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

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Abstract: Studies of dietary preferences of migratory species are of great importance as these species connect trophic webs of habitats across the route of migration and thus represent trophic relationships between the spatially disjointed communities. Here we describe dietary preferences of the White Sea threespine stickleback *Gasterosteus aculeatus* 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 nearshore, 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 Bayes 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 completely 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

Marine communities of the Arctic are vulnerable to climatic oscillations and anthropogenic pressures. Here the recent warming is occurring at a pace of 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 structure of communities inhabited by both boreal and arctic species. The White Sea is a mediterranean sea 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].

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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* is a 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 negligeable commercial significance and previously was targeted by fisheries only during the periods of high abundance. Sticklebacks occupy an important position in 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, genesis of particular organic matter in an ecosystem and type of ecosystem itself **[18–23]**. Values of $\delta^{13}C$ and $\delta^{15}N$ represent the major energy flow pathways at lower trophic levels, offers a time-integrated measure of the organism's trophic position, accounts for temporal and spatial variation in feeding at multiple levels of the food web, and detects 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 differentiatiation between cryptic species or revealing previously unknown aspects of their biology [26].

The present study is aimed on the assessment of trophic status of the White Sea threespine stickleback during spawning period in 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 nearshore communities of the White Sea. As the ongoing climate changes strongly affect Arctic marine ecosystems, studies of diet of widely distributed and highly abundant fish such as threespine stickleback spawning in the nearshore 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

The 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 during spawning season in June-July 2016 and in June 2019 (Figure 1). The first site - centre of Chupa Inlet entrance of Kandalaksha Bay (***CIE***) at several hundred meters from the shoreline and approx. 50 meters depth; other three sampling sites were situated near the shoreline and represented various types of spawning grounds (Table 1) [13,16,27,28].

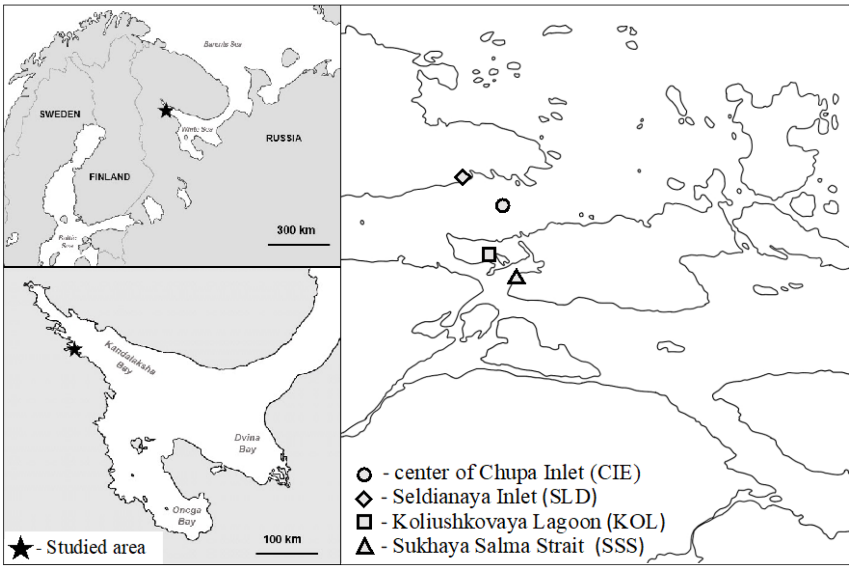

Figure 1. The study area

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

Variable	Seldianaya Inlet (*SLD*)	Koliushkovaya Lagoon (*KOL*)	Sukhaia 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 Zostera marina beds with dry biomass 1 kg/m2 and projective cover – up to 100 %	Eelgrass beds near the sea entrance with dry biomass up to 0.1 kg/m2 and projective cover up to 30 %, filamentous algae	Fucoids in the littoral, eelgrass with dry biomass up to 0.003 kg/m2 in sublittoral zone

The stickleback at spawning grounds were sampled in 2016 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 with hand nets. The stickleback in at the center of CIE were sampled in 2019 with surface twin trawl with characteristics similar to the beach seine. In 2016, simultaneously with collecting fish in the same sites, we also sampled zooplankton and zoobenthos to analyze stable isotopes of basal food organisms. Qualitive zooplankton samples in one replicate were collected with a plankton net (size 93 mm) by filtering the surface water. Benthic invertebrates were collected using benthic rectangular dredges.

