at the time of stickleback arrival

to the spawning sites in the inshore areas of Keret Archipelago. While the range and abundance of stickleback is currently increasing, summer survival of this species mostly depends on itself during the spawning season. During the summer, both males and females prefer to consume stickleback eggs, but females also forage for twenty benthic taxa. On the contrary, males spend most of the time guarding the nest and thus their diet is less variable. In the end of the spawning period, sticklebacks demonstrate a backward switch from benthic to planktonic feeding. Our study also demonstrates that stable isotope signatures ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) remarkably change during the spawning season, being a perfect indicator of diet preferences of this fish species in line with recent studies involving approach of analyzing the diet using DNA-metabarcoding [40].

**Author Contributions:** Conceptualization, methodology, writing—original draft preparation, A.G-Y.; sampling, N.P., M.I., T.I.; laboratory analysis, A.G-Y., A.T.; data analysis, A.G-Y., E.N.; writing—review and editing, E.G-Y, D.L., M.I.; funding acquisition, M.I., A.G-Y, E.G-Y.

All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Russian Science Foundation, grant number RSF 22-24-00956.

**Acknowledgments:** Authors would like to thank the staff of Education and Research station “Belomorskaya” of the Saint-Petersburg State University and our colleagues at the Department of ichthyology and hydrobiology of Saint-Petersburg State University for their support and collaboration.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

## References

1. Pörtner, H.-O.; Roberts, D.C.; Masson-Delmotte, V.; Zhai, P.; Tignor, M.; Poloczanska, E.; Weyer, N. The Ocean and Cryosphere in a Changing Climate. IPCC Spec. Rep. Ocean Cryosphere Chang. Clim. 2019.
2. Steffen, W.; Rockström, J.; Richardson, K.; Lenton, T.M.; Folke, C.; Liverman, D.; Summerhayes, C.P.; Barnosky, A.D.; Cornell, S.E.; Crucifix, M.; et al. Trajectories of the Earth System in the Anthropocene. Proc. Natl. Acad. Sci. 2018, 115, 8252–8259, doi:10.1073/pnas.1810141115.
3. Cusa, M.; Berge, J.; Varpe, Ø. Seasonal Shifts in Feeding Patterns: Individual and Population Realized Specialization in a High Arctic Fish. Ecol. Evol. 2019, 9, 11112–11121.
4. Fossheim, M.; Primicerio, R.; Johannessen, E.; Ingvaldsen, R.B.; Aschan, M.M.; Dolgov, A.V. Recent Warming Leads to a Rapid Borealization of Fish Communities in the Arctic. Nat. Clim. Change 2015, 5, 673–677.
5. Filatov, N.; Pozdnyakov, D.; Johannessen, O.M.; Pettersson, L.H.; Bobylev, L.P. White Sea: Its Marine Environment and Ecosystem Dynamics Influenced by Global Change; Springer Science & Business Media, 2007;
6. Lajus, D.L.; Dmitrieva, Z.V.; Kraikovski, A.V.; Lajus, J.A.; Alexandrov, D.A. Atlantic Salmon Fisheries in the White and Barents Sea Basins: Dynamic of Catches in the 17–18th Century and Comparison with 19–20th Century Data. Fish. Res. 2007, 87, 240–254.
7. Studenov, I.; Chupov, D.; Ustyuzhinskiy, G.; Tortsev, A. RESULTS OF ATLANTIC SALMON INVESTIGATIONS IN NORTHERN DVINA RIVER DURING FISHING FOR RESEARCH PURPOSES. Fisheries 2020, 2020, 64–70, doi:10.37663/0131-6184-2020-3-64-70.
8. Lajus, D.L.; Alekseeva, Y.I.; Lajus, J.A. Herring Fisheries in the White Sea in the 18th–Beginning of the 20th Centuries: Spatial and Temporal Patterns and Factors Affecting the Catch Fluctuations. Fish. Res. 2007, 87, 255–259.
9. Yershov, P.; Sukhotin, A. Age and Growth of Marine Three-Spined Stickleback in the White Sea 50 Years after a Population Collapse. Polar Biol. 2015, 38, 1813–1823.

