



# Spatial heterogeneity and temporal dynamics of protein-degrading activity and life-history traits in threespine stickleback *Gasterosteus aculeatus*

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Received: 29 February 2020 / Accepted: 31 August 2020 / Published online: 30 September 2020  
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**Abstract** The paper studies the spatial heterogeneity and temporal dynamics of life-history traits, parasite invasion, and biochemical variables (e.g., tissue protein content and calpain activity) in adult and juvenile threespine stickleback *Gasterosteus aculeatus*. Samples were collected on three spawning grounds in Kandalaksha Bay, on the White Sea, located within a few kilometers of each other. These sites featured differences in temperature regime, water exchange, feed organism composition, predation load, aquatic vegetation, and spawner density. Whereas we found no parasite-related or size heterogeneity in adult fish on these grounds at the beginning of the spawning season, muscle protein reserves and protease (calpain) activity patterns in adults showed significant spatial heterogeneity, which increased as spawning progressed and was particularly pronounced in females. Sticklebacks expend much energy during spawning and are worn out by the end of the spawning period. To maintain individual viability, skeletal muscle proteins degrade, and the resulting amino acids are oxidized to produce energy. Protein-degrading calpains play a key role in these processes, and also mediate intense protein metabolism in juvenile stickleback, showing higher values in individuals growing under more favorable conditions. Thus, by studying the heterogeneous life-history traits and biochemical characteristics of adult and juvenile stickleback, we discovered the effects of environmental factors on their physiology, biochemical variables, and growth.

**Keywords** *Gasterosteus aculeatus* · Calpain · Skeletal muscle · Growth · Spawning · Habitat · White Sea

## Introduction

Threespine stickleback (*Gasterosteus aculeatus* Linnaeus, 1758, Gasterosteidae) is widespread in both marine and fresh waters of the Northern Hemisphere. This species is able to adapt to a wide range of environmental conditions due to its physiological and behavioral plasticity and is characterized by high ecological, phenotypic, and genetic diversity (Conover et al. 2009; Hendry et al. 2009; Stuart et al. 2017). Sticklebacks epitomize a popular model of evolutionary biology and ecology (Scharsack et al. 2012; Barber 2013; Hendry et al. 2013; Gibbons et al. 2017). The phenotypic variation of marine populations, which winter in open parts of the sea and spawn in coastal zones, is associated with the heterogeneity of spawning habitats with respect to abiotic (temperature, salinity, sediment, wave exposure, etc.) and biotic (feeding objects, predators, parasites, aquatic vegetation) factors (Jones et al. 2012; Arnegard et al. 2014).

Environmental conditions affect the growth and development of fish. Thus, they influence the metabolism of skeletal muscles that comprise more than half of a fish's body mass and accumulate structural proteins. Skeletal muscle proteins, in turn, are a source of amino acids used physiologically as plastic and energetic substrates. The rate of protein degradation in skeletal muscles, which depends crucially on calcium-dependent protease, or calpain, activity (Goll et al. 2003; 2008; Seiliez et al. 2014), is recognized as an

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indicator of fish growth, protein metabolism, and the development of adaptive responses to environmental changes regulating these processes at all stages of ontogenesis from fertilization to senescence (Mommssen 2004; Overturf and Gaylord 2009; Nemova et al. 2010; Johnston et al. 2011; Lysenko et al. 2017). Fish calpains are also responsible for the development of compensatory or pathological transformations responding to abiotic environmental variables, such as salinity, temperature, pollutants, etc. (Nemova et al. 2010; Lysenko et al. 2018a).

Understanding the adaptive mechanisms of White Sea threespine stickleback is not only important from the perspective of their population biology, but it also has significant ecosystem implications. At present, stickleback is the most numerous fish species in the White Sea (Ivanova et al. 2016), and their notable increase in numbers over the past two decades is likely due to climate change (Lajus et al. 2020). Stickleback plays an important role in the White Sea ecosystem, participating in trophic (Demchuk et al. 2015; 2018; Bakhvalova et al. 2016), chorological (Rybkina et al. 2017), and host-parasite (Rybkina et al. 2016) interactions. Therefore, changes in their abundance can affect the entire marine ecosystem.