2.2 Laboratory analyses

All fish were weighed (±0.01 g), measured for total length (TL) (±0.1 mm) and sexed by observation of 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 food organisms were identified up to possible lowest taxonomic unit and counted (Qi). The best-preserved specimens of each taxon (up to 10 individuals) were measured with a micrometer eyepiece scale (up to 0.03 mm) for calculations of their biomass (Ii). The individual masses of prey organisms were determined based on their body length [29,30] or ready-average mass [31], and then summed to get the total mass of particular prey taxon.

For stable isotope analysis, we have analyzed 175 samples from coastal 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 analysed samples were taken from putative planktonic and benthic prey objects. Zooplanktonic samples collected at each site was pooled which were not taxonomically identified. Benthic organisms 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 ± 1μg. 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 δ-notation relative to international standard (vPDB for carbon and the atmospheric N2 for nitrogen) (1).

$$\delta(‰) = (Rsample \ / \ Rstandard - 1) \times 103 \quad (1)$$

where R =13C/12Cor15N/14N. 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 δ13C and δ15N values in the laboratory standard was ± 0.2‰

2.3 Data analysis

For fish, percent number (%Qi), percent biomass (%Ii), and percent frequency of occurrence (%Fi) were calculated, along with index of relative importance (IRIi) and percent IRI (%IRIi) of each their prey items [33,34] using the following equations (2, 3):

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

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

Based on the stomach content data we calculated the D–index [35].

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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). 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 Bayes mixing models, which were performed in MixSIAR package in R [37]. 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) [38].

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

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

where δ13C and δ15N are the carbon and nitrogen isotope values derived from the predator's tissue.

Statistical analyses were performed using standard spreadsheets software (MS Excel 2013) and STATISTICA v7.0. Separate factorial ANOVA analyzes were run on individual parameters. 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.

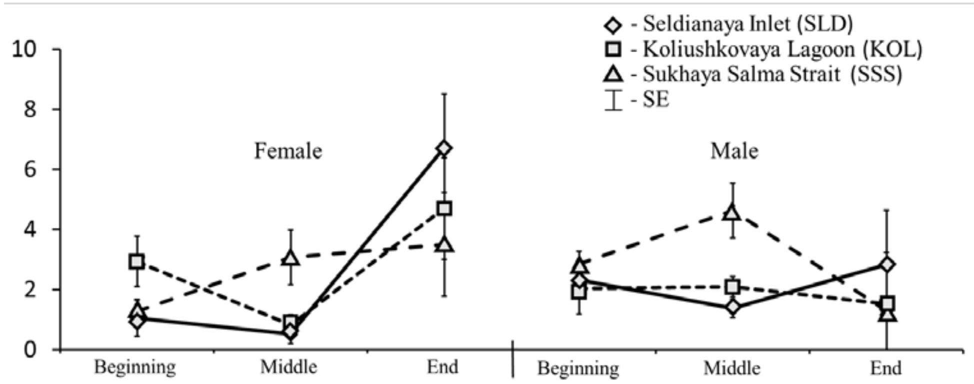

Figure 2. Feeding intensity of threespine stickleback during the spawning season. The Y-axis represent stomachs fullness index *FI* (‱). The dots and bars represent the sample means and standard errors (SE).

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, $F_{1\text{-}99}$=36.79, p<0.001). Inshore sites ***SSS*** and ***SLD*** representing the stickleback spawning grounds were characterized by notably higher D-index than in the open water site – about 2.5-fold for females and 2-fold for males.

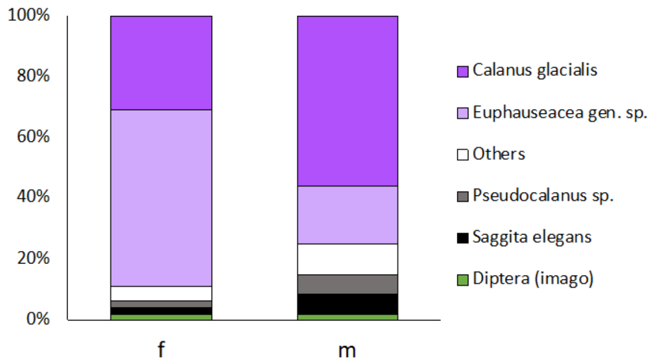

Figure 3. Stomach contents of female (F) and male (M) threespine stickleback in open waters of Chupa Inlet at the beginning of spawning period

During the spawning season the most important element of stickleback stomach content at the nearshore sites were stickleback eggs, comprising of up to 95% of stomach content in some individuals. *G. aculeatus* demonstrated a switch from a planktonic to a benthic feeding, preying on polychaetes (up to 80%), 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) in offshore and inshore zones.