10. Yurtseva, A.; Noreikiene, K.; Lajus, D.; Li, Z.; Alapassi, T.; Ivanova, T.; Ivanov, M.; Golovin, P.; Vesala, S.; Merilä, J. Aging Three-Spined Sticklebacks *Gasterosteus Aculeatus*: Comparison of Estimates from Three Structures. *J. Fish Biol.* 2019, 95, 802–811, doi:10.1111/jfb.14071.
11. Ivanova, T.S.; Ivanov, M.V.; Golovin, P.V.; Polyakova, N.V.; Lajus, D.L. The White Sea Threespine Stickleback Population: Spawning Habitats, Mortality, and Abundance. *Evol. Ecol. Res.* 2016, 17, 301–315.
12. Murzina, S.A.; Nefedova, Z.A.; Pekkoeva, S.N.; Lajus, D.L.; Nemova, N.N. Fatty Acids of the Three-Spined Stickleback (*Gasterosteus Aculeatus* L.) from the White Sea. *Appl. Biochem. Microbiol.* 2019, 55, 73–77, doi:10.1134/S0003683819010125.
13. Lajus, D.L.; Golovin, P.V.; Zelenskaia, A.E.; Demchuk, A.S.; Dorgham, A.S.; Ivanov, M.V.; Ivanova, T.S.; Murzina, S.A.; Polyakova, N.V.; Rybkina, E.V.; et al. Threespine Stickleback of the White Sea: Population Characteristics and Role in the Ecosystem. *Contemp. Probl. Ecol.* 2020, 13, 132–145, doi:10.1134/S1995425520020079.
14. Lajus, D.; Ivanova, T.; Rybkina, E.; Lajus, J.; Ivanov, M. Multidecadal Fluctuations of Threespine Stickleback in the White Sea and Their Correlation with Temperature. *ICES J. Mar. Sci.* 2021, 78, 653–665.
15. Cury, P.; Bakun, A.; Crawford, R.J.M.; Jarre, A.; Quiñones, R.A.; Shannon, L.J.; Verheye, H.M. Small Pelagics in Upwelling Systems: Patterns of Interaction and Structural Changes in “wasp-Waist” Ecosystems. *ICES J. Mar. Sci.* 2000, 57, 603–618, doi:10.1006/jmsc.2000.0712.
16. Bakhvalova, A.E.; Ivanova, T.S.; Ivanov, M.V.; Demchuk, A.S.; Movchan, E.A.; Lajus, D.L. Long-Term Changes in the Role of Threespine Stickleback (*Gasterosteus Aculeatus*) in the White Sea: Predatory Fish Consumption Reflects Fluctuating Stickleback Abundance during the Last Century. *Evol. Ecol. Res.* 2016, 17, 317–334.
17. Demchuk, A.; Ivanov, M.; Ivanova, T.; Polyakova, N.; Mas-Martí, E.; Lajus, D. Feeding Patterns in Seagrass Beds of Three-Spined Stickleback *Gasterosteus Aculeatus* Juveniles at Different Growth Stages. *J. Mar. Biol. Assoc. U. K.* 2015, 95, 1635–1643, doi:10.1017/S0025315415000569.
18. Fry, B. Food Web Structure on Georges Bank from Stable C, N, and S Isotopic Compositions. *Limnol. Oceanogr.* 1988, 33, 1182–1190.
19. Zanden, M.J.V.; Rasmussen, J.B. Primary Consumer  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and the Trophic Position of Aquatic Consumers. *Ecology* 1999, 1395–1404.
20. Post, D.M. Using Stable Isotopes to Estimate Trophic Position: Models, Methods, and Assumptions. *Ecology* 2002, 83, 703–718.
21. Golubkov, S.M.; Berezina, N.A.; Gubelit, Y.I.; Demchuk, A.S.; Golubkov, M.S.; Tiunov, A.V. A Relative Contribution of Carbon from Green Tide Algae *Cladophora Glomerata* and *Ulva Intestinalis* in the Coastal Food Webs in the Neva Estuary (Baltic Sea). *Mar. Pollut. Bull.* 2018, 126, 43–50, doi: <https://doi.org/10.1016/j.marpolbul.2017.10.032>.