Our study aims to understand what mechanisms are involved in the response of juvenile and adult threespine stickleback to different environmental conditions. We focused on such traits as body length and weight, physiological (condition factor) and biochemical (tissue protein content, intracellular protease activity) variables, and parasite loading.

## Material and methods

### Sampling sites

Samples were collected in three locations the Kandalaksha Bay in the White Sea situated within a few km from each other, notably differing in abiotic and biotic conditions (Table 1). The study used spawners of both sexes captured at the beginning (28-30 May) and end (7-10 July) of the 2016 spawning season and young-of-the-year stickleback captured at the spawning grounds on 31 July and 18 August 2017, using a 7.5 m long and 1.5 m high beach seine within 30 m of the shoreline.

### Temperature data

According to data from the White Sea Hydrology and Zooplankton Time-Series: Kartesh D1 (<https://www.st.nmfs.noaa.gov/plankton/time-series/ru-10101>), 66.3306°N, 33.6683°E, water temperatures (°C) in 2016 were close to normal, averaging 12.7 in June, 17.86 in July, and 15.91 in August. In 2017 the temperature increased slowly at the beginning of summer, averaging 7.92 in June, 15.48 in July, and 14.48 in August.

### Sample collection and processing

Immediately after capture, the fish were frozen in liquid nitrogen and stored until analysis began. After short-term defrosting at room temperature, total body length (TL cm; digital Vernier caliper, CHIZ Russia) and total mass (TM g; digital balance model SPX2202, OHAUS USA) were measured for each fish. For parasite analysis, the number of parasites was counted on ten females and ten males from each site during each period – sixty females and sixty males in total. Parasites included visceral nematodes and trematodes *Cryptocotyle* sp., which appear as cysts (“black spots”) on the body surface. Organ samples (liver, gills, skeletal muscle) taken from the adults were used for biochemical analyses, as were whole juvenile fish. The adult condition factor (K) was calculated according to the formula:  $K = 100 (TM/TL^3)$ .

### Biochemical analyses

In our work, chemical reagents, protease inhibitors (leupeptin, L5793, phenylmethylsulfonyl fluoride, P7626, and pepstatin, P5318), and proteins (casein, C7078, bovine serum albumin, A 2153) produced by Sigma-Aldrich (USA), and technical equipment in the Core Facility of the Karelian Research Centre of Russian Academy of Sciences were used.



**Table 1** Characteristics of abiotic and biotic conditions of the sampling locations (according to Bakhvalova et al. 2016; Dorgham et al. 2018; Golovin et al. 2019; Ivanova et al. 2019)

Variable	Seldianaya Inlet	Koliushkovaya Lagoon	Sukhaia Salma Strait
Geographical coordinates	66°33'80.66" N, 33°62'25.16" E	66°31'32.62" N, 33°64'59.53" E	66°31'16.96" N, 33°64'73.70" 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
Stickleback food composition	Adults: stickleback eggs, Chironomidae, Polychaeta, and Amphipoda. Juveniles: copepods <i>Temora longicornis</i> and <i>Microsetella norvegica</i> , ciliophora <i>Helicostomella subulate</i> , Oligochaetae, and Orthocladinae	Adults: stickleback eggs, Gastropoda, Chironomidae, Polychaeta, and Amphipoda. Juveniles: strong dominance of cladocera <i>Acartia longiremis</i> , copepods, Oligochaetae, and Orthocladinae	Adults: Diptera, Amphipoda, Polyhaetae. Juveniles – no data
Predatory fish	Atlantic cod <i>Gadus morhua</i> , European sculpin <i>Myoxocephalus scorpius</i> , navaga <i>Eleginus navaga</i>	almost absent	Atlantic cod <i>Gadus morhua</i> , European sculpin <i>Myoxocephalus scorpius</i> , navaga <i>Eleginus navaga</i>

*Extracting calpains from tissues.* The total fraction of both soluble and membrane-bound calpains was extracted from stickleback organs via homogenization of samples in 20 mM Tris-HCl-buffer (pH 7.5), with the addition of 150 mM NaCl, 5 mM EDTA-Na, 20 mM dithiothreitol (DTT), 0.1% non-ionic detergent of the X-100 triton, and protease inhibitor cocktail (1 mM PMSF, 1 µg/ml leupeptin, 1 µg/ml pepstatin) in a 1:10 ratio (weight/volume), using a tissue homogenizer (TissueLyser LT, Qiagen, Germany) and a microcentrifuge (5417R, Eppendorf, Germany) operating at 20,000 g for 20 min. The resulting supernatant, or enzyme-containing fraction, yielded the proteases was studied.