Taxa	Open waters		Spawning grounds	
	IRI, % (F)	*IRI, % (M)*	*IRI, % (F)*	*IRI, % (M)*
Diatomeae *gen. sp.*	3.2	0.3	1.5	0.0
Calanus glacialis	**20.7**	**61.6**	-	-
Oithona similis	2.5	0.2	-	-
Pseudocalanus sp.	9.3	**19.7**	-	-
Copepoditii Copepoda	**16.0**	3.0	-	-
Euphauseacea *gen. sp.*	**33.9**	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
Gasterosteus aculeatus eggs	-	-	**67.8**	**82.0**
Other planktonic food prey	12.7	3.4	5.6	9.3
Other benthic food prey	0.0	1.0	0.4	0.01

At spawning grounds, females tended to show more diverse diet comparing to males (25 vs 17 prey items) with greater variability of rare food organisms. The main food items (*IRI, %)* were completely the same in both sexes. In the open waters difference between sexes are greater for both rare and main food items (*IRI, %*) (Table 2), but still does not approach statistical significance.

3.3 Stable isotopes values in sticklebacks, benthic and planktonic invertebrates

In sticklebacks, δ13C values varied between –25.76‰ and –19.34‰ in males, and from –22.76‰ to –19.34‰ in females. The δ15N varied between 11.19 and 13.65‰ in females, and between 12.07 and 13.82‰ in males (Table 3). Difference in δ15N values between sexes was significant, differences of δ15N values in sticklebacks (sexes pooled together) between sites and periods of spawning season were not significant. Differences in δ13C 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 δ13C and δ 15N and trophic position (TP, mean ± SE) in males, females and eggs of *Gasterosteus aculeatus*.

Sex and spawning period	n	δ13C	δ15N	δ C13 range	δ N15 range	TP observed	TP expected
Threespine stickleback *Gasterosteus aculeatus*							
Females, beginning	15	-22.15 ± 0.101	12.58 ± 0.140	-22.76 to -21.51	11.19 to 13.24	5.22 ± 0.044	4.2
Females, middle	15	-21.02 ± 0.108	13.02 ± 0.087	-21.57 to -20.33	12.41 to 13.65	5.32 ± 0.027	4.2
Females, end	15	-20.16 ± 0.111	12.56 ± 0.135	-20.75 to -19.34	11.53 to 13.59	5.04 ± 0.042	3.8
Males, beginning	15	-22.05 ± 0.272	12.96 ± 0.138	-25.48 to -21.26	12.07 to 13.8	5.34 ± 0.043	4.4
Males, middle	15	-21.24 ± 0.131	13.05 ± 0.117	-22.1 to -20.64	12.11 to 13.75	5.33 ± 0.037	4.1
Males, end	15	-20.55 ± 0.052	13.09 ± 0.118	-20.85 to -20.07	12.11 to 13.82	5.21 ± 0.037	4.2
Stickleback eggs	3	-22.45 ± 0.315	12.63 ± 0.104	-22.81 to -21.95	12.47 to 12.75	5.2 ± 0.033	
Prey organism							
Amphipoda	15	-16.98 ± 0.249	4.86 ± 0.451	-18.93 to -15.67	2.4 to 8.61	2.66 ± 0.124	
Chironomidae	21	-18.47 ± 0.217	4.96 ± 0.421	-20.12 to -15.79	2.27 to 6.88	2.73 ± 0.125	
Gastropoda	12	-15.06 ± 0.341	4.91 ± 0.31	-16.97 to -13.21	3.2 to 6.13	2.65 ± 0.095	
Isopoda	3	-16.27 ± 0.166	5.76 ± 0.177	-16.49 to -16.03	5.6 to 6.05	2.86 ± 0.052	
Oligochaeta	3	-19.01 ± 0.36	7.56 ± 0.214	-19.52 to -18.51	7.31 to 7.9	3.56 ± 0.063	