22. Croizier, G.L.; Schaal, G.; Point, D.; Loc’h, F.L.; Machu, E.; Fall, M.; Munaron, J.-M.; Boyé, A.; Walter, P.; Laë, R. Stable Isotope Analyses Revealed the Influence of Foraging Habitat on Mercury Accumulation in Tropical Coastal Marine Fish. *Sci. Total Environ.* 2019, 650, 2129–2140.
23. Costalago, D.; Forster, I.; Nemcek, N.; Neville, C.; Perry, R.I.; Young, K.; Hunt, B.P.V. Seasonal and Spatial Dynamics of the Planktonic Trophic Biomarkers in the Strait of Georgia (Northeast Pacific) and Implications for Fish. *Sci. Rep.* 2020, 10, 1–12.
24. Zanden, M.J.V.; Cabana, G.; Rasmussen, J.B. Comparing Trophic Position of Freshwater Fish Calculated Using Stable Nitrogen Isotope Ratios ( $\Delta^{15}\text{N}$ ) and Literature Dietary Data. *Can. J. Fish. Aquat. Sci.* 1997, 54, 1142–1158, doi:10.1139/cjfas-54-5-1142.
25. Zanden, M.J.V.; Rasmussen, J.B. A Trophic Position Model of Pelagic Food Webs: Impact on Contaminant Bioaccumulation in Lake Trout. *Ecol. Monogr.* 1996, 66, 451–477, doi:10.2307/2963490.
26. Ravinet, M.; Ishikawa, A.; Kitano, J. Trophic Niche Differentiation and Phenotypic Divergence among Cryptic Species of Japanese Ninespine Sticklebacks. *Evol. Ecol. Res.* 2016, 17, 505–523.
27. Golovin, P.V.; Bakhvalova, A.E.; Ivanov, M.V.; Ivanova, T.S.; Smirnova, K.A.; Lajus, D.L. Sex-Biased Mortality of Marine Threespine Stickleback (*Gasterosteus Aculeatus* L.) during Their Spawning Period in the White Sea. *Evol. Ecol. Res.* 2019, 20, 279–295.
28. Ivanova, T.S.; Ivanov, M.V.; Bakhvalova, A.E.; Polyakova, N.V.; Golovin, P.V.; Kucheryavyy, A.V.; Yurtseva, A.O.; Smirnova, K.A.; Lajus, D.L. Homing Ability and Site Fidelity of Marine Threespine Stickleback on Spawning Grounds. *Evol. Ecol. Res.* 2019, 20, 297–315.
29. Chislenko, L.L. Nomograms for Determining the Weight of Aquatic Organisms by Body Size and Form (Sea Mesobenthos and Plankton); Nauka, 1968;
30. Alimov, A.F.; Bogatov, V.V.; Golubkov, S.M. Production Hydrobiology; Nauka, 2013;
31. Pertzova, N.M. Average Mass and Sizes of Abundant Zooplankton Species in the White Sea. *Oceanology* 1967, 7, 309–313.
32. Keough, J.R.; Sierszen, M.E.; Hagley, C.A. Analysis of a Lake Superior Coastal Food Web with Stable Isotope Techniques. *Limnol. Oceanogr.* 1996, 41, 136–146, doi: <https://doi.org/10.4319/lo.1996.41.1.0136>.
33. Hyslop, E.J. Stomach Contents Analysis—a Review of Methods and Their Application. *J. Fish Biol.* 1980, 17, 411–429, doi:10.1111/j.1095-8649.1980.tb02775.x.
34. Pinkas, L.; Oliphant, M.S.; Iverson, I.L.K. Food Habits of Albacore; Bluefin Tuna and Bonito in Californian Waters. *Calif. Fish Game* 1971, 152, 1–105.
35. Jost, L. Entropy and Diversity. *Oikos* 2006, 113, 363–375, doi:10.1111/j.2006.0030-1299.14714.x.
36. Hureau, J.-C. Biologie Cornpree de Quelques Poissons Antarctiques (Nototheniidae); Bull. Inst. Océanogr., 1970