*Analyzing the proteolytic activity of calpains.* The activity of calpains (EC 3.4.22.53), or the calcium-dependent caseinolytic activity sensitive to cysteine protease inhibitors, in the enzyme-containing fraction (Enns and Belcastro 2006) was tested. The reaction mixture with a total volume of 500 µl included 0.5 mg of protein substrate (alkali-denatured casein), 20 mM DTT, 200 µl of enzyme-containing fraction, and 2.5 mM CaCl<sub>2</sub> (Ca<sup>2+</sup>-dependent activity) or calcium ion chelator EDTA-Na (Ca<sup>2+</sup>-independent activity) in 50 mM Tris-HCl-buffer (pH 7.5). After 30 minutes of incubation at 28°C (CH-100 thermostat, BioSan, Latvia), the residual protein content in aliquots of 100 µl was determined using the Bradford method (Bradford 1976). A unit of calpain activity (AU) was defined as the amount of enzyme causing an increase in optical absorption at 595 nm by 0.1 OU during the reaction under the above conditions (measured with a CLARIOstar microplate reader, BMG Labtech, Germany). The specific activity of calpains was calculated per 1 mg of protein in the corresponding fraction.

*Analysis of protein content.* The concentration of soluble protein was determined following the Bradford (1976) technique using bovine serum albumin as a standard.

### Statistical processing

Raw data were initially checked for normality of distribution and homogeneity of variances using Kolmogorov-Smirnov and Levene's tests, respectively. Variables that did not have a normal distribution were log-transformed to fit the prerequisites of parametric tests. Multiple analysis of variance (MANOVA)



was used to evaluate the contribution of individual factors, such as the stage of the spawning period, sex, organ, spawning ground, and parasitic invasion, to the biochemical data. A multiple comparison post-hoc test (Tukey's HSD) was used to determine significance. In all analyses, results are provided as Mean  $\pm$  Standard Deviation and were considered significant when  $p < 0.05$ .

## Results

### Adults: life-history traits

At the beginning of the spawning period (28-30 May), stickleback density was highest in Seldianaya Inlet and lowest in Sukhaya Salma Strait; at the end of the spawning season, densities were much lower and more similar across the different sites (Table 2). The patterns of stickleback abundance and sex ratios observed during this field experiment were discussed in detail elsewhere (Demchuk et al. 2018; Dorgham et al. 2018; Golovin et al. 2019).

We found no significant differences in body mass and length among individuals of the same sex from different habitats (Table 2). Female average body mass and condition factor exceeded those of males at the beginning of spawning across all habitats. Female body mass decreased significantly by the end of the spawning season, evidently due to the release of eggs. Male body mass also decreased by the end of the spawning season in all locations, and this decrease was statistically significant in Koliushkovaya Lagoon. We observed no evident trend in total length changes during the spawning season for either sex.