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Polychaeta	5	-16.48 ± 0.64	8.3 ± 0.14	-18.11 to -15.71	8.12 to 8.65	3.2 ± 0.567
Zooplankton	22	-22.22 ± 0.373	7.26 ± 0.394	-24.64 to -16.65	5.36 to 12.14	3.39 ± 0.114

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

Variables	Factor		
	Sex	Period	Site
δ13C	0.12	**<0.01**	**0.03**
δ15N	**<0.01**	0.07	0.79
TP	**<0.01**	**<0.01**	0.86

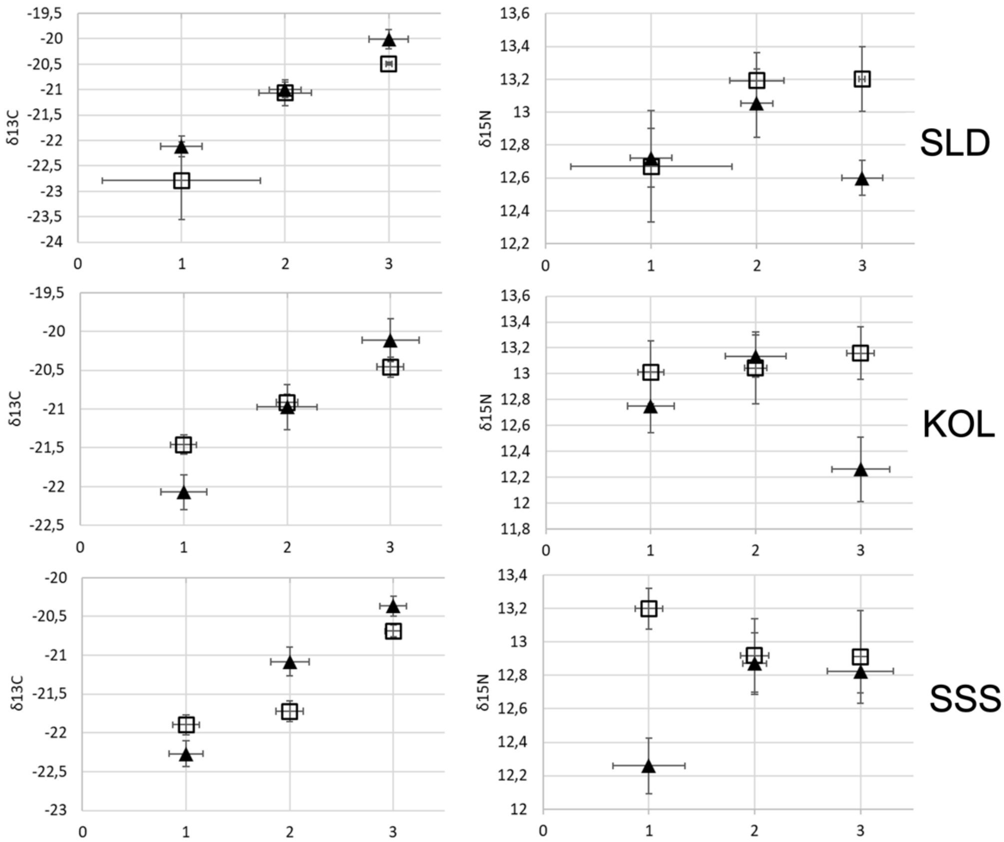

Figure 4. Mean (±SE) stable isotope values of carbon δ13C and nitrogen δ15N content of male (squares) and female (triangles) sticklebacks during three periods (1 – beginning, 2 – middle, 3 – end) of spawning season. See Figure 1 for site description.

Stable isotope values (δ13C) differentiated potential prey items for *G. aculeatus* into two groups corresponding to planktonic and benthic taxa respectively (Figure 5). Before the spawning season, both female and male sticklebacks sampled at the offshore site preferred consuming plankton taxa to benthic. However, during spawning period, the carbon δ13C 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

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Trophic position of stickleback significantly differed between sexes and spawning periods. Observed trophic position (TP) of male sticklebacks was slightly higher compared to female), 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).