37. Nilsen, M., Pedersen, T., Nilssen, E.M. and Fredriksen, S. Trophic studies in a high-latitude fjord ecosystem—a comparison of stable isotope analyses ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and trophic-level estimates from a mass-balance model. *Canadian Journal of Fisheries and Aquatic Sciences*, 2008, 65(12), pp.2791–2806.
38. Stock, B.C.; Jackson, A.L.; Ward, E.J.; Parnell, A.C.; Phillips, D.L.; Semmens, B.X. Analyzing Mixing Systems Using a New Generation of Bayesian Tracer Mixing Models. *PeerJ* 2018, 6, e5096.
39. Britton, J.R.; Busst, G.M.A. Stable Isotope Discrimination Factors of Omnivorous Fishes: Influence of Tissue Type, Temperature, Diet Composition and Formulated Feeds. *Hydrobiologia* 2018, 808, 219–234.
40. Jakubavičiute, E.; Bergström, U.; Eklöf, J.S.; Haanel, Q.; Bourlat, S.J. DNA Metabarcoding Reveals Diverse Diet of the Three-Spined Stickleback in a Coastal Ecosystem. *PLoS ONE* 2017, 12, 1–16, doi:10.1371/journal.pone.0186929.
41. Snowberg, L.K.; Bolnick, D.I. Assortative Mating by Diet in a Phenotypically Unimodal but Ecologically Variable Population of Stickleback. *Am. Nat.* 2008, 172, 733–739, doi:10.1086/591692.
42. Bolnick, D.I.; Snowberg, L.K.; Hirsch, P.E.; Lauber, C.L.; Knight, R.; Caporaso, J.G.; Svanbäck, R. Individuals' Diet Diversity Influences Gut Microbial Diversity in Two Freshwater Fish (Threespine Stickleback and Eurasian Perch). *Ecol. Lett.* 2014, 17, 979–987, doi:10.1111/ele.12301.
43. Rybkina, E.V.; Demchuk, A.S.; Lajus, D.L.; Ivanova, T.S.; Ivanov, M.V.; Galaktionov, K.V. Dynamics of Parasite Community during Early Ontogenesis of Marine Threespine Stickleback, *Gasterosteus aculeatus*. *Evol. Ecol. Res.* 2016, 17, 335–354.
44. Whoriskey, F.G.; FitzGerald, G.J. Sex, Cannibalism and Sticklebacks. *Behav. Ecol. Sociobiol.* 1985, 18, 15–18.
45. Rohwer, S. Parent Cannibalism of Offspring and Egg Raiding as a Courtship Strategy. *Am. Nat.* 1978, 112, 429–440.
46. Mehli, M.; Bakker, T.C.M.; Frommen, J.G. Nutritional Benefits of Filial Cannibalism in Three-Spined Sticklebacks (*Gasterosteus aculeatus*). *Naturwissenschaften* 2009, 96, 399–403.
47. Vadeboncoeur, Y.; Zanden, M.J.V.; Lodge, D.M. Putting the Lake Back Together: Reintegrating Benthic Pathways into Lake Food Web Models: Lake Ecologists Tend to Focus Their Research on Pelagic Energy Pathways, but, from Algae to Fish, Benthic Organisms Form an Integral Part of Lake Food Webs. *BioScience* 2002, 52, 44–54, doi:10.1641/0006-3568(2002)052[0044:PTLBTR]2.0.CO;2.
48. Kainz, M.J.; Hager, H.H.; Rasconi, S.; Kahilainen, K.K.; Amundsen, P.-A.; Hayden, B. Polyunsaturated Fatty Acids in Fishes Increase with Total Lipids Irrespective of Feeding Sources and Trophic Position. *Ecosphere* 2017, 8, e01753.
49. Reimchen, T.E.; Steeves, D.; Bergstrom, C.A. Sex Matters for Defence and Trophic Traits of Threespine Stickleback. *Evol. Ecol. Res.* 2016, 17, 459–485.
50. MacNeil, M.; Skomal, G.; Fisk, A. Stable Isotopes from Multiple Tissues Reveal Diet Switching in Sharks. *Mar. Ecol. Prog. Ser.* 2005, 302, 199–206, doi:10.3354/meps302199.