**Table 2** Size indicators and the condition factor of adult stickleback from three sampling sites in 2016

Indicator	Sampling site, females / males		
	Seldianaya Inlet	Koliushkovaya Lagoon	Sukhaya Salma Strait
<b>Beginning of the spawning season, 28-30 May</b>			
Abundance, ind/m <sup>2</sup>	69.5 / 31.9	16.5 / 27.9	2.7 / 1.6
Total body mass, g	3.97 $\pm$ 0.72 / 2.82 $\pm$ 0.44	3.75 $\pm$ 0.50 / 2.92 $\pm$ 0.33 <sup>a</sup>	4.41 $\pm$ 0.72 / 2.81 $\pm$ 0.45 <sup>a</sup>
Total body length, cm	6.97 $\pm$ 0.57 / 6.48 $\pm$ 0.41	6.75 $\pm$ 0.49 / 6.64 $\pm$ 0.33	7.09 $\pm$ 0.33 / 6.34 $\pm$ 0.40 <sup>a</sup>
Condition factor	1.17 $\pm$ 0.16 / 1.04 $\pm$ 0.20	1.22 $\pm$ 0.09 / 1.00 $\pm$ 0.13 <sup>a</sup>	1.24 $\pm$ 0.13 / 1.10 $\pm$ 0.09
<b>End of the spawning season, 7-9 July</b>			
Abundance, ind/m <sup>2</sup>	4.2 / 1.6	0.9 / 0.4	1.3 / 2.4
Total body mass, g	2.99 $\pm$ 0.82* / 2.64 $\pm$ 0.39	2.89 $\pm$ 0.81* / 2.29 $\pm$ 0.15	3.16 $\pm$ 0.9* / 2.71 $\pm$ 0.33 <sup>a</sup>
Total body length, cm	6.73 $\pm$ 0.38 / 6.6 $\pm$ 0.4	6.62 $\pm$ 0.54 / 6.38 $\pm$ 0.26	6.85 $\pm$ 0.58 / 6.55 $\pm$ 0.26
Condition factor	0.98 $\pm$ 0.09* / 0.92 $\pm$ 0.11	0.99 $\pm$ 0.11* / 0.89 $\pm$ 0.13	0.98 $\pm$ 0.11* / 0.96 $\pm$ 0.03

Note: n = 10 in all samples, (a) differences are significant compared to females of the same sample, \*differences are significant compared to the start of the spawning season,  $P \leq 0.01$ , Tukey post-hoc test

**Table 3** Protein content (mg/g tissue) in the organs of adult stickleback from three sampling sites in 2016

Organ	Sampling site, females / males		
	Seldianaya Inlet	Koliushkovaya Lagoon	Sukhaya Salma Strait
<b>Beginning of the spawning season, 28-30 May</b>			
Liver	151.40 $\pm$ 10.51 / 106.10 $\pm$ 5.59 <sup>a</sup>	122.46 $\pm$ 4.61 / 129.00 $\pm$ 5.43	132.40 $\pm$ 11.32 / 109.00 $\pm$ 6.90 <sup>a</sup>
Gills	82.20 $\pm$ 4.24 / 75.92 $\pm$ 2.32 <sup>a</sup>	74.87 $\pm$ 0.72 / 79.00 $\pm$ 2.83	79.64 $\pm$ 8.67 / 78.40 $\pm$ 3.36
Skeletal muscle	58.00 $\pm$ 3.18 / 57.70 $\pm$ 1.76	47.02 $\pm$ 3.41 / 53.70 $\pm$ 2.93 <sup>a</sup>	62.13 $\pm$ 2.49 / 78.70 $\pm$ 5.16 <sup>a</sup>
<b>End of the spawning season, 7-9 July</b>			
Liver	89.16 $\pm$ 4.99* / 108.09 $\pm$ 6.40 <sup>a</sup>	110.20 $\pm$ 15.64 / 99.70 $\pm$ 4.07*	94.77 $\pm$ 6.02* / 122.83 $\pm$ 1.75 <sup>a*</sup>
Gills	65.09 $\pm$ 3.47* / 64.23 $\pm$ 2.19*	65.56 $\pm$ 1.35* / 62.15 $\pm$ 2.15*	62.61 $\pm$ 2.21* / 58.99 $\pm$ 3.20 <sup>a*</sup>
Skeletal muscle	40.29 $\pm$ 1.86* / 45.99 $\pm$ 1.41 <sup>a*</sup>	9.38 $\pm$ 0.69* / 33.24 $\pm$ 2.01 <sup>a*</sup>	27.33 $\pm$ 4.15* / 35.68 $\pm$ 1.24 <sup>a*</sup>

Note: n = 10 in all samples, (a) differences are significant compared to females of the same sample; \* differences are significant compared to the same site during the previous period,  $P \leq 0.05$ , Tukey post-hoc test.