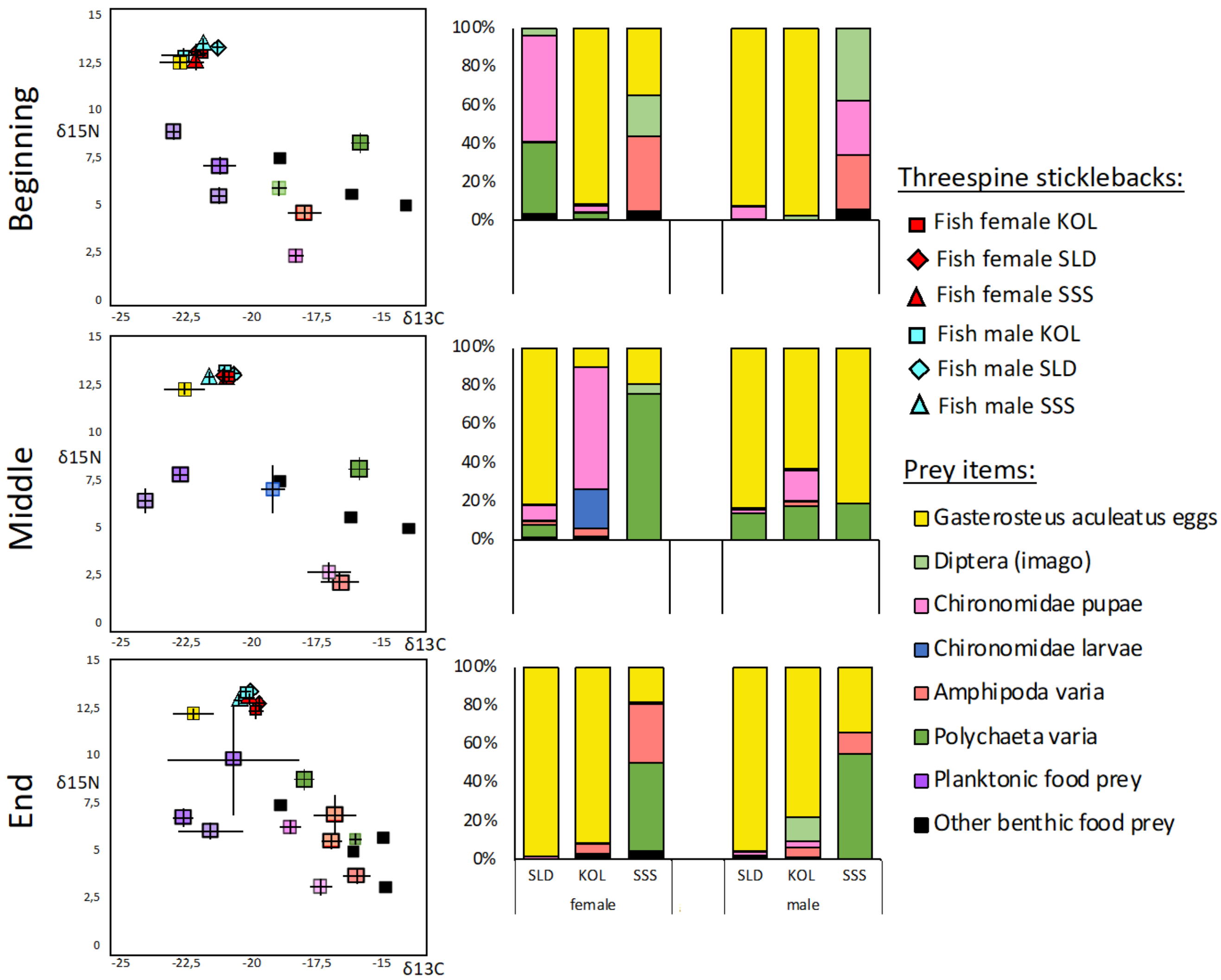

Figure 5. Mean (±SE) stable isotope values of carbon δ13C and nitrogen δ15N content (left column) and stomach contents (right column) of stickleback and their prey items during the spawning period (beginning, middle and end) at Seldianaya Inlet (SLD), Koliushkovaya Lagoon (KOL), Sukhaya Salma Strait (SSS) in 2016.

Results of Bayes 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 food 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

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of benthic (29.1% totally) and zooplanktonic prey (14.6%), whereas fish eggs remain practically the only food for males (99.9%).