51. Richert, J.E.; Galván-Magaña, F.; Klimley, A.P. Interpreting Nitrogen Stable Isotopes in the Study of Migratory Fishes in Marine Ecosystems. *Mar. Biol.* 2015, 162, 1099–1110, doi:10.1007/s00227-015-2652-6.
1. 52. Demchuk AS, Ivanov MV, Ivanova TS, Polyakova NV, Golovin PV, Lajus DL (2018) Feeding of the threespine stickleback *Gasterosteus aculeatus* (Linnaeus, 1758) in spawning grounds. *Trudy KNC RAN* 4:42–58. doi.org/10.17076/them818
53. Bentzen, P.; McPhail, J.D. Ecology and Evolution of Sympatric Sticklebacks (*Gasterosteus*): Specialization for Alternative Trophic Niches in the Enos Lake Species Pair. *Can. J. Zool.* 1984, 62, 2280–2286, doi:10.1139/z84-331.
54. Kristjánsson, B.K.; Skúlason, S.; Noakes, D.L. Rapid Divergence in a Recently Isolated Population of Threespine Stickleback (*Gasterosteus aculeatus* L.). *Evol. Ecol. Res.* 2002, 4, 659–672.
55. Reimchen, T.E.; Nosal, P. Replicated Ecological Landscapes and the Evolution of Morphological Diversity among *Gasterosteus* Populations from an Archipelago on the West Coast of Canada. *Can. J. Zool.* 2006, 84, 643–654, doi:10.1139/z06-036.
56. Kitano, J.; Mori, S.; Peichel, C.L. Sexual Dimorphism in the External Morphology of the Threespine Stickleback (*Gasterosteus aculeatus*). *Copeia* 2007, 2007, 336–349, doi:10.1643/0045-8511(2007)7[336:SDITEM]2.0.CO;2.
57. Spoljaric, M.A.; Reimchen, T.E. Habitat-Dependent Reduction of Sexual Dimorphism in Geometric Body Shape of Haida Gwaii Threespine Stickleback: REDUCTION IN SEXUAL DIMORPHISM IN STICKLEBACK. *Biol. J. Linn. Soc.* 2008, 95, 505–516, doi:10.1111/j.1095-8312.2008.01068.x.
58. Tاملander, T.; Renaud, P.E.; Hop, H.; Carroll, M.L.; Jr, W.G.A.; Hobson, K.A. Trophic Relationships and Pelagic–Benthic Coupling during Summer in the Barents Sea Marginal Ice Zone, Revealed by Stable Carbon and Nitrogen Isotope Measurements. *Mar. Ecol. Prog. Ser.* 2006, 310, 33–46.
59. Peltonen, H.; Vinni, M.; Lappalainen, A.; Pönni, J. Spatial Feeding Patterns of Herring (*Clupea harengus* L.), Sprat (*Sprattus sprattus* L.), and the Three-Spined Stickleback (*Gasterosteus aculeatus* L.) in the Gulf of Finland, Baltic Sea. *ICES J. Mar. Sci.* 2004, 61, 966–971, doi:10.1016/j.icesjms.2004.06.008.
60. Grey, J. Trophic Fractionation and the Effects of Diet Switch on the Carbon Stable Isotopic 'Signatures' of Pelagic Consumers. *Int. Ver. Für Theor. Angew. Limnol. Verhandlungen* 2000, 27, 3187–3191.
61. Healy, K.; Kelly, S.B.A.; Guillerme, T.; Inger, R.; Bearhop, S.; Jackson, A.L. Predicting Trophic Discrimination Factor Using Bayesian Inference and Phylogenetic, Ecological and Physiological Data. *DESIr: Discrimination Estimation in R*; PeerJ Preprints, 2017;
62. Brush, J.M.; Fisk, A.T.; Hussey, N.E.; Johnson, T.B. Spatial and Seasonal Variability in the Diet of Round Goby (*Neogobius melanostomus*): Stable Isotopes Indicate That Stomach Contents Overestimate the Importance of Dreissenids. *Can. J. Fish. Aquat. Sci.* 2012, 69, 573–586, doi:10.1139/F2012-001.

63. Beltran, R.S.; Peterson, S.H.; McHuron, E.A.; Reichmuth, C.; Hückstädt, L.A.; Costa, D.P. Seals and Sea Lions Are What They Eat, plus What? Determination of Trophic Discrimination Factors for Seven Pinniped Species. *Rapid Commun. Mass Spectrom.* 2016, 30, 1115–1122.