**Table 4** Specific calpain activity (AU) in the organs of adult stickleback from three sampling sites in 2016

Organ	Sampling site, females / males		
	Seldianaya Inlet	Koliushkovaya Lagoon	Sukhaya Salma Strait
Beginning of the spawning season, 28-30 May			
Liver	37.75±11.70 / 52.48±11.97	36.60±6.12 / 19.72±6.97 <sup>b</sup>	33.92±9.27 / 55.49±21.64
Gills	62.64±18.68 / 26.98±4.23	13.17±5.20 <sup>a</sup> / 41.80±15.06	67.97±22.40 / 49.86±14.02
Skeletal muscle	38.76±11.43 / 51.11±6.68	21.40±6.09 / 40.14±11.10	37.17±11.39 / 39.65±10.51
End of the spawning season, 7-9 July			
Liver	52.92±26.4 / 13.6±6.91	29.65±10.23 <sup>c</sup> / 46.93±10.8	47.14±19.94 <sup>c</sup> / 35.76±5.52 <sup>c</sup>
Gills	40.35±13.01 / 55.35±13.01	47.01±18.49 <sup>c</sup> / 28.93±9.37	103.85±60.86 / 49.01±17.59 <sup>c</sup>
Skeletal muscle	58.97±14.43 / 36.97±8.2	160.05±28.6 <sup>a*</sup> / 37.87±10.23	126.87±47.47* / 148.92±61.66 <sup>b*</sup>

Note: Sample size is 5-10 females and 5-10 males per site at each period, (a) differences are significant compared to Seldianaya females; (b) differences are significant compared to Seldianaya males, (c) differences are significant compared to the skeletal muscle in the same sample for the same sex, \* differences are significant compared to the same site during the previous period,  $P \leq 0.05$ , Tukey post-hoc test

#### Adults: protein content and calpain activity

Notable tissue protein losses observed if compare pre- and post-spawning individuals reached 1.3 and 1.7-fold in gills and liver, respectively, and were particularly pronounced, up to 5-fold, in skeletal muscle (Table 3). The protein content differed significantly between the organs ( $p \leq 0.05$ , Tukey post-hoc test) at the beginning, but not at the end of the spawning season.

Analysis of calpain activity in the organs of fish from different spawning grounds showed that, at the beginning of the spawning period, the “Sex” and “Organ” factors did not correspond with this parameter (Table 4) whereas the “Spawning ground” factor showed a significant effect ( $F = 3.79$ ,  $p = 0.025$ , MANOVA). In this period, fish from Koliushkovaya Lagoon generally had the lowest levels of calpain activity and showed statistically significant differences in male liver and female gills ( $p \leq 0.05$ , Tukey post-hoc test) compared to fish from Seldianaya Inlet. By the end of the spawning season, differences among the habitats lost statistical significance, mainly due to a substantial (up to 7-fold) increase in calpain activity in the skeletal muscle of post-spawning fish. At the same time, the effect of the “Organ” factor became significant ( $F = 7.14$ ,  $p = 0.001$ , MANOVA). Specific calpain activation in the skeletal muscle was observed in Koliushkovaya Lagoon—where it was most significant for female fish—and in Sukhaya Salma, but not in Seldianaya Inlet.

Calpain activity at the beginning of the spawning period (28-30 May) was significantly lower than at the end (7-9 July), revealing the effect of the “Stage of Spawning Period” factor ( $F = 10.48$ ,  $p = 0.001$ , MANOVA). The main contribution to this difference is from the progressive differentiation of calpain activity in organs, which was low at the beginning of the spawning period and became statistically significant at the end (the effect of the “Organ” factor,  $F = 4.74$ ,  $p = 0.025$ , MANOVA). Time-dependent increase in calpain activity in skeletal muscle was observed in fish from Koliushkovaya Lagoon and Sukhaya Salma Strait, but not in fish from Seldianaya Inlet: this determines the differences between samples. We found no clear differences between males and females in terms of calpain activity.

#### Adults: parasite infection and its association with calpain activity

At the beginning of the spawning season, we found sporadic cases of the infection with metacercaria *Cryptocotyle* spp. (5% in Koliushkovaya and 15% in Sukhaya Salma) and visceral nematodes (15% in Seldianaya Inlet). No significant difference in the intensity of parasite infection was found among the habitats. By the end of the spawning season, visceral nematode infestations had increased in proportion to 70% in Seldianaya Inlet, 55% in the Lagoon, and 35% in Sukhaya Salma. *Cryptocotyle* spp. infestation also increased to 40% in Koliushkovaya and 10% in Sukhaya Salma. These differences, however, were not statistically significant. Comparing infected and uninfected stickleback in different habitats at the beginning and the end of the spawning period showed no effect of the “Parasite Infection” factor on the calpain activity in stickleback organs, despite the fact that parasite invasion occurred only among individuals from Seldianaya Inlet early in the spawning season, and rose from 35 to 70% of individuals from the different habitats post-spawning.