Table 5. Predicted diet composition of threespine stickleback (%) in different stages of spawning period based on Bayes mixing model performed with MixSIAR package in R. Range from 2.5% and 97.5% quantiles is in numerator, mean value is in denominator. Food 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** **0.164**	<0.01 – <0.01 <0.001	<0.01 – <0.01 <0.001	<0.01 – <0.01 <0.001
Stickleback eggs	**0.97 – 1.00** **0.994**	**0.80 – 1.00** **0.948**	**<0.01 – 1.00** **0.563**	**0.99 – 1.00** **0.999**	**0.87 – 1.00** **0.985**	**0.99 – 1.00** **0.999**
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** **0.146**	<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 [39–41] . Our data show that diet of sticklebacks at the spawning grounds was more diverse than in the pelagic zone, that can be associated with the switch from pelagic to benthic feeding at the spawning sites. Based on the stable isotope analysis, *G. aculeatus* has the most stable trophic relationships during their life history with planktonic species. Stomach content analysis of fishes from CIE (Chupa inlet entrance) site corroborates these data, showing that before the spawning season the main food sources of sticklebacks are *Calanus glacialis* and Euphausiacea varia (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). Our data also demonstrates that during the summer in the spawning grounds, sticklebacks slightly change their $^{\delta13}$C isotope signature from planktonic to benthic species. Thus, both analyses indicate that the White Sea threespine stickleback *G. aculeatus* can be regarded as omnivorous fish species that feeds on the most available food source at the moment. Stable isotope signature changes in muscle tissues during the summer season when fishes mostly spend time and presumably forage in the intertidal and upper subtidal zones.

According to the stomach content analysis, sticklebacks demonstrated nearly 2-fold increase in diversity of prey objects in the nearshore area than in the offshore before the spawning season, and no planktonic taxa in stomach contents of the nearshore fishes were found. Increased diversity of prey objects thus reflects the migration of the sticklebacks to the nearshore habitats where they take another niche. Previous studies demonstrated that juvenile sticklebacks firstly prefer to feed on benthic intertidal chironomid larvae and then swith towards the planktonic diet before the migration to the offshore [17,42]

Diversity of food objects measured as D-index was found to be higher ca. 2.5-fold (1.4 - 3.8) in females and about 2-fold (1.7 - 3.7) 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 prey objects 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.

While benthic feeding was more diverse that the planktonic, in the middle of spawning season sticklebacks were mostly feeding on the eggs of the same species. Egg and larvae cannibalism is already well known in threespine stickleback [13,43]. Eggs are rich with protein and fat, and probably are close to optimal composition for their own species, presumably effectively restore the female energy balance after spawning [44]. Stickleback males feed on eggs, also removing the undeveloped ones [44,45], to sustain themselves during the guarding the nest. It is also plausible that switching from water column planktonic to bottom benthic feed in the middle of 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 spawning, yet at the end of season females feed more intensively that 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 lake flies (Chironomidae) and polychaetes.

Enrichment of δ13C 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 spawning period fishes slowly return to planktonic diet, switching from consuming benthic organisms. Secondly, significant difference of δ13C 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 nearshore 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]. δ15N 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 δ13C 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

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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) is 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 δ13C 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 δ13C values. These changes were clearly traced using the stable isotope analysis. Before the spawning, diet of both sexes was almost completely consisting of zooplankton and other pelagic prey [51]. In spawning period, the trophic position of males is higher than in females. According to IRI index, males prefer eggs of the same species and their diet include significant amount of other food sources only in particular sites. Male sticklebacks are known to guard nests and due to decreased range of individual activity thus have less access to various prey objects. 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 inshore 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 Bayes mixing models implemented in MixSIAR confirmed the initial assumption that the most important food source of adult sticklebacks during the whole spawning period are their own eggs (56-99% for females and 99% for males). According to the model, predicted proportion of food 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, Bayes 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 Bayes mixing model generally confirmed initial expectations of variability of diet 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 direct microscopic study we demonstrate that dietary preferences of White Sea threespine stickleback Gasterosteus aculeatus changes at least seasonally 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 nearshore 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 nearshore 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 up to 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 slowly restore planktonic feeding. Our study also demonstrates that stable isotope signatures (δ13C and δ15N) 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 [39].

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