**Table 5** Density and body mass of stickleback juveniles from sampling sites in 2017

Date, studied parameter	Sampling site		
	Seldianaya Inlet	Koliushkovaya Lagoon	Sukhaya Salma Strait
Jul 31, body mass, mg	34.1±9.2	77.2±17.2 <sup>a</sup>	79.8±19.4 <sup>a</sup>
Aug 18, density, ind/m <sup>2</sup>	2300	50	0.8
Aug 18, body mass, mg	81.8±4.4 <sup>*</sup>	82.9±14.0	59.9±11.6

Note: n = 10 in all samples, (a) differences are significant compared to juveniles from Seldianaya Inlet collected on the same date, \* differences are significant compared to the sample from the previous period;  $P \leq 0.01$ , Tukey post-hoc test.

**Table 6** Calpain activity (AU) in stickleback juveniles from sampling sites in 2017

Date	Sampling site		
	Seldianaya Inlet	Koliushkovaya Lagoon	Sukhaya Salma Strait
Jul 31	78.1±16.03	114.23±18.38	169.8±22.15 <sup>b</sup>
Aug 18	169.7±32.16 <sup>a</sup>	159.6±33.51	107.23±24.89

Note: n = 10 in all samples, (a) differences are significant compared to the sample from the same habitat during the previous period, b differences are significant compared to juveniles from Seldianaya Inlet collected on the same date;  $P \leq 0.01$ , Tukey post-hoc test.

### Juveniles: life-history traits

Juvenile density in the sampling locations showed a high range of variation – from 2300 (Seldianaya) to 0.8 ind/m<sup>2</sup> (Sukhaya Salma) (Table 5). Juveniles also showed high heterogeneity in terms of inter-individual variation in mass (standard deviation approached 25% of the average body mass). There were also notable differences between the samples: juveniles from Seldianaya Inlet collected Jul 31 were significantly smaller than fish from the other samples, where no differences were observed.

### Juveniles: calpain activity

In juveniles sampled on Jul 31, calpain activity from Sukhaya Salma Strait and Koliushkovaya Lagoon fish was significantly higher than for fish from Seldianaya Inlet ( $p \leq 0.05$  compared with Sukhaya Salma Strait; Table 6). Later samples (Aug 18) showed no habitat-related differences in calpain activity.

## Discussion

In the White Sea, stickleback spawning occurs after adults migrate inshore from their wintering areas, presumably situated away from the shoreline. Therefore, stickleback migrates to spawning grounds from wintering areas where they likely mix and shoals approaching the spawning grounds should be homogenous in their population characteristics. Indeed, we found no spatial heterogeneity in size characteristics or condition factor at the beginning of the spawning season. This result is in agreement with our previous observations in the same area (Dorgham et al. 2018; Lysenko et al. 2018b).

After they arrive inshore, stickleback competes for high-quality spawning grounds on dense seagrass beds, which are very limited in the White Sea (Ivanova et al. 2016). The competition that redistributes fish among the spawning grounds maximizes heterogeneity in size and some morphological characteristics until the middle of the spawning season (Dorgham et al. 2018). During the spawning period, fish expend a lot of energy, particularly males competing for nesting territory and guarding their offspring. By the end of the spawning season, the exhaustion results in increased mortality among males and reduced condition factor in survivors, as we observe here and in previous studies (for estimations, see Demchuk et al. 2018). At the end of the spawning season, fish gradually leave their spawning area, thus reducing competition and spatial heterogeneity (Dorgham et al. 2018). Females actively feed and leave the spawning grounds first, whereas males stay longer to guard their progeny (Demchuk et al. 2018; Dorgham et al. 2018; Golovin et al. 2019).

Previous studies of the White Sea stickleback-parasite interactions on juveniles showed higher infestation by the endoparasites *Brachyphallus* sp. and *Bothriocephalus* sp. and lower loading of metacercaria *Cryptocotyle* sp. in Seldianaya Inlet compared with Koliushkovaya Lagoon (Rybkina et al. 2016). In our study, we found a similar pattern in adult stickleback infestations, although it was not statistically significant, likely due to the small sample size.



This is the first study of calpains in the threespine stickleback to show that the proteolytic activity of these enzymes in stickleback organs, such as the liver, gills, and skeletal muscle, is low in comparison with other fish species, such as Atlantic salmon *Salmo salar*, brown trout *S. trutta* and others (Kantserova et al. 2017; Lysenko et al. 2017). The absence of statistically significant differences between calpain activity in different organs is consistent with the concept of calpain as a “house-keeping protein” synthesized by any organism cell as inactive precursors and in excess (Goll et al. 2003), while their gene expression is regulated by hormonal and metabolic signals (Nemova et al. 2010). Drastic exhaustion of the protein pool in the tissues of spawning stickleback is obviously associated with calpain activation. Protein degradation is predictably more extensive in the skeletal muscle as a depot of structural proteins which could be readily degraded to maintain fish viability under starvation and energy losses. The contribution of calpains to skeletal muscle protein degradation in mammals and fish is significantly higher than in other organs due to the low availability of myofibrillar proteins for degradation by other proteases, including proteasomal and lysosomal-autophagic systems, as well as the common regulatory pathways of muscle physiology and calpain activity mediated by calcium (Goll et al. 2008; Nemova et al. 2010; Seiliez et al. 2014). In fish, the protein-degradation capacity of calpains is positively correlated with both the rate of myofibrillar protein metabolism and the growth rate (Nemova et al. 2010; Kantserova et al. 2017; Lysenko et al. 2017).

Differences found in calpain activity among stickleback samples at both the beginning and the end of the spawning season can be explained by either local environmental conditions (temperature in particular) or the redistribution of fish in the coastal zone as a result of competition for spawning grounds (see Dorgham et al. 2018 for evidence of such redistribution in the study area). It is interesting that no sex-related differences were observed in this study, although another study in the same area reported a higher mortality rate during the spawning period for males compared to females (Golovin et al. 2019). Such a difference is probably caused by the higher energy expenses of males resulting in exhaustion, tissue degradation, and bodyweight reduction observed in this study and in the previous research (Demchuk et al. 2018). Higher calpain activity in male muscles could be expected in this situation, but this is not supported by field observations and analyses of this study.

Calpain activation observed in the muscles of post-spawning fish, most significantly in females from Koliushkovaya Lagoon, suggests a specific role for protein degradation in switching the physiological status of fish (Mommensen 2004; Kantserova et al. 2017; Lysenko et al. 2018b). Although migration, spawning, and competition for nesting grounds may exhaust the fish, the protease-dependent utilization of muscle proteins maintains individual viability by providing substrates for oxidation. Another study performed on the same samples of stickleback showed that glutathione content and antioxidant enzyme activities, such as glutathione-S-transferase and catalase, also increase during the spawning period likely to compensate oxidative stress (Smirnov et al. 2019). Degradation of proteins results in an increase of water in the body which, according to Mommensen (2004), is important for maintaining the body mass and external shape.

In juvenile stickleback, the size structure and growth on different spawning grounds depends on a number of factors, such as time of spawning, the temperature regime during the embryonic and larval periods, feeding conditions, predation pressure, parasites, cannibalism, and migrations both in the coastal zone and to the open sea (Demchuk et al. 2015; Bakhvalova et al. 2016; Rybkina et al. 2016; 2017; Lajus et al. 2020). Offshore migration of juvenile stickleback in the study area takes place from late July to September at a body length of 20-24 mm (Lajus et al. 2020). It is almost impossible to quantify and control all the above-mentioned factors. Thus, we can offer only generic explanations of the high degree of juvenile heterogeneity within and among the samples we observed. In general, juvenile stickleback density in the study area follows a known pattern, i.e. with the highest density reported for Seldianaya Inlet and lowest for Sukhaya Salma Strait. In 2017, juvenile density in Seldianaya was close to the maximum observed for this location due to unusual temperature patterns: low temperatures during the first half of the spawning period, which delayed the spawning, and a sharp temperature rise in the middle resulting in simultaneous spawning of different fish and therefore in the appearance of larvae at about the same time.

In late July, Koliushkovaya Lagoon juveniles were larger than juveniles from Seldianaya Inlet probably because of higher temperatures in the Lagoon (see above) that can cause both early spawning and faster juvenile development. The intermediate body length of Sukhaya Salma Strait juveniles was due to the presence of several small individuals. This can be explained by the mixture of juveniles in this sample, which may include fish from the local spawning grounds and out-migrating fish from the Lagoon. Note



that the maximum weight of juveniles in this study, about 80 mg, corresponds to the length of out-migrants observed in previous studies (calculations are based on the equation linking body length and mass according to A. Demchuk, unpublished).

In August, juveniles do not manifest significant heterogeneity among locations and are similar in size with fish from Koliushkovaya and Sukhaya Salma sampled in late July. This confirms observations that individuals reached a threshold size, leave inshore areas, and are absent from the samples. Since then, the average size of juveniles remaining at the study sites is about the same. A similar pattern, i.e. greater similarity in the size of Koliushkovaya and Seldianaya juveniles in late August than in late July, was also observed in 2012 (Rybkina et al. 2016).

Determined by the morphology, sediments, and aquatic vegetation of the study sites as well as their degree of isolation from the sea, environmental heterogeneity in the study area is apparently the main factor determining the life-history traits and variation in biochemical characteristics of stickleback juveniles we sampled. A complex of factors affects juveniles directly and indirectly via changes in the biota of the spawning habitats. In Seldianaya Inlet, the percentage of copepods consumed by juveniles is higher than in the Lagoon (Rybkina et al. 2017), resulting in higher tissue content of reserve triacylglycerols, structural phospholipids, cholesterol esters, and physiologically significant fatty acids (Lajus et al. 2020), which may benefit fish welfare and viability (Pickova et al. 1998). Our study demonstrates the close association of calpain activity and growth rate in juveniles inhabiting biotopes that differ in growth-related resources. Data supporting habitat-related growth rate differences and the activity of growth-associated intracellular proteases have also been reported for salmonids inhabiting rivers of the White Sea basin (Kantserova et al. 2017; Lysenko et al. 2017).

## Conclusions

This study addressed variations in stickleback life-history traits estimated from fish density, size, and condition factor, and the contributions of endogenous physiological factors – such as organ metabolic function, sex, spawning period stage, or growth rate – and exogenous factors – such as parasitic invasion and habitat – to the spatial heterogeneity of calpain activity in adult and juvenile stickleback in the White Sea. We associate the increase in protein losses and calpain-dependent protein degradation in adult skeletal muscles at the end of the spawning season with the high energy demand of reproduction, which may trigger mobilization of energy substrates, like the amino acids released via the extensive hydrolysis of skeletal muscle proteins. The size heterogeneity of juveniles from different habitats apparently reflects the isolation of water bodies and their temperature differences, resulting in different spawning times, rates of embryonic and larval development, and composition of feed organisms.

Summarizing, we have found that, in threespine stickleback in the White Sea, muscle protein reserves and calpain activity manifest significant dynamics primarily associated with key periods in their life cycle. In adults, notable changes occur during the spawning season, and in juveniles during intense growth periods in the coastal zone. In turn, stickleback life-history timing depends on local environments resulting in spatial heterogeneity of fish by their physiological status, including muscle protein turnover. Further studies on phenotypic and biochemical variability at different life cycle stages should yield important information on the life cycle, ecological plasticity, and population dynamics of threespine stickleback in the White Sea.

**Conflict of interest** The authors declare that they have no conflict of interest.

**Authors' contribution** DLL and TSI designed the experiment and collected field samples. NPK and EDT carried out the biochemical studies. DLL, LAL, NPK, and NNN analyzed and interpreted the data. DLL and LAL drafted the manuscript. All authors read and approved the final manuscript.

**Acknowledgments** The authors thank N.V. Polyakova, A.Y. Bakhvalova, P.V. Golovin, and M.V. Ivanov for their assistance in collecting the field samples, the administration of St. Petersburg State University station “Belomorskaya” for the possibility of year-round scientific work on the White Sea, the White Sea Biological Station of the Zoological Institute Russian Academy of Sciences for providing data on water temperatures and Karen Alexander for help with English improvement.

**Funding** The contribution of LAL, NPK, EDT, and NNN was supported by the budget funding allocated for the theme no. 0218-2019-0076. Participation of DLL and TSI was supported by the Russian Science Foundation (project no. 19-14-00092).





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